Detecting Subtle Effects of Diet Preservatives on European Corn Borer (Lepidoptera: Crambidae)[1]

D. A. Andow[2] and T. J. Stodola

Department of Entomology, University of Minnesota, St. Paul, MN 55108 USA

J. Entomol. Sci. 36(3): 285-296 (July 2001)

Abstract A wheat germ-casien-agar diet for rearing European corn borer, *Ostrinia nubilalis* Hübner, contains five preservatives, sorbic acid (0.055% w/w), para-hydroxybenzoic acid methyl ester (methyl paraben, 0.144% w/w), propionic acid (0.488% w/w), aureomycin (0.292% w/w), and phosphoric acid (0.084% w/w). We conducted studies to determine if the first four of these preservatives can be reduced. In the first experiment we eliminated simultaneously propionic acid and aureomycin and either retained all sorbic acid and methyl paraben or reduced them by 50% or eliminated them as well. The diet with full sorbic acid and methyl paraben and no propionic acid and aureomycin performed similar to the unchanged control. All other diets resulted in microbial contamination that reduced survival of larvae. In the second experiment, we compared 5 diets, the full complement of sorbic acid and methyl paraben with elimination or 50% reduction of both propionic acid and aureomycin, elimination of aureomycin and 50% reduction in propionic acid. The last diet had no aureomycin or propionic acid and 50% reduction in methyl paraben. Some of the replicate dishes with diets without any propionic acid or aureomycin had microbial contamination that reduced survival of larvae. Larval survival was similar for the remaining diets. The diet without aureomycin and 50% reduction in propionic acid produced large larvae that were about half as variable in size as those from the control diet, suggesting that a reduction in these preservatives would increase moth uniformity. No differences in development rate were observed among the diets.

Key Words Sorbic acid, methyl paraben, aureomycin, propionic acid, meridic diet, European corn borer

Bottger (1942) developed the first synthetic diet for rearing European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), which was based on casein, cellulose, agar, and corn leaf powder. This diet was first improved by Beck et al. (1949) and later by Becton et al. (1962) and Guthrie et al. (1965). Around the same time, a wheat germ-casein-agar meridic diet was developed by Vanderzant and Adkisson (Adkisson et al. 1960, Vanderzant et al. 1962), although Beck (1953) had experimented earlier with wheat germ in insect diets. The Vanderzant/Adkisson diet was adapted for *O. nubilalis* (Lewis and Raun 1966, Lewis and Lynch 1969), and this has become the standard diet for rearing it in culture (Guthrie 1974, Guthrie et al. 1985). Several preservatives have been used to reduce the effect of bacteria and

[1]Received 15 November 1999; accepted for publication 21 October 2000.

[2]To whom correspondence should be addressed at Dept. of Entomology, 219 Hodson Hall, Univ. of Minnesota, St. Paul, MN 55108 USA (dandow@tc.umn.edu).

fungi on the insects reared on the diet. Lewis and Raun (1966) used 0.2% sorbic acid while Lewis and Lynch (1969) used 0.05% sorbic acid as a preservative. By 1974, the wheat germ diet contained 6 preservatives—sorbic acid, para-hydroxybenzoic acid methyl ester (methyl paraben), propionic acid mixed with phosphoric acid, aureomycin (chlorotetracycline), and formaldehyde (Guthrie 1974, Guthrie et al. 1985). Although there are no experimental data that demonstrate the necessity for all of these preservatives, the several decades of successful rearing of *O. nubilalis* on the diet suggests that the preservatives are not very detrimental to *O. nubilalis*.

Each of the preservatives acts in a different way. Sorbic acid interferes with carbohydrate metabolism, binds to the –SH group of enzymes, and destabilizes the cell membrane, and methyl paraben inhibits absorption of essential nutrients and amino acids and destroys cell membranes (Lück and Jager 1997). Propionic acid is used as a preservative in bread in some countries. It has an unspecified mechanism of action (Lück and Jager 1997). Phosphoric acid inhibits microbial cells by providing a high concentration of H+ (Russell and Gould 1991). Aureomycin is synthesized by *Streptomyces*, and inhibits binding of tRNA to the small ribosomal subunit (Conte and Barriere 1988). Although it inhibits both eukaryotic and prokaryotic protein synthesis, most bacteria have an active transport system that results in a higher intra-cellular concentration compared to eukaryotic cells (Gale et al. 1981). At low concentrations (0.2% v/v) formaldehyde exhibits bacteriostatic properties (Ewart 1987), while at higher concentrations formaldehyde is lethal to bacteria, yeasts, and molds (Chapman 1987).

