THE EFFECT OF ERYNIA RADICANS ON FOOD CONSUMPTION, UTILIZATION AND FECUNDITY BY THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA.

Abdul K. A. Mohamed, Lucius Lewis and Denise Lewis.

Associate Professor and graduate students, respectively, Biology Department, Jackson State University, Jackson, MS 39217.

Abstract

In food consumption and utilization studies, observations were confined to a period of 5 days after which mortality of Erynia radicans larvae began to occur. Mortality due to the fungus was 76 and 83% in male and female larvae, respectively. In control larvae the pupation rate was over 95%. Control larvae of both sexes consumed significantly (P = 0.05) more food than fungus treated larvae on day 4 and 5. Treated as well as control larvae showed a continuous gain in body weight up to day 3, following which treated larvae of both sexes gained significantly (P = 0.05) less weight than the control. Fungus infected larvae were also less efficient in converting ingested and digested food to body substances (ECI and ECD) on day 4 and 5 in females and on day 5 in males. Pupae of both sexes from treated larvae weighed less than the control. Fecundity of adults arising from fungus treated larvae was reduced by approximately 36.4% compared with the control.

Introduction

Erynia radicans (=Zoophthora radicans = Entomophthora sphaerosperma) is among the more promising of microbial agents for the control of the spruce Budworm, Choristoneura fumiferana and has a potential for commercial development as a microbial insecticide (Weatherston and Retnakaran 1975; Vandenberg and Soper 1979). However, an important drawback of mycopathogens in the control of insect pests is the time taken to kill the host. Generally, mortality of infected larvae occurs in 4-8 days after treatment (Mohamed et al. 1982) during which larvae continue to feed, presumably causing damage. Therefore, it would be benificial if it could be ascertained that fungal infection of the host can reduce food consumption. This would aid in assessing the suitability of the pathogen for use in insect pest management, particularly in a forest situation where the foliage can tolerate moderate feeding damage.

Most reported studies dealing with the control of insects with pathogens have dealt mainly with the direct effect on the host and have been evaluated in terms of percent mortality. Little attention has been focused toward the evaluation of the effect of infection on food consumption and the general reduction of the biological potency of the host (Jasic 1961). Ramakrishnan and Chaudhari (1974) studied the effect of a nuclear polyhedrosis virus (NPV) on the consumption and utilization of food by the Egyptian cotton leaf worm, Spodoptera litura. They found that food consumption and weight gain of virus infected larvae were significantly lower than the control. Drake and McEwen (1959) reported that the cabbage looper, Trichoplusia ni showed decreased food consumption following infection with NPV. Thomson (1958) showed that the microsporidian, Perezia fumiferanae retarded larval and pupal development, reduced fecundity and adult longevity of C. fumiferana. Larvae of Heliothis zea infected with Nomuraea rileyi showed significantly less food consumption and utilization than the control group (Mohamed et al. 1982).

The purpose of this study was to determine the effect of E. radicans on food consumption, utilization and fecundity of C. fumiferana.

Materials and Methods

Larvae and Fungal Cultures. - Overwintering second instar spruce budworm larvae were obtained from the Insect Pathology Institute, Saulte Ste. Marie, Ontario, Canada. Larvae were reared on Bio-Serv Spruce Budworm diet #9769, dispensed in about 15-20 ml aliquots into 30 ml plastic cups. Four to six larvae were placed in each cup and reared at 22.5°C with a photoperiod of 16 hr light and 8 hr darkness.

The culture of Erynia radicans was obtained from the laboratory of Dr. R. S. Soper, U.S.D.A. ARS, Insect Pathology Unit, Boyce Thompson Institute, Ithaca, N.Y. The initial sample of this fungus was isolated from a field collected spruce budworm larvae (Vandenberg and Soper 1979). It was grown on 70% modified egg-yolk medium (EY) in darkness at 25°C according to Soper et al. (1975.) Sporulation was induced according to the method of Vandenberg and Soper (1979). Several 250 ml flasks each containing 75 ml of CYP liquid medium (4% corn syrup, 1% yeast extract, 1% phytone peptone in distilled water) were inoculated with mycelia grown on EY, and continuously agitated in a controlled environmental shaker (New Brunswick Scientific Co. Inc.) at 25°C. When the culture reached the exponential growth phase (4-5 days), 5 ml of each culture was transferred to fresh CYP and allowed to grow for 2-3 more days. Then the medium was filtered and the remaining mycelia rinsed several times with sterile distilled water. After the excess moisture was drained off with paper filters, mycelia were placed in plastic Petri dishes (90 x 15 mm) and exposed to continuous fluorescent light for 24 hr at 20°C to stimulate sporulation.

