Effect of Dietary Sodium Tetraborate on Adult Longevity and Fecundity of *Drosophila melanogaster* (Diptera: Drosphilidae)[1]

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J. Entomol. Sci. 51(4): 305–313 (October 2016)

Abstract The fruit fly *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) is often used in various biological, molecular, and toxicological studies. Sodium tetraborate, a boron compound, was added to the artificial diet of developing *D. melanogaster* to determine its effects on adult longevity and fecundity. Insects reared from first instars to adults on diets containing 10, 30, 150, 300, or 400 mg/liter sodium tetraborate showed that the highest concentration (400 mg/liter) significantly reduced female longevity (31.65 ± 4.02 [mean ± SD] d for the controls versus 1.87 ± 0.30 d for 400 mg/liter) and male longevity (32.80 ± 1.96 d for controls versus 3.57 ± 0.42 d for 400 mg/liter). Females from the control diet produced 9.46 ± 0.57 (mean ± SD) eggs per female, whereas those fed on a diet containing 300 mg/liter produced only 1.92 ± 0.30 eggs per females. These results expand our knowledge of the impact of sodium tetraborate on various insects and indicate that boron compounds should be further investigated to ascertain their potential as an alternative control tactic for pest insects.

Key Words sodium tetraborate, *Drosophila melanogaster*, longevity, fecundity

Traditional chemical insecticides have adverse impacts on the environment, nontarget organisms, and worker safety. Therefore, our interests are focused on alternatives for pest management (Büyükgüzel et al. 2007, 2011; Durmuş et al. 2008). Boric acid has been traditionally used for controlling indoor pests as an alternative to chemical insecticides. Dust and liquid formulations of boric acid and other boron derivatives have been previously reported for management of cockroach species (Blattodea: Blattidae) (Gore et al. 2004, Zurek et al. 2003). Habes et al. (2006) found that boric acid destroyed the midgut epithelium of the German cockroach, *Blattella germanica* (L.) (Orthoptera: Blattellidae); increased the level of the detoxification enzyme glutathione transferase; and inhibited acetylcholinesterase. Boric acid added to the diets of honey bee, *Apis mellifera* (L.) (Hymenoptera: Apidae), larvae at 2.5 and 7.5 mg/g resulted in larval mortality within 5 to 6 d at 2.5 mg/g and within 4 d at 7.5 mg/g (Cruz et al. 2009).

In our laboratory, we previously tested the effects of boric acid and sodium tetraborate on the survivorship and development of *Galleria mellonella* L.

[1]Received 06 April 2016; accepted for publication 24 June 2016.
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(Lepidoptera: Pyralidae) (Durmuş and Büyükgüzel 2008, Hyršl et al. 2007). Hyršl et al. (2008) found that exposure to dietary boric acid caused changes in the protein profiles in larval hemolymph and fat bodies, leading to synthesis of 45-kDa protein in the hemolymph. However, there is little information on the compatibility of these boron compounds on adult insects, such as was described for the preadult stages by Hyršl et al. (2007).

Sodium tetraborate has lower mammalian toxicity than other chemical insecticides used in pest management. Yet, when consumed by insects, boron compounds prevent food consumption and digestion by destroying linings of the foregut and midgut (Ebeling 1995). Sodium tetraborate also reportedly inhibits reproduction of insects (Zhou and le Patourel 1990).

In our current study, we expand our knowledge of the effects of sodium tetraborate and boron compounds to other insects. The fruit fly *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) is a commonly used species for ecotoxicological, ecophysiological, and genotoxicity studies, primarily because of its short life cycle, adaptation to different ecological conditions, and ease of maintenance in laboratory cultures (Bownes 1975). Previous studies have demonstrated that different concentrations of some organophosphorous insecticides and food dyes have toxic effects on survivorship of *D. melanogaster* (Çakır and Sarıkaya 2005, Doğan et al. 2005). Little, if any, information is available on the effects of boron and boron derivatives on this fruit fly. This study was therefore undertaken to determine the effects of sodium tetraborate on adult longevity and fecundity of *D. melanogaster* after rearing larvae on diets amended with sodium tetraborate.