Diet preservatives can have detrimental effects on insects when present at high concentrations, including sorbic acid (Clark et al. 1961, Ouye 1962, Power and Singh 1974), methyl paraben (Clark et al. 1961, Ouye 1962, Singh and House 1970b, Power and Singh 1974), and aureomycin (Ignoffo 1963, Singh and House 1970b). Although the effects of the preservatives on *O. nubilalis* may be slight when used at moderate concentrations, recent work by Warnock (1998) and Bolin (1999) has made us question this assumption. Warnock (1998) found it difficult to distinguish among different antifeedant chemicals in resistant sweet corn lines when using European corn borer reared on diet with preservatives, but possible to distinguish differences when the preservatives were excluded. Bolin (1999) was not able to select for resistance in European corn borer to Cry1Ac toxin when the preservatives were in the diet, but when excluded, resistance evolved rapidly. Singh and House (1970a) showed that diet preservatives that had no discernable effects on healthy larvae did have detrimental effects on the larvae when they are nutritionally stressed. Thus, when larvae are exposed to other toxins in the meridic diet, such as secondary plant compounds or Cry toxins, the preservatives could cause all larvae to perish, thereby masking the effects of the toxins.

Consequently, we decided to investigate if some of the diet preservatives can be reduced without jeopardizing the growth and development of larvae due to microbial contamination. At the acidic pH of the diet, sorbic acid and methyl paraben are effective at suppressing growth of molds, yeasts and bacteria, while propionic acid is less effective than either of these (Sofos 1989, Lück and Jager 1997). Aureomycin is commonly used to suppress bacteria, but at acidic pH, bacterial contaminants are usually less compared to molds and yeasts (Lück and Jager 1997). We focused our experimental effort to making larger reductions in propionic acid and aureomycin and smaller reductions in sorbic acid and methyl paraben.

Materials and Methods

European corn borers were obtained from a colony established from field collections near LeSueur, MN, during 1993 and reared on wheat germ-casein-agar diet in our lab for about 60 generations following the methods of Guthrie et al. (1985) with slight modifications. Our principle methodological modification is to monitor larval-pupal survival in the diet and use only dishes with >80% survival to propagate the colony, which reduces inadvertent selection on the colony. For example, we have not observed a reduction in ability to feed on corn by this colony, despite one published observation that feeding ability on corn deteriorated after only 15 generations of rearing on the diet (Guthrie et al. 1980). Despite our care in reducing selection on the colony, it is possible that the colony has adapted to the preservatives in the diet; consequently, we developed a sensitive method for detecting the effects of preservatives.

The composition of our present diet is the same as Guthrie's (1974) wheat germ diet except that we have not used formaldehyde for the past ~15 yrs (Table 1). Experimental methods followed our standard rearing procedures. Diet was prepared the day before use and surface-sterilized with sterilizing UV light for 1 h at a distance of 60 cm after dispensing into sterile dishes. Egg masses from the colony were randomly assigned at the black-headed stage to diet treatments. Each replicate dish had 65 ml of diet and was incubated at 27°C under 24 h light. Two days after the eggs hatched, the hatching rate was estimated, and any dish with less than 90% hatch was discarded. Nineteen days later (occasionally 20 d later), all larvae and pupae were counted, staged and weighed. Two sub-stages of fifth instars were distinguished—a darker, feeding stage, designated stage 1, and the creamy white, pre-pupae as stage 2. Although several stages of pupae can be distinguished (Gelman and Hayes 1982), we simplified the scheme, distinguishing only four of their stages. The pre-tanned stage is the first stage and lasts 1 d (stage 3); the eyespot stage lasts about a d after the pre-tanned stage (stage 4); and the wing-band stage is the last d of the pupal period (stage 6). About 80 to 90% of the population in a dish at the time of sampling was in the intermediate pupal stage (stage 5), which lasts for 4 to 5 d. Adults were stage 7, but less than 1% had emerged as adults. Adults were counted, but not weighed. The proportion of diet covered with fungal hyphae was estimated by visual examination. Odors from microbial contaminants were also noted.

Two experiments were conducted. The first experiment compared the standard diet containing preservatives with considerable reductions in aureomycin and propionic acid (Table 2). Between 6 to 8 replicate dishes starting with about 80 eggs per dish were used for each diet, resulting in at least 480 larvae tested on each diet. Based on the results of this experiment, the second experiment (trials 1 and 2) was designed to evaluate smaller reductions in preservatives (Table 2). There were 8 replicate dishes in each trial starting with 60 eggs per dish, resulting in 960 larvae tested in each diet. Replicate dishes were blocked within trials by source of eggs, location in rearing chamber, and evaluation date, resulting in an incomplete randomized block design within trials, and an incomplete split randomized complete block design for the entire experiment.