Treatments

1. Food consumption and utilization. - Fifth instar larvae were used in all tests. The age of the larvae was determined according to the method of Retnakaran (1973). Larvae were

sexed as described by Koller and Leonard (1981). One hundred 5th instar larvae of each sex were placed in plastic cups individually and starved for 8 hr to empty their gut contents of residual food. Eighty larvae from each sex group comprised the untreated control group. A similar number of larvae were treated with E. radicans spores as described by Vandenberg and Soper (1979). The dose was estimated by exposing Petri dishes (100 x 15 mm) containing 2.5% sterile distilled water agar to sporulating cultures identical to those used in larval treatments. Spore concentration was estimated (spore/mm^2) by counting a random field in each of 10 areas in the dish (Vandenberg and Soper 1979). In preliminary tests, several groups of larvae were exposed to sporulating cultures at varying times (15 to 90 min.) An exposure time of 45 min. giving 39 ± 5.7 spores/mm^2 was the most appropriate in terms of mortality and survival of the larvae.

After treatment, larvae were held in individual Petri dishes (100 x 15 mm) lined with filter paper moistened daily with sterile distilled water. Each treatment was replicated 4 times with 10 larvae. Test larvae were held in an environmental growth chamber at 22.5°C with a photoperiod of 16 hr light and 8 hr darkness.

Larvae were individually provided with preweighed 5 mm diet plugs which were replaced daily. Food consumption and weight gain were determined by recording the initial weight of the larvae and diet, followed by recordings of the larval weight, feces and diet consumed every 24 hr. Data from these observations were used to determine food consumption and utilization. Diet consumed was corrected for moisture evaporation as described by Brewer and King (1979). The indices (Waldbauer 1968) to determine the efficiency of utilization were as follows:
a) Approximate digestibility which is the percentage of food ingested that is retained or utilized by the larvae:

$$(AD = \frac{\text{wt of food ingested} - \text{wt of feces}}{\text{wt of food ingested}} \times 100)$$

b) Efficiency of conversion of ingested food to body substances which is an overall ability of the insect to utilize ingested food for growth:

$$(ECI = \frac{\text{wt gain}}{\text{wt of food ingested}} \times 100)$$

c) Efficiency of converstion of digested food to body substances decreases as the digested food metabolized for energy increases:

$$(ECD = \frac{\text{wt gained}}{\text{wt of food ingested} - \text{wt of feces}} \times 100)$$

The data were analyzed by the analysis of variance and the Duncan's New Multiple Range Test (1955).

2. Fecundity. - One hundred 5th instar larvae of each sex were treated with E. radicans and placed in plastic cups containing diet. A similar number comprised the untreated control. Larvae were reared as described earlier and observed daily for mortality and pupation. Pupae were weighed within 24 hr of pupation and placed in the original cups. Adults used for oviposition were reared in individual containers (Stehr 1954). Branches of balsam fir, Abies balsamea[1] were used for oviposition. Each test was conducted over a period of 4-6 days, with daily removal and counting of egg masses. The number of eggs was determined by measuring the length and width of each egg mass as described by Bean (1961).

Results and Discussion

1) Food consumption and utilization. - Mortality of E. radicans treated larvae started on day 4 post treatment. Thus observations for food consumption and utilization were done for 5 days. On day 6 most of the treated larvae were dead. Mortality in fungus treated larvae was 76% in males and 83% in females. In the control 95% of males and 97.5% of the females pupated.

Table 1 shows the amount of food consumed by treated and control larvae. The amount of food consumed by treated larvae was significantly (P = 0.05) lower on day 4 and 5 post treatment for both sexes. Male and female control larvae showed a constant increase in food intake throughout the observation period. The average daily intake for the treated and control female larvae was 50.2 and 77.06 mg and for the males it was 60.4 and 75.9 mg respectively.

Table 1. - The effect of Erynia radicans on food consumption (mg) of Choristoneura fumiferana larvae.[a/]

DP[b]	Control	Fungus treated
Female		
1	67.3 ± 19.0a	59.4 ± 27.0a
2	66.2 ± 4.0a	60.9 ± 3.0a
3	60.7 ± 7.0a	51.5 ± 8.0a
4	94.2 ± 18.0a	40.2 ± 7.0b
5	96.9 ± 16.0a	39.0 ± 10.0b
Male		
1	56.3 ± 12.0a	61.8 ± 10.0a
2	71.4 ± 12.0a	78.4 ± 10.0a
3	67.4 ± 2.0a	57.3 ± 10.0a
4	90.6 ± 7.0a	54.9 ± 9.0b
5	93.9 ± 5.0a	49.3 ± 6.0b

a/ Means followed by a common letter horizontally do not differ significantly from each other (P = 0.05).
b/ Days post treatment.

