

COMPARISONS OF ELEMENTAL PROFILES OF THE WESTERN
SPRUCE BUDWORM^{1/} REARED ON THREE HOST FOLIAGES
AND ARTIFICIAL MEDIUM.

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Western spruce budworm were reared on three host
foliages and artificial medium. Trace element
analyses showed large differences in elemental
concentrations between food sources and only
minor differences between insect life stages.
Discriminant analyses were carried out to test
the distinctiveness of adult chemoprints from
each rearing regime. Fe, Cu, and Zn were
distributed differentially between egg mass and
other parts of the female moths. Females reared
on artificial medium weighed more than
foliage-fed females.

Introduction

The usefulness of a trace element
"chemoprint" of an insect, as determined by
simultaneous multi-elemental analytical
techniques such as X-ray energy-dispersive
spectrometry (XES) (Bertin 1978), has relied on a
basic assumption that the elemental profile of an
insect reflects its host plant and thus
indirectly the soil and bedrock geology. The
western spruce budworm, Choristoneura
occidentalis Freeman, has been shown to have a
sufficiently distinctive area specific chemoprint
to distinguish adults from two stands 2.6 km
apart (McLean et al. 1979). In addition,
considerable among tree variation was also
reported. The objective of this study was to
determine if there was significant variation
between the chemoprints of western budworm reared
on three host foliages, Douglas-fir (DF),
Pseudotsuga menziesii (Mirb.) Franco, Engelmann
spruce (ES), Picea engelmannii Parry, Grand Fir
(GF), Abies grandis (Dougl.) Lindl.,

^{1/} Choristoneura occidentalis Freeman Lepidoptera:
Tortricidae)

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and artificial medium Bio-Mix #9769 .
Quantitative data were developed in this study to
overcome legitimate criticisms raised on the
reliability of elemental assignments in earlier
studies of salmon, snowgeese and ambrosia beetles
using X-ray energy spectrometry (Bowden et al.
1979).

Methods

Fourth instar western spruce budworm (WSBW)
larvae were collected on hand clipped from
infested trees at Oregon Jack Creek, British
Columbia, in June 1978. These larvae were
laboratory-reared on new DF, ES and GF foliages
as well as artificial medium. The DF and GF
foliage were collected fresh from the U.B.C.
Endowment Lands as required. The ES foliage was
collected from Rhododendron Flats in Manning Park
B.C. and stored at 3°C until required. Samples
of new (less than 4 months old) and old (more
than a year old) foliage, male and female WSBW
pupae and adults of both sexes were collected
from each rearing regime and freeze dried.

A subset of larvae were assigned randomly
to one of the four rearing regimes and reared
individually through their fifth and sixth
instars. Weight measurements were taken within
24 hours of moulting. Adults were allowed to
mate in pairs, egg counts were made, and
percentage hatch determined.

Samples for XES analysis were mixed with
Somar Mix^{4/} in a ratio of 2 parts sample: 1
part Somar-Mix and thoroughly ground in a
SPEX^{5/} mill for five minutes. Two 60 mg self-
supporting 13 mm diameter pellets were made in a
KBr die at a pressure of 1000 kg. Quantitative
programs were developed using standard addition
techniques on bulked DF foliage and female WSBW
material. Checks were made against U.S.
National Bureau of Standards (NBS) orchard leaves
(# 1571) and NBS bovine liver (# 1577).

All XES analyses were carried out by
irradiating each pellet with Mo-filtered X-rays
produced by a Mo X-ray tube at 35 keV and 0.2 Ma
for 400 sec. The count rate of the emitted
X-rays was 20K cps and they were detected by a

^{3/} Bioserv Inc. Frenchtown, N.J.

^{4/} Somar Laboratories Inc., New York, N.Y.

^{5/} SPEX Industries Inc., Metuchen, N.J.

Si-Li drifted detector which had a 185 eV resolution at 5.895 keV. The general apparatus has been described by D'Auria and Bennett (1975).

Three pellets of pure Somar-Mix were also analysed with each group of samples to provide an average "background" spectrum. This was proportionalized to the analyte spectrum and subtracted to produce a spectrum with a minimum of background. Regions of interest were defined on this background subtracted spectrum to obtain the number of counts for each element being considered. Each region of interest was expressed as a proportion of the Compton backscatter peak (before background subtraction) of the analyte spectrum for derivation of the quantitative proportionalities. A detection limit was assigned at the point where the negative confidence interval ($P = 0.05$) of the calibration curves intersected the X-axis, i.e. the point where the normalized variable computed from the XES spectrum was equal to the confidence interval.

