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REARING REDBAY AMBROSIA BEETLE, *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE), ON SEMI-ARTIFICIAL MEDIA

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Abstract

Semi-artificial diets consisting of redbay (*Persea borbonia* (L.) Spreng.; Laurales: Lauraceae) sawdust and various nutrients were tested for rearing *Xyleborus glabratus* Eichoff (Coleoptera: Curculionidae: Scolytinae) in vitro. Comparison of 2 media, modified and standard, adapted from Biedermann et al. (2009) showed that the more solid consistency of the modified medium resulted in greater rates of successful brood production in cultures. A 2-layered medium structure with a nutrient rich lower layer and a nutrient poor upper layer proved to be superior to a single-layered structure. Using a 2-layered structure, 72.5% of foundresses successfully produced brood, which was similar to or greater than success rates of *X. glabratus* under natural field conditions. The most successful media recipes used finely ground wood from redbay, but some successful brood production also occurred when wood from pondberry (*Lindera melissifolia*) (Walter) Blume; Laurales: Lauraceae) and California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.; Laurales: Lauraceae) were used instead of redbay. A 2-layered structure with nutrient levels slightly higher than those in the modified medium of Biedermann et al. (2009) is recommended for rearing *X. glabratus* in vitro.

Key Words: laurel wilt, *Raffaelea lauricola*, redbay, *Persea borbonia,* avocado

Resumen

Las dietas semi-artificiales que consisten del aserrín del laurel rojo (*Persea borbonia* (L.) Spreng; Laurales: Lauraceae) y varios nutrientes fueron probados para criar el *Xyleborus glabratus* Eichoff (Coleoptera: Curculionidae: Scolytinae) in vitro. Una comparación de los 2 medios, uno modificado y el otro estándar, adaptado de Biedermann et al. (2009) mostraron que la consistencia más sólida del medio modificado resultó en una mayor tasa de producción de cría éxitosa en las crías del laboratorio. Una estructura de 2 capas de medio con una capa abajo rica en nutrientes y una capa arriba pobre en nutrientes demostró ser superior a una estructura de una sola capa. Al utilizar una estructura de 2 capas, 72.5% de fundadoras produjeron cría con éxito, que fue similar o superior a la tasa de éxito de *X. glabratus* en condiciones de campo naturales. Las recetas de los medios mas éxitosas utilizan madera del laurel rojo molida finamente, pero una producción exitosa de cría también se produjo cuando se utilizó la madera de Pondberry (*Lindera melissifolia*) (Walter) Blume; Laurales: Lauraceae) y del laurel de California (*Umbellularia californica* (Hook. & Arn.) Nutt; Laurales: Lauraceae) en lugar de laurel rojo. Se recomienda una estructura de 2 capas con los niveles de nutrientes ligeramente más altos que aquellos en el medio modificado de Biedermann et al. (2009) para la cría *X. glabratus* in vitro.

Palabras Clave: marchitez del laurel, *Raffaelea lauricola*, laurel rojo, *Persea borbonia,* aguacate

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Like other ambrosia beetles in the subtribe Xyleborina, the redbay ambrosia beetle, *Xyleborus glabratus* Eichoff (Coleoptera: Curculionidae: Scolytinae), lives within the sapwood of stressed or dying trees and feeds only on mutualistic fungi (Kirkendall et al. 1997), but unlike most other ambrosia beetles *X. glabratus* also attacks living trees and carries a lethal fungus. In the case of *X. glabratus* the primary mutualistic fungus is *Raffaelea lauricola* Harrington, Fraedrich

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and Aghayeva (Harrington & Fraedrich 2010; Harrington et al. 2010), the laurel wilt pathogen killing redbay, *Persea borbonia* (L.) Spreng. (Laurales: Lauraceae), and other members of the Lauraceae in the southeastern United States (Fraedrich et al. 2008; Fraedrich et al. 2011). This lifestyle makes observation of the beetle in its natural habitat difficult and also poses an interesting challenge for culturing ambrosia beetles because culture conditions must be suitable for both the beetles and their symbiotic fungi.

The first ambrosia beetle successfully reared in vitro was *Xyleborus ferrugineus* Fabricus (Saunders & Knoke 1967), which was reared on a sawdust and agar based medium in glass culture tubes. Since that time, a variety of ambrosia beetle species have been reared with some success including: *Xyleborus pfeili* Ratzberg (Mizuno et al. 1997; Mizuno & Kajimura 2009), *Xyleborus dispar* F. (French & Roeper 1972), *Xyleborus affinis* Eichhoff, *Xyleborinus saxesenii* Ratzeburg (Biedermann et al. 2009), and *Xylosandrus germanus* Blandford (Biedermann et al. 2009; Castrillo et al. 2012). While working with *X. saxesenii*, Biedermann et al. (2009) achieved a success rate of 7.2% using previously established media recipes. By modifying the medium with a much greater concentration of wood dust they increased the percentage of foundresses that produced brood to 23.9%, a rate similar to that for *X. saxesenii* females in wood under field conditions.

