A sweet-tasting sugar drives aversive responses in caterpillars

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ABSTRACT:

Non-caloric artificial sweeteners are widely used in the modern human food and beverage industry to combat obesity and diabetes, increasing concerns about their long-term health impact. Here, we report that a sweet-tasting, naturally occurring, non-nutritional pentose, L-arabinose, is not a sweetener to caterpillars of the herbivorous insect species cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). We used behavioral, developmental, and enzymatic approaches to demonstrate that L-arabinose could inhibit the feeding, development, and activity of intestinal sucrase in *H. armigera* caterpillars. Further, the sensitivities of gustatory receptor neurons in peripheral taste sensilla to other substances, for example the sucrose, fructose and plant leaf saps, were also inhibited when mixed with L-arabinose, indicating the indirect detection of L-arabinose in peripheral coding is possible. In vitro, L-arabinose inhibits *H. armigera* intestinal sucrase in an uncompetitive manner. Altogether, these findings demonstrate that L-arabinsoe is a feeding inhibitor to caterpillars of *H. armigera*. To our knowledge, this is the first report of a sugar that tastes sweet to mammals but an inhibitor both to peripheral taste sensitivity and feeding behaviors.

Introduction

In the past few decades, non-caloric artificial sweeteners (NAS) have become widely used additives in human food products with the intention to combat obesity and diabetes, however little is known regarding their health impact from long-term consumption (DuBois and Prakash, 2012; Swithers, 2015; van Opstal et al. , 2019). Among them, L-arabinose, a naturally-occurring and widely present pentose, is known to be poorly absorptive (Halschoujensen et al. , 2015; Seri et al. , 1996; Shibanuma and Houda, 2011; Yamamoto, 2013) but elicits a sweet taste for humans (Moskowitz, 1971). It is one of the components of biopolymers such as cereal fiber and pectin of corn, wheat, barley, rye, and oats and is produced as 30–40% in its linked form (Bengtsson, 1990; Garleb et al. , 1989; Saulnier et al. , 1995) and up to 75% in the “hairy region” of beet pectin (Oosterveld et al. , 2000). However, L-arabinose has been recognized to have a selective inhibitory effect on intestinal sucrase activity and thereby reduced the digestion, absorption, and net energy yield of sucrose in mammals including rats (Osaki et al. , 2001; Sanai et al. , 1997), mice (Seri et al. , 1996), and humans (Inoue et al. , 2000; Shibanuma and Houda, 2011). Whether such impacts of L-arabinose could extend to other animals such as herbivorous insects are not yet known.

Gustatory receptor neurons (GRNs) are housed within a peripheral sensillum that can detect and discriminate among a diverse array of sugars and other chemicals (Dahanukar

et al. , 2001; Fujii et al. , 2015; Matsunami et al. , 2000; Sato et al. , 2011; Zhao et al. , 2003). In general, sweetness is a “good” taste, eliciting appetitive consumption of nutritive foods, whereas bitterness is a “bad” taste, driving aversive behavior to foods containing harmful substances. Although great progress has been made in the understanding of sugar chemoreception in some animals, such as mammals and insects (Dahanukar et al. , 2007; Li et al. , 2002; Liman et al. , 2014; Musso et al. , 2017; Nelson et al. , 2001; Wang et al. , 2004), how some sugars function as sweeteners to some animals whereas others function as non-sweeteners to other animals remains largely unknown.

In *Drosophila*, pre-feeding of adults with NAS resulted in an inability to learn the non-nutritional property of these sugars and form Caloric Frustration Memory (CFM) in the memory center through a post-ingestion mechanism, which lead to devaluing NAS during its subsequent representation (Musso et al. , 2017). This mechanism involves activity of the central brain processes, but not a peripheral taste state (Burke et al. , 2012; Burke and Waddell, 2011; Musso et al. , 2017). However, the NAS L-arabinose acts as a feeding sweetener and induces feeding preference behaviors in flies (Burke and Waddell, 2011; Dahanukar et al. , 2007; Marella et al. , 2006). Interestingly, these results indicate that the peripheral nervous system of flies regards L-arabinose as a sweetener, whereas the central brain recognizes this NAS as a negative sugar. It remains unknown whether the perceptual contradiction of L-arabinose between peripheral and central systems in flies also applies to

other insect species.

In this study, we investigated whether the peripheral coding may be contrary to the feeding behaviors and ingestion processes of the herbivorous insect species, the cotton bollworm *Helicoverpa armigera*. To determine whether *H. armigera* caterpillars could perceive L-arabinose as a negative sugar, we investigated whether L-arabinose could affect the feeding preferences of caterpillars for host plants and other phagostimulants. We then investigated whether L-arabinose could be perceived by the GRNs in the peripheral sensillum of caterpillars by measuring the responses to L-arabinose, phagostimulants, a feeding deterrent, and their mixtures. Additionally, we investigated the effects of L-arabinose on larval development by exposing larvae to L-arabinose and monitoring the activity of intestinal sucrase from midguts of *H. armigera* caterpillars *in vitro*. 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 as well. We report here that both the peripheral and the central nervous system of *H. armigera* caterpillars perceive the non-nutritional sugar as a sweetener.

