Inhibition of *Drosophila* development by β–aminobutyric acid, a plant defense priming compound.

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Introduction

β–aminobutyric acid (BABA) is a non-protein amino acid that has been the subject of much recent research due to its ability to enhance plant defences against a wide range of plant pathogens (Conrath, 2009). In plants, BABA-induced resistance appears to function via a number of molecular defence signalling pathways (jasmonic acid, salicylic acid, ethylene, and so forth), with the augmented defence response being appropriate to the type of pathogen (bacteria, fungi, virus, and so forth) that challenges the plant (Jakab *et al*., 2001; Ton *et al*., 2005).

It was recently demonstrated that application of BABA as a root drench to plants could impair the performance of insect herbivores such as aphids and lepidopteran larvae (Hodge *et al*. 2005, 2006). The mechanism of this BABA-induced inhibition was unclear: the insects did not avoid treated plants nor was their feeding behaviour disrupted in any way. The insects appeared to feed as normal, but their growth was severely reduced and reproduction rate lowered, possibly indicating their nutrition was somehow being depressed (Hodge and Powell, 2010). After treatment via the roots, unmetabolized BABA can be found in the plant's leaf tissue and phloem sap (Cohen and Gisi, 1994), and thus direct inhibition of the insects by BABA could potentially occur. Inclusion of BABA into an artificial lepidopteran diet, however, had no effect on the development of the moth *Plutella xylostella*, and even relatively high doses of BABA applied topically or ingested via sucrose solution appeared to have no deleterious effects on pea aphids (*Acyrthosiphon pisum*) (Hodge *et al*., 2005, 2006).

The aim of this study was to use *Drosophila* as a model species to examine further whether development of insects can be directly impaired by exposure of juvenile stages to BABA. By incorporating BABA in an "instant" *Drosophila* diet, the effects of chemical plant defences on inhibition of insect development can be excluded. Another isomer, γ–aminobutyric acid (GABA), was used as a control to account for the addition of an amino acid to the diet, along with any associated changes in osmolarity, pH, and so forth.

Methods

Experiments were carried out using a flightless (though winged) mutant of *Drosophila hydei* (Sturtevant), with flat-bottomed glass vials (75 mm × 25 mm diameter) plugged with a polyurethane foam bung as the experimental arenas. In each vial, 1 g of Instant *Drosophila* Medium (IDM; Blades Biological Ltd, UK) was mixed with 4 ml of the hydrating solution and allowed to set. Same-aged larvae were obtained by allowing females to lay eggs in Petri dishes of IDM for 6 hours. When the larvae where 2 d old they were introduced into the experimental vials (10 larvae per vial) and maintained in an incubator with constant lighting, temperature of 19^{o}C, and ambient relative humidity

of 55%. Survival of larvae and the mean development time (from larvae being introduced into the vials to adult emergence) were recorded.

Two assays were performed. In the first assay, 50 mM BABA (N = 8), 50 mM GABA (N = 4), and a water control (N = 8) were used to make up the *Drosophila* medium. In the second assay, a range of BABA concentrations from 0 to 50 mM was used (see Figure 2 for doses and replicates), and an estimate of adult body size was made by measuring the length of wing vein 3 from the cross vein to the wing tip.

Data were analyzed using one-way ANOVA, with treatments compared to the controls in a pair-wise manner using Dunnett's test.

Results

In the first assay, the inclusion of 50 mM BABA in the *Drosophila* diet severely reduced survival ($F_{2,17}$ = 450.9; P < 0.001) and extended the development time of those few individuals that did survive by over a day ($F_{2,13}$ = 4.4; P < 0.05) (Figure 1). There was no effect of adding 50 mM GABA to the diet.

In the second assay, there was again a negative effect of BABA on *Drosophila* development (Figure 2). Survival was reduced by BABA ($F_{9,14}$= 6.7; P < 0.001), but the effect was not as dramatic as in the first assay. Development time was extended by BABA in an almost linear fashion ($F_{9,14}$ = 13.2; P < 0.001; Figure 2b), whereas wing length of both males ($F_{9,13}$ = 4.1; P < 0.02; Figure 2c) and females ($F_{9,12}$ = 3.3; P < 0.03; Figure 2d) displayed only a weak negative relationship with BABA concentration. Dunnett's comparisons of the treatments with the control in pair-wise fashion suggested that all the parameters were significantly affected (P < 0.05) at only the highest concentration (50 mM).