One of the major issues limiting success in laboratory cultures of Xyleborina beetles is contamination, usually by undesirable fungi or bacteria (Biedermann et al. 2009). These contaminants become established at the mouth of a culture tube and can rapidly spread down through the medium, overwhelming symbiotic ambrosial species. In response to this, Mizuno et al. (1997) and Mizuno & Kajimura (2009) tested a multi-layered medium structure to slow or prevent the spread of undesirable microorganisms. They initially used a 3-layered structure, which contained progressively more nutritional material, namely yeast and starch, as depth into a culture tube increased (Mizuno et al. 1997), but later Mizuno & Kajimura (2009) reported that a 2-layered structure was as effective for inhibiting the spread of undesirable microorganisms. Compared with the single-layered structure, the 2-layer medium increased the success rate of *X. pfeili* foundresses from 60% to 90%.

Laboratory cultures of *X. glabratus* would be useful for studies of life cycle details, host compatibility, climatic interactions, and mutualistic fungi relationships. Lab cultures also have advantage over field-based methods of study in that they are able to provide homogenous and consistent conditions. Here we describe a series of trials to develop an effective rearing technique for *X. glabratus*.

Materials and Methods

Trial 1

Two artificial media were adapted from Biedermann et al. (2009) for testing. The standard medium contained 0.35 g streptomycin (Sigma Chemical Co., St. Louis, MO), 1 g Wesson's salt mixture (MP Biomedicals, Solon, Ohio), 5 g brewer's yeast (ACROS Organics, Fair Lawn, New Jersey), 5 g casein (ACROS Organics, Fair Lawn, New Jersey), 5 g corn starch, 10 g sucrose (Fisher Scientific, Waltham, Massachusetts), 20 g agar (Difco, Detroit, Michigan), 2.5 mL wheat germ oil (Puritan Pride, Oakdale, New York), 5 mL 95% ethanol (AAPER Shelbyville, Kentucky), 500 mL deionized water, and 75 g redbay sawdust. Sawdust was prepared by first processing debarked redbay sections through a wood chipper and then grinding the wood chips in a Thomas-Wiley laboratory mill (Model 4, Arthur H. Thomas Co., Philadelphia, Pennsylvania) fitted with a 1 mm final screen mesh, but this was slow. In subsequent trials sawdust was made by repeatedly cutting debarked redbay sections with a 10 inch power mitre saw fitted with a general purpose blade. This resulted in wood dust in which 36.9 percent of particles were < 0.42 mm, 39.2 percent were 0.42-0.84 mm particles, and 24 percent were > 0.84 mm by weight. All ingredients were mixed thoroughly, and the media was autoclaved at 121 °C for 30 min. Immediately after autoclaving, the media were transferred to a sterile bench and poured into borosilicate glass (18 × 150 mm) culture tubes (Carolina Biological Supply Co., Burlington, North Carolina), which were then fitted with plastic caps.

The modified medium contained 0.35 g streptomycin, 1.25 g Wesson's salt mixture, 10 g casein, 5 g corn starch, 5 g sucrose, 30 g agar, 2.5 mL wheat germ oil, 2.5 mL peanut oil, 580 mL deionized water, and 200 g of redbay sawdust. The modified medium had to be packed rather than poured into culture tubes before autoclaving because of its more solid consistency. Cotton plugs were placed into the mouth of the filled tubes. A Pyrex glass dish (~20 × 30 × 5 cm) with a 2 kg weight added was set on top of the upright tubes to hold the plugs in place in order to prevent the modified medium from expanding out of the tubes while autoclaving. After autoclaving the tubes were transferred to a sterile bench where the cotton plugs were removed and the tubes were immediately covered with plastic caps. This first trial consisted of 33 tubes of modified and 35 tubes of standard medium.