Results

Effects of L-arabinose on feeding behaviors

We tested the feeding preferences of the 5th instar caterpillars of *H. armigera* to investigate whether L-arabinose may affect the feeding preferences between tobacco leaf discs treated with pure distilled water (control) and leaves treated by L-arabinose (0.0001-

333 mM) using a dual-choice leaf disc assay. Preference of *H. armigera* caterpillars for low concentrations of L-arabinose-treated tobacco leaves did not differ from control tobacco leaves (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, leaves treated by concentrations L-arabinose ≥10 mM significantly deterred caterpillar feeding (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 found that L-arabinose also could inhibit the feeding preferences of *H. armigera* caterpillars for phagostimulants, e.g., sucrose and fructose. We examined feeding preferences of *H. armigera* caterpillars between tobacco leaves treated with pure distilled water (control) and tobacco leaves treated with mixtures of 200 mM sucrose plus different concentrations of L-arabinose in a dual-choice leaf disc assay. Compared with the significant preference for the tobacco leaves treated by 200 mM sucrose alone (Fig. 1B, 200 mM Su.+0 LA, one-sample t-test: $t (81) = 5.117$, $p < 0.0001$), caterpillars of *H. armigera* had a similar preferences for control leaves and for leaves treated by mixtures containing 200 mM sucrose plus L-arabinose (100, 300, and 400 mM; Fig. 1B, one-sample t-test: 200 mM sucrose + 100 mM L-arabinose, $t(154) = -0.048$, $p = 0.962$; 200 mM sucrose + 300 mM L-arabinose, $t(55) = -0.156$, $p = 0.877$; 200 mM sucrose + 400 mM L-arabinose, $t(56) = 0.138$, $p = 0.891$), whereas tobacco leaves treated by mixtures containing higher concentrations of L-arabinose (500 mM, 600 mM, and 800 mM) significantly deterred caterpillar feeding (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 in mixtures with sucrose counteract the phagostimulant effect of 200 mM sucrose in a concentration-dependent manner.

Since L-arabinose counteracted the feeding induction effect of sucrose (Fig. 1B), we examined whether caterpillars could distinguish between leaves treated with a phagostimulant and those with the addition of L-arabinose. For these experiments, we chose to use tobacco leaf discs with 10 mM sucrose treatment since this concentration was found to significantly induce feeding in *H. armigera* caterpillars over the water-treated tobacco leaves (One-sample t-test: $t(24) = 5.281$, $p < 0.0001$). Feeding preferences of *H. armigera* caterpillars were monitored between 10 mM sucrose–treated tobacco leaves and tobacco leaves treated with mixtures containing 10 mM sucrose plus L-arabinose at different concentrations. No feeding preference effects were observed between control 10 mM sucrose-treated tobacco leaves and leaves treated by mixtures containing low concentrations of L-arabinose (1.0–100 mM; Fig. 1C; one-sample t-test: 10 mM sucrose + 1 mM L-arabinose, $t(110) = 1.363$, $p = 0.176$; 10 mM sucrose + 10 mM L-arabinose, $t(105) = -0.301$, $p = 0.764$; 10 mM sucrose + 100 mM L-arabinose, $t(70) = -1.121$, $p = 0.266$). Caterpillars significantly preferred tobacco leaves treated by sucrose/L-arabinose mixtures containing L-arabinose from 200 mM to 400 mM (Fig. 1C; one-sample t-test: 10 mM sucrose + 200 mM 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, $t(77) = -13.09$, $p < 0.0001$). In contrast to the effects of L-arabinose alone, which inhibits feeding, the addition of sucrose combined with L-arabinose at concentrations ≥ 200 mM counteracted the feeding inhibitory effects of L-arabinose and produced phagostimulatory effects (Fig. 1C).

We also examined the effects of L-arabinose on another feeding stimulant, fructose, to determine whether effects of L-arabinose and a stimulant are structure-dependent. Caterpillars of *H. armigera* showed greater preference for 200 mM fructose-treated leaf discs than for water-treated control tobacco leaves (Fig. 1D; one-sample t-test: 200 mM fructose, $t(101) = 6.722$, $p < 0.0001$), whereas feeding preferences were not significant for mixtures of 300 mM or 400 mM L-arabinose and 200 mM fructose (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, $t(109) = -0.677$, $p = 0.500$). Caterpillars preferred control leaves over 200 mM fructose/L-arabinose mixtures ≥ 500 mM (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 whether the plant leaf medium had an impact on the feeding preferences, we substituted pepper leaves for tobacco leaves. The results show that the effects of L-arabinose on *H. armigera* caterpillars are independent of leaf material source. Caterpillars had similar preferences for control pepper leaves and pepper leaves treated by L-arabinose (0.001–1.0 mM; Fig. 2A; one-sample t-test: 0.01 mM L-arabinose, $t(62) = 0.124$, $p = 0.902$; 0.1 mM L-arabinose, $t(252) = -1.315$, $p = 0.190$; 1.0 mM l-arabinose, $t(214) = -1.493$, $p = 0.137$). Caterpillars significantly preferred control pepper leaves more than leaves treated by 10 mM or higher concentrations of L-arabinose (Fig. 2A; one-sample t-test: 10 mM L-arabinose, $t(325) = -2.251$, $p = 0.025$; 100 mM L-arabinose, $t(155) = -2.028$, $p = 0.044$; 200 mM L-arabinose, $t(115) = -2.244$, $p = 0.027$; 300 mM L-arabinose, $t(95) = -5.211$, $p < 0.0001$; 400 mM L-arabinose, $t(107) = -4.360$, $p < 0.0001$).

Similarly, when using pepper leaves as the medium, L-arabinose also could augment the inducing effects of 200 mM sucrose on *H. armigera* caterpillars. Caterpillars significantly

preferred to feed on pepper leaves treated with mixtures containing 200 mM sucrose and L-arabinose (100 mM, 300 mM, 500 mM, 600 mM; 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, $t(38) = 4.521$, $p = 0.0001$). When pepper leaf discs were treated with 200 mM sucrose/L-arabinose mixtures ≥ 600 mM, no feeding preference behaviors were 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, $t(75) = -0.851$, $p = 0.398$). At concentrations of 200 mM sucrose and 1000 mM L-arabinose, caterpillars significantly preferred the control leaves over leaves treated by the mixture (Fig. 2B; one-sample t-test: 200 mM sucrose + 1000 mM L-arabinose, $t(71) = -2.110$, $p = 0.038$). Therefore, data from feeding assays of both tobacco leaves and pepper leaves treated by L-arabinose alone or by mixtures containing L-arabinose suggest L-arabinose not only could inhibit the feeding of *H. armigera* caterpillars for plant leaves, but also could counteract the inducing effects of phagostimulants in a concentration-dependent manner.