Samples of artificial medium, new and old foliage, pupae and adults of both sexes from each rearing regime were analysed for K, Ca, Mn, Fe, Cu, Zn, Br, As, Rb and Sr. An additional experiment was carried out to determine if these same elements were equally distributed between egg masses and the residual body of female WSBW from the AM rearings. For this study one group of five samples of five

females each were freeze dried and analyzed to determine the overall ppm of each element. In a second group, five samples of five females each were taken, anaesthetized with CO₂, the ovaries and associated tissues removed from abdomens and the composited samples of the five egg masses and five residual carcasses freeze dried prior to XE analysis.

Comparisons of elemental data among life stages, and among whole insects, egg masses, and skeletons, were carried out using ANOVA in ^{6/}MIDAS. Stepwise discriminant analysis, BMD: P7M (Jennrich and Sampson 1977), was used to test whether the elemental profiles of the insects reared from each host foliage and the artificial medium were distinct.

Results and Discussion

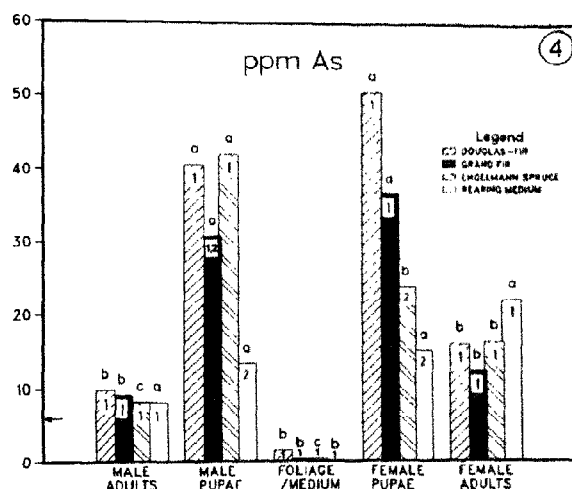
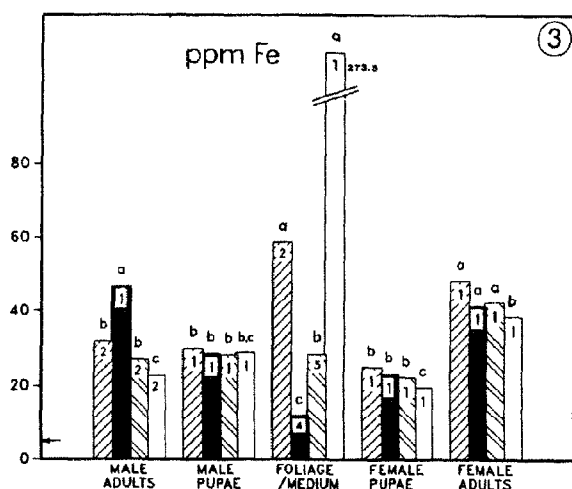
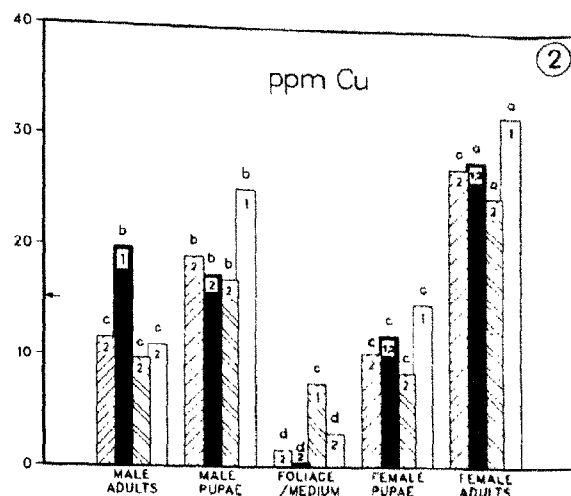
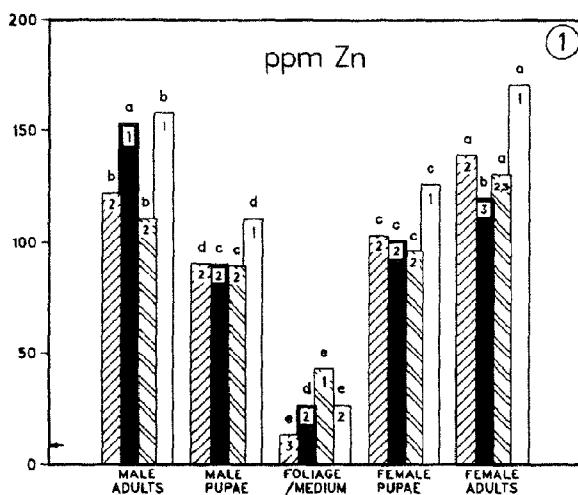
The WSBW feed primarily on new foliage. They are voracious feeders and the majority of their consumption of foliage occurs during the fifth and sixth instars (Brown 1973). During

^{6/}Michigan Interactive Data Analysis System (Fox and Guire 1976).