All beetles used for this trial were reared in the laboratory from logs naturally infested in the field so they contained the natural complement of mycangial fungi. Rearing bins were made from black plastic file boxes (38 × 30 × 25 cm; Spacemaker™,

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Newell Office Products Co, Madison, Wisconsin) with 10 × 10 cm sections cut from the box lids, which were covered with cotton cloth, to allow moisture to evaporate. Six cm-diam holes were cut into the box bottoms near one end and similar diameter holes were cut into the jar lids of clear plastic collection jars. The lids were attached to the bottom of the boxes with small screws so that the holes in the lids and bins were aligned. Moist cotton was placed into each jar to keep emerging beetles hydrated, and the jars were fitted onto the lids to collect beetles as they emerged. A single LED bulb was fixed below each plastic jar, and the bins were otherwise kept in dark conditions. Boxes were filled with several short sections of redbay wood that was naturally infested in the field and emerging beetles were collected from the plastic jars 3-5 times per wk. Emergence of *X. glabratus* from logs stored in this fashion continued for up to 4 months. After collection, beetles were stored in a refrigerator at 4 °C for no more than 2 wk until introduction into culture tubes.

Both media were allowed to dry for at least 4 days before introducing beetles. Immediately prior to introduction, beetles were surface sterilized with 95% ethanol for 5 to 10 s and then rinsed with sterile deionized water to reduce potential contamination by non-mycangial fungi or bacteria. Rapid transfer of beetles from refrigeration to ethanol seemed to result in reduced mortality compared with beetles ethanol rinsed at room temperature. A small hole was scratched into the medium surface in each tube to facilitate beetle boring, and a single adult *X. glabratus* female was introduced head first into this hole. Only actively moving beetles were selected for use in the experiment. Tubes were wrapped in paper to exclude light and stored in a dark incubator at 24 °C. Introductions of beetles into tubes took place between 28 Nov 2010 and 3 Feb 2011. This extended time period was due to initially low live beetle availability. Full examination of tubes was performed by cutting off the bottom of culture tubes using a Dremel rotary tool (Dremel Co., Racine, Wisconsin) fitted with an abrasive cutting disc. The medium was then pushed out of the tube using a wooden dowel rod. The medium, which maintained the form of the culture tube (Fig. 1A), was dissected, and the numbers of live adult females, dead adult females, males, pupae, larvae, and eggs were recorded. Subsets of tubes consisting of equal numbers of each medium were opened in this manner at 2, 3, and 4 month intervals. Tube dissections were performed between 2 Feb 2011 and 13 May 2011. Maximum tunnel or gallery length was also measured. This measure did not account for multiple branches of the tunnels so it was only a relative estimate of gallery size. A successful gallery was one in which more than 2 mature adults were recovered. Gallery productivity was defined as the total number of adults, pupae, and larvae produced per culture tube. The number of eggs was not included because some may have been missed during gallery dissection due to their small size and lack of movement.

Trial 2

The second trial compared beetles reared from naturally infested logs in the laboratory, as described above, to those reared on artificial media and recovered from the culture tubes to determine if artificial rearing affected performance. All tubes used in this trial contained the modified medium. Beetles reared from logs were inoculat-



Fig. 1. A) Example of *in vitro* medium with *Xyleborus glabratus* galleries after removal from the glass culture tube. Several adult *X. glabratus* are observable in the galleries.

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ed into 39 tubes and beetles reared from semi-artificial media were inoculated into 38 tubes. Beetles were introduced into tubes on 22 Apr 2011 and 3 May 2011. Subsets of tubes were opened as described above every 2 wk beginning at 1 month post introduction. Otherwise all methods were the same as trial 1.

Trial 3

This trial was performed to test the effect of a 2-layered medium structure and increased quantities of starch and yeast, as well as the effect of wood from 2 other species in the family Lauraceae, i.e., California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) and pondberry (*Lindera melissifolia* (Walter) Blume), on beetle success and productivity. Medium A was the same as the modified medium used in initial trials, and other media were based on this recipe (Table 1). All tubes were prepared with sawdust from freshly harvested wood except for media E-dry which used redbay dust prepared from bolts harvested several months prior and stored at room temperature. Using sawdust from dry wood would be advantageous since it is becoming more difficult to find fresh redbay. Likewise, if other lauraceous woods are equally effective it would make medium preparation easier.

Media were prepared using the same methods as in the previous trial. In 16 of the 32 medium A tubes, 3 adult female *X. glabratus* were simultaneously introduced into each. In all other tubes a single female was introduced as before. All media were made on either 11 or 15 Dec 2012. Moisture content of fresh redbay, dry redbay, and pondberry samples were measured by cutting a small sample of each from bolts before they were processed into sawdust. Samples were weighed then dried in an oven at 40 °C for 48 h then weighed again. Moisture content of the samples was: 39% for fresh redbay, 9.1% for dry redbay, and 41.5% for pondberry. All tubes in this trial were stored in a dark cabinet at room temperature until they were dissected between 44 and 51 days after introduction of beetles.