Inhibition of L-arabinose on gustatory sensitivity

Gustation plays an important role in the feeding behaviors of caterpillars (Hanson and Dethier, 1973; Zhang et al. , 2019) and gustatory perception in lepidopteran caterpillars are mainly dominated by two pairs of gustatory sensilla styloconica, the lateral sensilla and the medial sensilla, located on the maxillary galea of mouthparts (Dethier and Crnjar, 1982; Hanson and Dethier, 1973). In *H. armigera* caterpillars, a gustatory receptor neuron (GRN) in the lateral sensillum is sensitive to sucrose (Tang et al. , 2000), and the GRN in the medial sensillum is sensitive to sinigrin (Tang et al. , 2015). At least three GRNs in both lateral and medial sensilla are sensitive to host plant leaf saps (Tang et al. , 2014). We investigated whether GRNs in the maxillary sensilla styloconica of 5th instar caterpillars are

sensitive to L-arabinose and whether L-arabinose could inhibit the sensitivities of other GRNs: those sensitive to the phagostimulant sucrose, the bitter substance sinigrin, as well as host plant saps by using the single sensillum electrophysiological recording.

The medial sensillum and the lateral sensillum of the 5th instar *H. armigera* caterpillars are shown in Figs. 3A and 3B. Our results show that non-receptor neurons sensitive to L-arabinose were found in both the medial and the lateral sensillum (Fig. 3C). When L-arabinose was mixed with other stimuli (the phagostimulant sucrose, the bitter substance sinigrin, and host plant leaf saps), the sensitivities of GRNs to these other substances were inhibited. We tested the responses of the lateral sensillum in sequence to 10 mM L-arabinose, 1.0 mM sucrose, and to mixtures containing 1.0 mM sucrose plus different concentrations of L-arabinose as shown in 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. We then followed with increasing concentrations of L-arabinose in mixtures containing 1.0 mM sucrose and observed that the activity of GRN2 decreased gradually to the point of becoming completely inactive at sucrose mixture concentrations of L-arabinose ≥ 100 mM (Figs. 4A, 4B). When only the 1.0 mM sucrose was subsequently applied to the lateral sensillum, the activity of GRN2 recovered completely (Figs. 4A, 4B).

Similarly, the sensitivity of GRN2 in medial sensillum of *H. armigera* caterpillars induced by tobacco-leaf saps was also depressed gradually in the response to mixtures of tobacco leaf saps plus L-arabinose in a concentration-dependent manner (Fig. 5). Interestingly, with mixtures containing 1.0 mM sinigrin and increasing concentrations of L-arabinose, the strong sensitivities of GRN3 in the medial sensillum to sinigrin were also

depressed gradually (Fig. 6). These results suggest L-arabinose inhibits the gustatory sensitivities of GRNs to both phagostimulants and deterrents.

Larval development after exposure to L-arabinose

We investigated larval development time, larval mortality, 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). It shows that either the development times, the duration of pupation and the pupal weights were significantly different in caterpillars exposed to these artificial diets (one-way ANOVA: development times, $F(5, 473) = 48.928$, $p < 0.0001$; duration of pupation, $F(5, 379) = 5.384$, $p = 0.02$; pupal weights, $F(5, 466) = 11.125$, $p < 0.0001$) (Fig. 7). Developmental times of caterpillar instars consuming artificial diets containing 6.66 mM, 66.6 mM, and 199.8 mM L-arabinose were similar to caterpillars reared on the control diet (Scheffé Test: $p = 0.138$; Fig. 7A), whereas the developmental times were significantly prolonged in caterpillars reared on diets containing 333 mM and 500 mM L-arabinose (Scheffé Test: $p < 0.001$). Larval mortalities were not significantly affected in caterpillars reared on artificial diets containing L-arabinose (one-way ANOVA: $F(5, 18) = 0.283$, $p = 0.917$, Fig. 7B). Pupation duration times were prolonged significantly and the pupal weight decreased significantly when caterpillars were reared on artificial diets containing 500 mM L-arabinose (Figs. 7C and 7D; Scheffé Test: pupation duration times, $p = 0.005$; pupal weights, $p < 0.001$; Figs. 7C and 7D). The developmental assays demonstrate that consumption of L-arabinose results in delay of larval development.

Activity of digestive enzymes after exposure to artificial L-arabinose

In mammals, L-arabinose inhibits intestinal sucrase activity and thereby delays sucrose digestion by preventing sucrose hydrolysis to fructose and glucose (Sanai et al. , 1997; Seri et al. , 1996; Shibanuma and Houda, 2011). We similarly found the sucrase activity in midguts of *H. armigera* caterpillars could be inhibited by the exposure to artificial diets

containing L-arabinose across all caterpillar life stages. As anticipated, sucrose concentration increased and the glucose levels degreased in midguts L-arabinose-treated caterpillars (Fig. 8). The sucrase activities decreased in midguts of the fifth instar caterpillars in an L-arabinose concentration-dependent manner (6.66 mM – 500 mM; one-way ANOVA: $F(5, 134) = 26.755$, $p < 0.0001$). Sucrase activities from midguts of caterpillars exposed L-arabinose ≤ 66.6 mM did not differ from control activities (Scheffé's test, $p = 0.692$), and the sucrase activities were also not significantly different in caterpillars exposed to diets containing 199.8 mM, 333 mM, to 500 mM L-arabinose (Scheffé's Tests: $p = 0.902$). However, sucrase activities of caterpillars exposed to the low concentrations of L-arabinose (6.66 mM and 66.6 mM) were significantly higher than those exposed to 199.8 mM, 333 mM, or 500 mM L-arabinose (Scheffé's Tests: $p < 0.01$, all results; Fig. 8A).