The number of survivors was calculated as the total number of individuals surviving to when the dishes were scored. Survival could also be calculated as a proportion of the initial number of larvae, but this neither improves the analysis nor interpretation of results. Data were analyzed by ANOVA (Proc GLM, SAS 1988), after testing for the

Table 1. Wheat germ-casein-agar diet used to rear European corn borer, *Ostrinia nubilalis* containing the full complement of diet preservatives used in experiments

Component	Volume	Percent (w/w)
Distilled water	12.38 l	85.0
Agar	280 g	1.922
Wheat germ	520 g	3.570
Dextrose	400 g	2.746
Casein	440 g	3.021
Cholesterol	32 g	0.220
Salt mixture UPS XIII No 2*	144 g	0.989
Vitamin supplement**	92 g	0.632
Ascorbic acid	120 g	0.824
Fumidil B (500 ppm)	6.9 g	0.047
Sorbic acid	8 g	0.055
Methyl paraben	21 g	0.144
Propionic acid	72 ml	0.488
Phosphoric acid	7.2 ml	0.084
Aureomycin	42.5 g	0.292

* Composition of Salt Mixture (ICN Biomedicals, Inc., Percent): Calcium Biphosphate 13.58%, Calcium Lactate 32.69, Ferric Citrate 2.96, Magnesium Sulfate 13.70, Potassium Phosphate Dibasic 23.99, Sodium Biphosphate 8.73, Sodium Chloride 4.35.

** Composition of Vitamin Supplement (ICN Biomedicals, Inc.; gm/kg mixture): Vitamin Acetate 1.800, Vitamin D_2 0.125, DL-α-Tocopherol Acetate 22.000, Ascorbic Acid 45.000, Inositol 5.000, Choline Chloride 75.000, Menadione 2.250, p-Aminobenzoic Acid 5.000, Niacin 4.250, Riboflavin 1.000, Pyridoxine Hydrochloride 1.000, Thiamine Hydrochloride 1.000, Calcium Pantothenate 3.000, Biotin 0.020, Folic Acid 0.090, Vitamin B_{12} 0.00135.

homogeneity of error variance using Levene's test (Snedecor and Cochran 1980). Developmental speed was examined by constructing the cumulative distribution function of development stage, which is done by calculating the proportion of individuals that were of a particular developmental stage or younger. Data were analyzed by using the Kolmogorov-Smirnov one-sample, two-sided statistic to construct 95% confidence bands around each of the distribution functions and looking for lack of overlap of the bands (Daniel 1978). This enables detection of any kind of difference in development speed, including variance and skew.

Weights were not distributed normally, and no transformation appropriately stabilized error variance. Examination of the residual plots indicated that most dishes had a bimodal distribution of weights. To examine this distribution, eight additional dishes of diet 7 (Table 1) were prepared and infested with 60 neonates each. After 19 days, the larvae and pupae were counted, weighed and separated into vials. Each individual was allowed to eclose, and the sex of the adult moth was recorded. Cumulative

Table 2. Description of experimental diet treatments, showing the use of the various preservatives in each of the experimental diets as a percent of the control diet given in Table 1

Experiment	Diet	Sorbic acid (100 = 0.055%)	Methyl paraben (100 = 0.144%)	Aureomycin (100 = 0.292%)	Propionic acid mix (100 = 0.488%)
1	1	100	100	100	100
	2	0	0	0	0
	3	100	100	0	0
	4	50	50	0	0
2	1	100	100	100	100
	3	100	100	0	0
	5	100	100	50	50
	6	100	50	0	0
	7	100	100	0	50

distributions by weight were plotted for each dish, and we observed two clusters of individuals, with the smaller ones being males and the larger ones being females. The range of weight over which the male and female distributions overlapped, and the fraction of individuals within the overlapping range was recorded for each dish.

Because we confirmed that the larger individuals were females and the smaller ones were males, we used several descriptive statistics to characterize these weight distributions (O'Hagan 1994). These statistics enabled us to detect subtle effects of the preservatives on the weights of European corn borer. First, a distribution function of weights was constructed for each dish (Fig. 1a). From this distribution function we estimated the probability density associated with each weight. The probability density associated with a particular weight is equal to the slope of the distribution function at that weight, so we used the data in the distribution function to estimate the slope. At each point on the distribution function, we used that point and either the 1, 2, 3, 4, or 5 flanking points on each side of the point to estimate the slope. These correspond to 3-, 5-, 7-, 9-, and 11-point running slope estimates (Fig. 1b-d). A greater number of points results in a smoother probability density curve, which can mask some of the actual variability in density, while fewer points results in a more jagged probability density curve, which can overemphasize the influence of a single individual weight. Based on our inspection of many of these curves, we chose the 5-point density functions as that which revealed variation, but did not overemphasize single individuals. Heuristically, this makes sense because each individual is weighted 20% in the 5-point density functions, which allows individuals to have a significant effect while not dominating the slope estimate. We specified the two modes (ω_1 and ω_2—the highest points on the density function), measures of spread around those modes (ρ_1 and ρ_2), the difference between the modes (δ), and the proportion of individuals weighing less than the anti-mode ($F(\omega_3)$) (Fig. 2). We stabilized error variance of each variable with a square root transformation (Levene's test), and analyzed each mode and its spread