TABLE 1: Comparison of elemental levels in new and old foliages of the Douglas-fir, Engelmann Spruce and Grand Fir, used to rear the WSBW

Element	ppm ^{a/}					
	Df		ES		GF	
	Old	New	Old	New	Old	New
K	4106b	7300a	5800b	17000a	5000b	11400a
Ca	9700a	5017b	12706a	2103b	10145a	6100b
Mn	104.0a	80.3b	540.4a	124.8b	593.7a	244.1b
Fe	99.8a	58.6b	202.5a	28.6b	34.0a	12.1a
Cu	0.2a	1.6a	10.0a	7.6a	2.6a	0.4a
Zn	4.9a	13.2a	69.4a	42.9b	24.2a	26.4a
As	6.5a	1.5a	4.3a	0.4a	2.4a	0.3a
Br	18.0a	10.4a	10.8a	15.0a	7.4a	7.1a
Rb	5.5a	7.9a	7.0b	11.6a	9.2b	22.3a
Sr	53.7a	24.8b	82.4a	15.2b	50.6a	28.4b

^{a/}Means within each element by host category followed by the same letter not significantly different, t-test, $P < 0.05$



FIGURES 1-4: Results of the study showing levels of elements in western spruce budworm pupae and adults reared from three host foliages and artificial medium.

NOTE: Letters above bars indicate significant differences among life stages reared on each host; numbers within bars indicate significant differences within the same life stages taken from each rearing regime, Scheffe's Test, $P < 0.05$; detection limit indicated by horizontal arrow on Y-axis.

this period, tremendous growth takes place. For these reasons it was assumed that there would be sufficient opportunity for the different food sources to produce an effect on the elemental profile of reared pupae and adults. There were wide differences in element concentrations between new and old foliage with higher concentrations of K in all new foliages and higher Rb levels in new foliage for ES and GF. There were lower concentrations of Ca, Mn, Fe (except for GF) and Sr in new foliage as compared to old foliage (Table I). The remaining elements (Cu, As, Br) did not vary in concentration between old and new foliage except Zn which showed a minor variation with higher levels in old ES foliage.

Two of the important enzyme co-factors in living tissue, Zn and Cu, showed elevated levels in WSBW pupae and adults as compared to the new foliage (Figs. 1, 2). Even though ES foliage has the highest Zn levels amongst the foliages and AM, it was the insects reared on the artificial medium which had highest levels. Zn levels were similar in male and female pupae but there was considerable variation among the adults (Fig. 1). A similar pattern of increasing Cu levels was found from new foliage to female pupae and female adults. Although Cu levels of male pupae were elevated, they decreased during metamorphosis to the adult (Fig. 2). No explanation can be given for this phenomenon. It should also be noted that neither the oxidation state nor the chemical

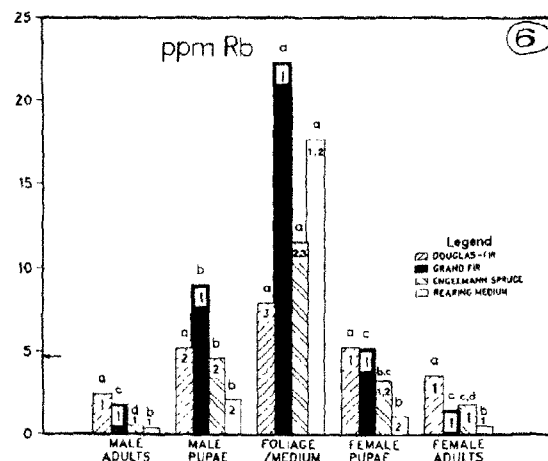
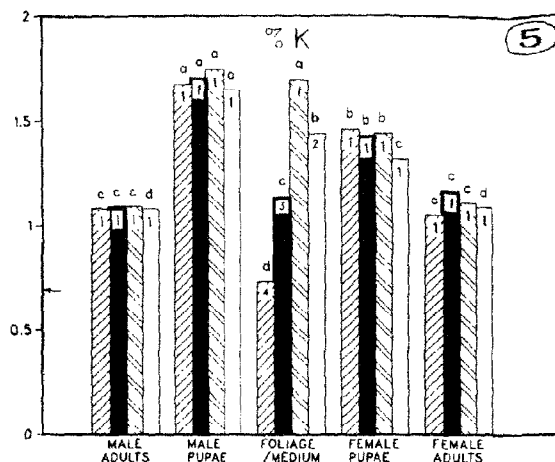
environment of the element in the insect is determined during XES analysis. Levels of Fe varied widely among foliages and artificial medium but all pupae had similar concentrations (Fig. 3). Adults also had similar levels except GF male WSBW reared on GF which had higher Fe levels than other males. These results suggest that Fe is probably under homeostatic regulation.