The weight gain of treated and control larvae is presented in Table 2. Fungus treated larvae of both sexes showed significantly (P = 0.05) less weight gain on day 4 and 5. The average daily weight gain for the treated and control

1/ Specially ordered for us by a local garden and nursery center.

female larvae was 4.38 and 8.64 mg respectively. Similarly for the males the average weight-gain was 3.98 mg for the treated and 7.38 mg for the control group. The amount of feces produced in the treated larvae was significantly lower for both sexes on days 3, 4 and 5 (Table 3).

Table 2. - The effect of Erynia radicans on weight gain (mg) of Choristoneura fumiferana larvae.[a/]

DP[b]	Control	Fungus treated
Female		
1	5.8 ± 3.0a	4.9 ± 0.2a
2	6.4 ± 1.0a	7.5 ± 2.0a
3	6.3 ± 5.0a	4.8 ± 1.0a
4	10.9 ± 3.0a	2.3 ± 1.0b
5	13.8 ± 2.0a	2.4 ± 1.0b
Male		
1	3.7 ± 0.9a	3.0 ± 1.0a
2	7.4 ± 2.0a	6.1 ± 1.0a
3	8.5 ± 0.3a	5.2 ± 1.0a
4	7.6 ± 1.0a	2.9 ± 1.0b
5	9.7 ± 4.0a	2.7 ± 1.0b

a/ Means followed by a common letter horizontally do not differ significantly from each other (P = 0.05).
b/ Days post treatment.

Weight gain and fecal production are directly related to the amount of food consumed (Ramakrishnan and Chaudhari 1975). The decrease in the values of these parameters in treated

Table 3. - The effect of Erynia radicans on feces production (mg) by larvae of Choristoneura fumiferana.[a/]

DP[b]	Control	Fungus treated
Female		
1	4.1 ± 1.8a	4.7 ± 1.0a
2	7.3 ± 1.4a	6.9 ± 2.6a
3	12.0 ± 1.5a	4.6 ± 1.0b
4	15.0 ± 2.0a	2.7 ± 1.0b
5	17.6 ± 2.0a	2.2 ± 1.0b
Male		
1	4.5 ± 1.0a	4.0 ± 1.4a
2	4.3 ± 2.0a	4.4 ± 3.0a
3	7.5 ± 2.9a	4.3 ± 2.0b
4	13.2 ± 2.0a	3.6 ± 1.0b
5	20.0 ± 5.0a	3.7 ± 2.0b

a/ Means followed by a common letter horizontally do not differ significantly (P = 0.05).
b/ Days post treatment.

larvae can be attributed to reductions in food consumption of the larvae (Table 1). Reduction in food consumption, weight gain and fecal production in infected larvae have been previously reported by Mohamed et al. (1982) for H. zea infected with N. rileyi and by Ramakrishnan and Chaudhari (1975) in S. litura infected with NPV.

A more direct relationship between these parameters can be observed if we consider the total food consumption, total weight gain and total fecal production (Table 4). The total food consumption in male and female was significantly lower (P = 0.05) than that of the control. This

Table 4. - Total food consumption, weight gain and fecal production (mg) by larvae of Choristoneura fumiferana infected with Erynia radicans.[a/]

Parameter[b/]	Female		Male	
	Con	FT	Con	FT
FC	385.3a	251.0b	379.5a	302.0b
WG	43.2a	21.9b	36.9a	20.1b
FP	56.5a	21.1b	59.5a	20.0b

a/ Means followed by a common letter horizontally for male and female separately do not differ significantly (P = 0.05).
b/ Con = Control, FT = Fungus treated, FC = Food consumption, WG = Weight gain, FP = Feces production.

reduction in food consumption was approximately 20 and 35% for the treated male and female larvae respectively. Similarly, the total weight gain and fecal production were significantly lower (P = 0.05) in the treated larvae of both sexes indicating that these indices were directly related to the amount of food consumed.

2) Food utilization. - The approximate digestibility (AD) values are summarized in Table 5.