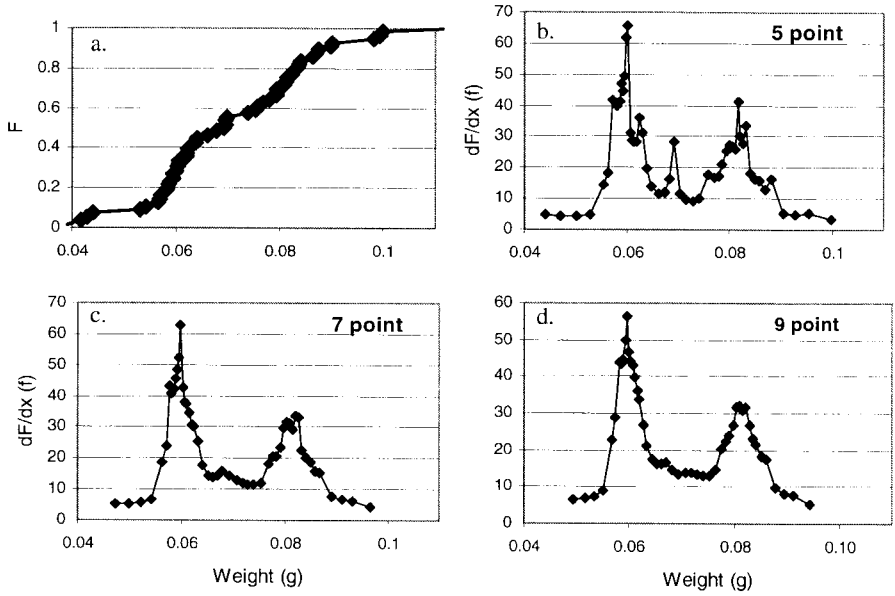

Fig. 1. Example of weights observed in a single illustrative replicate dish (Trial 1, Dish 5-2). F is the cumulative distribution of weights, and dF/dx is the probability density function (f) for the weights. The probability density function was estimated by estimating the instantaneous slope of F using 3-, 5-, 7-, 9- or 11-point running sequences of data from F. We used the 5-point running slope to determine essential characteristics about the distribution of weights. The curves corresponding to the 3- and 11-point running slope are not shown. The 3-point curve was not smooth enough, and the 11-point curve was no more informative than the 9-point curve. a) Cumulative distribution function; b) 5-point running slope estimate of density function; c) 7-point running slope estimate of density function; d) 9-point running slope estimate of density function.

measure with MANOVA (ω_1 and ρ_1; ω_2 and ρ_2), and the other two measures by ANOVA. All analysis was done using Proc GLM (SAS 1988).

Results and Discussion

Experiment 1. All dishes with diet 2 (no preservatives) had an excessive amount of fungal growth, and very few larvae reared on the diet completed development (mean 0.1/dish). All dishes of diet 4 had at least some hyphal growth, and only 24.4 larvae/dish survived in this treatment. Only 3 of the 14 dishes of the other two diets had any hyphae growth and produced larval numbers of 63.3 to 74.9 larvae/dish (Table 3). These results indicate that we can reduce aureomycin and propionic acid without suffering visual fungal contamination as long as we retain sufficient sorbic acid and methyl paraben in the diet.