Highly variable levels of As were found in the insect life stages even though concentrations in the foliage and AM were extremely low (Fig. 4). Accumulations of these high levels suggest bioaccumulation may have occurred in the field before the laboratory rearing was undertaken. There was a significant decrease in As concentrations in the emerged adults.

Levels of the important macronutrient K were significantly different among the rearing medium and new foliages (Fig. 5). The levels within each life stage were not significantly different, suggesting that this element is also under homeostatic regulation. Levels in adults were significantly less than those of the pupae. A potassium substitute of some interest is Rb and it has been used as a marker element for dispersal studies (see recent review by Raulston 1979). The levels of Rb in the foliages and AM were relatively low and there were decreasing levels recorded in pupae and adults (Fig. 6).

The elements Ca and Sr were present in comparatively high concentrations in the foliages and artificial medium but only small amounts were taken up by the insects (Figs. 7, 8). Br levels, on the other hand, have undergone bioaccumulation, especially in those insects reared on DF and GF (Fig. 9). It is possible that salt spray accumulated on foliage samples collected from the University Endowment Lands and that once Br was within the larvae it was not excreted. Of all the elements, Mn was one of the most abundant in foliage samples but was barely detectable in the WSBW life stages (Fig. 10). It would appear that this element is either not taken up or is rapidly excreted by the WSBW.

How was the development of the WSBW affected by the various diets? The larvae which fed on the artificial medium showed greater weight gains in the females at all life stages (Table 2). There were no significant differences in weight gain (as a proportion of L6 weight) for male pupae or adults (Table 2) among food sources. The numbers of eggs laid by females reared on the host foliages were similar although significantly fewer eggs were laid by females reared on AM. Percentage hatched was also significantly greater for the eggs from the three host foliage reared females (Table 2). These results suggest that mating may not have been successful for the insects reared on artificial medium.



FIGURES 5-6: Results of western spruce budworm rearing study showing variations in elemental concentrations among host foliages and insect life stages. See note on Figs. 1-4 for explanation of significance levels indicated.

TABLE 2: Summary of development data for field collected western spruce budworm reared on new foliages of three host tree species and artificial medium

Stage ^{b/}	Males				Females			
	DF ^{a/}	ES	GF	AM	DF	ES	GF	AM
Live weight (mg)								
Initial L6 weight	25.3	40.4	46.3	31.1	40.5	66.9	54.0	41.7
(No.)	(6)	(4)	(5)	(4)	(3)	(6)	(6)	(7)
Live weight as a proportion of L6 weight								
Initial pupal weight	2.9a	2.5a	1.8a	3.0a	1.9b	2.2b	1.9b	5.0a
Final pupal weight	2.5a	2.0a	1.5a	2.6a	1.6b	2.1b	1.7b	4.4a
Adult weight	1.5a	1.5a	0.8a	1.5a	1.2b	1.4b	1.2b	3.4a
Fecundity (eggs per female)					120.8ab	143.3a	102.8ab	68.4b
(n)					(10)	(9)	(9)	(9)
Mean percent hatch					98.6a	91.2a	89.4a	32.1b

^{a/}Hosts indicated as DF = Douglas-fir, ES = Engelmann Spruce, GF = Grand Fir and AM = artificial medium.

^{b/}Weights determined within 24 hours of moulting to minimise variation resulting from feeding.

