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Dosage Effects Between Dietary Niacin and Other B Vitamins on Larval Development of *Ceratitis capitata* (Diptera: Tephritidae)

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ABSTRACT The nutritional interaction between niacin (nicotinic acid) and a group of nine other B vitamins in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), larval diet was evaluated. Four doses of niacin (0, 0.2, 2.0, and 20 ppm) and four doses of a mixture of nine other B vitamins (0, 7.07, 70.7, and 707 ppm) in the diet were cross-tested. The other B vitamins consisted of thiamin, riboflavin, pantothenic acid, pyridoxine, folic acid, biotin, inositol, choline chloride, and p-amino benzoic acid. Diets with high doses (≥70.7 ppm) of the other B vitamins and low doses (≤0.2 ppm) of niacin resulted in no pupal recovery. However, a delayed larval duration and reduced pupal recovery resulted when no niacin and 7.07 ppm or lower of the other B vitamins were compared with those from a complete diet that contained 20 ppm niacin and 707 ppm of the other B vitamins. With omission of the other B vitamins, larval duration was delayed but was not dose dependent on niacin. Pupal recovery was not affected if niacin levels were maintained at or >0.2 ppm and the other B vitamins were absent. With 20 ppm of niacin in the diet, the larval duration and pupal recovery were dependent on the dose of the other B vitamins. Larval durations were shortened with elevated doses of the other B vitamins, whereas pupal recoveries increased with elevated dose of the other B vitamins. Therefore, the other vitamins reduced the larval duration when niacin was maintained at ≥2 ppm. When the niacin levels decreased to ≤0.2 ppm, doses of the other vitamins has to be reduced <7.07 ppm to maintain normal larval development.

KEY WORDS niacin, nicotinic acid, diet, larval diet

NICOTINIC ACID, ALSO KNOWN as niacin or vitamin B_3, is important for the normal function of many physiological processes. Like other B vitamins, niacin is water soluble. It plays an essential role in animals in the process of turning food into energy, as well as in the metabolism of fats and carbohydrates, along with other vitamins such as riboflavin and pyridoxine, niacin helps keep skin, digestive tract, and nervous system of vertebrates functioning properly. Niacin has been reported as a hypolipidemic agent for affecting the growth and development of nematodes in vivo and in vitro (Ritter and Smith 1989) and as a feeding stimulant for twospotted spider mite, *Tetranychus urticae* Koch (Dabrowski 1974). Many investigations have shown the effect and importance of dietary niacin and other vitamins on insect growth and development (Saksena and Perti 1971, Eymann and Friend 1985, Jang 1986, Ritter and Smith 1989, Zang and Ma 1991, Bashan and Emre 1992, Levinson et al. 1992). Studies have shown that diets lacking niacin can induce physiological malfunctions such as shortened oviposition period (Ozalp and Emre 1992), reduced egg hatch (Tsiropoulos and Hagen 1987), lowered reproduction rate (Levinson et al. 1992), and decreased lipid content (Ritter and Smith 1989). There are, however, very few published studies dealing with the biological or biochemical interaction between niacin and other vitamins. This study evaluated the interaction between various doses of niacin and a group of nine other B vitamins present in a meridic larval diet previously developed by Chang et al. (2001) for *Ceratitis capitata* (Wiedemann).

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Materials and Methods

Insects. Eggs of *C. capitata* were obtained from adult colonies of the Maui Med-93 strain maintained at USDA–ARS and Pacific Basin Agricultural Research Center in Honolulu, HI. This colony has been reared on a modified diet of Tanaka et al. (1969). Newly hatched larvae (within 12 h) were used as the starting stage for bioassay in this study.

Diets. *C. capitata* #1 meridic larval diet described by Chang et al. (2001) was used as the base diet in this study. The vitamin compositions of this diet are listed in Table 1. Niacin and a cocktail of the other nine vitamins (thiamin, riboflavin, pantothenic acid, pyridoxine, folic acid, biotin, inositol, choline chloride, p-amino benzoic acid) were used throughout this study.

Table 1. Composition of vitamins in *C. capitata* #1 meridic larval diet

Item	Vitamins	mg/50 g diet
1	Niacin (nicotinic acid)	1.00
2	Thiamin	1.00
3	Riboflavin	1.00
4	Pantothenic acid	1.00
5	Pyridoxine	1.00
6	Folic acid	0.25
7	Biotin	0.10
8	Inositol	10.00
9	Choline chloride	20.00
10	ρ-Amino benzoic acid	1.00