Table 5. - Approximate digestibility (AD) of the larvae of Choristoneura fumiferana infected with Erynia radicans.[a/]

DP[b/]	Control	Fungus treated
Female		
1	93.9 ± 11.4a	92.2 ± 7.6a
2	89.0 ± 10.8a	88.7 ± 12.7a
3	80.2 ± 11.8a	91.1 ± 16.7a
4	83.5 ± 8.3a	93.3 ± 11.2a
5	81.8 ± 12.8a	94.5 ± 13.6a
Male		
1	92.0 ± 8.4a	93.5 ± 11.2a
2	94.0 ± 8.7a	94.4 ± 14.9a
3	88.9 ± 11.1a	92.2 ± 13.7a
4	85.4 ± 12.6a	93.4 ± 9.8a
5	78.7 ± 6.9a	92.5 ± 6.2b

a/ Means followed by a common letter horizontally do not differ (P = 0.05).
b/ Days post treatment.

No significant difference (P = 0.05) was observed between the control and the treated group in the female larvae. In males the AD for the control was significantly lower on day 5. The gross efficiency (ECI) and the net efficiency

(ECD) showed an identical trend (Table 6, 7). In the female treated larvae, both ECI and ECD values were significantly lower (P = 0.05) than the control group on day 4 and 5, while in the males the ECI and ECD of treated larvae were significantly lower on day 5 only.

Table 6. - Gross efficiency (ECI) of the larvae of Choristoneura fumiferana infected with Erynia radicans.[a/]

DP[b/]	Control	Fungus treated
Female		
1	8.6 ± 3.7a	8.2 ± 2.2a
2	9.7 ± 9.3a	12.3 ± 5.0a
3	10.4 ± 6.3a	9.3 ± 6.4a
4	11.6 ± 3.4a	5.7 ± 3.5b
5	14.2 ± 8.6a	6.0 ± 4.2b
Male		
1	6.6 ± 2.3a	6.3 ± 3.0a
2	10.4 ± 4.2a	7.8 ± 5.6a
3	12.6 ± 3.4a	9.0 ± 4.1a
4	8.4 ± 2.0a	5.3 ± 3.5a
5	10.3 ± 3.3a	5.5 ± 3.9b

a/ Means followed by a common letter horizontally do not differ (P = 0.05).
b/ Days post treatment.

Table 7. - Net efficiency (ECD) of the larvae of Choristoneura fumiferana infected with Erynia radicans.[a/]

DP[b/]	Control	Fungus treated
Female		
1	9.2 ± 3.8a	8.6 ± 2.2a
2	10.9 ± 5.8a	13.9 ± 6.1a
3	10.7 ± 5.8a	10.2 ± 4.4a
4	13.9 ± 4.1a	6.2 ± 1.6b
5	17.4 ± 8.9a	6.4 ± 3.9b
Male		
1	7.1 ± 2.3a	6.8 ± 1.0a
2	11.0 ± 4.6a	8.2 ± 3.3a
3	14.2 ± 3.5a	9.8 ± 5.4a
4	9.8 ± 2.6a	5.7 ± 3.8a
5	13.1 ± 4.8a	5.9 ± 4.7b

a/ Means followed by a common letter horizontally do not differ (P = 0.05).
b/ Days post treatment.

3) Fecundity. - The purpose of this portion of this study was to determine a dosage that would produce a chronic and a sublethal effect that might result in the weakening of the host. Consequently, such an effect may cause a general disruption of the vital functions which could be manifested in reduced reproductive potential and shortened longevity (Gaugler and Brooks 1975). However, it was difficult to achieve such a sublethal dose. We tried various spore concentrations (6.3 to 38.1 spore/mm^2) as measured by the amount of time larvae were exposed (Vandenberg and Soper 1979) but we were unable to obtain sufficient number of adults for fecundity study due to low rate of emergence even though in some of the treatments (lower dosages) there was a high percent of pupation. Table 8 summarizes the results from one of the successful tests. In the fungus treated group the mortality was 55.6 and 63.2% in male and female respectively. Weight of control pupae was 75.2 ± 1.6 mg in males and 111.4 ± 2.6 mg in females as compared to 59.7 ± 2.0 mg for males and 97.7 ± 2.0 mg for females in the treated group. Fecundity expressed as mean number of egg/female was 92.8 in the control (9 pairs) and 59.0 in the treated group (2 pairs only).