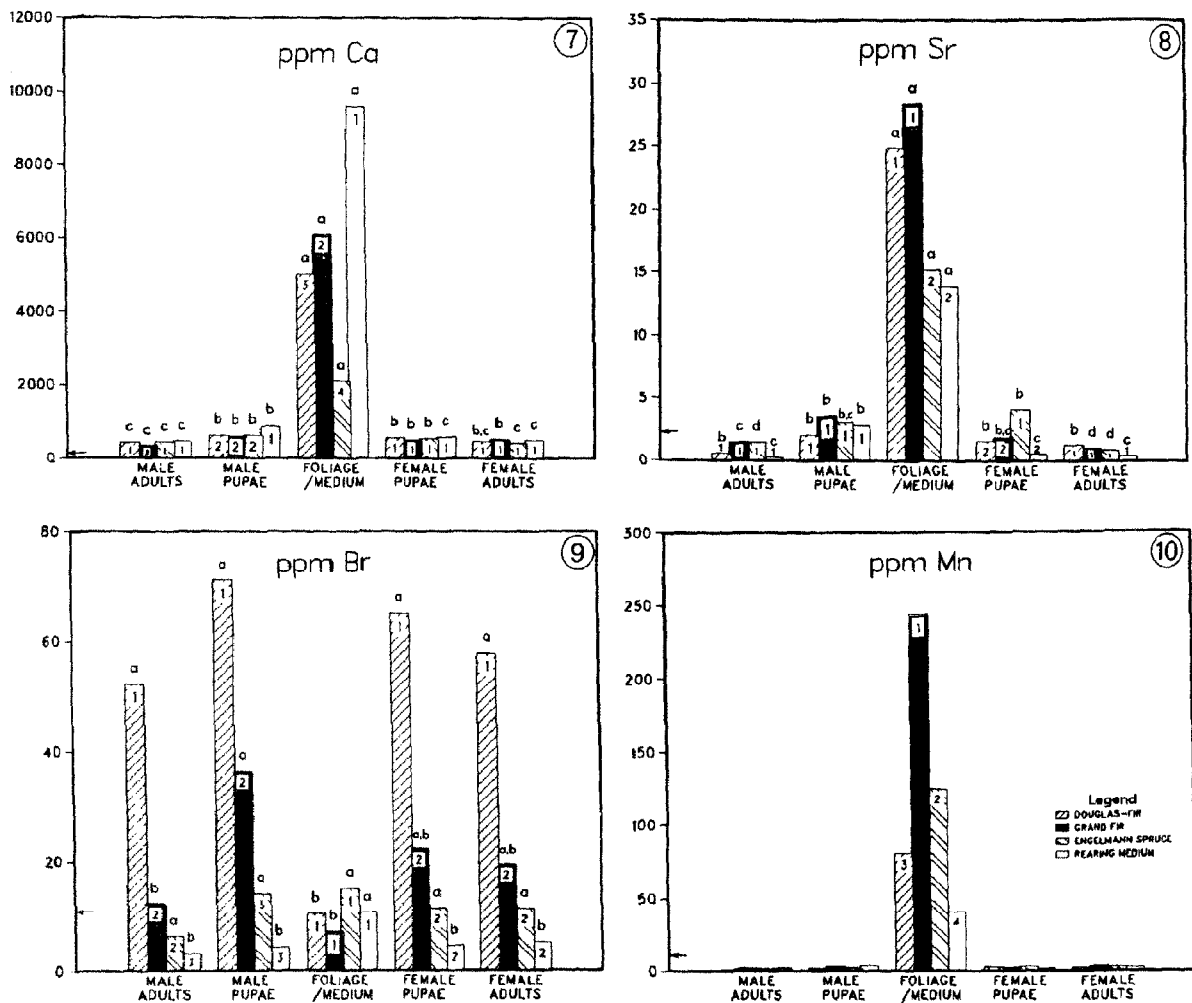
Comparisons of the levels of Fe, Cu, and Zn in carcasses and egg masses showed higher Fe and Cu levels in the carcasses and higher Zn levels in the egg masses (Table 3). Thus, the amount of oviposition by females will affect residual levels of these elements which could be of consequence in population studies of female WSBW involving chemoprinting. Fluctuations associated with oviposition or feeding may be circumvented by using a portion of the body not associated with oviposition such as a wing as Turnock *et al.* (1980) suggested in their study of the red turnip beetle, *Entomoscelis americana* Brown. In retrospect a more reliable chemoprint for female WSBW might be obtained by removing abdomens before analysis.