All diets were prepared as follows: a mixture of 18 amino acids and the vitamin mixture were each prepared in bulk. They were combined with other ingredients: sugar (McCollum & Davis, Aurora, OH), salt mixture no. 185, cholesterol, methylparaben, sodium benzoate, streptomycin, oxytetracycline HCl, citric acid, and ribonucleic acid (ICN Pharmaceuticals, Costa Mesa, CA). All dry materials including 12 g of corncob bulking agent (Mt. Pulaski Proudcts, IL) were weighed into sterile polyethylene Stomacher blender bags (80 ml, 16 by 10 cm) and mixed in a Stomacher laboratory blender (400-ml capacity) (Daigger and Company, Lincolnshire, IL) at normal-speed setting (230 rpm) for 60 s. The diet mixture was mixed in a Stomacher blender with 33 ml of hot water (55°C) for an additional 120 s at high speed (260 rpm). Diet mixtures were prepared in each sampling bag, respectively, and stored at 4°C. Before use, diets were transferred from refrigeration to a room kept at 24 ± 1°C and 65 ± 1% RH, where the diets were mixed at a normal-speed setting for 60 s.

Three niacin dilutions (0.2, 2, and 20 ppm) tested in this study were prepared by adding 10 mg of niacin to 10 ml of distilled water to obtain a solution of 1 mg/ml followed by 10-fold serial dilutions with distilled water to make 0.1 and 0.01 mg/ml solutions. One milliliter of 0.01, 0.1, or 1.0 mg/ml solutions was each added into 50 g of diet mix to produce 0.2, 2.0, and 20 ppm of niacin in the diet mix, respectively. Three dilutions for nine other B vitamins in a group (7.07, 70.7, and 707 ppm) were made in a similar manner. All vitamins except niacin were weighed in a ten-fold dose (353.5 mg in total) and mixed in 10 ml water (55°C) to make a solution of 35.35 mg of vitamin mix/ml, which was then diluted serially by tenfold to give 3.535 and 0.3535 mg/ml. One milliliter of solution of 35.35, 3.535, or 0.3535 mg/ml was each added into 50 g of diet mix to produce 707, 70.7, and 7.07 ppm of the other nine B vitamins in diet mix, respectively.

Bioassays. Fifty newly eclosed larvae were randomly selected from 1 ml of eggs by using a fine brush and were transferred onto a strip of blotting paper on top of 50 g of diet inside a sterile polyethylene bag by using a fine brush. Four bags of each test diet were prepared individually. Each bag was stapled and maintained at 24 ± 1°C, 65 ± 1% RH, and a photoperiod of 12:12 (L:D) h. When larvae reached third instars, polyethylene bags were opened and placed in a 1-liter waxed cup with vermiculite for pupariation. Pupae were counted and weighed as soon as brown puparia were formed. Pupae recovered from each diet were expressed as percentage of recovery of neonate larvae used at the start of the study. Daily pupal weights were totaled and divided by the total number of pupae from each diet to calculate mean pupal weight. The larval development period was measured from egg hatch to the first day of pupation. The mean larval developmental period was calculated by using a weighed arithmetic mean obtained from the sum of the daily pupal collections times the number of days to pupation divided by total number of pupae (Sanders 1990).

Data and Statistical Analysis. The relative mean pupal recovery and pupal weight were calculated in relation to those from the control. Mean relative percentages were obtained by pooling and averaging all treatments/batches together, and expressing them as relative percentages. Thus, controls were considered as 100% in all calculations. Each insect batch was replicated at least four times in each treatment.

The percent data were normalized using PROC Univariate, and differences in the insect batch/treatment data were determined by analysis of variance (ANOVA or PROC Univariate), with the honest significant difference (HSD) value calculated as Tukey's statistic at $\alpha = 0.05$ (SAS Institute 2001).

Results and Discussion

Dose interaction between niacin and nine other B vitamins (as a group) in the larval diet for *C. capitata* were evaluated in this study based on larval duration, pupal recovery, adult emergence, and adult flight ability (whether the adult flier can fly) (Chang et al. 2000).

Larval Duration. Larval duration was defined as the duration from egg hatch to the onset of pupariation. Larval duration from larvae reared on the diet with 2 ppm of niacin and 707 ppm of the other vitamins was not significantly different from that on the diet with 20 ppm of niacin and 707 ppm dose of the other B vitamins (control) (8.97 ± 0.17 and 8.81 ± 0.21 d, respectively) (Fig. 1). Without niacin or with 0.2–20 ppm of niacin in the diet, larval duration was extended with decreased doses of other B vitamins (Fig. 1). With 7.07 ppm of other B vitamins, larval duration was prolonged with an increase in niacin concentrations (0–20 ppm). Without any other B vitamins in the diet, larval duration was significantly extended with any change in niacin concentrations (Fig. 1). There was no significant difference in larval duration between control and treated groups when doses of the other B vitamins were ≥70.7 ppm, and 20 ppm of niacin was maintained in the diet (Fig. 1). From our results, larval duration was influenced by the doses (0–707 ppm) of the other B vitamins when niacin doses were ≥2 ppm. With ≤0.2 ppm of niacin and ≥70.7 ppm of other vitamins in the diet, no flies survived (Fig. 1). Further dose-response tests, especially with individual B vitamins other than niacin, should be examined.