Trial 4

Another trial was performed to test the effect of the 2-layered medium structure and increased quantities of starch and yeast on gallery success and beetle production. Media were prepared using the same methods as in the previous trial. Medium A was identical to the modified medium used in initial trials, and other media differed only as indicated in Table 2. After beetles were introduced into tubes, all 240 tubes were stored at room temperature in a 45 liter ice chest sterilized with bleach (5.95% NaClO) prior to the experiment to reduce contamination from mites and other fungi. Media were made on 23 May, 24 May, 30 May, and 6 Jun 2012. Beetles were introduced into tubes on 30 May, 5 Jun, and 11 Jun 2012. Half of the tubes of each medium were dissected 41-44 days after beetle introduction, another 10 of each medium were dissected from 68-69 days after beetle introduction, and the remaining tubes were dissected from 77-91 days after introduction. Upon dissection cultures were recorded as contaminated if bacteria or undesirable fungi were present in the galleries in amounts that seemed sufficient to negatively alter the quality of the culture.

Statistical Analysis

The SAS statistical software package was used for all analyses (SAS 1985). For the first 2 trials the Chi-squared test in the FREQ procedure was used to evaluate differences in success rates between media and foundress collection method, respectively. The t-test procedure was used to examine differences in productivity measured as total progeny (adults + pupae + larvae / tube) and gallery length.

TABLE 1. COMPONENTS OF THE DIFFERENT MEDIA TESTED IN TRIAL 3. MEDIUM A WAS IDENTICAL TO THE MODIFIED MEDIUM OF BIEDERMANN ET AL. (2009) USED IN INITIAL TRIALS AND OTHER MEDIA VARY FROM IT AS INDICATED.

Medium	Base material	Layers*	Amount of yeast	Amount of starch	N
A (1 ♀)	Fresh *P. borbonia* dust	Single	1x	1x	16
A (3 ♀)	Fresh *P. borbonia* dust	Single	1x	1x	16
B	Fresh *P. borbonia* dust	Single	3x	6x	32
C	Fresh *P. borbonia* dust	2	1x	1x	32
E	Fresh *P. borbonia* dust	2	3x	6x	32
E-dry	Dry *P. borbonia* dust	2	3x	6x	17
Calif. Bay	Fresh *U. californica* dust	2	3x	6x	37
Pondberry	Fresh *L. mellisafolia* dust	2	3x	6x	32

*In 2-layered media the lower 12 cm of each tube was filled with medium as indicated and a 1.5 cm layer of depleted medium that consisted of 75 g redbay dust, 220 ml di H_2O, 3 g sucrose, and 11 g agar was added above.

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TABLE 2. MEDIA USED IN TRIAL 4. COMPOSITION 1 IS IDENTICAL TO THE MODIFIED MEDIUM OF BIEDERMANN ET. AL. (2009) USED IN INITIAL TRIALS AND OTHER MEDIA VARIED FROM IT AS INDICATED.

Medium Composition	Layers*	Amount of Yeast	Amount of Starch	Amount of Sucrose	N
1	Single	1x	1x	1x	39
1	2	1x	1x	1x	40
2	Single	2x	2x	1x	41
2	2	2x	2x	1x	38
3	Single	4x	4x	3x	38
3	2	4x	4x	3x	40

*In 2-layered media the lower 12 cm of each tube was filled with medium as indicated and a 1.5cm layer of depleted medium that consisted of 75 g redbay dust, 220 ml di H_2O, 3 g sucrose, and 11 g agar was added above.

For trial 3 differences in overall female success rates were analyzed using logistic regression (PROC Logistic) to compare success probabilities among media types and least square means were separated using Tukey's HSD test. Differences in productivity among media were analyzed using Poisson regression in the generalized linear model procedure (PROC GENMOD). Because the data were over-dispersed (higher deviance from the model than expected), the "scale=deviance" option was used to correct it. Counts of offspring were adjusted so they were counts per female for medium tubes that had more than 1 female added. Tukey's HSD was used for means separation. Success rates and mean productivity are presented in the results.

Trial 4 success rates and rates of contamination of medium tubes were also analyzed using logistic regression. We compared success probability across media layers (1 or 2) and compositions which consisted of the 3 combinations of starch, yeast and sucrose used with both 1 and 2 layer media (Table 2). There was no significant interaction between layers and composition so the final models had no interaction term. Differences in productivity among all females were analyzed using Poisson regression. Like trial 3, the data were over-dispersed so the "scale=deviance" option was used to correct it. When only successful females were included in the analysis the total productivity data were normally distributed following log (x+1) transformation so they were analyzed using the GLM procedure and Tukey's HSD was used for means separation. Means and standard errors are presented in the results.

