

JOURNAL OF THE LEPIDOPTERISTS' SOCIETY

Volume 51

1997

Number 4

Journal of the Lepidopterists' Society
51(4), 1997, 277-287

INTOXICATED LEPIDOPTERANS: HOW IS THEIR FITNESS AFFECTED, AND WHY DO THEY TIPPLE?

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ABSTRACT. Butterflies imbibing fluids at fallen, rotting fruits sometimes show signs of intoxication. Fallen fruits as well as woody plant sap-flows undergo natural fermentation, which may result in frothy brews containing up to perhaps 3% ethanol. Many lepidopterans are attracted to volatile fermentation products, but studies of actual consumption are lacking. In laboratory choice tests, adults of *Choristoneura fumiferana* (Clemens) neither favored nor shunned 1% ethanol in plain or sweetened water for imbibing. Adults imbibing up to 1% ethanol were unimpaired in six of seven monitored fitness factors. One fitness factor, fertility, defined as the proportion of pairs reproducing, declined incrementally starting at concentrations of 0.5% ethanol. Two hypotheses are presented to explain lepidopteran intoxication in nature.

Additional key words: *Choristoneura fumiferana*, Tortricidae, imbibing, fermentation, ethanol.

Butterflies may become sluggish and more easily captured while imbibing fluids at fallen, rotting fruits, and collectors often use fermenting brews as baits (Norris 1936, Utrio & Eriksson 1977). Because the sweet fluids of fallen fruits and woody plant sap-flows may ferment (Janzen 1977), it is assumed that lepidopterans imbibing them become intoxicated from fermentation products such as ethanol. Although the attractancy of fermentation products to certain lepidopterans has been experimentally documented (Utrio & Eriksson 1977, Utrio 1983), studies of actual ferment consumption are lacking. Neither lepidopterans nor other insects are among the invertebrates featured in Winterstein's (1919) classic treatise on narcosis.

Unlike butterfly intoxication, moth intoxication does not seem to have been reported despite the fact that most of the experimental work on ferment attractancy utilized moths. Moths typically feed at dusk or after dark, times when they are difficult to observe in the wild. Also, in the era before sex lures, attractancy research was done to support prescriptions for trapping and monitoring lepidopteran pests in fruit orchards

(Dethier 1947, Green et al. 1960, Madsen & Morgan 1970), a context in which consumption and intoxication were irrelevant. Species in four moth families have nevertheless been recorded at natural sap flows, namely Noctuidae, Sphingidae, Geometridae, and Tortricidae (Norris 1936, Foster & Tate 1966).

Fermentation is the chemical alteration of carbohydrates by microorganisms. The microorganisms involved are usually yeast fungi and their close relatives, of which more than 500 species in 54 genera are recognized (Phaff et al. 1978). Different yeasts give rise to different fermentation products, and naturally produced ethanol is thought to be fairly common. It is not unusual for imbibed ferments to be described as frothy (Wilson 1926, Foster & Tate 1966, Simon & Enders 1978). Frothiness results from the co-production of carbon dioxide with ethanol (Phaff et al. 1978). Yeasts are believed to be introduced by insects to fallen fruits and woody plant sap-flows (do Carmo-Sousa 1969, Phaff et al. 1978). Woody plant sap-flows result from wounding by a variety of biotic and physical agents, as well as from unknown causes (Wilson 1926, Ohman & Kessler 1964, Simon & Enders 1966, Radwan 1969).

Here I examine whether or not individual *C. fumiferana* adults in the laboratory prefer diets for imbibing that contain 1% ethanol. I also compare the following seven fitness factors between groups of adults receiving diets spiked with concentrations of 0.1–5% ethanol: fertility, lifespan, preoviposition period, oviposition period, time to 80% oviposition, fecundity, and egg hatch. In designing and conducting this study, I drew heavily on previous personal experience with adult feeding in *C. fumiferana* (Miller 1987, 1989).