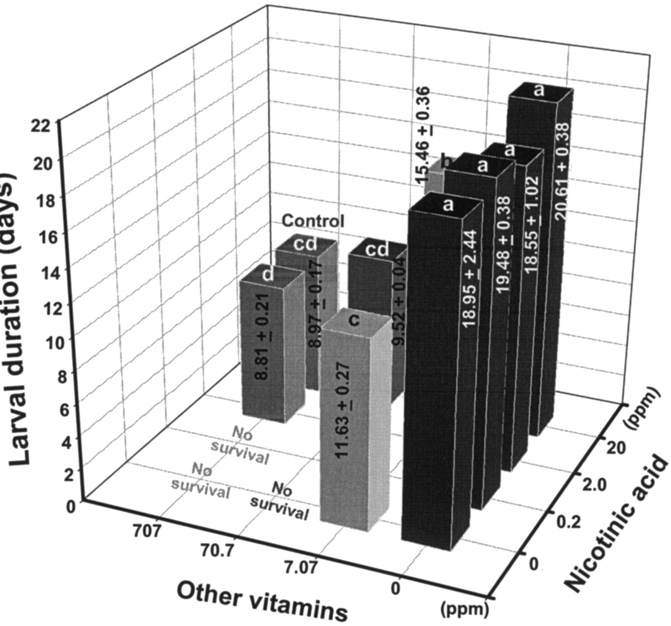

Fig. 1. Mean larval duration from *C. capitata* larvae reared on diets with various dose combinations of niacin and the other vitamins. Different letters on the top of bars are statistically different ($\alpha = 0.05$). Means ± SE on the side of bars expressed mean larval duration.

Pupal Recovery. Pupal recovery was defined as total number of pupae recovered from the number of seeded eggs. No pupal recovery was observed from larvae reared on a diet containing niacin at 0.2 and 707 ppm of the other B vitamins. Chang et al. (2000) suggested that at least 2 ppm of niacin is required in larval diet for normal larval development. However, the interrelated effect of dietary niacin and other vitamins on pupal development was not examined in that study. In the current investigations, no pupal recovery was obtained from larvae reared on the diet without niacin and with 70.7 and 707 ppm of the other B vitamins as well as those from diets containing 0.2 ppm of niacin and 707 ppm of the other B vitamins (Fig. 2). This finding suggests that high doses of the other vitamins do not interact with niacin when lower doses (≤0.2 ppm) of niacin are present in the diet, resulting in toxic-like or other metabolic adverse effects. Pupal recoveries increased as niacin dose increased up to 0.2 ppm and then remained constant when the other B vitamins were omitted (Fig. 2). This indicates that niacin plays a more important role than the other B vitamins because the larvae can still grow without other B vitamins although larval duration is delayed. Interestingly, when both niacin and the other B vitamins were deleted from the diet, pupal recoveries were ≈66% of the control and were not significantly different from the diet with 7.07 ppm of the other B vitamins. This finding suggests that when there was lack of dietary niacin, larvae could only tolerate a low dose of the other vitamins (Fig. 2). With ≥2.0 ppm of niacin in the diet, the higher doses of the other B vitamins resulted in higher pupal recoveries, although it was not significantly different statistically. When both niacin and the other vitamins were at ≥2.0 or ≥70.7 ppm, pupal recoveries were not different from control. These results demonstrated that both niacin and the other vitamins are indispensable for optimal larval development. With our test range in this study, the higher the doses, the more comparable larval development to that of the control.

Pupal Weight. Chang et al. (2000) stated that there were neither beneficial nor detrimental effects on *C. capitata* larval development reared on diets with 20–320 ppm of niacin. The current study tested four doses of niacin (0, 0.2, 2, and 20 ppm) and confirmed that 20 ppm of niacin and ≥70.7 ppm of other vitamins are not affecting the pupal weight. Pupae gained normal weight as long as both the dose of niacin and the other B vitamins were ≥2.0 ppm and 70.7 ppm (Fig. 3). Without the other nine dietary vitamins, pupal weight was significantly lighter than that of the control group but was not dependent on niacin dose. Mean pupal weights from larvae reared on diets without the other vitamins were not significantly different from those with different doses of niacin (0, 0.2, 2.0, and 20 ppm) in the diet (Fig. 3). However, with a 7.07 ppm of the other B vitamins in the diet, pupae from larvae reared on diet with 20 ppm niacin were heavier than those from diet without niacin in the diet. Moreover, with a constant dose of niacin (0, 2, or 20 ppm), the higher the content of other vitamins in the diet, the heavier the pupae.