Sucrose concentrations increased (one-way ANOVA: $F(5, 113) = 39.750$, $p < 0.0001$) and the glucose level decreased (one-way ANOVA, $F(5, 230) = 17.590$, $p < 0.0001$) in midguts of caterpillars reared on artificial diets containing L-arabinose (6.66 mM, 66.6 mM, 199.8 mM, 333 mM, and 500 mM) (Fig. 8B). Compared with control caterpillars, the sucrose content in midgut of caterpillars reared on artificial diets containing 6.66 mM and 66.6 mM L-arabinose tended to increase, but was not significant (Scheffé's Tests: $p = 0.106$), whereas the concentration of sucrose in caterpillars exposed to artificial diets containing 199.8 mM was significantly higher than caterpillars reared on control diet (Scheffé's Tests: $p = 0.023$). As expected, after caterpillars were exposed to diets containing high concentration of L-arabinose (333 mM and 500 mM), the concentrations of sucrose in midguts were significantly higher than caterpillars exposed to control diet and diets containing low concentration of L-arabinose (Scheffé's Tests: $p < 0.0001$; Fig. 8B).

Glucose levels in midguts of caterpillars exposed to artificial diet containing 66.6 mM L-arabinose decreased, but not significantly (Scheffé's Tests: p = 0.688), whereas midgut glucose concentrations of caterpillars reared on artificial diets containing 199.8 mM, 333 mM, or 500 mM L-arabinose decreased significantly (Scheffé's Tests: $p < 0.01$; Fig. 8C). These results suggest consumption of L-arabinose results in the inhibition of sucrase activity, resulting in accumulation of midgut sucrose, reductions in midgut glucose, and reduced sucrose digestion efficiency.

Kinetic analysis of sucrase inhibition by L-arabinose

Similar to the action of L-arabinose on mammalian intestinal sucrase (Krog-Mikkelsen et al. , 2011; Seri et al. , 1996), we also found that L-arabinose inhibits *H. armigera* intestinal sucrase in an uncompetitive manner *in vitro*. Lineweaver-Burk analysis yielded parallel lines for the different concentrations of L-arabinose (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). L-arabinose at concentrations of 6.66 and 9.99 mM resulted in 12.24% and 26.81% inhibition of sucrase activity, respectively, at Vmax with an apparent K_i of 30.66 mM.

We also tested a known alpha-glucosidase inhibitor, acarbose, against caterpillar midgut sucrase and observed competitive inhibition of sucrase activities. 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 increasing inhibitor concentrations (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 (Kotake et al. ,

2016). Although it has been recognized to have selective inhibitory effects on intestinal sucrase activity in mammals (Krog-Mikkelsen et al. , 2011; Sanai et al. , 1997), no findings have been reported regarding the effects on digestive enzymes of insect species, especially the herbivorous insects. In our present study, we show that L-arabinose not only inhibits the feeding preferences and gustatory responses of *H. armigera* to host plants and other sugars, but also inhibits intestinal sucrase activities. The inhibiting effects of L-arabinose also appear to be reflected by increased larval development times. Collectively, these data indicate that peripheral gustatory sensitivity, feeding behaviors, development, and the intestinal digestive sucrase activities are inhibited by L-arabinose.

To our knowledge, there are no prior reports of L-arabinose, a sugar that tastes sweet to mammals, functioning as both a peripheral taste sensitivity inhibitor and feeding behavior inhibitor in other animals. As a non-nutritional sugar, L-arabinose is still a sweetener to *Drosophila* that activates proboscis extension responses similar to that observed with other non-caloric sweeteners, such as L-glucose (Burke et al. , 2012; Huetteroth et al. , 2015; Murata et al. , 2017; Musso et al. , 2017). Ingestion of L-arabinose or L-glucose could lead to caloric frustration memory by devaluing these sugars (Musso et al. , 2017) through a post-ingestion process (Musso et al. , 2017; Musso et al. , 2015). Our results indicate that L-arabinose does not activate peripheral responses of the GRNs in maxillary sensilla of *H. armigera* caterpillars, but inhibits the activities of peripheral GRNs sensitive to other

substances, suggesting that peripheral coding in *H. armigera* caterpillars for L-arabinose is different from that in *Drosophila*. Considering the inhibition on feeding preferences for host plant leaf and phagostimulants, this indicates L-arabinose is not a sweetener for caterpillars of *H. armigera*. 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 eusocial insect species, the termite *Reticulitermes chinensis* (Isoptera: Rhinotermitidae) (unpub.).

In both mammals and insects, most sugars and bitters can be detected by corresponding gustatory receptors in GRNs, the sensitivities of which contribute to the decision of food selection (Jiang et al. , 2015; Matsunami et al. , 2000; Nelson et al. , 2001; Shim et al. , 2015; Thorne et al. , 2004). Our data show no specific L-arabinose-sensitive GRNs were found in the maxillary sensillum of *H. armigera* caterpillars, but the activities of GRNs to other substances, including GRNs sensitive sucrose, host leaf saps, and even the deterrent sinigrin, could be inhibited when combined with L-arabinose, suggesting the inhibition effect of L-arabinose is non-specific and not due to L-arabinose-sensitive *GRN*s in the maxillary sensilla. In contrast to findings in *Drosophila* where preference for L-arabinose is related to the presence of sweet gustatory receptor (McGinnis et al. , 2016; Miyamoto et al. , 2013), our electrophysiological and behavioral data suggest that L-arabinose is not a