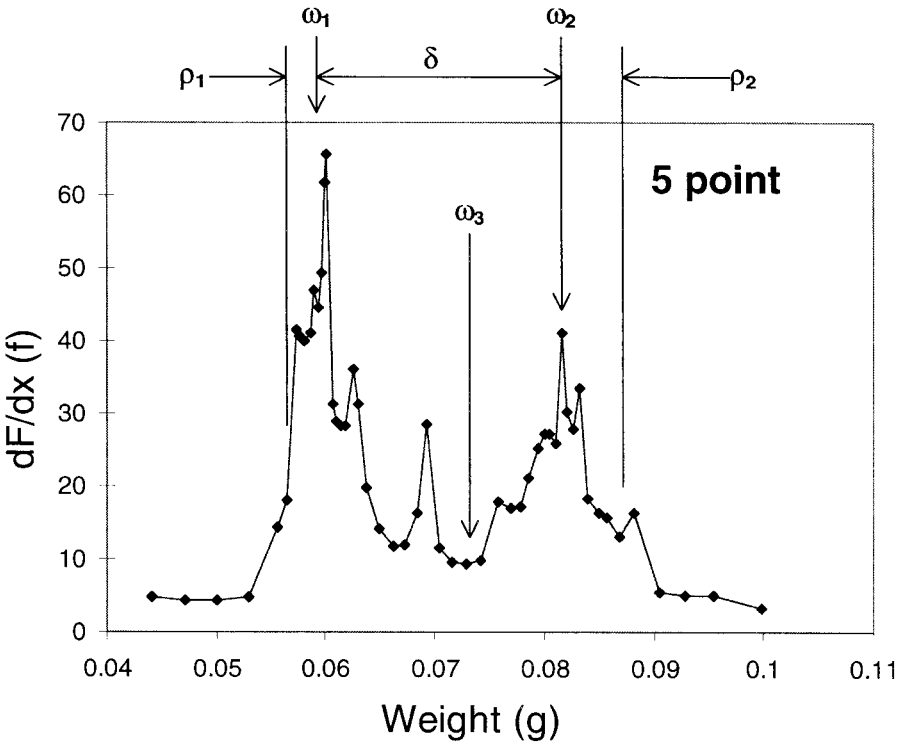

Fig. 2. Descriptive statistics derived from the 5-point density functions of weight. ω_1 is the mode of the lower peak, which corresponds to male larvae. ρ_1 is the difference in weight between the lower mode and the 50% percentile of data to the left of the mode. This is the lower interquartile range of the mode, and is a measure of variation associated with that mode. ω_2 is the mode of the upper peak, which corresponds to female larvae. Similar to ρ_1, ρ_2 estimates the upper interquartile range of the upper mode, and is a measure of its variation. δ is the weight difference between the two modes. ω_3 is the antimode between the two modes, and approximately separates males and females, and $F(\omega_3)$ is approximately equivalent to the sex ratio (proportion of males).

Experiment 2. Fungal hyphae grew in 18 of the 32 dishes of diets 3 and 6, and a smaller number of larvae survived in these treatments (Table 3). This suggests that some aureomycin and/or propionic acid is needed in the diet. The number of surviving larvae in the other three diets ranged from 52.5 to 57.3/dish, which were not significantly different. No dishes in these treatments had visual or olfactory signs of contamination. These results suggest that aureomycin is not needed in the diet (diet 7). There were no significant differences in development rate among the diets (Fig. 3).

Weights of the immature stages had a bimodal distribution within almost every dish. Based on the rearing experiments, we determined that the larger individuals

Table 3. Mean (±SE) number of survivors per diet dish in each experimental diet. The diets are described in Table 2. Dashes indicate diets that were not tested in a trial

Experiment		Diet 1	2	3	4	5	6	7
1	Survivors	74.9	0.1	63.3	24.4	—	—	—
	S.E.	1.8	0.1	5.1	13.2			
	n	8	8	8	5			
2	Survivors	57.3	—	54.3	—	55.3	49.5	52.5
Trial 1	S.E.	3.5		3.7		2.1	3.2	4.1
	N	8		8		8	8	8
2	Survivors	53.6	—	49.0	—	57.5	45.1	54.4
Trial 2	S.E.	3.2		6.4		4.2	3.2	5.7
	n	8		8		8	8	7

corresponded to mostly females, and the smaller ones to mostly males. In dishes where the male and female distributions overlapped, the difference in weight between the largest male and the smallest female was 0.0042 ± 0.0013 grams, which represents 6.9 ± 2.2% of the total range of larval weights. The proportion of males and females in the overlapping weight range was 0.14 ± 0.05 of the total population of a dish.

The MANOVAs on weight indicated that there was a significant effect of diet on both modes. For the first and second mode, Wilk's $\lambda = 0.796$ and 0.762, $F_{8,136} = 2.05$ and 2.48, and $P = 0.045$ and 0.015, respectively. The modal size of small immature *O. nubilalis* (ω_1) was slightly less in diet 3 than the other diets, and the modal size of large immature *O. nubilalis* (ω_2) was slightly less in diets 3 and 6 than the other diets (Fig. 4). Diet 6 had more variable immature weights associated with the small mode (ρ_1), and diet 7 had the least variability associated with the large mode (ρ_2) (Fig. 4), with a reduction in variability of about half. Thus, it appears that diets 3 and 6 produce smaller and/or more variable insects compared to the other diets, and diet 7 produces less variable insects compared with diets 1 and 5.

There were no significant differences in the weight difference between the modes (δ) among the diets ($P = 0.066$), which averaged 0.016 g. In addition, there were no differences in the proportion of small individuals ($\mathbf{F}(\omega_3)$) among the diets ($P = 0.788$), although on average 52.9% were small individuals.

