Aversive responses to L-arabinose in caterpillars of *Helicoverpa armigera* (Hübner)

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ABSTRACT

Background: One of the naturally non-nutritive artificial sweeteners, L-arabinose, is widely used in the modern human food industry to combat obesity and diabetes because of its inhibition effect on the intestinal sucrase activity in mammals. However, whether it shows impact on herbivorous insects remains largely unknown. In the present study, we investigated the effects of L-arabinose on the feeding behaviors, gustation, development and intestinal sucrase activity of an herbivorous insect species, the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae).

Results: The results show that both the feeding preferences of *H. armigera* caterpillars for host plants and for phagostimulants were inhibited by L-arabinose in a dose-dependent manner. Gustatory receptor neurons (GRNs) sensitive to L-arabinose were not found in the peripheral gustatory sensilla, but the sensitivities of GRNs sensitive to other plant metabolites were suppressed by L-arabinose. Exposure of dietary L-arabinose to caterpillars resulted in prolonged larval developmental duration, suppressed activity of intestinal sucrase and reduced glucose level in midguts. *In vitro*, L-arabinose inhibits activity of intestinal sucrase of *H. armigera* caterpillars in an uncompetitive manner.

Conclusion: Taken together, these findings demonstrate that L-arabinose is a feeding and physiological inhibitor to caterpillars of *H. armigera* caterpillars. To our knowledge, this is the first report of a non-nutritive sweetener perceived by an animal as an inhibitor from both peripheral taste coding and feeding decisions, which should provide a physiological and behavioral basis for the future possible application of L-arabinose in controlling the herbivores pests.

Keywords: L-arabinose, inhibitor, *Helicoverpa armigera*, feeding preference, taste sensitivity, sucrase activity

Introduction

L-arabinose, a naturally-occurring and widely present pentose, is known poorly absorptive [1-4] but elicits a sweet taste for humans [5]. Therefore it is widely used additives in human food products with the intention to combat obesity and diabetes [6-8]. L-arabinose is one of the components of biopolymers such as cereal fiber and pectin in a variety of crops, corn, wheat, barley, rye, and oats, 30–40% produced in its linked form [9-11] and up to 75% in the “hairy region” of beet pectin [12]. However, the impacts of L-arabinose on invertebrates, such as the herbivorous insects, are largely not yet known.

In general, sweetness is a “good” taste, eliciting appetitive consumption of nutritive foods, whereas bitterness is a “bad” taste, driving aversive behavior to foods those containing harmful substances[13-16]. In flies, the NAS L-arabinose acts as a peripheral feeding phagostimulant and induced feeding preference behaviors [17-19]. Similarly, *Drosophila* pre-fed with L-arabinose resulted in an inability to learn the non-nutritional property of the NAS but could form caloric frustration memory through a post-ingestion learning mechanism, which involves activity of the central brain processes, but not a peripheral taste state [19-21]. Interestingly, these results indicate that the peripheral nervous system of flies detects L-arabinose as a sweetener, whereas in the brain as a negative sugar.

In this study, we investigated the effects of L-arabinose on the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and the underlying mechanisms. *H. armigera* is a widely distributed agricultural pest in the world, feeding on a variety of crops, including corn, wheat, pepper, tobacco [22]. Our results demonstrate that L-arabinose not only inhibits the peripheral taste activities of caterpillars but also inhibits feeding preferences, development, and intestinal sucrase activities, suggesting *H. armigera* caterpillars perceive this deterrent-like pentose as an inhibitor both from peripheral and

central nervous systems. Our finding provides a new insight to understand the evolution of sensory coding of herbivores to negative non-nutritive sweeteners.

Results

Inhibition of L-arabinose on feeding behaviors

To examine the effect of L-arabinose on the feeding preference of 5th instar caterpillars of *H. armigera*, L-arabinose at different concentrations from 0.0001mM to 333 mM was added on the tobacco leaf discs and the tobacco leaf discs treated with pure distilled water was used as control. At the low concentrations of L-arabinose, *H. armigera* showed no significant preference (0.0001-1.0 mM; one-sample t-test: 0.0001mM, $t(107)= 0.409$, $p = 0.686$; 0.001mM, $t(86) = -0.935$, $p = 0.352$; 0.01mM, $t(160)= -1.157$, $p = 0.249$; 0. 1mM, $t(117) = -1.319$, $p = 0.190$; 1.0 mM, $t(224) = -1.679$, $p = 0.095$; Fig. 1A). In contrast, the high concentrations of L-arabinose ≥ 10 mM significantly deterred the feeding of caterpillars (Fig. 1A;one-sample t-test: 10mM, $t(272) = -2.77$, $p = 0.006$; 66.6mM, $t(243) = -3.491$, $p = 0.001$; 133mM,$t(190) = -5.88$, $p< 0.0001$; 200mM,$t(311) = -6.359$, $p< 0.0001$; and 333mM, $t(100) = -5.743$, $p< 0.0001$).

In addition, we examined feeding preferences of *H. armigera* caterpillars to the mixtures of 200mM sucrose plus different concentrations of L-arabinose in a dual-choice leaf disc assay. At the concentrations of 100, 300, and 400 mM, L-arabinose showed no effect on the preference of sucrose (Fig. 1B,200mM Su.+0 LA, one-sample t-test: $t (81) = 5.117$,$p< 0.0001$; one-sample t-test: 200mM sucrose + 100mM L-arabinose, $t(154) = -0.048$, $p = 0.962$; 200mM sucrose +300mM L-arabinose, $t(55) = -0.156$, $p = 0.877$; 200mM sucrose +400mM L-arabinose, $t(56) = 0.138$, $p= 0.891$). In contrast, the high concentration of L-arabinose (500 mM, 600 mM, and 800 mM) significantly deterred feeding of caterpillars to sucrose (Fig. 1B; one-sample t-test: 200mM sucrose + 500 mM L-arabinose, $t(83) = -4.224$, $p= 0.0001$; 200 mM sucrose + 600 mM L-arabinose, $t(81) = -3.118$, $p= 0.0025$; 200

mM sucrose + 800 mM L-arabinose, $t(23) = -2.876$, $p = 0.0085$). These results indicate that L-arabinose counteracts the phagostimulant effect of 200 mM sucrose in a dose-dependent manner.

Next, we examined whether caterpillars could distinguish between leaves treated with phagostimulants and those with L-arabinose. Feeding preferences of *H. armigera* caterpillars to the tobacco leaves treated with mixtures containing 10 mM sucrose plus L-arabinose at different concentrations were performed. No feeding preference were observed at low concentrations of L-arabinose (1.0–100 mM; Fig. 1C; one-sample t-test: 10 mM sucrose + 1mM L-arabinose, $t(110) = 1.363$, $p = 0.176$; 10 mM sucrose + 10mM L-arabinose, $t(105) = -0.301$, $p = 0.764$; 10 mM sucrose + 100mM L-arabinose, $t(70) = -1.121$, $p = 0.266$). From mixtures containing L-arabinose from 200mM to 400mM, caterpillars significantly preferred control leaves over mixture-treated leaves (Fig. 1C; one-sample t-test: 10 mM sucrose + 200mM L-arabinose, $t(69) = -3.362$, $p = 0.001$; 10 mM sucrose + 300 mM L-arabinose, $t(46) = -4.396$, $p < 0.0001$; 10 mM sucrose + 400 mM L-arabinose, $t(77) = -13.09$, $p < 0.0001$). These results indicate that the addition of L-arabinose to sucrose produces inhibiting effects and counteracts the feeding attracting effects of sucrose (Fig. 1C).

The examination of effects of L-arabinose on another feeding stimulant, fructose, was also performed. The feeding preferences were not significant affected by L-arabinose at the concentrations of 300mM or 400mM (Fig. 1D; one-sample t-test:200 mM fructose + 300 mM L-arabinose, $t(1012) = -1.911$, $p = 0.059$; 200 mM fructose + 400 mM L-arabinose, $t(109) = -0.677$, $p = 0.500$), but increased by L-arabinose at the concentrations ≥ 500mM (Fig. 1D; one-sample t-test: 200 mM fructose+ 500 mM L-arabinose, $t(113) = -4.115$, $p = 0.0001$; 200 mM fructose + 600 mM L-arabinose, $t(100) = -0.3.572$, $p = 0.0005$).