MATERIALS AND METHODS

The adults used here were collected as pupae from balsam fir (*Abies balsamea* [L.] Mill.) and white spruce (*Picea glauca* [Moench] Voss) in two successive years at three sites within 6 km of Hovland, Cook Co., Minnesota. The pupae were sexed using the guide of Jennings and Houseweart (1978). Sexed pupae were placed one pair per container in 1-pint (0.48 l) round cardboard ice cream cartons whose bottoms and tops were replaced with Petri dish bases and lids. Male and female pupae were matched developmentally so as to maximize eclosion synchrony. Pairs were assigned sequentially to different ethanol concentration treatments within fitness experiments so that early and late eclosing pairs would be equally distributed throughout. A fresh sprig of balsam fir 5–8 cm long was placed in each pair container as an oviposition substrate. Containers were kept on a table in a laboratory maintained at 25°C on a 12L:12D fluorescent lighting schedule. Diets for imbibing were provided to moths by means of saturated 3–4 cc³ synthetic

sponges. In the fitness experiments, there was one sponge per container, and the diet was renewed at intervals of 1–2 days. In the choice experiment, diets were provided only during tests.

The choice experiment consisted of placing adults individually in a round 1-gallon (3.8 l) 17.5 cm diameter cardboard ice cream carton with a glass cover, and observing each one for 20 min. Moth placement was at the center of the arena floor, 8 cm equidistant from two sponges on opposite sides of the floor, one soaked in a 1% solution of ethanol in either plain or sweetened water, the other soaked in the nonalcoholic equivalent. Sweetened water was 10% honey (v/v). If imbibing occurred, the time to its start was recorded. Each moth of each pair assigned to the choice experiment was used once daily in a test near midday under regular laboratory lighting.

In the fitness experiment with water-based diet, five concentrations of ethanol (0–5%) were provided, and in the experiment with 10% honey, three concentrations (0–1%) (v/v) were provided. In both experiments, data were collected from pair containers once daily near midday. Records were made of female and male eclosion dates, number of eggs laid daily, and dates of male and female deaths. Foliage was inspected for eggs with a 9 cm diameter reading glass. Eggs were removed from moth containers daily, counted under a stereomicroscope, and placed in labeled Petri dishes. Egg dishes were checked once daily to count the numbers of eggs hatching. Pairs were deemed fertile if the female laid any viable eggs. At death, females were stored in a freezer until they could be dissected for counting unlaidd mature eggs. Size and low stainability with methylene blue were the criteria by which unlaidd eggs were deemed chorionated and thus mature or ripe (Miller 1987).

RESULTS

Diet-choice experiment. Thirteen female and 13 male adults were observed 92 times in the choice arena. Because choice results with plain and sweetened water diets were virtually identical, they were pooled. The pooled results show that no choice was made 43 out of 92 times, an outcome consistent with previous findings that the moths do not always imbibe when given the opportunity (Miller 1989). Among the 49 choices made, the chosen diet was as often nonalcoholic as alcoholic (Fig. 1). Adults choosing the nonalcoholic diet took 5.5 min (SD = 6.1 min) on average to make a choice, while those choosing the alcoholic diet took 5.4 min (SD = 5.7 min). The moths that promptly made a choice walked directly to the sponge, often turning around once in place first. The preponderance of females over males in Fig. 1 is due to longer female lifespans.