RESULTS

Trial 1

Only 3 of 34 introduced females produced adult brood in the standard medium, equating to a success rate of 8.8%, which was significantly lower ($\chi^2 = 6.370$, $P = 0.01$, df = 1) than the modified medium in which 33.3% of females produced offspring. Average tunnel length was also lower in the standard medium ($t = 4.36$, df = 66, $P < 0.0001$) with a mean of 18.5 mm compared with 60.9 mm in the modified medium (Table 3). Female productivity was higher on modified medium ($t = 2.72$, df = 66, $P < 0.008$).The maximum productivity from a single female on the modified medium was 34 mature females, 3 pupae, 9 larvae, and 5 eggs at the time of dissection while the maximum from a female on the standard medium was 10 mature females, 1 larva and 7 eggs.

Trial 2

Success rate ($\chi^2 = 0.7014$, $P = 0.45$, df = 1), productivity ($t = -1.00$, $P = 0.33$, df = 75), and tunnel length (t = -0.77, $P = 0.44$, df =75) were similar in modified medium tubes started with foundresses collected from naturally infested logs and those started with females collected from *in vitro* cul-

TABLE 3. RESULTS FROM TRIAL 1 COMPARING THE STANDARD MEDIUM AND MODIFIED MEDIUM OF BIEDERMANN ET. AL. (2009) FOR REARING *XYLEBORUS GLABRATUS*. SUCCESS RATE, PRODUCTIVITY, AND TUNNEL LENGTH WERE ALL SIGNIFICANTLY LOWER IN THE STANDARD MEDIA.

Medium	No. tubes	% Successful[a]	Total productivity per successful tube mean ± SE[b]	Tunnel length (modified medium) mean ± SE[b]
Modified	33	33	20.82 ± 4.63	60.91 ± 8.60
Standard	34	8.8	9.00 ± 1.53	18.51 ± 4.86

[a]Significantly different based on χ^2 test ($P < 0.01$).
[b]Significantly different based on T-test ($P < 0.05$).

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ture tubes. Nine females from logs were successful and produced an average of 13.3 (SE = 2.6) progeny/tube in 43.7 mm of gallery/tube (SE = 5.4), while 12 females from trial 1 modified medium cultures were successful and produced 14.6 progeny (SE = 2.1) and 50.5 mm of gallery/tube (SE = 7.0).

Trial 3

Females on media A, C and E had higher probabilities of successfully producing brood than those on the medium prepared using California bay laurel wood but not the other media tested (Fig. 2). Likewise, females in media C and E had significantly higher productivity than all other media types except A (Fig. 2). Some successful galleries were seen in media made with both of the alternate species of wood, pondberry and California bay laurel, but female productivity was low.

Trial 4

Overall, 111 of the 236 tubes in this trial successfully produced adult beetles. Layers had a significant effect on success and total productivity for all females combined (Fig. 3B) and for the subset of females that produced brood (Fig. 3A). Females in single layer media were less successful (38%) compared 2 layer media (55.9%; $P = 0.0041$). Females had a higher probability of success on media with lower nutrient levels (compositions 1 and 2) than those on media with the highest nutrient levels (composition 3) regardless of whether they had 1 or 2 layers ($P = 0.0012$). The highest rates of successful females occurred

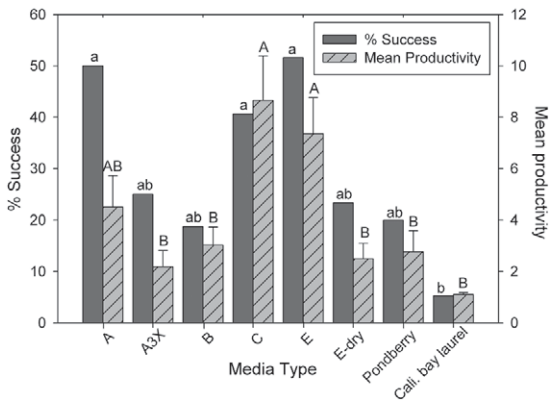


Fig. 2. Results for trial 3 comparing various media for rearing *Xyleborus glabratus*. Media details are provided in Table 1. Solid bars are the percent of females that produced brood by medium, and striped bars are mean (+SE) productivity (adults + pupae + larvae) per tube by medium. Columns with the same letters are not significantly different ($P > 0.05$).

on the 2-layered media. These had 72.5 and 60.5% likelihood of success for composition 1 and 2, respectively. The medium with the lowest likelihood of success was the single-layer medium with the highest nutrient composition (21.6%) which was significantly less than the other single-layer media that had lower nutrient levels.

