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Development of Western Spruce Budworm on Douglas-fir Callus Tissue

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Abstract

The success of feeding and development of western spruce budworm *(Choristoneura occidentalis* Freeman) on callus tissue of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) was determined. Fewer insects died when fed pure callus tissue than when fed on standard diet or callus incorporated into the standard diet. The final weight of insects fed callus was less, however, than that of insects fed the standard diet. This demonstrates that either pure callus or callus incorporated in a standard artificial diet can be used to rear budworm. This technique has potential as a bioassay system to determine host susceptibility or resistance to insects.

Keywords: Callus tissue, western spruce budworm, *Bacillus thuringlensis,* bioassay, Douglas-fir.

Introduction

Rearing insects on callus diets is useful for several reasons. Callus tissue has been used to study susceptibility or resistance of host genotypes (Williams and others 1985,1987). The promise of introducing new resistant genes by genetic engineering will require insect bioassays for levels of resistance. In many gene-insertion systems, it is easier to produce transgenic callus than regenerated plants; for example in Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco), callus containing introduced marker genes has been produced (Dandekar and others 1987); however, no transgenic plants have been reported.

Insertion of foreign genes that code for novel toxins into plant genomes shows promise in controlling insect pests. The procedure has, so far, been successful on herbaceous plants such as tomato and tobacco (Barton and others 1987, Fischoff and others 1987, Vaeck and others 1987). In these species, the toxin-coding gene from *Bacillus thuringiensis* (Bt) has been introduced into the plant genomes and been shown to inhibit certain lepidopterous pests; for example, tobacco hornworm *(Manduca sexta* (L.)), tobacco budworm *{Heliothis virescens* (F.)), and the corn earworm *(Heliothis zea* (Boddie)).

Development of a feeding bioassay to monitor Bt gene expression in plant tissue and its effects on the insects is prerequisite to developing resistant trees. The gene coding for the toxin could be introduced into tree cells by any of several methods, and these cells could be cultured to form callus. The callus might then be fed directly to insects as has been done with herbaceous plants (Williams and others 1985, 1987) or be incorporated into artificial diets (Isenhour and Wiseman 1988). Defoliating Lepidops, to our knowledge, have not been reared on the callus tissue of their coniferous hosts.

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In another group of insects (bark beetles), success on host callus has varied (Mott and others 1978, Cook and others 1990). When regenerated transgenic plants eventually are available, entire plants or fresh foliage could replace callus tissue for bioassay.

This paper evaluates the suitability of rearing western spruce budworm *(Choristoneura occidentalis* Freeman) larvae on callus tissue derived from Douglas-fir cotyledons. We found that western spruce budworm larvae can be successfully reared on either pure callus or callus incorporated into an artificial diet.

Materials and Methods

The western spruce budworms used in the study were obtained from a disease-free laboratory colony of strain COC-FS-01 maintained for more than 30 generations at Corvallis. The larvae were reared from second instars (after emerging from diapause) to adult emergence by using a modified diet incorporation technique (Dulmage and others 1976) as one of the treatments.

Callus was produced from cotyledon explants taken from Douglas-fir seedlings 2 to 3 weeks after germination. The cotyledons were surface-sterilized in 0.525 percent sodium hypochlorite for 30 minutes and rinsed three times in sterile, double-deionized water. The tip portions were removed with a sterile scalpel and the remaining explants placed on about 25 milliliters of callus-forming medium in 100- by 15-millimeter plastic petri dishes. The medium contained the mineral salts and vitamins, as described by Schenk and Hildebrandt (1972), plus 0.1 grams/liter myo-inositol, 30 grams/liter sucrose and 8 grams/liter agar. In addition, 5.0 micromolar alpha-naphthaleneacetic acid (NAA) and 0.5 micromolar benzylaminopurine (BA) were added, and the pH adjusted to 5.6 before autoclaving. Cultures were maintained under cool white fluorescent light (40-60 micromoles per square meters per seconds) and subcultured every 4 to 6 weeks. All callus had been in culture for 2 to 8 months before use.