sweetener to *H. armigera* caterpillars. Activity of the deterrent sinigrin on sinigrin-sensitive GRNs was also inhibited when mixed with L-arabinose; therefore, we posit that L-arabinose could activate neither sweet *GRN*s nor bitter *GRN*s in the maxillary sensilla. Rather, L-arabinose likely inhibits the activities of GRNs in the maxillary sensilla by a special process. While the exact inhibition mechanism remains unclear, it seems to be a form of indirect inhibition of the peripheral coding in *H. armigera* caterpillars. Since non-L-arabinose sensitive GRNs were found in the maxillary sensillum, the inhibition effects exclude the narrow sense of activity integration between GRNs sensitive to bitters and sugars (Bernays and Chapman, 2000, 2001; Cocco and Glendinning, 2012; Delventhal and Carlson, 2016). In *Drosophila* (Meunier et al. , 2003) and the honeybee, *Apis mellifera* (de Brito Sanchez et al. , 2014), bitter substances could also be detected by peripheral coding through bitter GRN activation. We hypothesize that the inhibition process may be related to one or more of these mechanisms, such as membrane depolarization (Kumazawa et al. , 1986; Oami, 1998), the G-proteins (Lindemann, 2001; Naim et al. , 1994), GPCR kinases (Varkevisser and Kinnamon, 2000; Zubare-Samuelov et al. , 2005), transduction pathway activation (Kim et al. , 2010; Ozeck et al. , 2004), binding of extracellular proteins present in the endolymph (Jeong et al. , 2013), or neurotransmitter mediation (Chu et al. , 2014; Wang and Bradley, 1993).

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 through which insects remember that sweet-tasting sugars are not nutritious (Behmer et al. , 1999; Burke et al. , 2012; Musso et al. , 2017; Musso et al. , 2015; Wright et al. , 2010). Our experiments did not appear to involve a learning process of *H. armigera* caterpillars. However, during the feeding process, some *H. armigera* caterpillars vomited after consumption of the L-arabinose-treated plant leaves. This suggests 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 30 min to 6 h, we surmise that post-ingestive feedback may also play a prominent role in governing *H. armigera* feeding behaviors.

It is natural that *H. armigera* caterpillars perceive non-nutritional L-arabinose as a negative compound for at least two possible reasons. First, our investigations *in vitro* 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 time is prolonged through all instar stages on artificial diets containing 5% L-arabinose. Such impact is similar to that

of the nicotine and caffeine on the development of other herbivorous insect caterpillars (Glendinning, 2002; Usher et al. , 2011). Furthermore, we found that almost all of the neonate caterpillars died after 3% L-arabinose was added into the artificial diet with a low content of sucrose in our preliminary experiments, but most caterpillars survived if exposed to the same artificial diet without L-arabinose, suggesting that L-arabinose has a fatal impact on caterpillars if the diet is short of caloric sugars. Unlike the peripheral coding of mammals (Burke and Waddell, 2011; Dahanukar et al. , 2007; Marella et al. , 2006; Moskowitz, 1971; Yamamoto, 2013) and *Drosophila*, which regard the non-nutritive pentoses as a sweetener (Musso et al. , 2017), our results indicate that *H. armigera* caterpillars could perceive this NAS as a non-sweetener both from peripheral coding and central brain activity.

Insects have stood out as one of the most remarkable lineages in the 3.5-billion-year history of life (Engel, 2015), which is a much longer history than mammals. We suppose that some insects have evolved better mechanisms of adaptation than mammals to the toxin-like L-arabinose sugar. Studies of insect feeding adaptation 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 phytotoxic effects of L-arabinose on caterpillars for the purposes of pest control should be addressed in future studies.

Methods

Insects

H. armigera were from our established laboratory colony, which were reared on an standard artificial diet (Jiang et al. , 2010) prepared from the following ingredients: wheat bran (150 g) (Jinyuan Flour Induster Co., Ltd, Zhengzhou, China), soybean powder (80 g) (Quanruixing Foods Co., Ltd, Wuzhi County, China), yeast powder (25 g), casein (40 g) and sorbic 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 controlled conditions by photoperiod (L16:D8), temperature (27 ± 1°C) and 75% relative humidity. When we investigated the effects of L-arabinose on the development and the intestinal physiology of *H. armigera* caterpillars, L-arabinose (Sigma-Aldrich) was added into the standard artificial diet as described above.

Feeding choice assay

The dual-choice plant leaf disc bioassay was used to test the feeding preference of *H. armigera* caterpillars that were exposed 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) or 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 treatment 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 mM D-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 were treated by two different solutions (four of each). 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 among 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. 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 mM KCl (Beichen Fangzhen 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 (Tang et al. , 2014).

The analysis of electrophysiological responses of caterpillar sensilla styloconica to different tastants is similar to that 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 exposure to L-arabinose

Neonate caterpillars were exposed to standard artificial diets for *H. armigera* caterpillars (Jiang et al. , 2010) amended with L-arabinose (0.1%, 1%, 3%, 5%, or 7.5%) until the final

caterpillar instar. The developmental duration and the mortality of caterpillars were recorded. Caterpillars were maintained through pupation to adult emergence, and pupal weights and emergence rates were recorded.

Digestive physiology

Neonate caterpillars were exposed to standard artificial diets with or without (control group) different concentrations of L-arabinose (0.1%, 1%, 3%, 5%, or 7.5%) 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).

Kinetic analysis of sucrase inhibition by L-arabinose

Midguts of the fifth instar caterpillars of *H. armigera* were obtained 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 from 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 (7.35 μM or 11.79 μM; Sigma-Aldrich). Selected concentrations of these sugars were determined based on preliminary experiments.

The standard reaction assay volume was 90 μL. After the reaction mixtures were incubated at 37°C for 5 min, reactions were terminated by incubation 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 frequency in 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 ($p < 0.05$).

Developmental parameters and midgut enzyme activities were log-transformed for uneven sample sizes. Means were compared by one-way ANOVA followed by a Scheffé Test (threshold for $p < 0.05$). All statistical analyses were conducted using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA).

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Figure legends

Fig. 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 ($p < 0.05$).

Fig. 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 by different concentrations of L-arabinose (LA) and water-treated leaves (control). **B:** Preference between water-treated pepper leaves (control) and pepper leaves treated by mixtures of 200 mM 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 ($p < 0.05$).

Fig. 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 a galea. **C:** Responding curves of the lateral sensillum and the medial sensillum to series concentrations of L-arabinose in 2.0 mM KCl.

Fig. 4 Representative traces and effects 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 ($p < 0.05$).

Fig. 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 ($p < 0.05$).