Fitness experiments. Moths receiving 5% ethanol became ex-

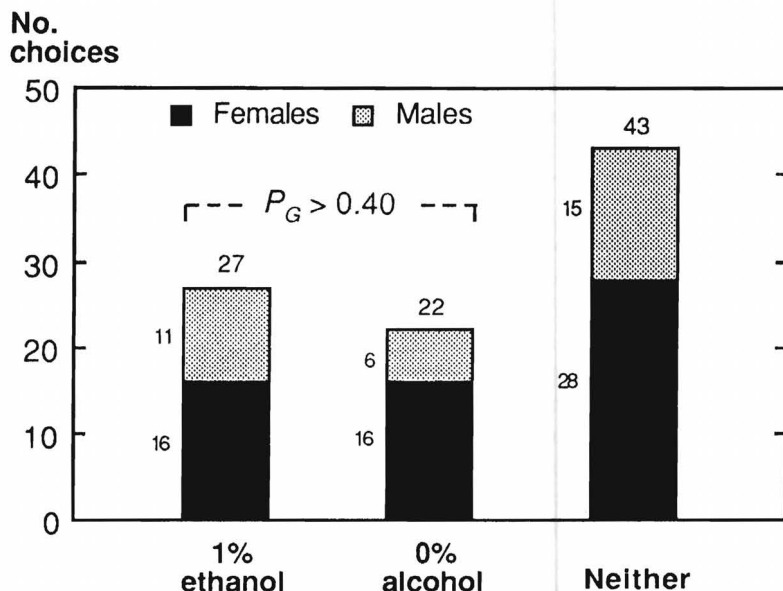


FIG. 1. Diet choices made within 20 min by individual *Choristoneura fumiferana* adults placed in the center of a 1-gallon (3.8 l) container with alcoholic and nonalcoholic diet sponges on opposite sides of the 17.5 cm diameter bottom. The alcoholic diet was 1% ethanol in either plain water or 10% honey water, and the nonalcoholic diet the same without ethanol. Results for the two experiments with different diet bases are pooled. The frequencies were tested for independence in a 2×2 contingency table using the G statistic.

tremely intoxicated. They were lying on their backs within minutes after imbibing. Fourteen out of 16 did not reproduce, and although these remained alive for a few days, they always appeared comatose. At 1% ethanol, no signs of intoxication were evident.

Fertility results from the two fitness experiments were pooled because of similar reproductive fractions in each experiment (Table 1). Fertility is broadly defined as the proportion of pairs reproducing (reproductive fraction). Pooled fertility dropped from 67% to 12% on diets of 0% to 5% ethanol, respectively (Fig. 2). Fertility of 67% at 0% ethanol is typical of normal laboratory fertility of *C. fumiferana* (Outram 1971, Miller 1987, 1989). Distinct fertility reduction started at 0.5% ethanol.

Results other than fertility from the two fitness experiments were not pooled because of the underlying differential effects of plain and sweetened water (Table 1; also Miller 1987, 1989). Within each experiment, preoviposition period, oviposition period, time to 80% oviposition, and lifespans of the sexes did not differ significantly among ethanol concentrations 0–1% (Table 1). It must be emphasized that these results represent only the fertile pairs at each ethanol concentration; results based on

TABLE 1. Performance of reproducing moth pairs receiving diets with different ethanol concentrations. Means are followed by SD's in parentheses. Only reproductive fractions pooled from the two experiments differ significantly among ethanol concentrations (Fig. 4); differences in other factors among ethanol concentrations of 0–1% are not significant ($P_F > 0.20$). Results for 5% ethanol were not included in the statistical analysis because of the small reproductive fraction.

Factor	Ethanol concentration				
	0%	0.1%	0.5%	1%	5%
Water-based diet					
Reproductive fraction, pairs/pairs	12/16	11/17	10/16	10/17	2/16
Mean preoviposition period (days)	2.8 (0.8)	2.3 (0.7)	2.8 (1.1)	3.1 (1.8)	1.0 (0.0)
Mean oviposition period (days)	7.4 (2.2)	5.0 (2.2)	5.0 (2.9)	5.1 (3.1)	3.5 (0.7)
Mean period female emergence to 80% oviposition (days)	7.2 (1.7)	6.0 (1.6)	6.3 (1.9)	6.6 (2.2)	4.5 (0.0)
Mean lifespan (days)					
female	10.2 (1.9)	7.2 (2.4)	7.8 (2.5)	7.8 (2.8)	4.5 (0.7)
male	8.7 (2.5)	7.0 (2.3)	8.1 (1.8)	6.7 (1.6)	5.5 (2.1)
10% honey-based diet					
Reproductive fraction, pairs/pairs	12/20	—	8/21	6/21	—
Mean preoviposition period (days)	2.2 (0.6)	—	2.2 (0.9)	2.5 (1.0)	—
Mean oviposition period (days)	13.6 (3.4)	—	14.1 (6.5)	17.3 (3.6)	—
Mean period female emergence to 80% oviposition (days)	9.2 (1.9)	—	8.8 (3.4)	9.8 (1.7)	—
Mean lifespan (days)					
female	15.8 (3.2)	—	16.4 (6.6)	19.8 (3.1)	—
male	12.4 (4.1)	—	10.5 (4.9)	11.3 (6.2)	—

all pairs would differ moderately among ethanol concentrations. It is clear without statistical analysis that the two reproducing pairs receiving 5% ethanol (Table 1) were severely impaired. These moths may have survived because of weak imbibing tendencies.

