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Radioisotope Technique for Estimating Lady Beetle[1] Consumption of Tobacco Budworm[2] Eggs and Larvae

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ABSTRACT

Females of the tobacco budworm, *Heliothis virescens* (F.), were fed Phosphorus-32 (in $NaH_2{}^{32}PO_4$) in a 10% sucrose solution. Eggs and larvae of the moths were assayed for radioactivity before feeding them to the adult lady beetles, *Coleomegilla maculata* DeGeer. The adults were assayed for radioactivity each day for 10 days after consumption of the eggs or larvae. Radioactivity levels in the lady beetles showed a gradual decline each day after consumption of egg or larvae.

Prediction equations from regression analysis indicate that the number of eggs or larvae eaten by adult *C. maculata* can be predicted in laboratory or field studies after determining radioactivity of *H. virescens* eggs or larvae prior to lady beetle consumption.

Hines et al. (1973) showed that eggs and larvae of the tobacco budworm, *Heliothis virescens* (F), could be labeled with ^{32}P by feeding $NaH_2{}^{32}PO_4$ in a sugar solution to female moths. Their work was the 1st step in a program to study the potential for using a radioisotope labeling technique for investigation of the predator-prey relationship between *Heliothis* spp. and various predators. Reported here is a further development of this technique and its use in studying consumption of *H. virescens* eggs and larvae by a spotted lady beetle, *Coleomegilla maculata* DeGeer.

Methods and Materials.—Two methods of feeding female moths were tested. The 1st method was identical to that reported by Hines et al. (1973). The 2nd method was developed to eliminate the high experimental error reported by Hines et al. (1973) when loop feeders were employed. They found that the loop feeders varied from 12–25 µl of solution/loop calibrated to hold ca. 19 µl.

In the 2nd method, a micrometer was used to deliver 18.2 µl of ^{32}P solution through a syringe to the point of a No. 16 needle. The drop on the needle point was placed on a loop and fed to the moth. Since variation in the levels of radiation in the eggs and larvae also might partially be attributed to physiological differences in the moths, female *Heliothis* pupae were weighed and only those weighing 291±50 mg were used in the tests. After moths were fed, they were handled in the same manner as described by Hines et al. (1973).

Each test consisted of 5 treatments replicated 4 times for each female moth. Treatments were 16, 8, 4, 2, or 1 egg(s) or larva(e) fed to an adult lady beetle. Each replication per test required 31 eggs or larvae. A test was usually completed in 4 days since 4 egg batches were used, but in some cases, the number of days required to obtain 124 eggs or larvae for the 4 replications was greater than 4 because a treated moth failed to lay the required 31 eggs on some days.

A test consisted of 1 mated, ^{32}P treated female moth set up for use in studying consumption of eggs or larvae by adult spotted lady beetles. Eggs were those laid between the 3rd and 10th day after the moth was fed ^{32}P. Eggs laid by the treated moths were used either in a test of egg consumption by the lady beetles or in a test of larval consumption by the lady beetles. Twenty eight tests were conducted, 8 tests with eggs and larvae from moths fed by the 1st method and 8 tests with eggs and 4 tests with larvae from moths treated by the 2nd method.

Since a majority of the experiments was performed in the winter months, hibernating lady beetles were field collected on the Delta Branch Experiment Station at Stoneville, Miss., and immediately placed on an artificial diet similar to that of Atallah and Newsom (1966). Lady beetles were held in pint plastic containers in a 24-h light regime at room temperature and humidity. Ca. 100 lady beetles were held in each pint container.

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Each container contained 2–3 vials of diet and 1 vial of distilled water plugged with absorbent cotton. Diet was replaced every 7–10 days. The lady beetles showed no apparent loss in viability up to a period of 45–60 days.

The specified number of *Heliothis* eggs or larvae for each treatment were selected randomly from the oviposition cages (Hines et al. 1973) and placed in planchets and assayed for radioactivity. Then an adult lady beetle was placed in each planchet and secured within by a piece of Glad Wrap®. After each lady beetle consumed the eggs or larvae placed in the planchets, it was removed and held in a vial containing diet. Lady beetles were assayed for radioactivity each day for 10 days after consuming labeled eggs or larvae.

Radioactivity was measured by means of a Tracerlab FD-1 Geiger flow counter with a 1.25-in. mono mal resin, aluminum-coated window (EON Corp., Brooklyn, N. Y.), having density of 125 $\mu g/cm^2$.

All cpm taken for the eggs and larvae and for the lady beetles were corrected for background radiation and adjusted to 0 time level of radioactivity for ^{32}P. The fraction of radiation remaining for the number of days elapsed after 0 time level of radioactivity was obtained from a ^{32}P Decay Table. The following formula can be applied:

$$NCPM = \frac{GCPM - BR}{FR_N}$$

where

GCPM = gross counts per minute of eggs, larvae or lady beetles;

BR = background radiation;

FR_N = fraction of ^{32}P remaining (Decay Table);

NCPM = net counts per minute of eggs, larvae or lady beetles.