In the discriminant analysis of males from the four rearing regimes, 16/17 (94%) were correctly assigned to their feeding group in the jackknife classification procedure (Table 4). All group means were significantly separated from each other. For the females, the problem of partitioning Zn, Fe and Cu between carcasses and egg masses was allowed for by designating these elements a "secondary" set in the discriminant analysis. This required that selection of significant variables be made from among the other seven elements first and only when none of these met the criterion for entry ($F = 4.0$) would selection be made from the secondary set. In this case, the results were identical with and

TABLE 3: Comparisons of Fe, Cu and Zn levels in whole insects, egg masses and residual carcasses of WSBW reared on artificial medium

Element	ppm ($\bar{x} \pm s.d., n=5$) ^{a/}		
	Whole Insect	Skeleton	Egg Mass
Fe	27 $\pm$ 3b	49 $\pm$ 5a	20 $\pm$ 5c
Cu	12 $\pm$ 3b	22 $\pm$ 7a	7 $\pm$ 4b
Zn	153 $\pm$ 9ab	132 $\pm$ 18b	168 $\pm$ 31a

^{a/}Means within rows followed by the same letter, not significantly different, $P < 0.05$, Scheffe's Test.



FIGURES 7-10: Results of the study showing levels of elements in western spruce budworm pupae and adults reared from three host foliages and artificial medium. See note with Figs. 1-4 for explanation of significance levels indicated.

without the designation of a secondary set of elements with only Zn and Br being included in the discriminant function. The only group means not statistically distinct were those of female WSBW reared on the ES and GF. (Table 5). In the jackknife classification procedure only 11/18 (61%) of the females were correctly assigned to rearing regime.

House (1974) reviewed the roles of minerals in insect nutrition tabulating data for Ca, Cu, Fe, K and Zn (of the elements considered in this study). He also described the area of mineral requirement of insects as probably the most neglected area of research in insect nutrition.

Quantitative multi-elemental procedures, such as XES, will add greatly to our understanding of normal element concentrations and allow us to more fully appreciate the normal homeostatic mechanisms operating during an insect's metamorphosis and will also, hopefully, indicate which elements are suitable for geographical characterization of populations. Three promising candidates appear to be As, Br and Rb. Some of these, especially Rb, might be manipulated to mark a forest defoliator population, as has been done with several agricultural insects (Raulston 1979). More reliable chemoprinting results for female WSBW might be obtained by removing abdomens prior to analysis and by so doing avoid variations related to oviposition.

TABLE 4: Results of discriminant analysis of male adult WSBW reared on Douglas-fir, Grand Fir and Englemann Spruce foliages, and artificial medium (DF, GF, ES and MED respectively)

Step Variable Entered	Coefficients for Canonical Variable		Element
	1	2	
1	-0.21	0.22	Br
2	0.12	-0.06	Fe
3	0.11	0.06	Zn
Constant	-11.18	-0.18	

Eigenvalues: 7.82, 6.78

Cumulative proportion of total dispersion: 0.47, 0.88

Canonical Correlations: 0.94, 0.94

Matrix of F-values for testing group means:

Group	DF	GF	ES
GF	15.09**		
ES	27.30**	10.72**	
AM	23.20**	24.54**	24.20**

Jackknife classification matrix:

Origin of	No. Classified as				Total
	DF	GF	ES	AM	
Moths					
DF	5	0	0	0	5
GF	0	4	1	0	5
ES	0	0	5	0	5
AM	0	0	0	2	2

94.1% of calibration group correctly assigned to host food source.

a/
Probability level indicated, ** = $P < 0.01$

Acknowledgements

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TABLE 5: Results of discriminant analysis of female adult WSBW reared on foliage from Douglas-fir, Grand Fir, Englemann Spruce and artificial medium (DF, GF, ES and AM respectively)

Step Variable Entered	Coefficients for Canonical Variable		Element
	1	2	
1	-0.08	0.03	Zn
2	0.01	0.07	Br

Constant 11.15 -5.47

Eigenvalues: 3.35, 2.40

Cumulative proportion of total dispersion: 0.58, 1.00

Canonical Correlations: 0.88, 0.84

Matrix of F-values^{a/} for testing group means:

Group	DF	GF	ES
GF	11.38**		
ES	13.08**	0.96	
AM	17.65**	18.43**	13.03**

Jackknife classification matrix:

Origin of	No. Classified as				Total
	DF	GF	ES	AM	
Moths					
DF	2	1	1	0	4
GF	1	3	0	0	4
ES	2	1	2	0	5
AM	1	0	0	4	5

61.1% of calibration group correctly assigned to host food source.

a/Probability level indicated, ** = $P < 0.01$

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