Numbers of eggs produced, laid, and hatched showed no significant differences among concentrations of 0–1% ethanol (Figs. 3, 4). Here again, the two pairs that reproduced on 5% ethanol clearly underperformed (Fig. 3). The absence of increase in fecundity of females on alcoholic compared with the nonalcoholic diets suggests that alcohol was not metabolized for energy.

DISCUSSION

Technically, "intoxication" refers to behavior while "toxication" refers to deeper effects such as fitness. Throughout this paper I use the more familiar term to refer to both. Gomez's (1973) description of intoxication in a female *Opsiphanes cassiae* L. (Nymphalidae), quoted below, appears to be the most detailed for a butterfly. The butterfly accidentally flew indoors and alighted near an uncorked bottle of wine containing

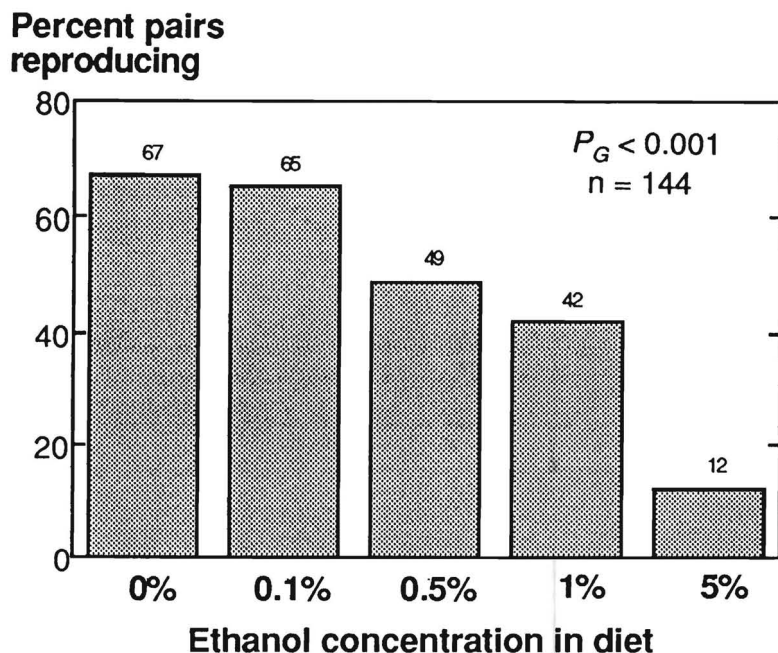


FIG. 2. Fertility or reproductive fraction of pairs of *Choristoneura fumiferana* adults receiving different ethanol concentrations for imbibing. Results for the two experiments with different diet bases are pooled.

12% ethanol. Imbibing a drop of proffered wine, it began to act abnormally within 5 min.

"First, some very slow up and down flapping of wings, followed by forewings being lowered and directed forward with brisk movements several times, hindwings remaining upright . . . [and moving] forward . . . until . . . propped far ahead of their normal resting position. Antennae were lowered until they touched the table. . . . Movement of fore-, hindwings and antennae were repeated several times. . . . After a brief period of inactivity, a hopping spastic side-walking took place alternating with wing and antennae motions as well as a tremulous and agitated moving of the legs. More wine was offered to the insect which sipped it directly from my fingertip. . . . Another sequence of the behaviour described above was observed until all wings were placed flat on the table. . . . A few forward strokes of forewings followed by a very fast vibratory flapping preceded a period of inaction. A few minutes later the butterfly took flight in a close-spiralling pattern towards an incandescent light, hitting the hot bulb several times, alighting and again attempting flight to the light . . . close to which it finally perched. After a few hours it resumed normal behaviour and flew away the next day."