To incorporate the callus into the standard diet (Lyon and others 1972), the callus tissue was macerated in a microblender and mixed with the diet just before gelling. The amount of fresh tissue added was based on the dry weight of the tissue versus the dry weight of the diet ingredients from the regression

$$y = 0.715 + 0.098901x;\ r^2 = 0.94.$$

The regression was developed by weighing 25 pieces of fresh callus ranging in fresh weight from 144 to 691 milligrams, oven-drying at 65 °C for 48 hours, and reweighing the dried material. Insects were fed one of the following diets:

1. 0 percent callus (standard diet)
2. 27 percent callus (27 percent dry weight of callus/dry weight of diet)
3. 100 percent callus

The 100-percent callus .treatment was achieved by placing small-pieces-of callus on the callus-forming medium containing 15 grams/liter agar. This higher agar concentration was necessary to prevent excess .water from hindering larval movement, feeding and survival

The larvae were randomly assigned to the test diets within 16 hours of emergence from hibernacula. Fifty larvae per treatment were reared individually in small petri dishes. Insects that died during the first 24 hours were replaced. The dishes were maintained at 26 °C in a standard environmental cabinet. Relative humidity was near the saturation point because of the moisture content of the diet. All dishes were examined daily to determine mortality and pupation date. All pupae were weighed to the nearest milligram 24 hours after pupation to compensate for initial hardening and to prevent damage. Larval development times and pupal weights for all test animals successfully completing emergence were analyzed by ANOVA; significant differences were determined by Tukey's w-procedure (Steel and Torrie 1960) and using SAS (SAS Institute Inc. 1987).

Results and Discussion

Mortality during rearing ranged from 6 percent on pure Douglas-fir callus tissue to 28 percent on the callus and diet mixture (table 1). Most of the mortality occurred during the last instar or the pupal stage. The three larvae that died when fed pure callus tissue did so in the last instar. Mortality in the pupal stage usually occurred during adult emergence.

Mean larval development times ranged from 18.4 to 20.2 and 20.7 to 23.4 days for males and females, respectively (table 2). The ranges were similar to those obtained in a previous experiment on laboratory temperatures.[1] For both sexes, the larvae reared on the callus and diet mixture took significantly longer ($P < 0.05$) to develop than those reared on the standard diet or on the pure callus tissue.

Mean pupal weights ranged from 90.4 to 119.1 and 134.6 to 176.5 milligrams for males and females, respectively (table 2). Male pupae were significantly heavier ($P < 0.05$) when raised on the standard diet than on the pure callus tissue or on the diets containing the callus tissue. Female pupae were significantly lighter ($P < 0.05$) when fed pure callus tissue. The heaviest female pupae were produced when fed the standard diet, but the weights were not significantly different from those of pupae fed the diet including callus tissue.

Our data showed that either pure Douglas-fir callus tissue or tissue that has been incorporated into an artificial diet can be used to successfully rear western spruce budworm larvae. The low level of mortality that occurred during development from the third to fifth instars indicated that these would be appropriate stages for bioassays of transgenic callus tissue. Increased development time and reduced insect weight may be more sensitive measures of toxicity than direct insecticidal activity.

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[1] Unpublished data on laboratory rearing of the western spruce budworm. On file with: R.C. Beckwith, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, Oregon 97331.

Table 1—-Survival and larval development of western spruce bud-worm reared on Douglas-fir callus tissue

Treatment (percent callus)	Number started	Proportion of total insects in each category		
		Died	Pupated	Emerged
0	50	0.18	0.94	0.82
27	50	.28	.86	.72
100	50	.06	.94	.94

Table 2—Mean larval development and pupal weights of western spruce bud-worm raised on various percentages of Douglas-fir callus tissue

Sex	Callus tissue	Number of insects	Mean larval deviation time[a]	Standard deviation	Mean pupal weight[a]	Standard deviation
	Percent		*Days*		*Milligrams*	
Male	0	21	18.7a[a]	1.5	119.1a[a]	12.0
	27	25	20.2b	1.5	98.1b	20.0
	100	19	18.4a	1.5	90.4b	8.7
Female	0	21	20.7a	1.5	176.5a	23.2
	27	11	23.4b	1.6	163.5a	18.1
	100	27	21.4a	1.9	134.6b	10.0

[a] Means followed by the same letter within sex are nor significantly different (Tukey's w-procedure) ($P > 0.05$).

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