To determine the possible effect of plant leaf, the feeding preferences was also examined by using pepper leaves instead of tobacco leaves. The treated pepper leaves with L-arabinose at low concentrations showed no effect on the feeding preference (0.001–1.0 mM; Fig. 2A; one-sample t-test: 0.01mM L-arabinose, $t(62) = 0.124$, $p = 0.902$; 0.1mM L-arabinose, $t(252) = -1.315$, $p = 0.190$; 1.0 mM L-arabinose, $t(214) = -1.493$, $p = 0.137$), whereas those treated with high concentrations inhibited the feeding preference (Fig. 2A; one-sample t-test: 10mM L-arabinose, $t(325) = -2.251$, $p = 0.025$; 100mM L-arabinose, $t(155) = -2.028$, $p = 0.044$; 200mM L-arabinose, $t(115) = -2.244$, $p = 0.027$; 300mM L-arabinose, $t(95) = -5.211$, $p < 0.0001$; 400mM L-arabinose, $t(107) = -4.360$, $p < 0.0001$). The results show that the effects of L-arabinose on *H. armigera* caterpillars are independent of leaf material source.

Similarly, at concentration of 100mM, 300mM, 500mM and 600mM, L-arabinose showed no effect on the preference of *H. armigera* caterpillars to 200mM sucrose (Fig. 2B;one-sample t-test: 200 mM sucrose, $t(62) = 5.718$, $p < 0.0001$; 200 mM sucrose + 100 mM L-arabinose, $t(33) = 4.630$, $p = 0.0001$; 200 mM sucrose + 300 mM L-arabinose, $t(41) = 2.899$, $p = 0.0060$; 200 mM sucrose + 500 mM L-arabinose, $t(38) = 4.521$, $p = 0.0001$). At the concentrations of 600mM and 800mM, no feeding preference was observed (Fig. 2B; one-sample t-test: 200 mM sucrose + 600 mM L-arabinose, $t(76) = -0.564$, $p = 0.574$; 200 mM sucrose + 800 mM L-arabinose, $t(75) = -0.851$, $p = 0.398$), whereas at concentration of 1000 mM of L-arabinose in mixture, caterpillars significantly preferred control leaves over mixture-treated leaves (Fig. 2B; one-sample t-test: 200 mM sucrose + 1000 mM L-arabinose,$t(71) = -2.110$, $p = 0.038$), also suggesting L-arabinose counteracts the effects of the phagostimulant sucrose in a dose-dependent manner.

Inhibition of L-arabinose on gustatory sensitivity

Gustatory perception in *H. armigera* caterpillars are mainly dominated by two pairs of gustatory sensillastyloconica, the lateral sensilla and the medial sensilla, located on the maxillary galea of mouthparts (Figs. 3A and 3B). The results of single sensillum recording show that non-receptor neurons housed in both the medial and the lateral sensillum are sensitive to L-arabinose (Fig. 3C).

We then investigated the effects of L-arabinose on the sensitivity of GRNs sensitive to other plant metabolites, such as the phagostimulant sucrose, the deterrent sinigirn and host plant tobacco leaf saps, by testing the responses of sensillum induced by mixtures containing plant metabolites and a series concentration of L-arabinose, respectively. Firstly, we tested the responses of a single lateral sensillum to different stimulli in sequence (Fig. 4). The primary two stimuli, 2.0 mM KCl and 10 mM L-arabinose, only induced weak responses of a salt-like GRN4 in the lateral sensillum, however 1.0 mM sucrose induced a strong response of the sucrose-sensitive GRN2 in the lateral sensillum. The activity of GRN2 decreased gradually to the point of becoming completely inactive at sucrose mixture concentrations of L-arabinose ≥ 100mM (Figs. 4A, 4B). When only the 1.0 mM sucrose was applied, the activity of GRN2 recovered completely (Figs. 4A, 4B).

Similarly, the sensitivity of GRN2 in medial sensillum of *H. armigera* caterpillars to mixtures of tobacco leaf saps plus L-arabinose was also depressed gradually in the response in a dose-dependent manner (Fig. 5). Interestingly, the strong sensitivities of the deterrent-sensitive GRN3 in the medial sensillum to 1.0 mM sinigrin were also depressed gradually with increasing of the L-arabinose concentrations in mixtures (Fig. 6). These results suggest L-arabinose inhibits gustatory sensitivities of GRNs sensitive to both phagostimulants and deterrents.

Larval development prolonged after exposure to L-arabinose

The larval development duration, larval mortality, puapl development duration, pupal weight, and emergence rate of adults reared from the neonatal on artificial diets containing serial concentrations of L-arabinose (6.66 mM– 500 mM) were also examined. The results show that L-arabinose has significant effects on the larval developmental duration, the pupal developmental duration and the pupal weights of *H. armigera* (one-way ANOVA: larval developmental duration, $F(5, 473) = 48.928$, $p < 0.0001$; pupal developmental duration, $F(5, 379) = 5.384$, $p = 0.02$; pupal weights, $F(5, 466) = 11.125$, $p < 0.0001$) (Fig. 7). At the concentrations of 333 mM and 500 mM of L-arabinose, the larval developmental duration was significantly prolonged (Scheffé Test: $p < 0.001$; Fig. 7A). Larval mortalities were not significantly affected by L-arabinose (one-way ANOVA: $F(5, 18) = 0.283$, $p = 0.917$, Fig. 7B). At the concentration of 500 mM, L-arabinose significantly prolonged the pupal duration and reduced the pupal weight (Figs. 7C and 7D; Scheffé Test: pupation duration duration, $p = 0.005$; pupal weights, $p < 0.001$; Figs. 7C and 7D). The developmental assays demonstrate that consumption of L-arabinose results in delaying of larval development.

Suppressed activity of digestive enzymes after exposure to L-arabinose

After dietary exposures of caterpillars from neonates to the 5th instar stage to different concentration of L-arabinose, the sucrase activity in midguts of the 5th instar *H. armgiera* caterpillars was suppressed in a dose-dependent manner (6.66 mM – 500 mM; one-way ANOVA: $F(5, 134) = 26.755$, $p < 0.0001$) (Fig. 8A). The sucrase activities of caterpillars exposed to 6.66 mM and 66.6 mM dietary L-arabinose were not significantly different from that of the control caterpillars (Scheffé's test, $p = 0.692$), while the sucrase activities of caterpillars exposed to 199.8 mM, 333 mM and 500 mM dietary L-arabinose were significantly lower than those of caterpillars exposed to 6.66 mM and 66.6 mM dietary L-arabinose and the control caterpillars (Scheffé's Tests: $p < 0.01$, all results; Fig. 8A),

demonstrating the inhibition effects increase with the concentration of L-arabinose up in the diets.

As expected, in midguts of caterpillars exposed to dietary L-arabinose from neonates to the 5th instar stage, the sucrose concentration increased in a dose-dependent manner (one-way ANOVA: $F(5, 113) = 39.750$, $p < 0.0001$) (Fig. 8B). Sucrose concentration of caterpillars exposed to 199.8 mM L-arabinose was not significantly different from that of that of caterpillars exposed to 6.66 mM and 66.6 mM L-arabinose (Scheffé's Tests: $p = 0.926$; Fig. 8B), whereas was significantly higher than that of the control caterpillars (Scheffé's Tests: $p=0.023$; Fig. 8B). After exposed to 333 mM and 500 mM dietary L-arabinose, the sucrose concentration in midguts were significantly higher than that of caterpillars exposed to low dietary L-arabinose and the control diet (Scheffé's Tests: $p < 0.0001$, all results; Fig. 8A).

Correspondingly, the glucose levels in midguts of L-arabinose-fed caterpillars decreased in a dose-dependent manner with increasing of the dietary L-arabinose (one-way ANOVA, $F(5, 230) = 17.590$, $p < 0.0001$). The glucose levels in caterpillars exposed to 199.8 mM dietary L-arabinose were significantly lower than that in control and caterpillars to 6.66 mM dietary L-arabinose (Scheffé's Tests: $p < 0.01$; Fig. 8C). The glucose levels of caterpillars exposed to 333 mM and 500 mM L-arabinose were even lower than that of caterpillars to 199.8 mM dietary L-arabinose (Scheffé's Tests: all $p < 0.05$; Fig. 8C).

Kinetic analysis of sucraseinhibition by L-arabinose

We also found that L-arabinose inhibits *H. armigera* intestinal sucrase in an uncompetitive manner *in vitro* (Fig.9). Vmax was decreased from 7.31 to 5.85 mmol/min/mg protein and Km decreased from 7.24 to 5.15 mM with increasing L-arabinose concentration (0 to 9.99 mM L-arabinose). At concentrations of 6.66 and 9.99 mM, L-arabinose reduced sucrase activity 12.24% and 26.81%, respectively.

The inhibition of sucrase activities by the alpha-glucosidase inhibitor, acarbose, was also examined. The Vmax was similar from 8.94 to 8.89 mmol/min/mg protein and Km increased from 2.14 to 4.71 mM with concentrations increasing (0 to 9.99 μM acarbose; Fig.9B). The apparent K_i was calculated as 54.08 μM.