The only variable found to be significant during the Poisson regression model selection process was layers. Females on 2-layer media had significantly higher ($P = 0.0003$) expected productivity (total adults + pupae + larvae) of 12.0 offspring (expected range 10.7-13.5) compared to single-layer media which had an expected productivity of 5.9 offspring (expected range 4.9-6.9). The composition or nutrient content of the media had no effect on productivity (Fig. 3B).

When only females that produced brood were included in the analysis (Fig. 3A), females on 2-layer media had significantly higher productivity than those on single-layer media ($P = 0.01$). Females on single-layer media averaged 16 offspring/female (SE = 2.76) while those on 2-layer media produced 21.9 offspring/female (SE = 2.69). Although chances of success were lower on the high nutrient medium (Fig. 3B, composition 3), females that were successful produced significantly more brood ($P = 0.0002$) on high nutrient media than females on the lower nutrient media in both single- and 2-layer media (Fig. 3A).

The 2-layer media were less likely to have visible or high levels of contamination at the time of dissection than the single-layered media (Fig. 4; $P < 0.0001$). All 2-layer media had less than 20% likelihood of visible contamination while single-layer media had 34-48% likelihood of contamination. The nutrient composition of the media had no significant effect on levels of contamination ($P = 0.81$).

Tubes dissected at different times had similar numbers of adults ranging from an average of 4.7 (SE = 0.49) at 41-44 days to 7.4 (SE = 1.35) at 68-69 days and 5 (SE = 1.52) adults at 77-91 days. Immatures (larvae+pupae) were highest in tubes dissected at 68-69 days (7.7 ± 2.18 immatures) and lowest in tubes dissected at 77-91 days (0.8 ± 0.34) indicating that holding tubes longer than 82 days would not increase yield much.

Of the 60 most productive tubes, 42 were 2-layered cultures, and these were evenly distributed between the three 2-layer media compositions. The greatest number of adults recovered from any tube was 63 (all females) from a tube containing 2-layer medium with an intermediate level (composition 2) of nutrients. The single most productive foundress produced 51 adult females, 1 adult male, 26 pupae, 63 larvae, and 28 eggs (169 total progeny) at the time of dissection on the 2-layer medium with the high nutrient composition. All male broods occurred in 4 tubes, and 4-5 adult male beetles were found in each of these. A

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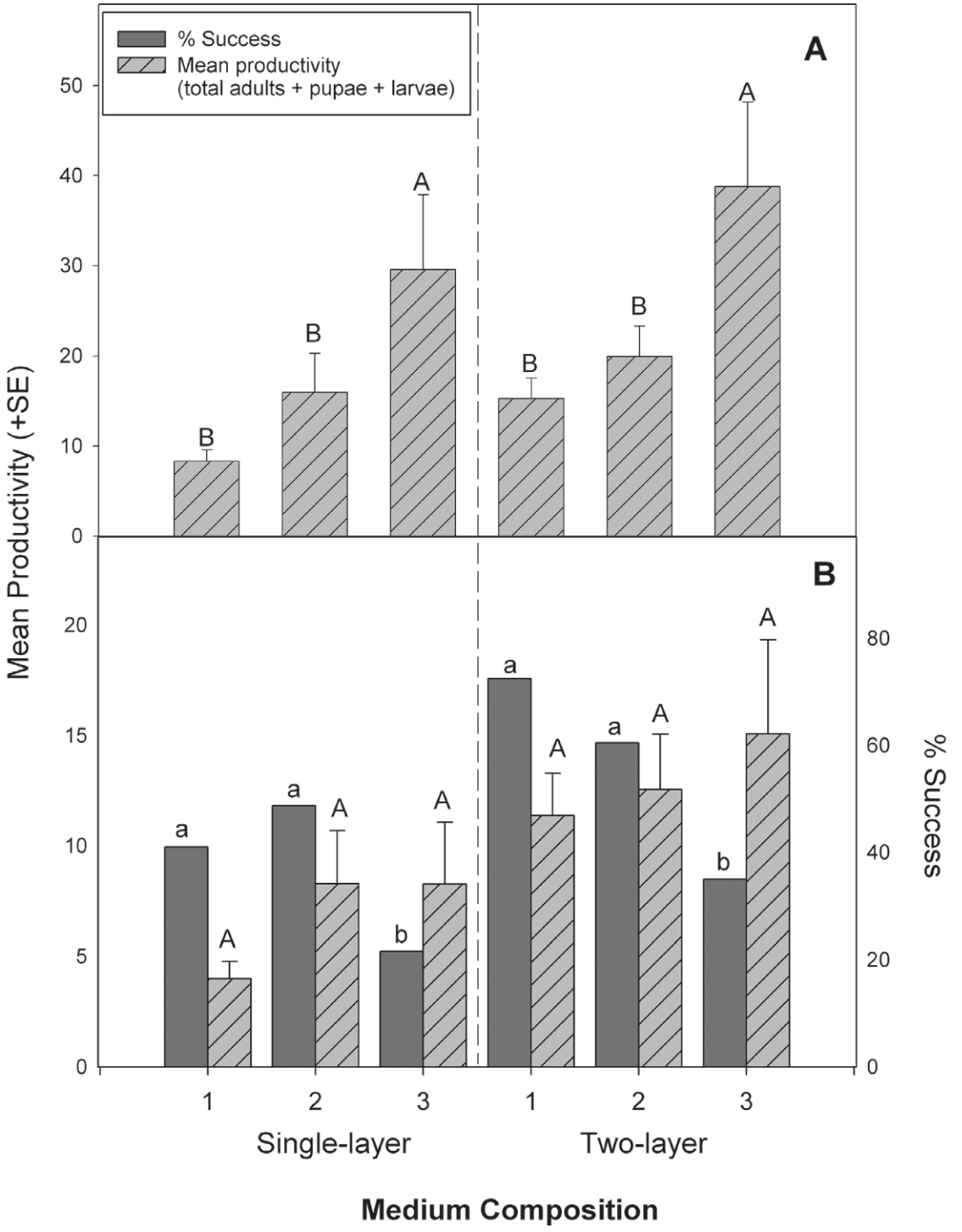