Several studies demonstrated niacin and other B vitamins are essential components of the insect diet. However, there are few reports about the mode of

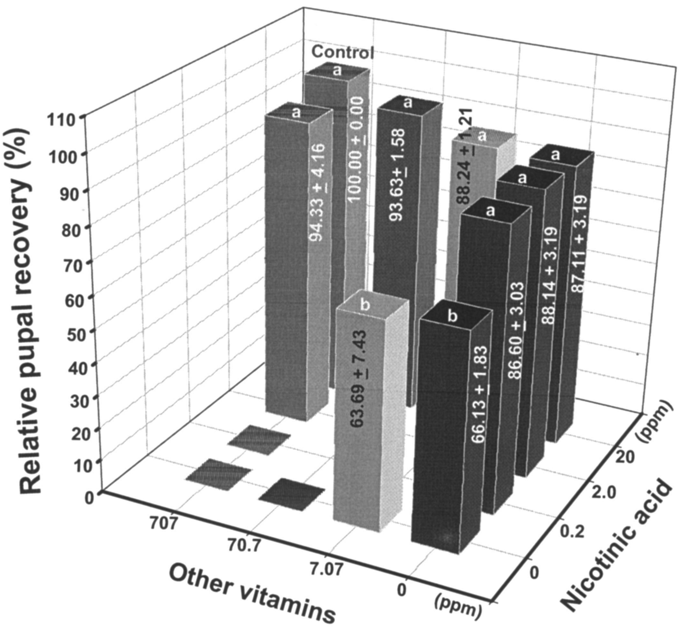

Fig. 2. Relative pupal recovery from *C. capitata* larvae reared on diets with various dose combinations of niacin and the other vitamins. Different letters on the top of bars are statistically different ($\alpha = 0.05$). Means ± SE on the side of bars expressed mean relative pupal recovery to those from the control diet.

action of niacin and the interaction between niacin and other vitamins in the diet.

Wicker (1984) reported that niacin could be synthesized in the weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Jang (1986) reported that deficiency of niacin affected development and growth in the Mediterranean fruit fly resulting in high larval mortality, reduced pupation, increased developmen-

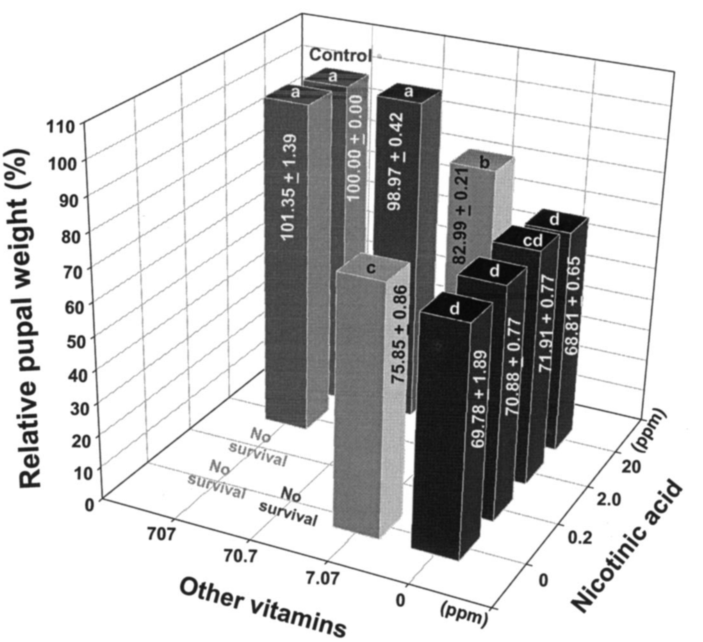

Fig. 3. Mean pupal weight from *C. capitata* larvae reared on diets with various dose combinations of niacin and the other vitamins. Different letters on the top of bard are statistically different ($\alpha = 0.05$). Means ± SE on the side of bars expressed mean pupal weight.

tal period, and decreased adult emergence. Niacinamide and nicotinamide adenine dinucleotide phosphate replacement could overcome the niacin deficiency (Jang 1986). Zang and Ma (1991) suggested that the essential need of dietary niacin is due to association of niacin and niacinamide with some proteins under enzymatic action and formation of NADP-enzyme during fifth instar development of *Bombyx mori* (L.). Tsiropoulos and Hagen (1987) reported that picolinic acid, an antimetabolite of niacin reduced hatchability of *Rhagoletis completa* Cresson. Dabrowski (1974) discovered that niacin at 1 μg/ml distilled water acted as feeding stimulants for *T. urticae,* but lost its simulating effect at 0.1 μg/ml.

In this study, there were only three set of dose combinations (0, 2, and 20 ppm) were used. Our results showed a clear trend although more tests need to be done to confirm this finding. However, our results confirmed our earlier theory that both niacin and the other vitamins are indispensable to maintain a normal weight in *C. capitata* larval diet.

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