Vandenberg and Soper (1979) showed that mortality of larvae of the spruce budworm treated with Erynia radicans occured in 3 to 6 days. Similarly, in our study larval death occurred in 4-6 days. During the infection stage, in this study, the food comsumption and utilization were significantly lowered in the fungus treated larvae as compared with the control on day 4 and 5. Also, pupae from infected larvae weighed less than untreated ones. Such effects are probably a result of depleted nutritional reserves and a reduced ability to assimilate food efficiently (Thomson 1958). Consequently, larvae will be less efficient at forming nutrient reserves for the pupae and adult stages.

In insect species such as the spruce budworm, where the adults do not consume food, nutritional reserves are dependent on the amount of nutrients accumilated by the larvae for the entire metabolism of the individual (Jasic 1961). Thus, if the reduction in food consumption and utilization in infected larvae occurs under natural conditions, adult females arising from diseased larvae will have even smaller reserves, since the individual must use a portion of the fat stored for general activity, thereby diverting a large portion of this reserve from egg production. This is important when considering the utilization of E. radicans in the management of an insect pest like the spruce budworm in a forest situation where the foliage can tolerate some feeding without serious economic damage.

Table 8. - The effect of Eyrinia radicans on the spruce budworm larval mortality, pupation, emergance and fecundity.[a/]

Treatments	% Larval Mortality		% Pupation		Pupal wt (mg)		% Emergance		Eggs/per Female[b/]
	M	F	M	F	M	F	M	F	
Fungus treated	55.6	63.2	44.4	36.8	59.7 ± 2.0	87.7 ± 2.0	9.4	8.0	59.0
Control	0.0	0.0	100.0	100.0	75.2 ± 1.6	111.4 ± 2.6	98.0	100.0	92.8

a/ Rate of larval spore exposure was estimated at 8.4 spore/mm^2.
b/ Based on the mean of only 2 pairs in the fungus treated group and 9 pairs in the control.

Acknowledgment

This work was funded by a program sponsored by the Forest Service, USDA, entitled "Canada/U.S. Spruce Budworm Program".

References

Bean, J. L. 1961. A method for estimating the number of spruce budworm eggs per egg mass. J. Econ. Entomol. 54:1064.

Brewer, F. D. and E. G. King. 1979. Consumption and utilization of soyflour-wheat germ diets by Heliothis spp. Ann. Entomol. Soc. Am. 72: 415-17.

Drake, E. L. and F. L. McEwen. 1959. Pathology of nuclear polyhedrosis of the cabbage looper, Trichoplusia ni. J. Insect Pathol. 1:281-93.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

Gaugler, R. R. and W. M. Brooks. 1975. Sublethal effects of infection by Nosema heliothidis in the corn earworm, Heliothis zea. J. Invertebr. Pathol. 26:57-63.

Jasic, J. F. 1961. Microsporidia as a factor in reducing the fecundity in insects. J. Invertebr. Pathol. 3:103-11.

Koller, N. and D. Leonard. 1981. Comparison of energy budget for the spruce budworm on black and white spruce. Oecologia (Berl) 49:14-20.

Mohamed, A. K. A., F. W. Brewer, J. V. Bell and R. J. Hamalle. 1982. Effect of Nomuraea rileyi on consumption and utilization of food by Heliothis zea Larvae. J. Georgia Entomol. Soc. 17:356-63.

Ramakrishnan, N. and S. Chaudhari. 1974. Effect of nuclear polyhedrosis disease on consumption, digestion and utilization of food by the tobacco caterpillar, Spodoptera litura. Indian J. Ent. 36:93-7.

Retnakaran, A. 1973. Hormonal induction of supernumerary instars in the spruce budworm, Choristoneura fumiferana (Lepidoptera: Torticidae). Can. Ent. 105:459-61.

Soper, R. S., F. R. Holbrook, I. Majchrowica and C. C. Gordon. 1975. Production of Entomophthora resting spores for biological control of aphids. Univ. of Maine Life Sci. Agric. Exp. Stn. Tech. Bull. 76, 15pp.

Stehr, G. 1954. A laboratory method for rearing the spruce budworm, Choristoneura fumiferana. Can. Ent. 83:423-28.

Thomson, H. M. 1958. The effect of a microsporidian parasite on the development, reproduction and mortality of the spruce budworm, Choristoneura fumiferana. Can. J. Zool. 36:499-511.

Vandenberg, J. D. and R. S. Soper. 1979. A bioassay technique for Entomophthora sphaerosperma on the spruce budworm, Choristoneura fumiferana. J. Invertebr. Pathol. 33:148-54.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5:229-82.

Weatherston, I. and A. Retnakaran. 1975. The potential of autocides and micoorganisma as ecologically acceptable agents for the regulation of spruce budworm infestations. J. Environ. Quality. 4:294-303.