We detected a subtle effect of diet preservatives. Larger, probably female, larvae were more uniform in size when aureomycin was eliminated and propionic acid was reduced 50% compared to the standard diet. This subtle effect could be detected only by using the statistical methods we developed to analyze the bimodal weight data. It should, however, be expected that the effects of the preservatives, if any, would be subtle. Singh and House (1970a) showed that the effects of preservatives could depend on the nutritional content of the diet. Large effects of preservatives on insects

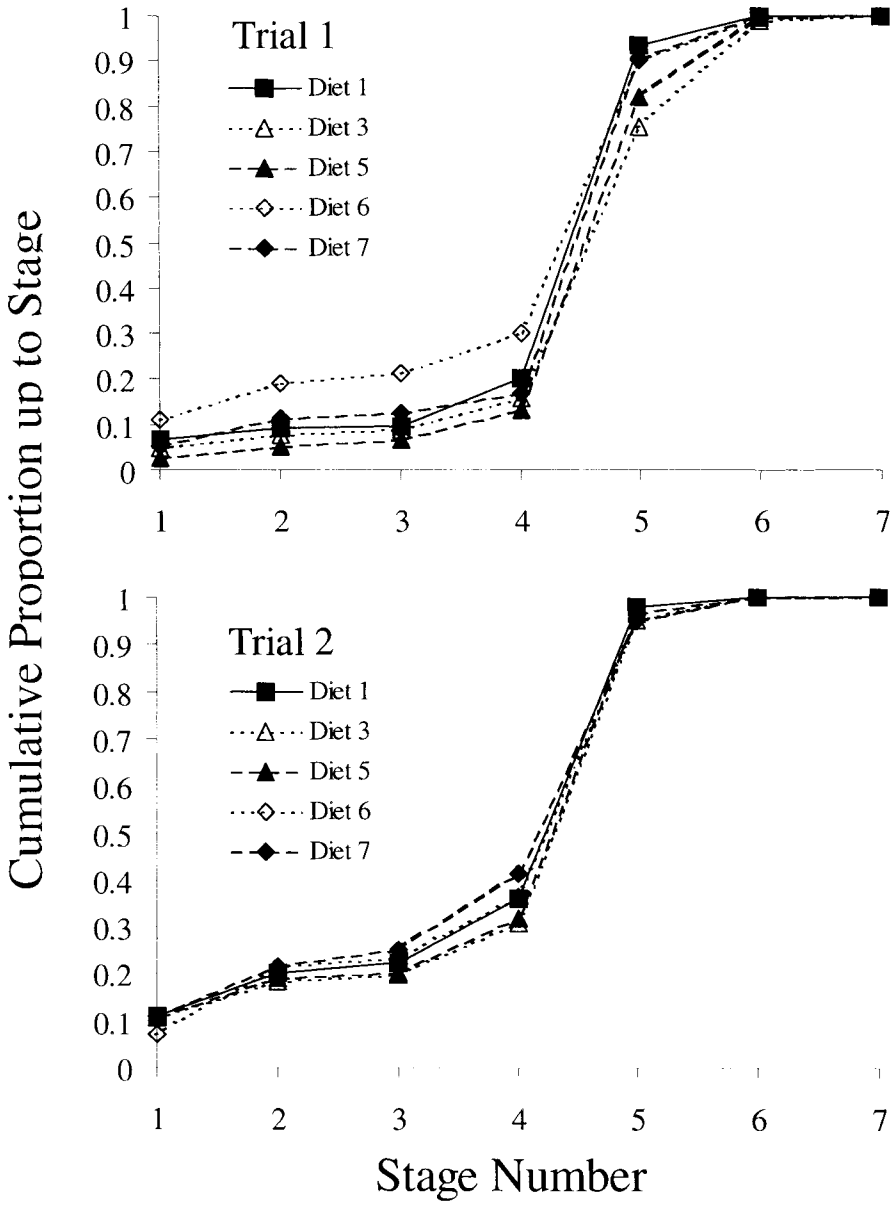

Fig. 3. Distribution of developmental stages of European corn borer for the experimental diets in Experiment 2. The stages are: (1) fifth instar dark, feeding stage, (2) fifth instar creamy white, pre-pupal stage, (3) pre-tanned pupal stage, (4) the eyespot pupal stage, (5) intermediate pupal stage, (6) the wing-band pupal stage, and (7) adult.