Discussion

L-arabinose is a plant-specific saccharide that is not found in animals [23]. Although it has been demonstrated to have a selective inhibitory effect on sucrase activity in mammal intestine [24, 25], the effects on digestive enzymes of insect species, especially the herbivorous insects, were not examined. In the present study, we found that L-arabinose inhibits the feeding behaviors, the gustatory sensitivity, the larval development, and the intestinal physiology of the herbivorous *H. armigera* caterpillars. The feeding preferences of caterpillars for both host plants and phagostimulants were inhibited by L-arabinose in a dose-dependent pattern. Similarly, sensitivity of the peripheral GRNs of caterpillars to host leaf saps and phagostimulants were also inhibited by L-arabinose in similar dose-dependent manners. The difference of inhibiting effects between series concentration of L-arabinose on feeding behaviors is consistent with that on sensitivity of peripheral GRNs, suggesting the impacts of L-arabinose on peripheral gustatory coding may contribute to determining the rejection of L-arabinose during feeding processes.

The impacts of L-arabinose on behaviors and gustation could be further reflected by the prolonged larval development and the suppressed intestinal sucrase activity of *H. armgiera* caterpillars after L-arabinose ingestion. The depressed sucrase in midguts resulted in the accumulations of sucrose, the decreasing of glucose level and thereby delayed the sucrose ingestion. As a result, the developmental duration of caterpillars could be prolonged as found in our present work. The prolonged duration by L-arabinose in our work is similar to that in other caterpillars after the digestion of toxic substances or deterrents [26-29]. Thus, it

is not surprising that caterpillars of *H. armigera* perceive this pentose as a deterrent-like inhibitor both in peripheral gustatory coding and in feeding decisions. Consequently, our results from behaviors, gustation, development and intestinal physiology are consistent and demonstrate L-arabinose is an inhibitor for the polyphagous herbivorous insect *H. armigera*.

It is generally believed that food acceptance or rejection in animals is finally regulated by the central nervous system, which integrates external and internal feeding-relevant information [30-32]. Our results show caterpillars of *H. armgiera* not only reject feed L-arabinose-treated host plant leaves, but also rejected leaves treated by mixtures of phagostimulant and L-arabinose in a dose-dependent manner, suggesting the evaluation of L-arabinose with different concentration is possible. Such evaluations behaviors are always mediated by the central nervous system, where the complex information could be integrated at different level [33-36]. Our previous study found that the difference of host-plant preference of *H. armigera* caterpillars for cotton and pepper leaves [37] was consistent with the difference in GNR activity in response to the two host plants [38]. And sensory axons derived from GRNs in the peripheral gustatory sensillum of *H. armigera* caterpillars were projected to the suboesophageal ganglion and further to the tritocerebrum [39], where the chemosensory information is primarily integrated [40-42]. Accordingly, the combining results suggest the rejection of L-arabinose in *H. armigera* caterpillars is mediated by the central nervous system.

To our knowledge, there are no reports of sweeteners functioning as inhibitors to both peripheral gestation and feeding behaviors of animals. In *Drosophila*, both L-arabinose and another non-nutritional sugar, L-glucose, were perceived as sweetener by peripheral gustatory system with proboscis extension responses [20, 21, 43, 44], and the detection of L-arabinose were mediated by sweet gustatory receptors [45, 46]. Our results indicate that L-arabinose couldn’t activate any specific peripheral GRNs in *H. armigera* caterpillars, but

activities of GRNs sensitive to phagostimulants or deterrent were inhibited by L-arabinose if in mixtures, demonstrating the peripheral coding of L-arabinose in *H. armigera* caterpillars is different from that in *Drosophila*. The fitness may partly contribute to the difference of L-arabinose coding between *H. armigera* and *Drosophila.* Caterpillars of *H. armigera* are phytophagous with chewing mouthparts and the foods always contain cereal fibers where L-arabinose always enriches [9, 11, 23]. *H. armigera* caterpillars may have evolved chemosensory mechanisms of perceiving this deterrent-like pentose as a deterrent, just as the adaptation ability of this species to toxic secondary plant metabolites of host plants [47-49]. Compared to lepidopteran caterpillars, wild *Drosophila* always feed on decaying or fermented fruits with less L-arabinose, and *Drosophila* would not has evolved the ability of perceiving L-arabinose as an inhibitor. Even in closed insect species, the adaptation ability to toxic compounds is different and it is always result from the difference of fitness, such as ecological diversities [50-54].

The inhibiting mechanism of L-arabinose on peripheral GRNs activities of *H. armigera* caterpillars excludes the narrow sense of activity integration between GRNs sensitive to bitters and sugars [55-58], since non- L-arabinose sensitive GRNs were found in the peripheral maxillary sensillum. Through indirect detection of sweeteners have not yet reported in herbivores, detection of deterrents by suppressing the activity of sugar GRNs have been founded in *Drosophila* [59] and the honeybee *Apis mellifera* [60]. We hypothesize that the inhibition process of L-arabinose on the GRN activity of *H. armigera* caterpillars may be related to one or more of these mechanisms, such as membrane depolarization [61, 62], the G-proteins [63, 64], GPCR kinases [65, 66], transduction pathway activation [67, 68], binding of extracellular proteins present in the endolymph [69], or neurotransmitter mediation[70, 71].

It has been reported that insects can learn to devalue toxic compounds or sweet non-nutritional sugars by a dopamine signaling-modulated post-ingestion process, which insects could remember the sweet-tasting sugars are not nutritious [20, 21, 72-74]. Our experiments did not appear to involve a learning process of *H. armigera* caterpillars. However, during the feeding process, some caterpillars vomited after consumption of the L-arabinose-treated plant leaves, suggesting that L-arabinose can evoke an emesis response, perhaps related to digestive disruption, which may be related to decreased midgut sucrase activity. During our investigations, we placed eight leaf discs around the glass plate bottom: four L-arabinose-treated discs and four control discs. Preference behaviors ceased only after at least two discs of one type (control discs or treatment discs) had been consumed. Since the feeding period of *H. armigera* caterpillars varied from 30min to 6h, we surmise that post-ingestive feedback process may also mediate the feeding behaviors of *H. armigera* caterpillars.

To determine whether these effects were unique to *H. armigera*, we also investigated whether the inhibition effects could be observed in other insect species. Indeed, our preliminary experiments suggest L-arabinose could also inhibit the feeding preferences for foods in another *Helicoverpa* species, the tobacco budworm *H. assulta*, and the eusocialinsect species, the termite *Reticulitermes chinensis* (Isoptera: Rhinotermitidae) (unpublished data).

Insects are one of the most remarkable lineages in the 3.5-billion-year history of life [75], which is a much longer history than mammals. We suppose that some insects have evolved smart mechanism than mammals in perceiving the toxin-like L-arabinose sweetener. Studies of insect feeding decision to L-arabinose should be broadened in an effort to extend this evolution-based assumption by overserving its effects in different herbivorous insect species. Important questions remain with regard to the inhibiting mechanisms of L-

arabinose on the sensitivities of other GRNs in the maxillary sensillum; the physiological mechanisms and the ecological context may be complicated. The potential applications of deterrent-like effects of L-arabinose on caterpillars for the purposes of pest control should be addressed in future studies.

Finally, as a matter of fact, it is natural that *H. armigera* caterpillars perceive the non-nutritional L-arabinose as a deterrent-like compound for at least two possible reasons. First, our investigations both *in vitro* and *in vivo* show that L-arabinose could inhibit the activity of intestinal sucrase and thereby result in a reduction of glucose level in midgut homogenate, which may have vital impact on the energy utilization of caterpillars. Second, the larval development duration is prolonged after exposure to dietary L-arabinose. Furthermore, we found that almost all the investigated neonates died after 3% L-arabinose was added into the artificial diet containing a low content of sucrose in our preliminary experiments (unpublished data), suggesting that L-arabinose has a fatal impact on caterpillars if the diet is short of caloric sugars. Unlike the peripheral gustatory coding of mammals [1, 5, 17-19] and *Drosophila* [20], which regard the non-nutritive pentose as a sweetener, our results indicate that caterpillars of *H. armigera* could perceive this NAS as a non-sweetener inhibitor both from peripheral coding and central brain activity level.