Fig. 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 ($p < 0.05$).

Fig. 7 Effects of L-arabinose on the development of *H. armigera*. Comparison of larval developmental times (**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 duration times (**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 ($p < 0.05$).

Fig. 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 ($p < 0.05$).

Fig. 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

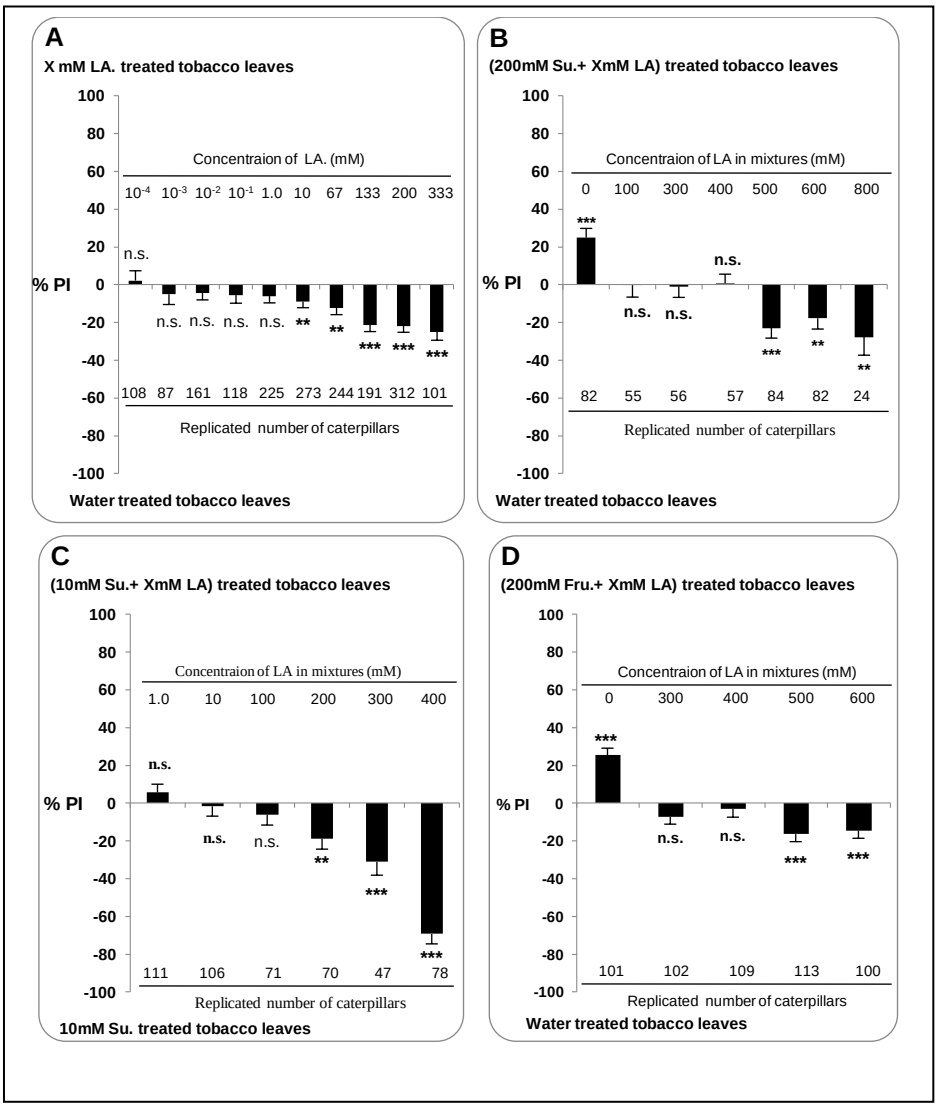


Figure 2

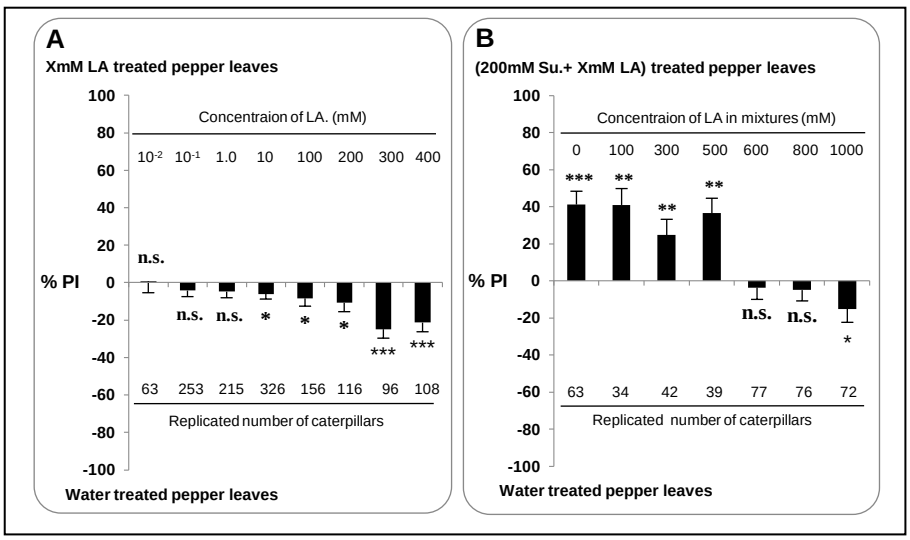

A
XmM LA treated pepper leaves
Concentraion of LA. (mM)
10-2 10-1 1.0 10 100 200 300 400
% PI
100 80 60 40 20 0 -20 -40 -60 -80 -100
n.s. n.s. n.s. * * * *** ***
63 253 215 326 156 116 96 108
Replicated number of caterpillars
Water treated pepper leaves
B
(200mM Su.+ XmM LA) treated pepper leaves
Concentraion of LA in mixtures (mM)
0 100 300 500 600 800 1000
% PI
100 80 60 40 20 0 -20 -40 -60 -80 -100
*** ** ** ** n.s. n.s. *
63 34 42 39 77 76 72
Replicated number of caterpillars
Water treated pepper leaves

