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Leafy spurge cell culture as the base of an artificial diet for *Aphthona flava*

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Introduction

Aphthona flava is currently one of the insects being most actively studied as a biological control agent for leafy spurge. It has been released in field sites in five Canadian providences as well as in the United States (Harris *et al*., 1985). The adults feed on the leaves of leafy spurge, and the larvae mine the primary and secondary roots and the root hairs, thereby disrupting the vascular tissues and depleting the carbohydrate reserves of the plant (Leafy Spurge News, 1989). APHIS and the USDA-ARS are cooperating on a project to develop an artificial diet for the mass rearing of the insect. This approach is an alternative to expensive and time-consuming collection of the insects from their native habitat in Europe and their subsequent processing (to remove parasites and diseases) in quarantine facilities. Successful mass rearing in the laboratory could provide the numbers of insects required for large releases in spurge-infested rangelands.

Background research

Initial experiments on the development of an artificial diet were done last year at the Western Regional Research Center in Albany, California and reported on by Dr. Gary Manners at the Leafy Spurge Symposium in Rapid City last July. Briefly, eleven established diets for chrysomelid beetles did not support growth of *Aphthona flava* larvae. Chemical extracts of leafy spurge roots or leafy spurge root powders incorporated into an agar base were also completely ineffective in supporting growth or maturation of the larvae (Tables 1 and 2).

Table 1. Effect of extracts of leafy spurge on survival of larvae of *Aphthona flava.**

EXTRACT	YIELD (% dry wt.)	SURVIVAL (Mean no. of days)
HEXANE	10.9	1.6[a]
ACETONE	1.4	1.4[a]
METHANOL	4.4	1.8[a]
WATER	0.6	0.8[a]
HEXANE + ACETONE		0.6[a]
METHANOL + WATER		0.6[a]
CONTROL DIET		3.8[b]

*2nd Instar Larvae, 10 replicates/treatment of 2 larvae/container.

Table 2. Effect of leafy spurge root powders in agar* on survival of larvae of *Aphthona flava*.

CONC. (mg root powder/ml)	SURVIVAL (Mean no. of days)
100	4.2[a]
50	3.8[a]
10	3.0[a]
5	2.2[b]

*Freeze-dried secondary roots in 1.7% agar.

Significant survival of first instar larvae was observed last year on freeze-dried leafy spurge roots over the course of one month (Fig. 1). Preliminary experiments with vacuum-filtered fresh suspension culture cells of leafy spurge tissue showed similar duration, although a lower percentage of larval survival when fed unammended cultures. This was the point in time at which molting of the head capsule and entrance into the second instar stage was expected, but never observed. The larvae did eat the suspension culture cells and grew to be 3-5 times the length of the neonates.

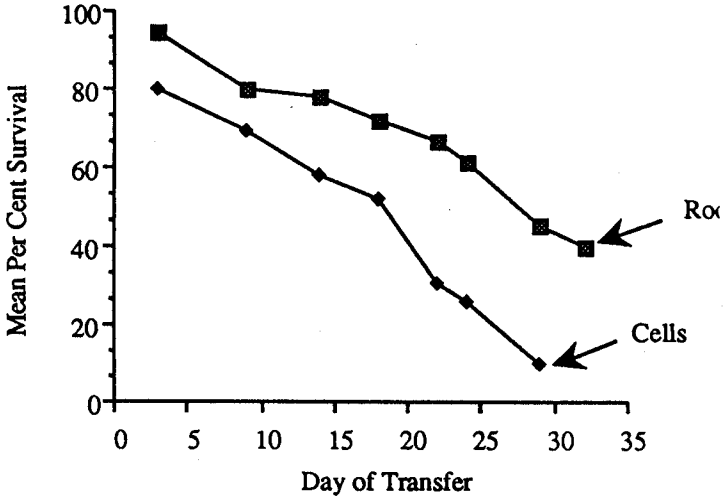

Figure 1. Survival of larval transfers of *Aphthona flava* on freeze-dried leafy spurge roots vs. cell suspension cultures.

Current research

Preliminary results this season show that larvae will also feed on freeze-dried (lyophylized) cell suspension cultures, presented on 0.8% agar containing 0.5% chloramphenicol. The protocol we have devised provides sterile freeze-dried cells, which are subsequently spread on 9 cm chloramphenicol agar plates. The agar provides enough moisture for the cells to rehydrate themselves sufficiently, without becoming so wet that the neonates drown. This limited moisture environment reduces the possibility of fungal contamination. The eggs are sterilized in 1% bleach, followed by 5% sodium thiosulfate and sterile water. Hatched larvae (50-72%) are transferred to fresh cells every three to five days.

Good survival rates have been obtained using the protocol described. Larvae can be maintained on a nutrient-rich, sterile environment in which they burrow, feed, and grow. Larvae survive 20-30 days on unammended freeze-dried leafy spurge suspension culture cells. The latest experiments have been aimed at defining a potential unknown factor(s) responsible for inducing larval molt. Amendment experiments have been set up using commercially available insect molting hormone (20-hydroxyecdysone) and chemical extractives from fresh leafy spurge roots. These amendments are added back to the freeze-dried cells at known concentrations and the effect on larval feeding and survival is observed.

Thus far, survival and significant growth has been observed on 20-hydroxyecdysone, presented at up to 10 ppm in freeze-dried cells. The oldest larvae have survived for 30 days on this treatment, but no molting has yet been observed.

Cell-cultured based diet

The current focus of the project is to develop a successful artificial diet for *A. flava* using cell suspension cultures of the host plant tissues as the basic dietary constituent. This approach differs in a number of ways from the development of a classical empirical insect diet (Table 3):

Literature cited

Harris, Peter, Paul H. Dunn, Dieter Schroeder, and Ronald Vonmoos**.** 1985**.** Biological Control of Leafy Spurge in North America. *In:* Leafy Spurge. Monograph Series of the Weed Science Society of America, Number 3, Alan K. Watson (editor), Chapter 8, pg. 79-92.

Leafy Spurge News. Volume XI, Issue 1, March 1989. Russell Lorenz (editor), (insert on Biological Control of Leafy Spurge - pg. 1 of 2).

Table 3. Comparison of classical vs. cell culture-based insect diets.

	Classical Diet		Cell Culture-Based Diet
1)	usually in an artificial support base (i.e. agar blocks). *A flava* will not burrow into agar.	1)	uniform, natural environment. Allows larvae to follow burrowing instinct.
2)	more labor intensive to produce large amounts and not as easy to extract for chemical analysis.	2)	technology in place to generate large quantities.
3)	based on nutrient requirements for generalist feeders.	3)	may be chemically analyzed for phagostimulants attractive to specialist feeders such as *A. flava*.
4)	artificial system - no means to examine nutritionally important products of plant metabolism.	4)	living system - can incorporate precursors to important nutrients and determine effect of metabolism on available compounds.
5)	introduction of phagostimulants by trial-and-error experiments.	5)	chemical nature of cells may provide clues as to phago stimulants. Optimization of the levels of these compounds may provide a more effective diet.
6)	eleven formulations have failed to support *A. flava* larvae.	6)	a novel approach, with positive data from initial experiments.