Fig. 3. Results for trial 4 testing various media (detailed in Table 2; i.e, amounts of yeast were 1-fold, 2-fold and 4-fold in 1, 2 and 3, respectively) for rearing *Xyleborus glabratus* considering only tubes where the female produced brood (A) or all tubes (B). In graph A, means within a layer type (single- or 2-layer) with same letter are not significantly different (P > 0.05). In graph B, solid bars are percentage of females that successfully produced brood and striped bars are mean total productivity/female. Success (solid bars) and productivity (striped bars), within a layer type, with the same letter are not significantly different ($P > 0.05$).

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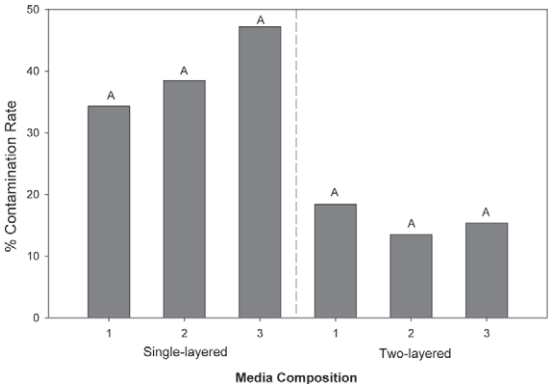


Fig. 4. Rates of visually observable microbial contamination of culture tubes of differing media composition for trial 4. See Table 2 for media composition details. Means within a layer type (single or 2 layer) with the same letter are not significantly different ($P > 0.05$).

total of 1,155 adult females and 129 adult males were recovered from the culture tubes in trial 4. In addition to the beetles recovered from individual culture tubes, 241 females and 48 males had escaped from the tubes and were found loose within the ice chest. Therefore, the total number of adults was 1,396 females and 177 males (8:1 female: male).

Discussion

Female tunneling activity often occurred along the walls of the culture tubes making observation of the colony possible without disturbing the culture media (Fig. 1). Eggs were usually laid individually or in small clusters (≤ 10 eggs) near the end of a short tunnel branching from 1 of the primary tunnels, or simply within a primary tunnel. Galleries excavated larger than the tunnel diam were rare. Galleries consisting of simple tunnels all the same diam are common to *Xyleborus* spp. but contrast with the disc-shaped brood chambers often created by other Xyleborini genera (Biedermann et al. 2009).

Mature adults were usually produced within 30 days following introduction of a female, and all adults found at this time except the foundress were teneral adults. Live adults along with eggs and larvae were found within tubes dissected up to 106 days after initiation. In some cases the introduced females were found alive and boring throughout the media for as long as 90 days without depositing any eggs and, in these cases, very little fungal growth was seen within the tunnels. This is consistent with the behavior of other ambrosia beetles that will not oviposit until a successful ambrosia garden has been established (Peer & Taborsky 2007). Females of other ambrosia beetle species are known to guard the entry point of a gallery with their bodies (Biedermann et al. 2009), but this behavior was not seen in *X. glabratus* in culture.