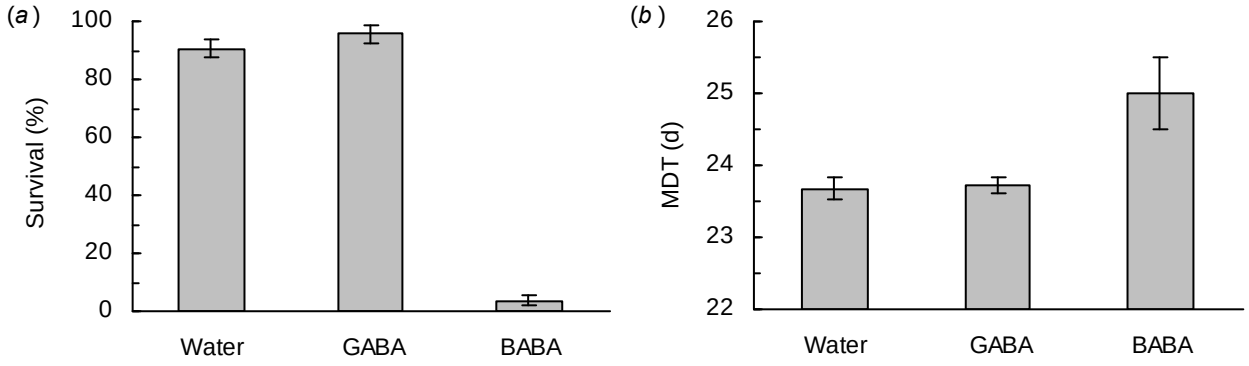

Figure 1. The effect of reconstituting instant *Drosophila* diet with 50 mM BABA and GABA solution on (*a*) survival and (*b*) mean development time of *Drosophila hydei* (mean ± se).

Discussion

Overall, the results of these assays illustrated that BABA has a negative impact on the development of *Drosophila hydei*: development time was extended, body size reduced, and the proportion of larvae reaching adulthood lowered. Interestingly, the γ-isomer had no such effects,