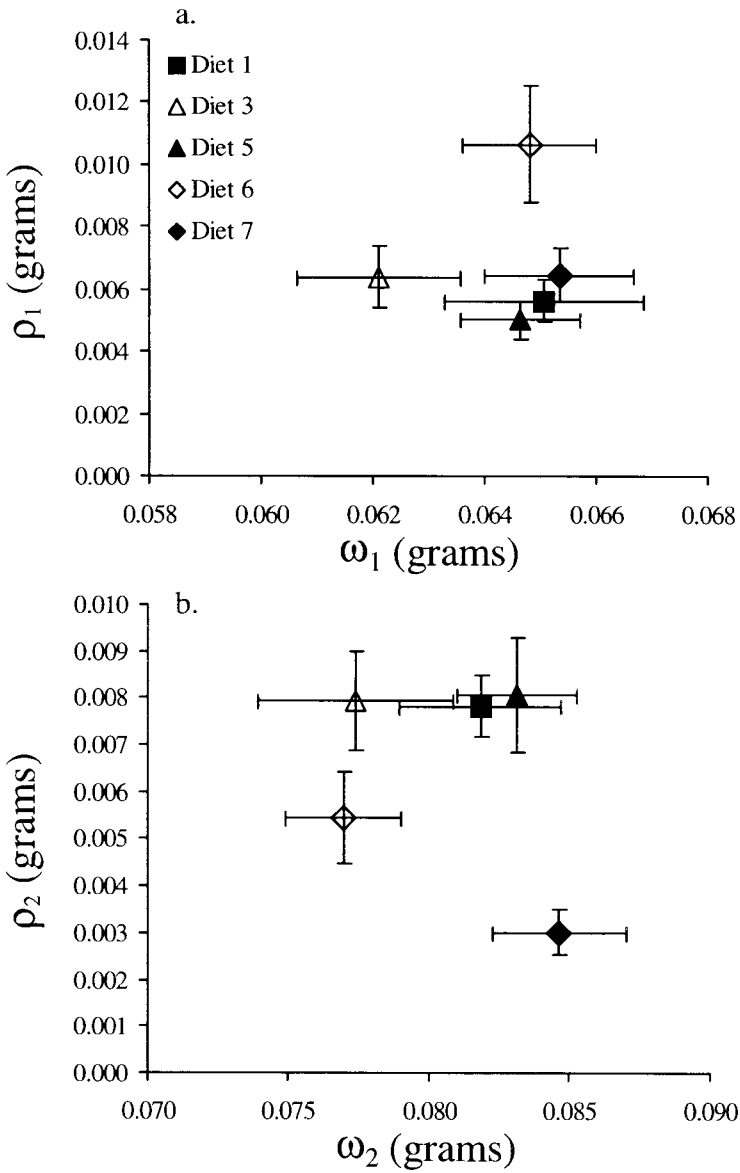

Fig. 4. Interaction between modal weight (ω) and a measure of variation around the mode (ρ) for the experimental diets in experiment 2 for both trials combined, showing standard errors of the untransformed means for the (a) smaller, first mode and the (b) larger, second mode.

feeding on diets with good nutritional content only occurred at very high concentrations of preservatives. According to this hypothesis, European corn borers feeding on the nutritious wheat germ-casein-agar diet should be little affected by the preservatives. Our observed slight effects are consistent with this hypothesis.

We conclude that diets 1, 5, and 7 produced similar sized individuals that developed at a similar rate with a similar level of mortality. Diet 7, however, produced slightly less variable large insects. Consequently, it is possible to reduce the amounts of aureomycin and propionic acid in the diet without detrimentally affecting larval growth and development, and possibly improving the uniformity of production. We have now reared *O. nubilalis* since 19 January 1999 (18 generations) using diet 7, which has a 50% reduction in propionic acid and no aureomycin, without loss of production or visible microbial contamination.

Although the 0.055% sorbic acid and 0.144% methyl paraben was needed in all of the diet combinations we tried, we cannot be sure that both are necessary at their present concentrations. Both are effective suppressants of molds and yeasts at low pH. Because sorbic acid is more effective at suppressing microbes at pH 4 than methyl paraben (Lück and Jager 1997), it may be possible to reduce the concentration of methyl paraben as long as sorbic acid and propionic acid are present at 0.055% and 0.244%, respectively.

Acknowledgments

We thank T. Kurtti and J. Harmon and the rest of the Insect Ecology lab for their comments on this work. Funding came in part from USDA Regional Research Project NC-205. This is publication 99-117-0006 of the University of Minnesota Agricultural Experiment Station.

References Cited

Adkisson, P. L., E. S. Vanderzant, D. L. Bull and W. E. Allison. 1960. A wheat germ medium for rearing the pink bollworm. J. Econ. Entomol. 53: 759-762.

Beck, S. D. 1953. Nutrition of the European corn borer, *Pyrausta nubilalis* (Hübn.). III. An unidentified dietary factor required for larval growth. J. Gen. Phys. 36: 317-325.

Beck, S. D., J. H. Lilly and J. F. Stauffer. 1949. Nutrition of the European corn borer, *Pyrausta nubilalis* (Hbn.). Ann. Entomol. Soc. Amer. 42: 483-496.

Becton, A. J., B. W. George and T. A. Brindley. 1962. Continuous rearing of European corn borer larvae on artificial diet. Iowa State J. Sci. 37: 163-172.

Bolin, P. C. 1999. Durability of *Bacillus thuringiensis* transgenic corn for management of the European corn borer (Lepidoptera: Crambidae), 185 pp. Ph.D. Diss., Univ. Minnesota, St. Paul.

Bottger, G. T. 1942. Development of synthetic food media for use in nutrition studies of the European corn borer. J. Agric. Res. 65: 493-500.

Chapman, D. G. 1987. Preservatives Available for Use. *In* Board R. G., M. C. Allwood and J. G. Banks (eds.), Preservatives in the Food, Pharmaceutical and Environmental Industries. Blackwell Scientific Publications, Boston.