Data were subject to multiple regression analysis in which there were 2 independent and 1 dependent variables. The 2 independent variables were X_1 (the number of eggs or larvae consumed by the lady beetles) and X_2 (the cpm of the eggs or larvae prior to lady beetle consumption). The dependent variable, $\hat{Y}$, represents the predicted cpm of the lady beetles after the consumption of the eggs or larvae for a particular day after consumption. The prediction equation was in the form of

$$Y = b_0 + b_1X_1 + b_2X_2.$$

RESULTS.—The standard partial regression coefficients for the cpm of eggs prior to lady beetle consumption (X_2) are higher than the standard partial regression coefficients for the cpm of the number of eggs consumed by the lady beetles (X_1). This signifies that the cpm of the eggs consumed was more important in predicting the cpm of the lady beetles after consumption than was the number of eggs eaten. The R square values decrease as time from egg consumption increased. This was expected since increased variation between the radiation levels in the lady beetles occurred with time due to varying individual rates of elimination. After converting the R square value for 1st day after consumption to percent, over 97% of the total variation was explained by the regression by either the 1st or 2nd method. The remaining 3% of the variation in the data were unexplained.

The standard deviation of mean cpm of eggs and larvae was computed for methods 1 and 2, and a comparison showed that the 2nd method was more accurate. Method 2 was then examined in greater detail.

The prediction equations in Table 1 are for lady beetles that consumed eggs labeled by the 2nd method of moth feeding. The R square values decreased with time after consumption.

Table 2 gives the prediction equations for the lady beetles that consumed larvae labeled by the 2nd method of moth feeding. The X_2 variable is again more important in predicting $\hat{Y}$ than is the X_1 variable. Radiation levels in the lady beetles were measured for only 5 days after consumption of the larvae in these tests.

DISCUSSION.—These equations may be used in predicting the number of *H. virescens* eggs or larvae consumed by adult *C. maculata* in special laboratory or field studies. It is essential that the cpm of the eggs or larvae be determined for each test. This is due to the variation that occurs among moths. The physiological

Table 1.—Prediction of ^{32}P levels in *C. muculata* after consumption of *H. virescens* eggs labeled by Method 2.

Day after egg consumption	Prediction equation $\hat{Y} = b_0+b_1X_1+b_2X_2$	Coefficient of determination R^2	Standard partial regression coefficients: No. eggs consumed X_1	CPM of eggs consumed X_2
1	$\hat{Y}_1 = -147.3339-1052.7221X_1+.9526X_2$	0.979	−0.337	1.320
2	$\hat{Y}_2 = -405.1799-1393.4931X_1+1.0056X_2$	.982	− .463	1.443
3	$\hat{Y}_3 = -156.3014-1108.9431X_1+.8929X_2$	.977	− .395	1.375
4	$\hat{Y}_4 = -55.4678-1019.3265X_1+.8609X_2$	.964	− .964	1.342
5	$\hat{Y}_5 = -99.4788-749.396X_1+.7571X_2$	.966	− .290	1.267
6	$\hat{Y}_6 = +196.4396-988.9737X_1+.7598X_2$	.964	− .420	1.392
7	$\hat{Y}_7 = +235.8787-1027.9446X_1+.7401X_2$	.946	− .456	1.420
8	$\hat{Y}_8 = +67.4903-1018.4850X_1+.7169X_2$	.930	− .467	1.420
9	$\hat{Y}_9 = -211.1504-1220.9750X_1+.7443X_2$	.901	− .572	1.507
10	$\hat{Y}_{10} = -464.4711-1503.6260X_1+.7980X_2$	.880	− .710	1.628

Table 2.—Prediction of ^{32}P levels in *C. maculata* after consumption of *H. virescens* larvae labeled by Method 2.

Day after larvae consumption	Prediction equation $Y = b_0+b_1X_1+b_2$	Coefficient of determination R^2	Standard partial regression coefficients	
			No. larvae consumed X_1	CPM of larvae consumed X_2
1	$Y_1 = -274.2039-3005.5426X_1+1.0771X_2$	0.981	−1.045	2.032
2	$Y_2 = -127.1447-741.0715X_1+.6065X_2$	.965	−0.285	1.267
3	$Y_3 = 10.7179-776.8923X_1+.5929X_2$	.965	−0.312	1.294
4	$Y_4 = -260.0408-938.0210X_1+.6036X_2$	.957	−0.392	1.369
5	$Y_5 = -542.1910-1393.3128X_1+.6748X_2$	.958	−0.600	1.578

differences among moths plus the differences in the amount of ^{32}P fed each moth are the primary reasons for the variations in the cpm in eggs or larvae. Nevertheless, the prediction equation, $\hat{Y} = b_0 + b_1X_1 + b_2X_2$, explained the relationship between expected cpm for lady beetles and cpm for eggs or larvae consumed, and the loss and degradation are accounted for adequately.

In the future, experimenters will be interested in predicting the number of *H. virescens* eggs or larvae consumed by adult *C. maculata*. A mean cpm per egg or larva from females fed ^{32}P would be the only known information since the number of eggs or larvae would be unknown. Eggs or larvae would then be subjected to predation and 1 day later radiation levels in the lady beetles could be determined.

Solving for X_1, the new equation would become: after transposing,

$$X_1 = \frac{Y - b_0}{b_1 + b_2X_2}.$$

For instance, consider a hypothetical mean cpm per moth egg as 4319. Several eggs are subjected to predation. After 24 h, lady beetles are collected and assayed for radioactivity. Assume that the cpm of a lady beetle was found to be 11410. These values are then substituted into the equation (see Table 1) and the number of eggs consumed by the lady beetle (X_1) is determined.

$$X_1 = \frac{Y - b_0}{b_1 + b_2X_2}$$

$$X_1 = \frac{11410-(-147.339)}{-1052.7221+(.9526)(4319)}$$

$$X_1 = 3.8 \text{ eggs}$$

Thus it is estimated that 4 eggs were consumed by the lady beetle in the 24-h period.

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