Where adults are short lived, as in *Choristoneura fumiferana*, intoxication is more appropriately viewed in a fitness than behavioral context because reproduction is the predominant activity. The most ethanol-sensitive fitness factor proved to be fertility, broadly defined as the proportion of pairs reproducing (reproductive fraction). Fertility declined sharply from 49% at 0.5% ethanol to 12% at 5% ethanol (Fig. 2). It is

Mean no. eggs per female

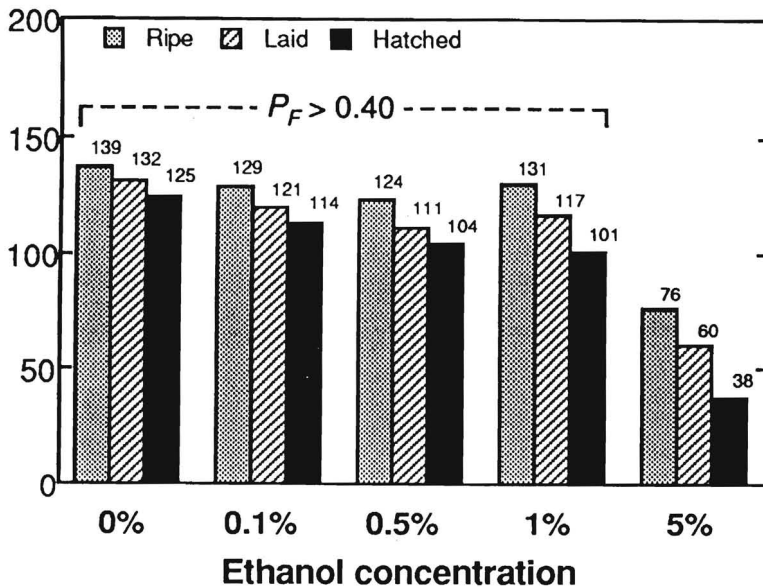


FIG. 3. Fecundity, oviposition, and egg hatch for *Choristoneura fumiferana* pairs receiving different ethanol concentrations in plain water for imbibing. Results represent fertile pairs only. F -tests were done separately for each egg category.

unclear at which interval fertilization was disrupted in the reproductive sequence of assembly, copulation, spermatophore transfer, sperm storage, and sperm use. However, the fact that females, and presumably males, are not known to imbibe until the third day of adulthood (Miller 1989) suggests disruption following spermatophore transfer, an interval usually occurring on the first or second day of adulthood (Outram 1971). Nonfertile females were not dissected for spermatophores.

No effects on *C. fumiferana* fitness were evident at 0.1% ethanol (Table 1, Figs. 2–4). However, it must be noted that actual quantities of ethanol ingested in this study are unknown. Previously, females were found to imbibe a mean of 4.5 mg of fluid per feeding (range of 0.9–10.0 mg) (Miller 1989).

One prominent instigator of sap-flows in forests inhabited by *C. fumiferana* is the yellow-bellied sapsucker, *Sphyrapicus v. varius* (L.). This bird pecks squarish holes through the bark to the sap-conducting phloem in many species of woody dicotyledons and gymnosperms (Foster & Tate 1966). Interiors of the holes are shaped so that sap collects in them. Sapsuckers and many other animals, including lepidopterans,

Mean no. eggs per female

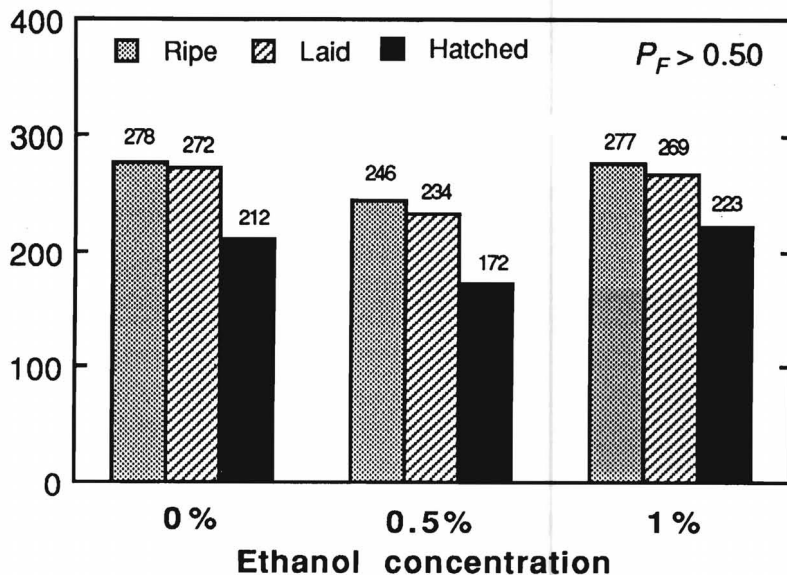


FIG. 4. Fecundity, oviposition, and egg hatch for *Choristoneura fumiferana* pairs receiving different ethanol concentrations in 10% honey for imbibing. Results represent fertile pairs only. *F*-tests were done separately for each egg category.