Figure 3

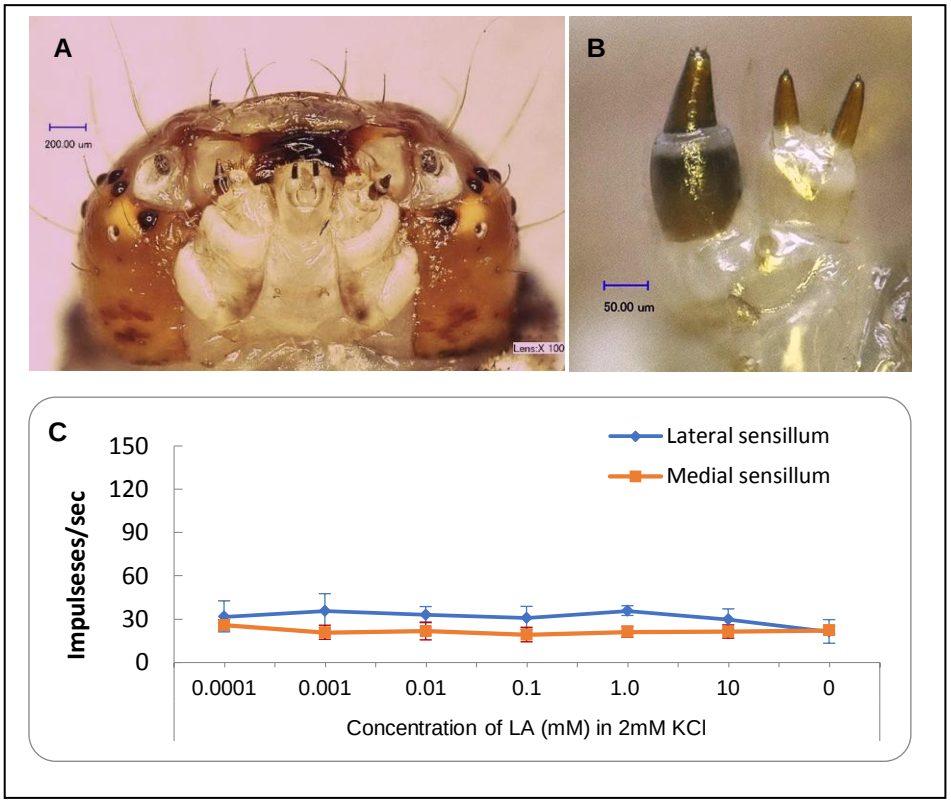

A
200.00 um
Lens:X 100
B
50.00 um
C
Impulseses/sec
150
120
90
60
30
0
Lateral sensillum
Medial sensillum
0.0001
0.001
0.01
0.1
1.0
10
0
Concentration of LA (mM) in 2mM KCl