Materials and Methods

Insect culture. Wild-type (W1118, Oregon R strain) *D. melanogaster* from a laboratory culture were used in this study. The stock culture was xenically maintained by rearing first instars to adults on an artificial diet (Lesch et al. 2007, Rogina et al. 2000). The colony was maintained and the tests were conducted at 25 $\pm$ 2°C, 60–70% relative humidity, and on a photoperiod of 12:12 (L:D) h. Newly emerged adults were used to maintain the stock culture. Insects were reared in 15-ml vials (26.5 $\times$ 58.7 mm, Yıldız Chemistry Co., Ankara, Turkey) with $\sim$5 ml of artificial diet. The standard diet was composed of 8 g of agar-agar ultrapure (Merck, Darmstadt, Germany), 20 g of sucrose (BioUltra, $\geq$%99, Sigma Chemical Co., St. Louis, MO), 11.78 g of dried powder yeast (Dr. Oetker Food Co., Torbalı-İzmir, Turkey), 0.8 g of ascorbic acid (BioUltra, $\geq$%99, Sigma), 7.72 ml of nipagine (SigmaUltra, *p*-hydroxybenzoic acid methyl ester, crystal), 36 g of mashed potatoes (Knorr, Unilever Co., Ümraniye, İstanbul, Turkey), and 1,000 ml of distilled water. Diet in liquid form was poured into the individual vials, and the vials were maintained at room temperature for 30 min to allow the diet to solidify. Ten or 15 males and fertilized females of the same age were placed in individual vials and maintained for 24 h, the time necessary for oviposition. Vials were plugged with cotton to prevent escape of the flies and to ensure adequate ventilation. After 24 h, adults were transferred into new vials with the control and amended diets according to treatment group as per Reis (2016). Transfers occurred every 3 d. Methods used to prepare and dispense diets and the rearing of the first instars are described in detail in Roberts (1986).

Feeding experiments. Sodium tetraborate decahydrate (crystalline form, 99%, borax, $Na_2B_4O_7{\cdot}10H_2O$, Eti Mine Works General Management, Ankara, Turkey) was directly incorporated into diets at concentrations of 10, 30, 150, 300, and 400 mg/liter diet while the diets were in liquid state. These concentrations were based on previous studies with sodium tetraborate and certain boron derivatives studied using *D. melanogaster* and other insects (Ali et al. 2006, Cisneros et al. 2002, Espinoza-Navarro et al. 2009, Gore et al. 2004, Xue et al. 2006, Yang et al. 2000a). Controls had no sodium tetraborate. Neonates were placed onto the respective diets by using a fine brush.

Adult longevity. First instars were reared until adult emergence on the artificial diets amended with given concentrations of sodium tetraborate. Newly emerged adults were removed from the diets and transferred to vials with fresh diets for determining longevity. The diets were changed every 3 d in the control and experimental groups. Sex determination was made according to morphological sexual dimorphism characters (Baker and Belote 1983). To determine average adult longevity, the number of dead adults in each of the treatment groups was counted and recorded daily until all adults had died. Experiments were replicated four times with 15 male and fertilized females at the same age per replicate.

Fecundity. Fecundity was determined by recording the number of eggs oviposited by the females in each treatment. Neonate larvae were reared to the adult stage on the diets containing sodium tetraborate. Females were allowed to oviposit in cups, and eggs were transferred into Petri dishes by using a fine brush. Egg counts were performed in the Petri dish on a black background. Females were provided with fresh diet daily during the oviposition period. Each experiment was replicated four times with 15 male and fertilized females per replicate. Egg production was monitored continuously from the first day of oviposition until experiments were completed. Egg production was calculated as eggs produced per female per day, hereafter termed fecundity.