Clark, E. W., C. A. Richmond and J. M. McGough. 1961. Artificial media and rearing techniques for the pink bollworm. J. Econ. Entomol. 54: 4-9.

Conte, J. E., Jr. and S. L. Barriere. 1988. Manual of antibiotics and infectious diseases, 6th edition. Lea and Febiger, Philadelphia, PA.

Daniel, W. W. 1978. Applied nonparametric statistics. Houghton Mifflin, Boston.

Egan, H., R. S. Kirk and R. Sawyer. 1981. Pearson's Chemical Analysis of Foods. Churchill Livingstone, NY.

Ewart, J. M. 1987. Preservation of milk samples. *In* Board, R. G., M. C. Allwood and J. G. Banks (eds.), Preservatives in the food, pharmaceutical and environmental industries. Blackwell Scientific Publications, Boston.

Gale, E. F., E. Cundliffe, P. E. Reynolds, M. H. Richmond and M. J. Waring. 1981. The molecular basis of antibiotic action. John Wiley and Sons, London.

Gelman, D. B. and D. K. Hayes. 1982. Methods and markers for synchronizing maturation of fifth-stage larvae and pupae of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 75: 485-493.

Guthrie, W. D. 1974. Techniques, accomplishments and future potential of breeding for resistance to European corn borers in corn, Pp. 359-380. *In* F. G. Maxwell and F. A. Harris (eds.), Proc. Summer Institute on Biol. Cont. of Plant Insects and Diseases. Univ. Press of Mississippi, Jackson, MS.

Guthrie, W. D., E. S. Raun, F. F. Dicke, G. R. Pesho and S. W. Carter. 1965. Laboratory production of European corn borer egg masses. Iowa State J. Sci. 40: 65-83.

Guthrie, W. D., F. A. Onukogu, W. H. Awadallah, J. C. Robbins and M. Lodholz. 1980. Changes in survival and development of cultures of European corn borers, *Ostrinia nubilalis,* reared in the laboratory on a meridic diet. Iowa State J. Sci. 56: 35-46.

Guthrie, W. D., J. C. Robbins and J. L. Jarvis. 1985. *Ostrinia nubilalis,* Pp. 407-413. *In* P. Singh and R. F. Moore, eds., Handbook of insect rearing, Volume 2. Elsevier, NY.

Ignoffo, C. M. 1963. A successful technique for mass-rearing cabbage loopers on a semisynthetic diet. Ann. Entomol. Soc. Amer. 56: 178-182.

Lewis, L. C. and R. E. Lynch. 1969. Rearing the European corn borer, *Ostrinia nubilalis* (Hübner), on diets containing corn leaf and wheat germ. Iowa State J. Sci. 44: 9-14.

Lewis, L. C. and E. R. Raun. 1966. Consumption and utilization of laboratory diets by European corn borer. Iowa State J. Sci. 41: 173-180.

Lück, E. and M. Jager. 1997. Antimicrobial food additives: Characteristics, uses, effects, 2nd edition. Springer Verlag, NY.

O'Hagan, A. 1994. Kendall's advanced theory of statistics, Volume 2B, Bayesian inference. Halsted Press, NY.

Ouye, M. T. 1962. Effects of antimicrobial agents on micro-organisms and pink bollworm development. J. Econ. Entomol. 55: 854-857.

Power, R. J. B. and P. Singh. 1974. Laboratory rearing method for the stem weevil, *Hyperodes bonariensis* (Coleoptera: Cuculionidae). New Zealand J. Zool. 1: 531-536.

Russell, N. J. and G. W. Gould. 1991. Food preservatives. Van Nostrand Reinhold, NY.

SAS. 1988. SAS/STAT user's guide, Release 6.12. SAS Institute Inc., Cary, NC.

Singh, P. and H. L. House. 1970a. Effects of streptomycin and potassium sorbate levels in relation to nutrient levels on the larvae of *Agria affinis.* J. Econ. Entomol. 63: 449-454.

1970b. Antimicrobials: "Safe" levels in a synthetic diet of an insect, *Agria affinis.* J. Insect Physiol. 16: 1769-1782.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical methods, 7th edition. Iowa State Univ. Press, Ames, IA.

Sofos, J. N. 1989. Sorbate food preservatives. CRC Press, Boca Raton, FL.

Vanderzant, E. S., C. D. Richardson and S. W. Fort, Jr. 1962. Rearing of the bollworm on artificial diet. J. Econ. Entomol. 55: 140.

Warnock, D. F. 1998. Bioassay-identified ear tissue resistance to European corn borer, *Ostrinia nubilalis* (Hübner), in sweet corn and its relationship to field resistance, Ph.D. Diss., Univ. Minnesota, St. Paul, MN. 200 p.