Methods

Insects

H. armigera were reared in laboratory on an standard artificial diet [76] prepared from the following ingredients: wheat bran (150 g) (Jinyuan Flour IndusterCo., Ltd, Zhengzhou, China), soybean powder (80 g) (Quanruixing Foods Co., Ltd, Wuzhi County, China), yeast powder (25 g), casein (40 g) andsorbic acid (3 g) (Aobox Biotechnology, Beijing), ascorbic acid (3 g) and acetic acid (4 ml) (Yongda Chemical Reagent Co., Ltd, Tianjin, China),

sucrose (10 g)(Sigma-Aldrich), agar (20 g)(Beijing Solarbio Science & Technology Co., Ltd, Beijing), vitamin composite powders (8 g) (Huixing Chemical Reagent Co., Ltd, Shanghai), and distilled water (1500 ml). Adults were supplied with a 10% v/v solution of sucrose in distilled water with vitamins. The colony was maintained in the laboratory under conditions of photoperiod (L16:D8), temperature (27 ± 1°C) and 75% relative humidity. The effects of L-arabinose on the development and the intestinal physiology of *H. armigera* caterpillars were examined by adding L-arabinose (Sigma-Aldrich) into the standard artificial diet as described above.

Feeding choice assay

The dual-choice bioassay by using plant leaf disc was performed to test the feeding preference of *H. armigera* caterpillars to artificial diets containing different concentrations of L-arabinose. Leaf discs (10 mm diameter) were punched from fresh leaves of tobacco *Nicotiana tabacum*“K326” (Solanaceae) and leaves of pepper *Capsicum frutescens* L., “Yu-Yi” (Solanaceae) using a conventional hand-held punch (7-1, Yuncheng Co., Ltd, Nantong, China) which then were immersed in different solutions for 5 min. Solutions and leaf mediums used in the feed choice assays were as follows: (1) water-treated tobacco leaves versus tobacco leaves treated with differing concentrations of L-arabinose (0.0001 - 333 mM); (2) water versus 200 mM sucrose with differing concentrations of L-arabinose (0, 100 - 800 mM) treated tobacco leaves;(3) 10 mM sucrose versus 10 mM sucrose with differing concentrations of L-arabinose (1 - 400 mM) treated tobacco leaves; (4) water versus 200 mMD-fructose (Sigma-Aldrich) with differing concentrations of L-arabinose (0, 300 - 600 mM) treated tobacco leaves; (5) water versus different concentrations of L-arabinose (0.01 - 400 mM) treated pepper leaves; (6) water versus 200 mM sucrose with differing concentrations of L-arabinose (0, 100 - 1000 mM) treated pepper leaves.

Eight leaf discs with two different solutions (four of each) were used for each test. For example, four discs treated by water and four discs by 1 mM L-arabinose solution were arranged in an ABABABAB fashion around the circumference of a Petri dish (12 cm diameter) on moist medium-speed qualitative filter paper (Φ11cm, Jiaojie, China). At test initiation, a single caterpillar was placed in the middle of a dish and the leaf disc consumption was observed at regular intervals until at least two of the four disks of either treatment (A or B) had been consumed. Remaining areas of all discs consumed were measured using transparency film (PP2910, 3M Corp.). The feeding preference index was calculated as follows:

Preference index= (area of treatment-disc consumed- area of control-disc consumed) / (area consumed of all discs)

For each kind of choice scenario, the mean consumption of all caterpillars was calculated.

Electrophysiological recordings

The electrophysiological sensitivity of gustatory neurons in the sensilla styloconica on the galea of *H. armigera* larvae was investigated using the single sensillum recording [77, 78]. Excised caterpillar heads were mounted on a silver wire electrode which was connected to the input of a pre-amplifier (Syntech Taste Probe DTP-1, Hilversum, The Netherlands). Glass micropipettes were filled with stimulus solutions in a concentration series of L-arabinose or mixtures of L-arabinose with other compounds. Amplified signals were digitized by an A/D-interface (Syntech IDAC-4) and sampled into a personal computer. Electrophysiological responses were quantified by counting the number of spikes in the first 1000 ms after the start of stimulation. Spikes were counted visually by the experimenter with the aid of Autospike v. 3.4 software (Syntech, Hilversum, The Netherlands). The lateral sensillum of caterpillars was used to test the responses to the following solutions: L-

arabinose from 0.001 mM to 10 mM in 2.0 mMKCl (BeichenFangzhen Chemical Reagent Factory, Tianjin, China), mixtures of 1.0 mM sucrose with a concentration series of L-arabinose from 10 mM to 300 mM, mixtures of L-arabinose with either tobacco leaf saps or pepper leaf saps. The medial sensillum was used to test responses to mixtures of 1.0 mM sinigrin with a concentration series of L-arabinose from 0.1 mM to 100 mM, as well as with mixtures of L-arabinose and tobacco leaf saps. The leaf saps were extracted as per our previous work [38].

The analysis of electrophysiological responses of caterpillar sensilla styloconica to different tastants is performed as described by Tang et al. (2016). In total, impulse waveforms were classified into different groups according to the amplitude, waveform (shape), and phasic temporal pattern, as well as by visual inspection, and then counted with the aid of Autospike version 3.7 software (Syntech, The Netherlands). The mean impulse frequency to one tastant of one kind of sensillum styloconica in the first second ($spk.s^{-1}$) was calculated.

Larval development after exposureto L-arabinose

Neonate caterpillars were fed on standard artificial diets for *H. armigera* caterpillars[76] with L-arabinose (6.66 mM, 66.6 mM, 199.8 mM, 333 mM, or 500 mM) until the final caterpillar instar. The larval developmental duration and the mortality of caterpillars were recorded. Caterpillars were maintained through pupation to adult emergence, the pupal developmental duration, the pupal weights and the emergence rates of adults were recorded.

Enzymatic activity

Neonate caterpillars were fedon standard artificial diets with different concentrations of L-arabinose (6.66 mM, 66.6 mM, 199.8 mM, 333 mM, or 500 mM) until the second day of the

fifth instar. Midguts of second-day fifth instar caterpillars were extracted and weighted. Midguts were then hand homogenized in PBS (pH 7.4) at a ratio of 1.0 g midguts to 6.0 ml PBS, then centrifuged at 2500 g for 10 min at 4°C. Supernatants were used to analyze sucrase activity, sucrose concentration, and glucose concentration by manufacturer protocols (No. A082-2 for Sucrase activity, No.A099 for Sucrose, and No.F006 for glucose; Nanjing Jiancheng Bioengineering Institute, Nanjing, China).Neonate caterpillars fedon standard artificial diets without L-arabinose were used as control group.

Kinetic analysis of sucraseinhibition by L-arabinose

Midguts of the fifth instar caterpillars of *H. armigera* were dissected and rinsed with ice-cold saline (10%) followed by weighing and hand-homogenization. Homogenized tissues were centrifuged at 2500 rpm for 10 min at 4°C. Supernatants were transferred to a fresh tube and stored at -20°C until use.

For the kinetic studies of the inhibitory effects of L-arabinose on sucrase activity in caterpillars, midgut supernatants prepared as above were incubated with increasing concentrations of sucrose (0, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM) in the absence or presence of L-arabinose (6.66 mM or 9.99 mM) or acarbose (7.35μM or 11.79 μM; Sigma-Aldrich). Selected concentrations of these sugars were determined based on preliminary experiments.

The volume of standard reaction assay was 90 μL. After the reaction mixtures incubated at 37°C for 5 min, reactions were terminated by incubating in boiling water for 5 min. After cooldown, the D-glucose concentration was measured using a Glucose Oxidase Method (No. F006; Nanjing Jiangcheng Bioengineering Company, Nanjing, China). Specific activity was calculated as micromoles of substrate hydrolyzed per milligram protein per min. Each assay was repeated at least three times and results were analyzed by Lineweaver-Burk plots.

Data analysis and statistics

All data are presented as mean ± SEM. A one-sample t-test was used to compare the means of the preference indices of caterpillars between for control leaves and treatment leaves.The levels of significance were as follows: NS (non-significant): $p \geq 0.05$, *: $p < 0.05$,**: $p < 0.01$, ***: $p < 0.001$. For statistical analysis of neuronal firing response to stimuli, the original data of the mean impulse frequencyin response to each stimulus were square-root transformed if the variances were not equal. A one-way ANOVA followed by a Scheffé test was used to compare the mean impulse frequency of the same GRN to different stimulations ($p < 0.05$).

The data of developmental parametersand midgut enzyme activities were log-transformed for uneven sample sizes. Means were compared by one-way ANOVA followed by aSchefféTest (threshold for $p < 0.05$). All statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA).

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Author Contributions

QT, XZ, YM and FY designed the research. LS and ZH performed most of the experiments. YM, WH and LL performed some of the behavioral and electrophysiological experiments.

YM, LS, ZH analyzed the data. QT, XY and CW wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Declaration

The authors have declared that no competing interest exists.