Statistical analysis. Longevity and fecundity data were analyzed using a one-way analysis of variance. Where significance ($P < 0.05$) was detected, means were compared by the least significant difference (LSD) test (SPSS Inc. 1997).

Results and Discussion

The highest concentration of sodium tetraborate (400 mg/liter) significantly reduced adult longevity and fecundity of *D. melanogaster* in these tests. Longevity (mean $\pm$ SD) of females in the control was 31.65 $\pm$ 4.02 d, whereas longevity after feeding on diets containing 400 mg/liter sodium tetraborate decreased to 1.87 $\pm$ 0.30 d (Fig. 1). Similar results were observed with male longevity; the control group lived 32.80 $\pm$ 1.96 d and those fed on diets amended with 400 mg/liter sodium tetraborate lived only 3.57 $\pm$ 0.42 d (Fig. 1).

Fecundity responded in like manner, with females in the control group ovipositing 9.46 $\pm$ 0.57 (mean $\pm$ SD) eggs/d. Those females fed diets amended with 300 mg/liter sodium tetraborate oviposited only 1.92 $\pm$ 0.30 eggs/d (Fig. 2). No females

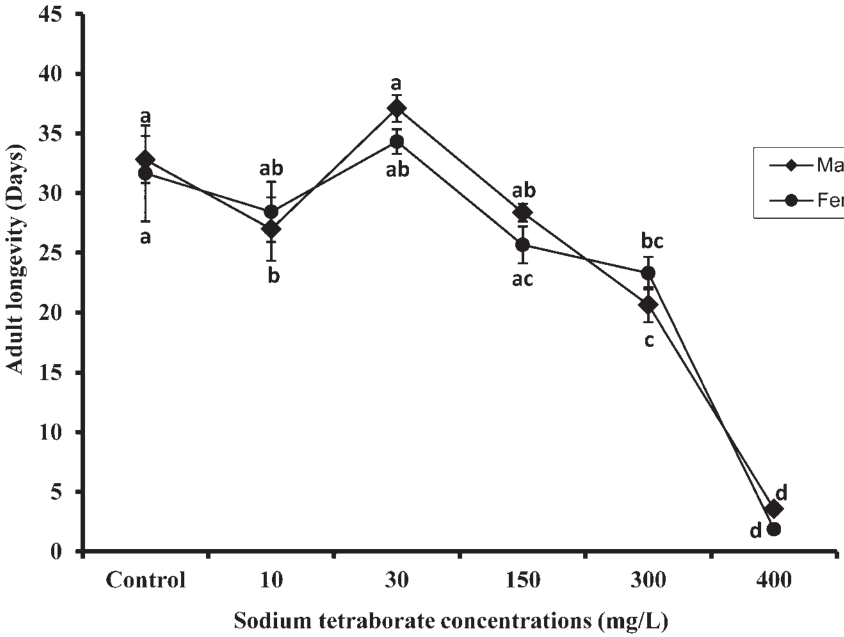

Fig. 1. Male and female *Drosophila melanogaster* adult longevity after feeding on artificial diets amended with sodium tetraborate. Data points with the same lowercase letter are not significantly different ($P < 0.05$) (LSD test; four replicates, with 15 insects per replicate).

were available after feeding on the highest concentration of sodium tetraborate (400 mg/liter); therefore, no fecundity data are available for that treatment.

These results show that consumption of sodium tetraborate by *D. melanogaster* larvae impacts the biological fitness of adult flies, with higher concentrations of 300–400 mg/liter diet reducing life expectancy and reproductive potential. Although the effects of sodium tetraborate seem related to dietary impairment, these results also support our objective that a secondary mechanism can be responsible for effects of the compound on adult physiology. Similar responses to sodium tetraborate and other boron compounds have been reported for *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) and *G. mellonella* (Durmuş and Büyükgüzel 2008, Hyršl et al. 2007, Yang et al. 2000b). Additional studies are needed to define the mode of action involved in the observed impairment of biological fitness.