feed at these reservoirs. Insects that visit them comprise much of the sapsucker diet (Foster & Tate 1966). *Choristoneura fumiferana* adults are also part of the sapsucker diet (Tate 1973), and presumably are among the tortricids at sapsucker feeding holes (Foster & Tate 1966). Intoxication signs sometimes noted in the birds themselves are attributed to ingestion of fermentation products (Pearson 1936, Foster & Tate 1966). Sugar concentration in the sap of feeding holes is typically 1–6%, sometimes higher (Tate 1973). Ethanol concentrations of ferments in nature apparently have not been measured, but typical sugar concentrations in sapsucker feeding holes would yield ethanol concentrations no higher than perhaps 3%, or one-half of the sugar concentration, assuming complete fermentation. However, fermentation may be self-limiting and thus incomplete (Jorgensen & Hansen 1948). Another source of fermentable sugars in forests is aphid honeydews, a food resource adult lepidopterans are also known to exploit (Pittioni 1923, Zoebelein 1956, Johnson & Stafford 1985).

In certain *Drosophila* species, ethanol tolerance and metabolism are well developed and based on specific enzymes. Such species also exten-

sively exploit ethanol as a habitat cue (Chawla et al. 1981, Parsons 1981). By contrast, there is neither evidence for *C. fumiferana* adults metabolizing ethanol for energy, nor evidence for hormesis or small-dose enhancement (Clarke 1990). Whether enzyme-based mechanisms of ethanol tolerance exist in any lepidopteran is unknown.

As to why lepidopterans with imbibing capability tipple in the wild on ethanol or other fermentation products, I offer two hypotheses.

First, lepidopterans that are neither attracted to nor repelled by ferments have life systems that lack the capability to use fermentation products as cues for finding adult feeding opportunities. Tiny amounts of fluids suffice for these small moths to imbibe, and their fitness is increased by water intake with or without dissolved sugars (Norris 1934, Kira et al. 1969, El-Sherif et al. 1979, Miller 1987, 1988). Lepidopterans in this group orient to the water at feeding sites, and intoxication is accidental, *C. fumiferana* being an example.

Second, lepidopterans that are drawn to ferments have life systems with the capability to use fermentation products as cues for finding adult feeding opportunities. Members of this group are large bodied and require ample nutrient fluids containing dissolved sugars and perhaps other ingredients (Portier & de Rorthays 1940, Lukefahr & Martin 1964, Utrio 1983). If these lepidopterans become intoxicated from foraging at ferments, the cost is tolerable given the food value of the unfermented fraction and possibly of the fermented fraction also. *Opsiphanes cassiae*, the intoxicated butterfly described earlier (Gomez 1977), is an example. Other examples include Nymphalidae in the fruit-and-sap-feeding subfamilies Brassolinae, Morphinae, Satyrinae, and Nymphalinae (Young 1979), and many stout-bodied Noctuidae (Utrio 1983). Young (1979) has argued that eye spots on the wings of butterflies that forage at fallen fruits evolved as a defense to offset their greater vulnerability to predation while on the ground, perhaps also while intoxicated.

Future investigations toward understanding lepidopteran intoxication should include more species, more fermentation products, and the composition of ferments at feeding sites.

ACKNOWLEDGMENTS

This work was supported by the Research Apprentice Program of the Cooperative State Research Service, U. S. Dept. of Agriculture, and by the Young Scholars Program of the College of Biological Sciences, University of Minnesota. I thank T. Do and D. Drekonja for laboratory assistance, and C. L. Boggs, J. E. Rawlins, A. M. Fallon, K. A. Mesce, and S. J. Weller for useful manuscript reviews.

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Received for publication 13 February 1996; revised and accepted 9 December 1996.