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641

642

Figure legends

Figure 1 Feeding preference of *H. armigera* caterpillars for tobacco leaves treated with different sugars. Columns represent the mean preference indices ± SEM of fifth instar caterpillars for tobacco leaves treated with one sugar or water (control) in dual-choice assays. **A:** Preference between leaves treated by different concentrations of L-arabinose (LA) or water-treated leaves (control). **B:** Preference between water-treated leaves (control) and leaves treated by mixtures of 200 mM sucrose plus different concentrations of L-arabinose. **C:** Preference between 10 mM sucrose-treated leaves and leaves treated with mixtures of 10 mM sucrose plus different concentrations of L-arabinose. **D:** Preference between water-treated leaves (control) and leaves treated by mixtures of 200 mM fructose (Fru.) plus different concentrations of L-arabinose. A one-sample t-test was used to compare the means of the preference indices for treatment leaves and for control leaves in each experimental setting ($p < 0.05$).

Figure 2 Feeding preference of *H. armigera* caterpillars for pepper leaves treated with different sugars. Columns represent the mean preference indices ± SEM of fifth instar caterpillars for pepper leaves treated with one sugar or water (control) in dual-choice assays. **A:** Preference between for leaves treated bydifferent concentrations of L-arabinose (LA) and for water-treated leaves (control). **B:** Preference between for water-treated pepper leaves (control) and for pepper leaves treated by mixtures of 200mM sucrose plus different concentrations of L-arabinose. A one-sample t-test was used to compare the means of the preference indices for treatment leaves and for control leave in each experimental setting ($p < 0.05$).

Figure 3 Morphologies and electrophysiological responses of the sensilla styloconica of *H. armigera* caterpillars to L-arabinose. A: Ventral view of the head showing the mouthparts of fifth instar caterpillars. **B:** Close-up view of the medial and the lateral sensillum styloconicum located on agalea. **C:** Responding curves of the lateral sensillum and the medial sensillum to series concentrations of L-arabinose in 2.0 mM KCl.

Figure 4 Representative traces andeffects of L-arabinose on the sensitivity of sucrose-GRN in the lateral sensillum. A: Representative traces (200 ms) obtained from a single lateral sensillum in the same caterpillar to different stimuli in order as shown: GR1, GR2, and GR4 represent the identified neurons from traces by using Autospike software. **B:** Comparison of response frequency ± SEM of the classified neurons to 1.0 mM sucrose (Su.) and to the mixtures of 1.0 mM sucrose with different concentrations of L-arabinose (LA.+Su.). Scheffé's post hoc test for one-way ANOVA was used to compare the mean response frequency of GR1 to different stimuli (p <0.05).Columns having no lowercase letters in common differ significantly (p< 0.05).

Figure 5 Representative traces and effects of L-arabinose on the GRNs in the medial sensillum sensitive to tobacco leaf saps. A: Representative traces (200 ms) obtained from a single medial sensillum in the same caterpillar to different stimuli in order as shown: GR1, GR2, GR3, and GR4 represent the identified neurons from electrophysiological traces by using Autospike software. **B:** Comparison of response frequency ± SEM of the classified neurons to tobacco saps (Tob.) and to tobacco saps with different concentrations of L-arabinose (LA.+Tob.). Scheffé's post hoc test for one-way ANOVA was used to compare the mean response frequency of GR2 and GR3 to different stimuli (p< 0.05). Columns having no lowercase letters in common differ significantly (p< 0.05).

Figure 6 Representative traces and effects of L-arabinose on the sensitivity of sinigrin-GRN in the medial sensillum. A: Representative traces (200 ms) obtained from a single medial sensillum in the same caterpillar to different stimuli in order as shown: GR1, GR3, and GR4 represent the identified neurons from electrophysiological traces by using Autospike software. **B:** Comparison of response frequency ± SEM of the classified neurons to 1.0 mM sinigrin (Sin.) and to mixtures of 1.0 mM sinigrin with different concentrations of L-arabinose (LA.+Sin.). Scheffé's post hoc test for one-way ANOVA was used to compare the mean response frequency of GR3 to different stimuli ($p < 0.05$). Columns having no lowercase letters in common differ significantly ($p < 0.05$).

Figure 7 Effects of L-arabinose on the development of *H. armigera*. Comparison of larval developmental duration (**A**) and larval mortality rate (**B**) after rearing larvae on artificial diets containing different concentrations of L-arabinose from neonate until pupation. Comparison of pupal developmental duration (**C**) and pupal weight (**D**) from caterpillars reared on artificial diets containing different concentrations of L-arabinose from neonate until pupation. Scheffé's post hoc test for one-way ANOVA was used to compare the means of developmental parameters of caterpillars reared on different L-arabinose-containing diets ($p < 0.05$). Columns having no lowercase letters in common differ significantly ($p < 0.05$).

Figure 8 Effects of L-arabinose on the intestinal physiology of *H. armigera* caterpillars. Comparison of the sucrase activity (**A**), sucrose concentration (**B**), and the glucose concentrations (**C**) in midguts of the fifth instar caterpillars after rearing on artificial diets containing different concentrations of L-arabinose from neonate until pupation. Scheffé's post hoc test for one-way ANOVA was used to compare the means of physiological parameters of caterpillars exposing to different concentrations of L-arabinose ($p < 0.05$). Columns having no lowercase letters in common differ significantly ($p < 0.05$).

Figure 9 Kinetic analysis of sucrase inhibition by L-arabinose and acarbose. Supernatants extracted from caterpillar midgut homogenates were incubated with increasing concentrations of sucrose in the absence or presence of inhibitor: (**A**) L-arabinose 6.66 mM (□) or 9.99 mM (△) and the control (**B**) acarbose 7.53 μM (□) or 11.79 μM (△). Assays without inhibitor: (○). Results represent Lineweaver-Burk analysis. R^2 is the coefficient of determination which predicts the goodness of fit for the linear regression model.