In a previous study with *G. mellonella*, sodium tetraborate incorporated into the larval diet at 0.3% (w/w) reduced survivorship, delayed development, and inhibited egg production (Durmuş and Büyükgüzel 2008). That study showed that *G. mellonella* exhibits a broad tolerance to high sodium tetraborate concentrations (0.005–0.3 g/100 g) before exhibiting symptoms of impact on biological fitness parameters. In contrast, our current study with *D. melanogaster* showed higher susceptibility to the compound, with negative impacts on biological fitness at lower concentrations (10–400 mg/liter). Similar effects are reported with boric acid when

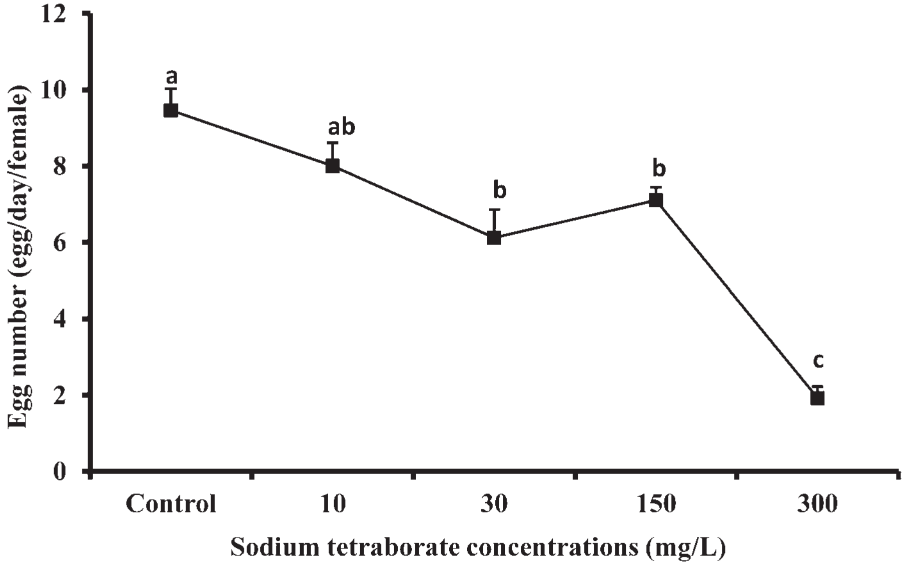

Fig. 2. Fecundity of *Drosophila melanogaster* after feeding on artificial diet amended with sodium tetraborate. Data points followed by the same lowercase letter are not significantly different ($P < 0.05$) (LSD test; four replicates, with 15 insects per replicate).

fed to *D. melanogaster* (10–300 mg/liter) (Güneş et al. 2015) and *G. mellonella* (156–2,500 ppm) (Hyršl et al. 2007). Collectively, results from these studies indicate that the response of the insects to the boron compounds varies with species but that the compounds had deleterious impacts on the biological fitness of all tested insects. Observed differences in tolerance to the compounds might be attributed to diet source, feeding behavior, and physiological activities associated with digestion and absorption in the midgut. Grenier et al. (1986) also indicated that different developmental stages of an insect species may have different physiological requirements. As suggested by Durmuş and Büyükgüzel (2008), high dietary concentrations of sodium tetraborate may have had a phagoattractive effect, leading to increased larval feeding rates and thereby higher consumption of the boron compound. Coincidental observations of larval growth in our study indicated that *D. melanogaster* larvae fed on diets with higher concentrations of sodium tetraborate seemed larger than larvae fed on diets with lower concentrations; however, this observation was not measured or otherwise verified.