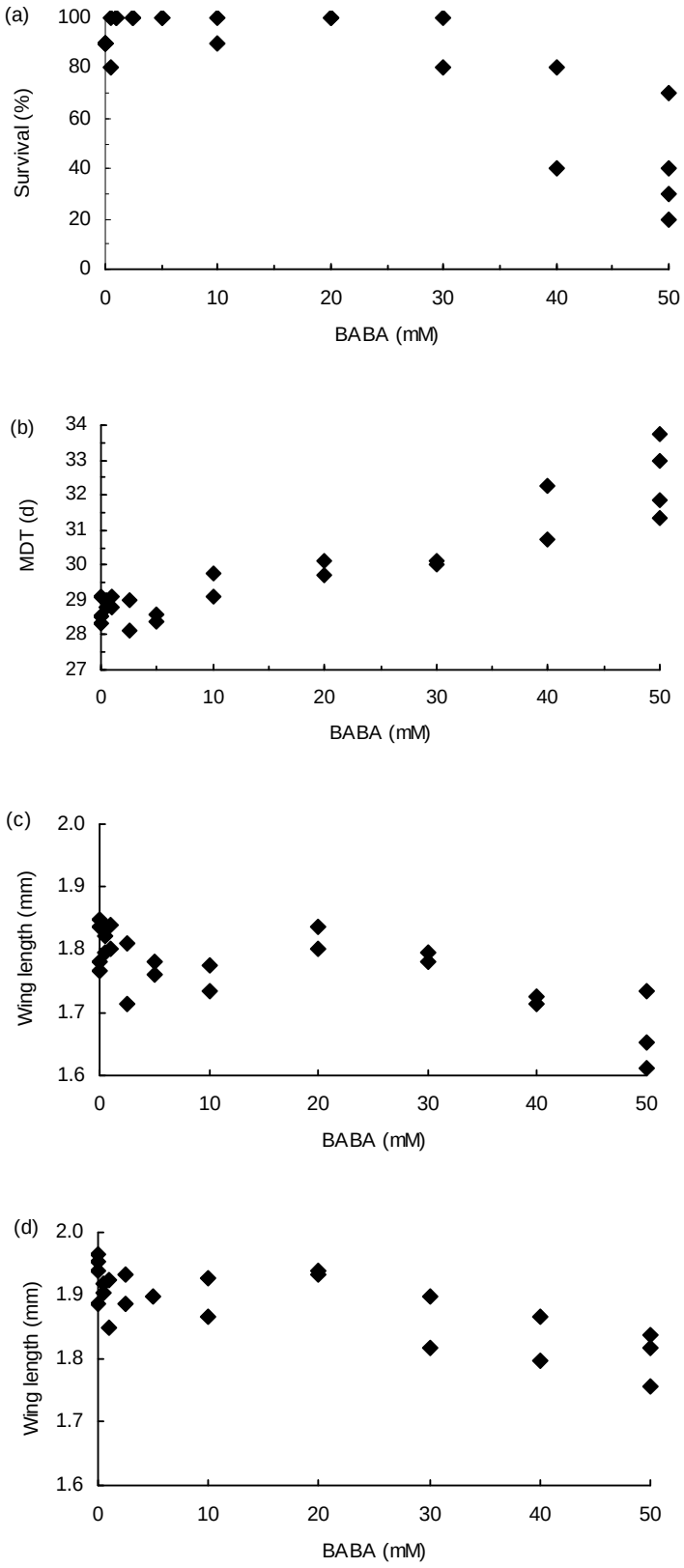

Figure 2. The effect of BABA concentration in instant *Drosophila* diet on (*a*) survival, (*b*) development time, and wing length of (*c*) male and (*d*) female *Drosophila hydei*.

suggesting there are some unique properties of β-isomer that are responsible for its inhibition of insect development (see also Hodge and Powell, 2010).

In these assays, the mode of action of BABA in reducing insect performance appears to have two potential routes through which it could act: (1) direct effects on the physiology of the developing insect (by ingestion or contact), or (2) acting via disruption of the microorganism populations that constitute the larval food source. There has been a recent example of BABA directly inhibiting a fungal plant pathogen (Fischer *et al*., 2009), so there may be scope for this compound to inhibit directly the dietary yeasts included in the diet medium that often provide the primary protein source of the larvae.

Nuclear magnetic resonance analysis has identified the presence of unmetabolized BABA in aphids reared on BABA-treated host plants (S. Hodge and J. Ward, 2010, *unpub. data*) suggesting that BABA could be having direct disruptive effects on insect development. The compound is also toxic to plants at high doses, and it has been suggested that it may interfere with amino acid metabolism, possibly by blocking amino acid transport systems. It is unknown whether inhibition of insect growth and development might also be occurring by this route.

Regardless of the mechanism(s) responsible, the results of these assays provide evidence that BABA can inhibit insect performance in the absence of plant-derived defence compounds. The results suggest that further research is required to elucidate how BABA reduces the rate of insect development, both when feeding on BABA-treated plants and when the compound has been incorporated into artificial diets. Investigating the effects of BABA on other species of *Drosophila* and Diptera, and other orders of insects, will provide further indication on the generality of these findings. In the face of these new findings, the lack of direct effects previously observed on aphids and lepidopteran larvae requires re-examination (Hodge *et al*., 2005, 2006).

References: Cohen, Y., and U. Gisi 1994, Phys. Mol. Plant. Path. 45: 441-456; Conrath, U., 2009, Adv. Botanical Res. 51: 361-395; Fischer, M.J.C., S. Farine, J. Chong, P. Guerlain, and C. Bertsch 2009, Crop Protection 28: 710-712; Hodge, S., T.W. Pope, M. Holaschke, and G. Powell 2006, Annals Appl. Biol. 148: 223-229; Hodge, S., and G. Powell 2010, Bulletin IOBC/WPRS (in press); Hodge, S., G.A. Thompson, and G. Powell 2005, Bull. Ent. Res. 95: 449-455; Jakab, G., V. Cottier, V. Touquin, G. Rigoli, L. Zimmerli, J-P. Metraux, and B. Mauch-Mani 2001, Eur. J. Plant Path. 107: 29-37; Ton, J., G. Jakab, V. Toquin, V. Flors, A. Lavicoli, M.N. Maeder, J-P. Metraux, and B. Mauch-Mani 2005, Plant Cell 17: 987-999.

Species abundance and sex ratios of *Drosophila melanogaster* and *Zaprionus indianus* in two different habitats of the Tropical Dry Forest of Alamos, Mexico (Diptera; Drosophilidae).

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Introduction

Alamos, Sonora, Mexico lies in the foothills of the Sierra Madre Occidental where the Sonoran desert meets the tropical dry forest. We investigated the sex ratio and abundance of the local Drosophilidae community during September 2010, which is the rainy season. Two species