Figures

Figure 1

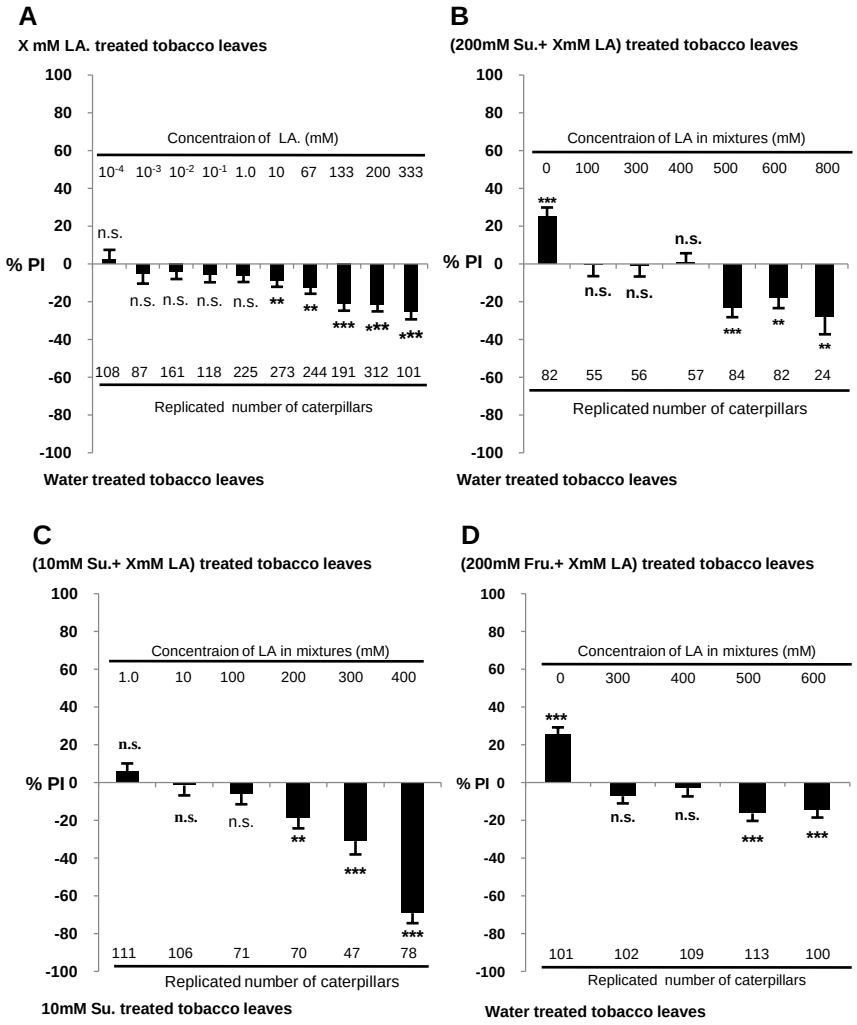


Figure 2

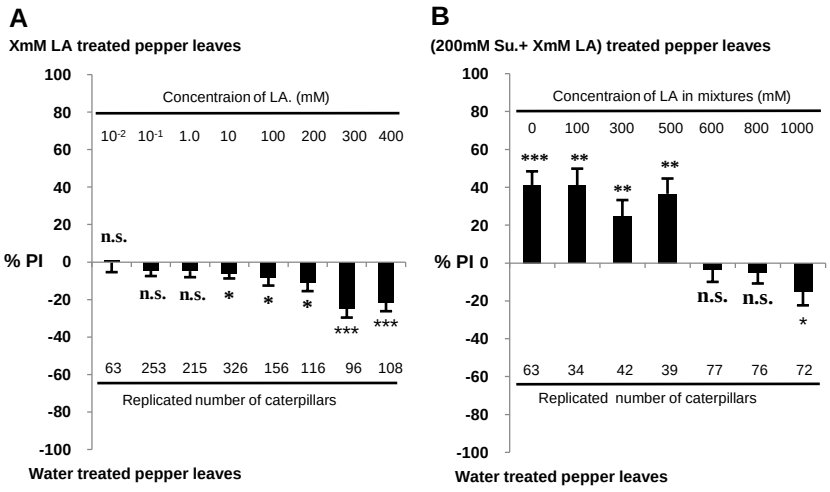

A
XmM LA treated pepper leaves
Concentraion of LA. (mM)
10-2 10-1 1.0 10 100 200 300 400
% PI
n.s.
n.s. n.s. * * * *** ***
63 253 215 326 156 116 96 108
Replicated number of caterpillars
Water treated pepper leaves
B
(200mM Su.+ XmM LA) treated pepper leaves
Concentraion of LA in mixtures (mM)
0 100 300 500 600 800 1000
% PI
*** ** ** **
n.s. n.s. *
63 34 42 39 77 76 72
Replicated number of caterpillars
Water treated pepper leaves

Figure 3

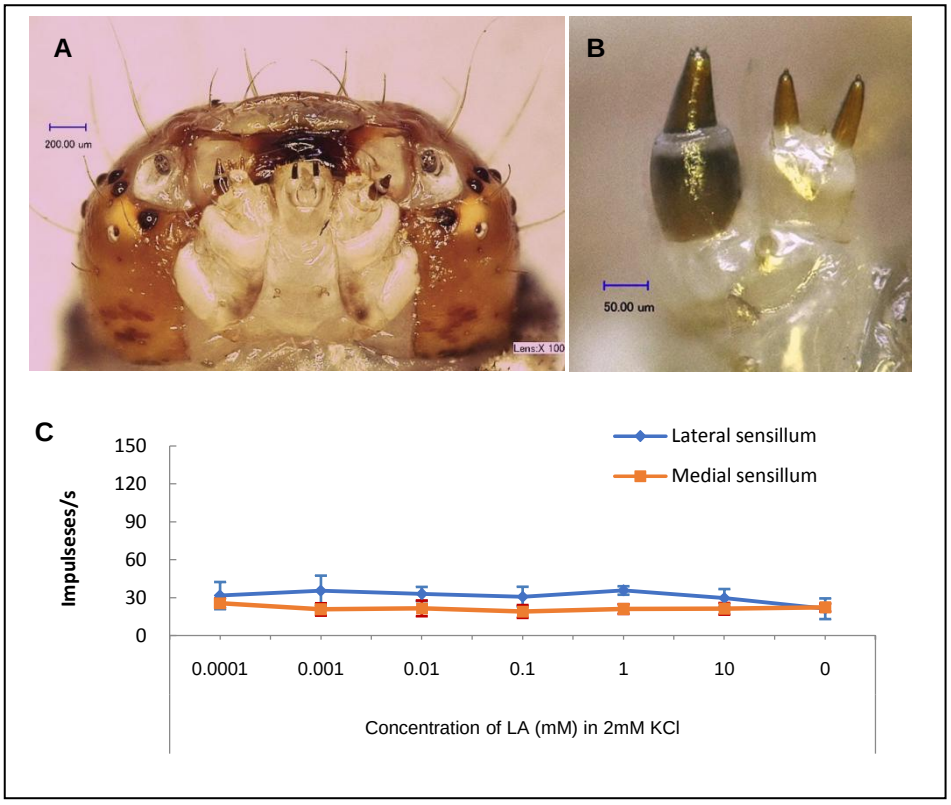

A
200.00 um
Lens:X 100
B
50.00 um
C
Lateral sensillum
Medial sensillum
Impulseses/s
150
120
90
60
30
0
0.0001
0.001
0.01
0.1
1
10
0
Concentration of LA (mM) in 2mM KCl

Figure 4

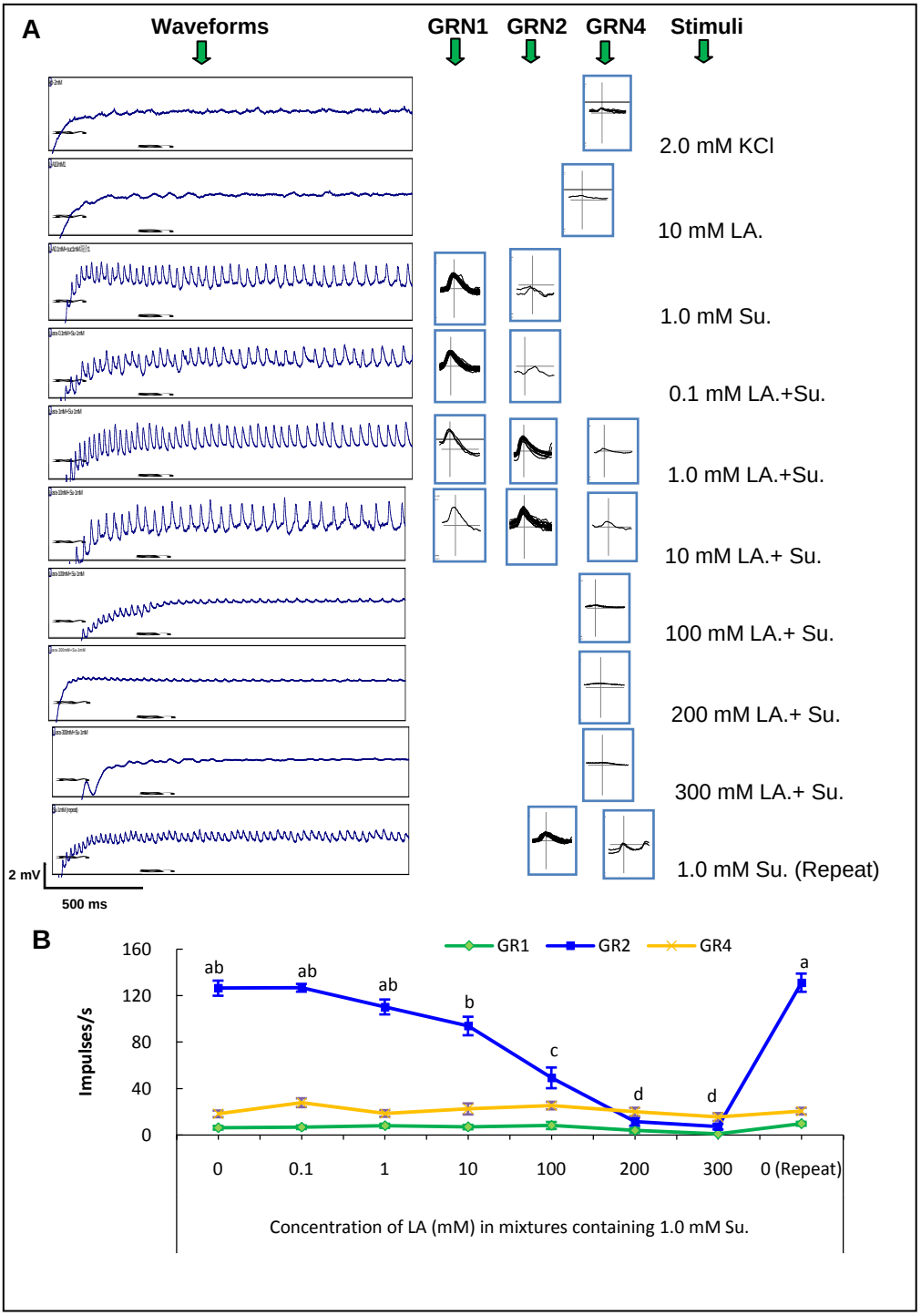

A
Waveforms
GRN1
GRN2
GRN4
Stimuli
2.0 mM KCl
10 mM LA.
1.0 mM Su.
0.1 mM LA.+Su.
1.0 mM LA.+Su.
10 mM LA.+ Su.
100 mM LA.+ Su.
200 mM LA.+ Su.
300 mM LA.+ Su.
1.0 mM Su. (Repeat)
2 mV
500 ms
B
GR1
GR2
GR4
Impulses/s
160
120
80
40
0
ab
ab
ab
b
c
d
d
a
0
0.1
1
10
100
200
300
0 (Repeat)
Concentration of LA (mM) in mixtures containing 1.0 mM Su.