Our findings further corroborate the impact that boron compounds have on insects. Boric acid incorporated into diets at high concentrations rendered the diets nutritionally unsuitable, in turn, leading to increased mortality and developmental periods in *B. germanica* and *G. mellonella* (Durmuş and Büyükgüzel 2008, Gore and Schal 2004, Gore et al. 2004, Hyršl et al. 2007). Boric acid at 1% in the bloodmeal of the mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) resulted in 98% mortality of the adult mosquitoes (Xue and Barnard 2003). Sodium tetraborate at high dietary concentrations increased adult mortality of the tropical fruit fly *A.*

suspensa (Yang et al. 2000b). Disodium octaborate tetrahydrate at 1 and 2% reduced survival of the flies *Musca domestica* L. (Diptera: Muscidae) and *Fannia canicularis* (L.) (Diptera: Muscidae) (Mullens and Rodriguez 1992). A high concentration of boric acid (0.25%) also significantly decreased adult longevity of *G. mellonella* (Hyršl et al. 2007).

Our previous studies with other insects showed that the deleterious effects of a non-nutritive supplement in the larval diet on adult fitness may depend on its interaction with dietary nutrients and thus alter the consumption rate of larvae (Büyükgüzel and Kalender 2008, Hyršl et al. 2007). These interactions might impair larval consumption rates, as suggested by Büyükgüzel (2001) after amending larval diets with antibiotics. Furthermore, as postulated in studies with the moths *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Cisneros et al. 2002) and *G. mellonella* (Durmuş and Büyükgüzel 2008), we conclude that consumption of increased levels of boron compounds impairs ingestion and digestion. Yang et al. (2000a) also indicated that a concentration of 0.5% sodium tetraborate in the larval diet of *A. suspensa* reduced midgut proteinase enzymes activities, further corroborating our findings.

Sublethal effects of ingested boron compounds on egg production observed in our study also corroborate results reported in other studies. For example, Yang et al. (2000b) reported that sodium tetraborate at 0.1% significantly decreased egg production and hatch, whereas higher concentrations completely inhibited oviposition by *A. suspensa*. Disodium octaborate and sodium tetraborate at 1 and 2% in diets reduced egg hatch to $<$25% for *M. domestica*, *F. canicularis*, and *Callosobruchus analis* (F.) (Coleoptera: Bruchidae) (Khan et al. 1996, Mullens and Rodriguez 1992). Significant reduction in egg production suggests that a physiological effect is impacting egg development in females. Indeed, some chemicals may affect egg production in the ovaries by altering the levels of juvenile hormone and ovarian ecdysteroids that regulate oocyte growth, yolk deposition, and embryonic development in insects (Gäde and Hoffmann 2005, Šula et al. 1987). Zhou and le Patourel (1990) demonstrated that toxicity of some boron derivatives includes premature drop of oothecae and reduced hatching, effects that may also contribute to the observed decreased fecundity and fertility of *B. germanica*. It is also reasonable to suggest that sodium tetraborate at high dietary concentrations acts in neuroendocrine hormones via the nervous system, as suggested by Habes et al. (2006) who reported that boric acid exerted a neurotoxic behavior as evidenced by poisoning and also inhibited acetylcholinesterase enzyme activity. They also demonstrated that boric acid increased glutathione transferase in the midgut epithelium tissue, suggesting that consumption of boric acid results in oxidative stress.

Reproductive success is generally accepted as being inversely related to longevity in insects and that increased reproduction capacity has negative effects on longevity with the concomitant expending of energy (Cords and Partridge 1996, White and Bell 1993). However, our results showed that high concentrations sodium tetraborate in larval diets resulted in decreased adult longevity and decreased egg production in *D. melanogaster*. It is reasonable to suggest that adult fitness may have been impaired by high energy demands of increased detoxification metabolism of the sodium tetraborate. Pro-oxidative effects in response to dietary boron compunds have been expressed as alterations in life table parameters

(Büyükgüzel et al. 2013, Hyršl et al. 2007). Although our current results indicate that adult longevity and fecundity are proper biological fitness criteria for assessment of boron compound effects on insects, further study is required to determine the mode of action of sodium tetraborate and other compounds in larval diets on adult insect biological fitness.

Acknowledgments

This study was supported by Research Fund of Bülent Ecevit University (project 2012-10-06-11). The National Boren Institute (Ankara, Turkey) provided the sodium tetraborate used in this study.

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