The initial trial testing the standard medium versus the modified medium showed that the more solid modified medium gave better results. This could be because the modified medium more closely resembles actual wood and therefore better simulates the beetle's natural habitat. Rearing beetles in media did not affect their productivity, at least after 1 generation, since they were just as productive as those collected from naturally infested wood.

Keeping cultures free of contamination proved to be a critical part of successfully rearing *X. glabratus*. Mites in particular resulted in many culture failures. Another factor that caused some difficulty was keeping the media from overflowing the tubes during autoclaving. The solid consistency of the modified medium prevented it from being poured into tubes; so, it had to be packed in by hand using a wooden dowel prior to autoclaving. The media expanded upon heating, and greater concentrations of starch and yeast seemed to result in greater expansion. However, tubes could be autoclaved while capped without cracking which aided in keeping the expansion of the media under control. Although none of our tubes broke, it is possible they could so we suggest placing the tubes in autoclavable bins and covering them with a cloth during autoclaving and cooling to contain the glass in case tubes break.

The ability of beetles to escape underneath the tube caps was a problem. Caps could be sealed with Parafilm or wax, but beetles would likely chew through it. Cork, rubber, or cotton stoppers could be used instead of caps, and this has been done in other studies (Saunders & Knoke 1967; Mizuno et al. 1997; Mizuno & Kajimura 2009). Another option is to use a fine mesh screen beneath the plastic caps to allow some air exchange and prevent beetle escape (P. H. W. Biedermann, personal communication). This behavior of the beetles to leave the tubes can be taken advantage of by attaching emergence cages to the tubes and collecting beetles as they emerge. Previous researchers (Saunders & Knoke 1967) collected beetles from culture tubes by inverting the tubes en masse over a collection tray or they could be placed into a rearing container similar to that used for rearing beetles from logs.

Trial 4 resulted in successful brood production by 47% of the females when considering all media and 72% for the 2-layer medium with the lowest nutrient composition (Fig. 3). This is similar to or possibly better than the roughly 50% successful gallery formation rate seen for *X. glabratus* naturally infesting trees in the field (Maner et al. in press). Biedermann et al. (2009) achieved *in vitro* success rates for *X. saxesenii* of about 20%

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TABLE 4. RECOMMENDED RECIPE FOR REARING THE REDBAY AMBROSIA BEETLE, *XYLEBORUS GLABRATUS IN VITRO*. THE RECIPE PRODUCES ENOUGH MEDIUM TO FILL APPROXIMATELY 36 CULTURE TUBES (18 × 150 MM).

Ingredient	Lower layer (12 cm)	Depleted upper layer (1.5 cm)
Streptomycin	0.35 g	0 g
Wesson's salt mixture	1.25 g	0 g
Yeast	8 g	0 g
Starch	8 g	0 g
Casein	10 g	0 g
Agar	30 g	5.5 g
Sucrose	5 g	1 g
Wheat germ oil	2.5 mL	0 g
Peanut oil	2.5 mL	0 g
95% ethanol	5 mL	0 g
Redbay sawdust	200 g	38 g
Di H_2O	580 mL	110 mL

which was similar to the rate of successful gallery formation for that species in the field. Both Weber & McPherson (1983) and Biedermann et al. (2009) reported success rates for *X. germanus* to be about 30%. Castrillo et al. (2012) also reared *X. germanus* on artificial media based on wood from several different host trees achieving success rates of about 70%. They found gallery productivity but not success rates varied with species of wood. *Xyleborus dispar* was reared by French & Roeper (1972) who found that 47% of introduced beetles successfully produced progeny on a media based on α-cellulose rather than wood dust. *Xyleborus ferrugineus* and *X. affinis* have both been reared with success rates of up to 90% (Saunders & Knoke 1967; Biedermann et al. 2009). Therefore, the results achieved here for *X. glabratus* are within the range of success rates reported for in-vitro production of ambrosia beetles in other studies.

Two-layered media similar Mizuno & Kajimura (2009) resulted in relatively high *X. glabratus* success rates and productivity with less contamination. Increasing nutrient levels did not increase productivity and the highest level of nutrients reduced female success, but if females were successful on them they produced more brood. We suggest that an appropriate media recipe for rearing of *X. glabratus* would be a 2-layer medium with intermediate levels of nutrients. Based on our results a recommended recipe for rearing *X. glabratus* in vitro is given in Table 4.

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