Figure 5

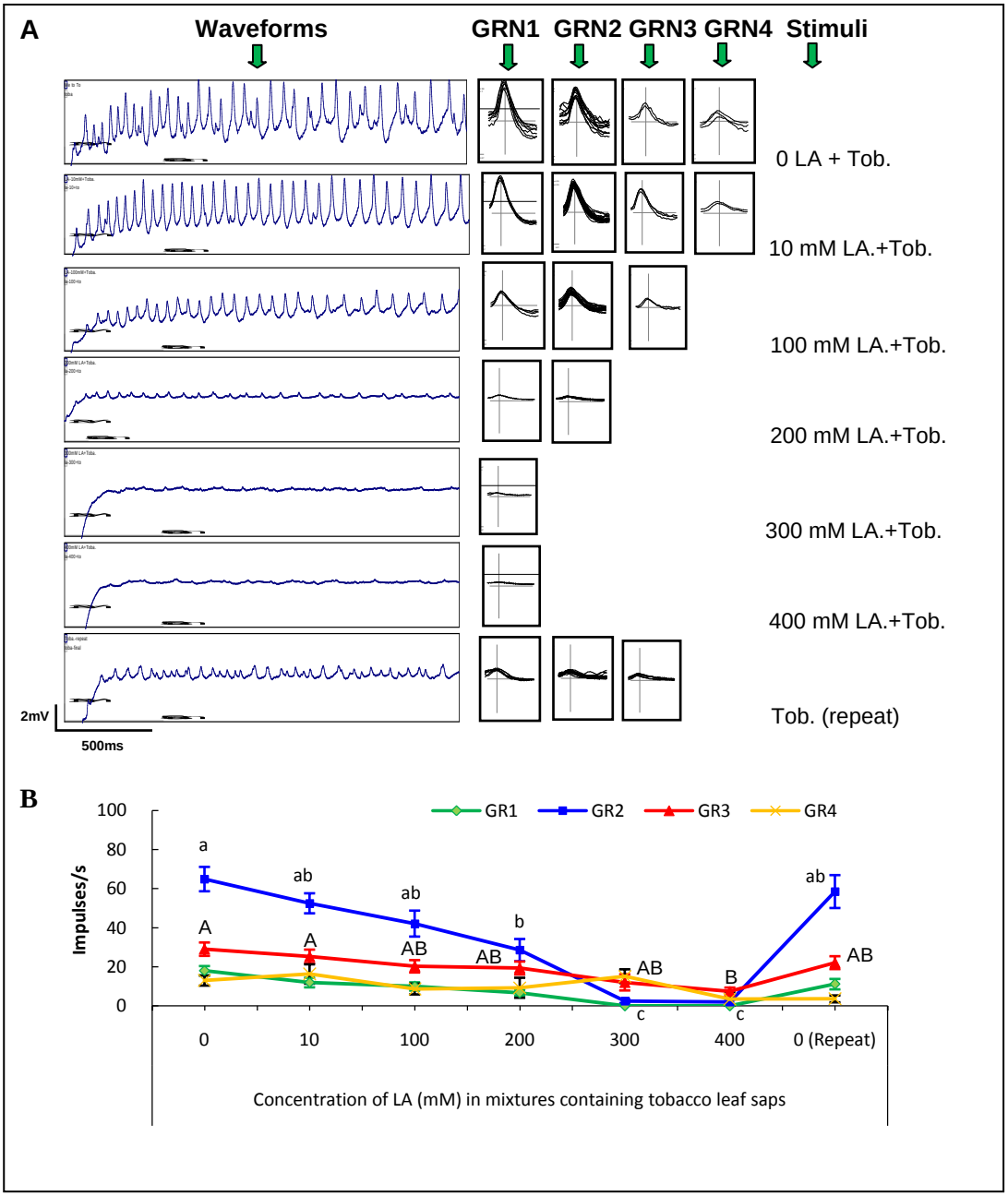

A
Waveforms
GRN1
GRN2
GRN3
GRN4
Stimuli
0 LA + Tob.
10 mM LA.+Tob.
100 mM LA.+Tob.
200 mM LA.+Tob.
300 mM LA.+Tob.
400 mM LA.+Tob.
Tob. (repeat)
2mV
500ms
B
GR1
GR2
GR3
GR4
Impulses/s
100
80
60
40
20
0
a
ab
ab
b
c
c
ab
A
A
AB
AB
AB
B
AB
0
10
100
200
300
400
0 (Repeat)
Concentration of LA (mM) in mixtures containing tobacco leaf saps

Figure 6

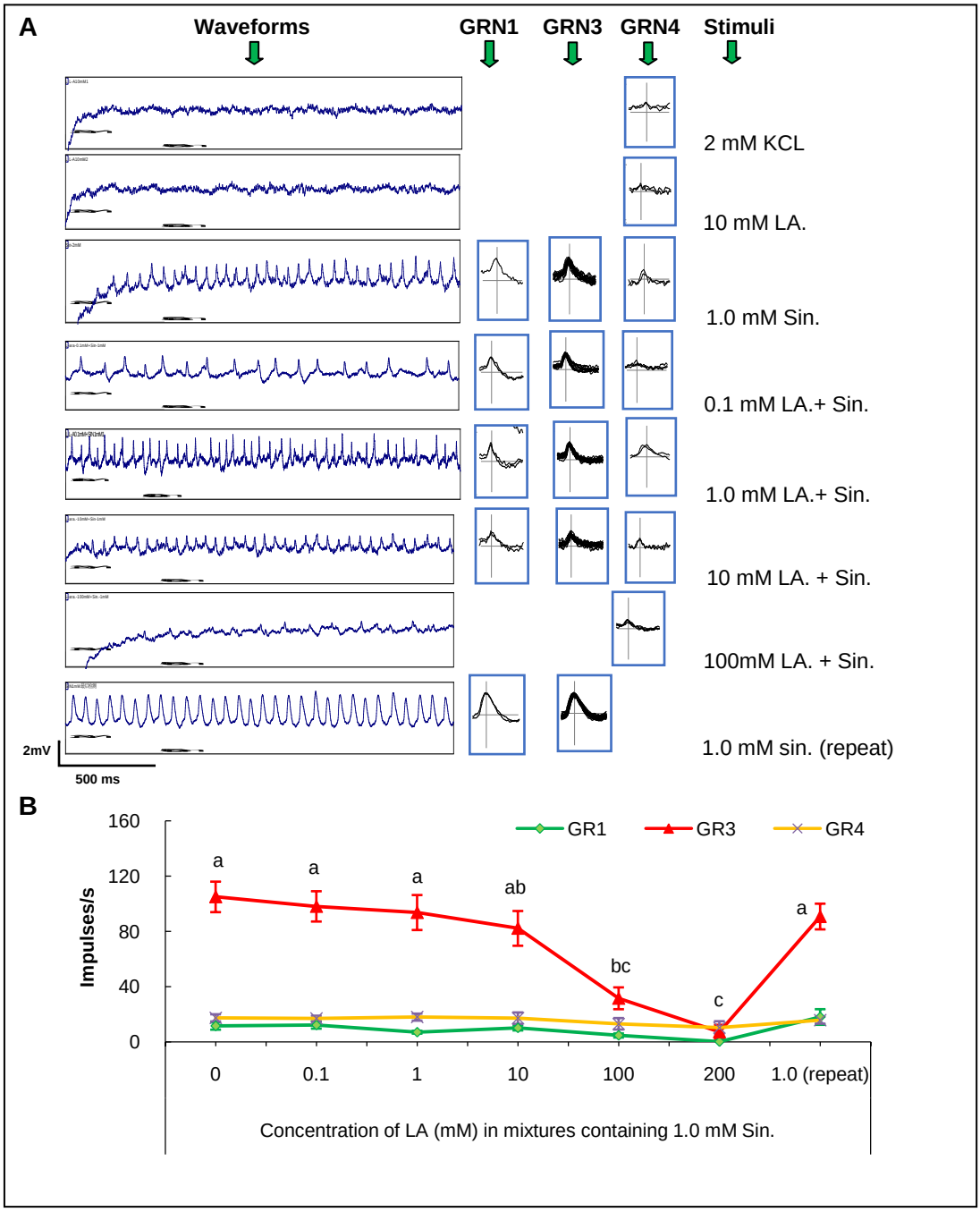

A
Waveforms
GRN1
GRN3
GRN4
Stimuli
2 mM KCL
10 mM LA.
1.0 mM Sin.
0.1 mM LA.+ Sin.
1.0 mM LA.+ Sin.
10 mM LA. + Sin.
100mM LA. + Sin.
1.0 mM sin. (repeat)
2mV
500 ms
B
GR1
GR3
GR4
Impulses/s
160
120
80
40
0
a
a
a
ab
bc
c
a
0
0.1
1
10
100
200
1.0 (repeat)
Concentration of LA (mM) in mixtures containing 1.0 mM Sin.

Figure 7

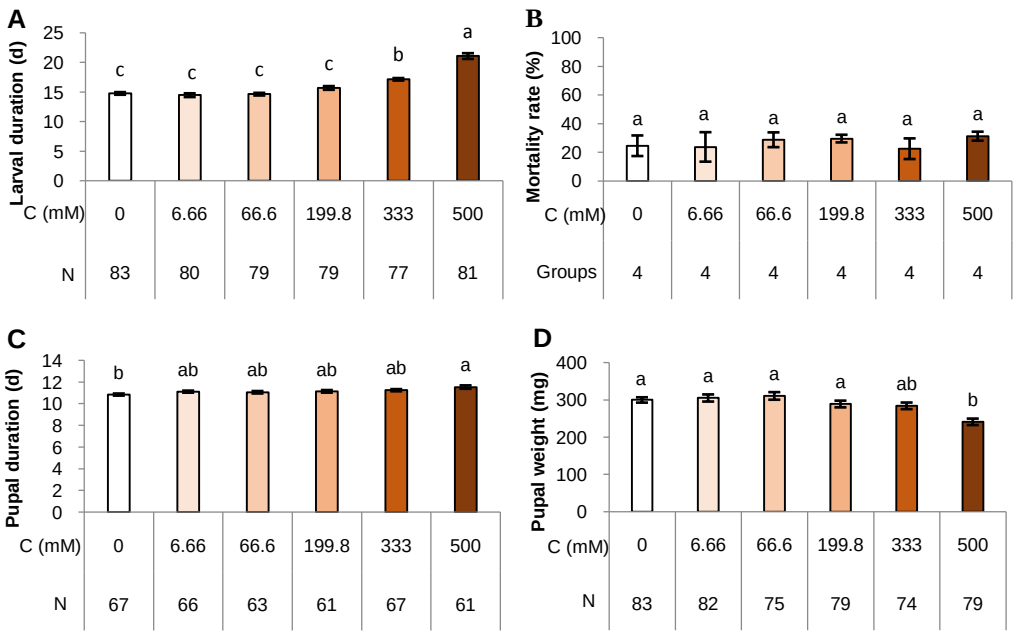

A
Larval duration (d)
25
20
15
10
5
0
c
c
c
c
b
a
C (mM)
0
6.66
66.6
199.8
333
500
N
83
80
79
79
77
81
B
Mortality rate (%)
100
80
60
40
20
0
a
a
a
a
a
a
C (mM)
0
6.66
66.6
199.8
333
500
Groups
4
4
4
4
4
4
C
Pupal duration (d)
14
12
10
8
6
4
2
0
b
ab
ab
ab
ab
a
C (mM)
0
6.66
66.6
199.8
333
500
N
67
66
63
61
67
61
D
Pupal weight (mg)
400
300
200
100
0
a
a
a
a
ab
b
C (mM)
0
6.66
66.6
199.8
333
500
N
83
82
75
79
74
79

Figure 8

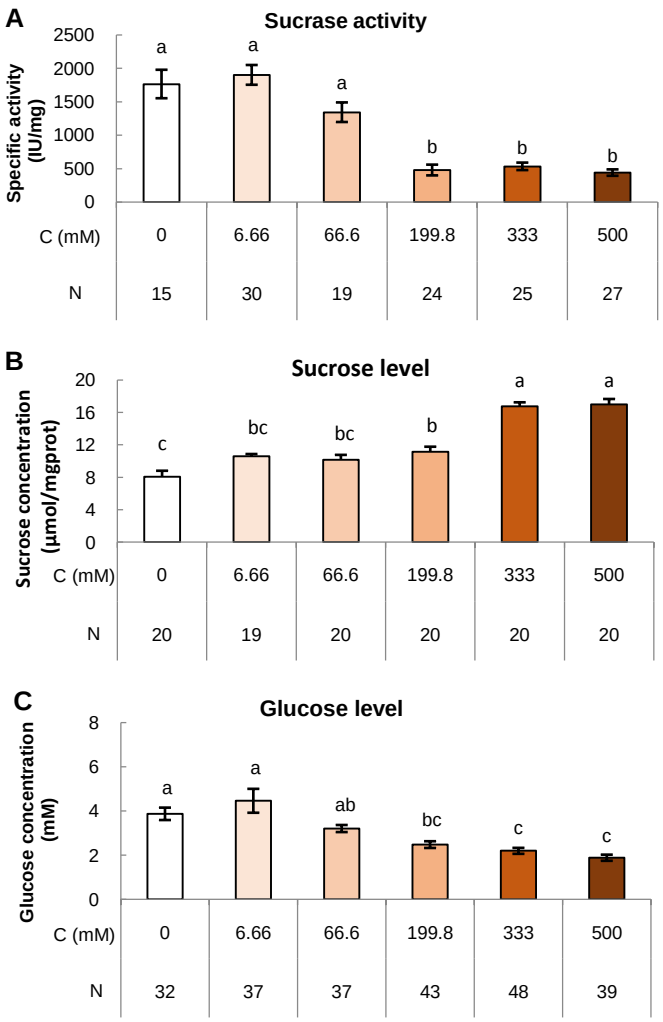
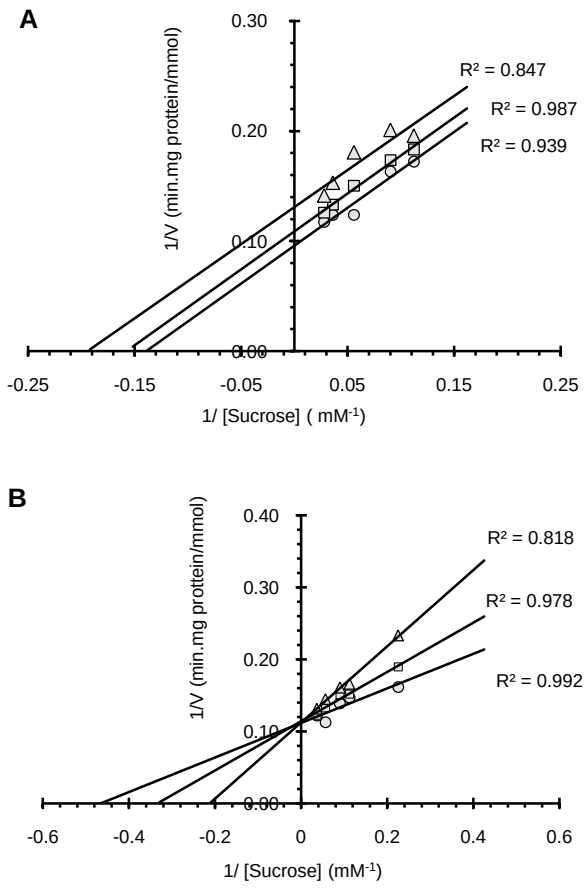

A
Sucrase activity
Specific activity (IU/mg)
2500
2000
1500
1000
500
0
a
a
a
b
b
b
C (mM)
0
6.66
66.6
199.8
333
500
N
15
30
19
24
25
27
B
Sucrose level
Sucrose concentration (µmol/mgprot)
20
16
12
8
4
0
c
bc
bc
b
a
a
C (mM)
0
6.66
66.6
199.8
333
500
N
20
19
20
20
20
20
C
Glucose level
Glucose concentration (mM)
8
6
4
2
0
a
a
ab
bc
c
c
C (mM)
0
6.66
66.6
199.8
333
500
N
32
37
37
43
48
39

Figure 9


A
1/V (min.mg prottein/mmol)
0.30
0.20
0.10
0.00
R² = 0.847
R² = 0.987
R² = 0.939
-0.25
-0.15
-0.05
0.05
0.15
0.25
1/ [Sucrose] (mM-1)
B
1/V (min.mg prottein/mmol)
0.40
0.30
0.20
0.10
0.00
R² = 0.818
R² = 0.978
R² = 0.992
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
1/ [Sucrose] (mM-1)