Figure 4

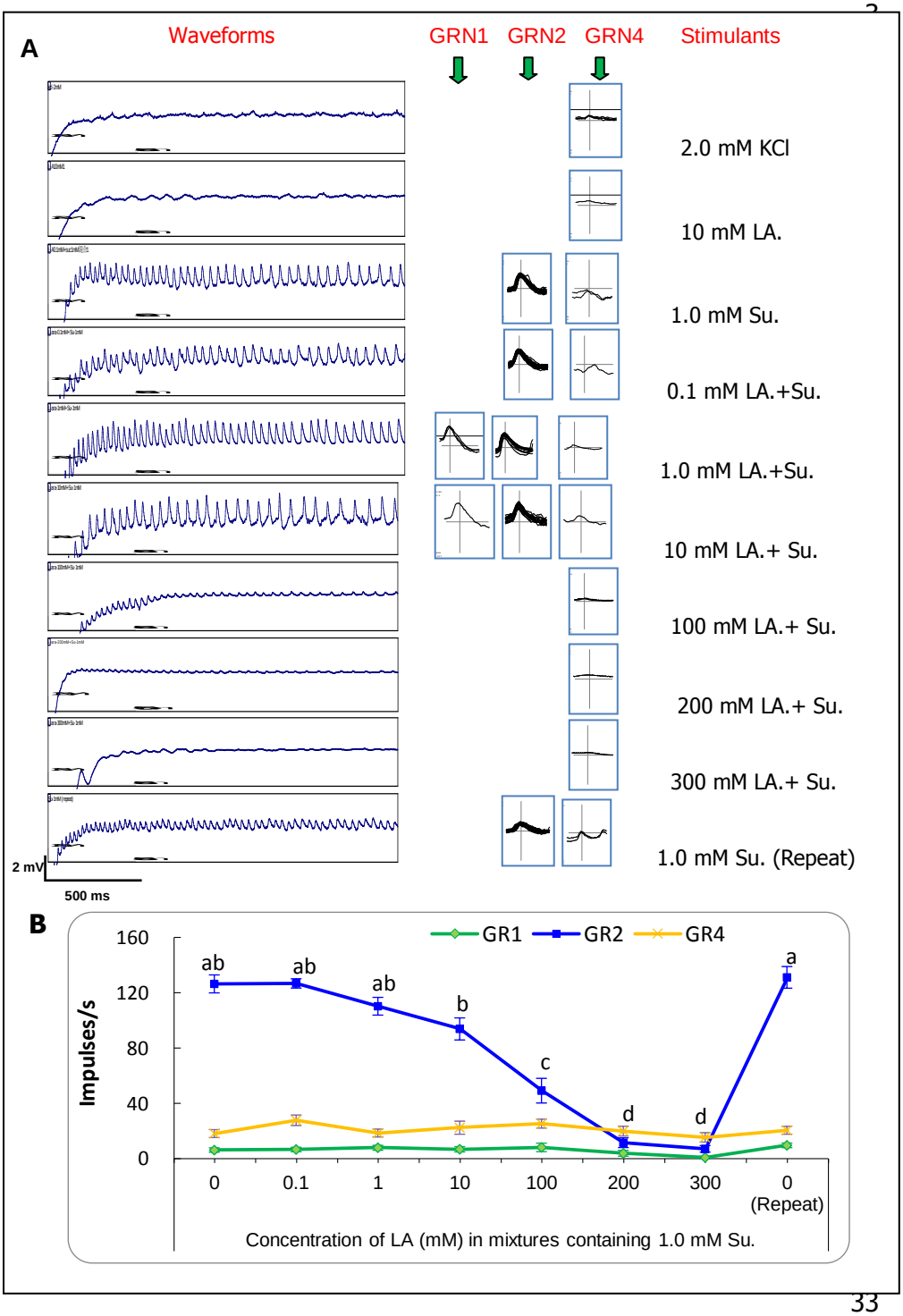

A
Waveforms
GRN1
GRN2
GRN4
Stimulants
2.0 mM KCl
10 mM LA.
1.0 mM Su.
0.1 mM LA.+Su.
1.0 mM LA.+Su.
10 mM LA.+ Su.
100 mM LA.+ Su.
200 mM LA.+ Su.
300 mM LA.+ Su.
1.0 mM Su. (Repeat)
2 mV
500 ms
B
GR1
GR2
GR4
Impulses/s
160
120
80
40
0
ab
ab
ab
b
c
d
d
a
0
0.1
1
10
100
200
300
0
(Repeat)
Concentration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

Figure 5

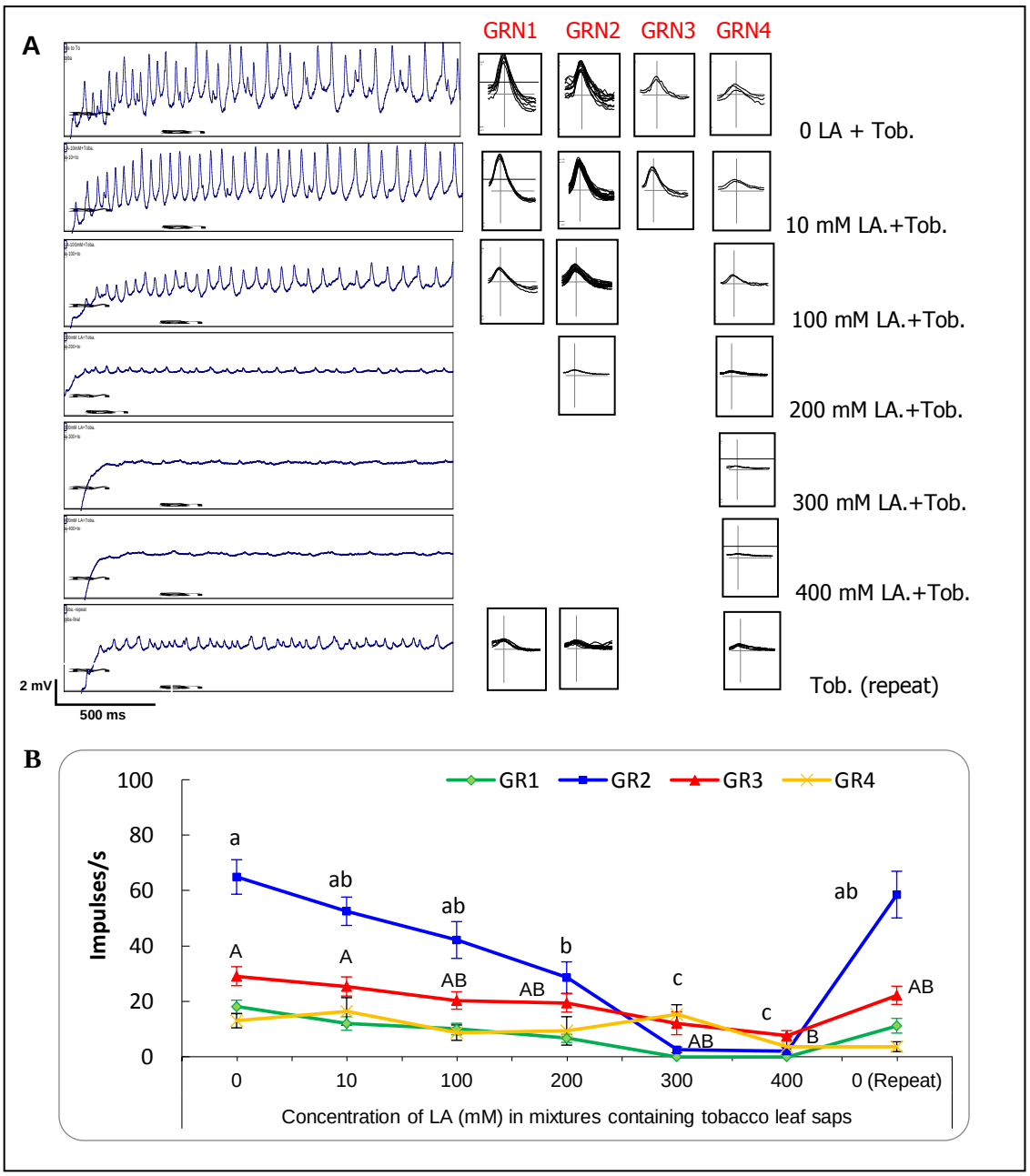

A
GRN1
GRN2
GRN3
GRN4
0 LA + Tob.
10 mM LA.+Tob.
100 mM LA.+Tob.
200 mM LA.+Tob.
300 mM LA.+Tob.
400 mM LA.+Tob.
Tob. (repeat)
2 mV
500 ms
B
GR1
GR2
GR3
GR4
Impulses/s
100
80
60
40
20
0
a
ab
ab
b
c
c
ab
A
A
AB
AB
AB
B
AB
0
10
100
200
300
400
0 (Repeat)
Concentration of LA (mM) in mixtures containing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

Figure 6

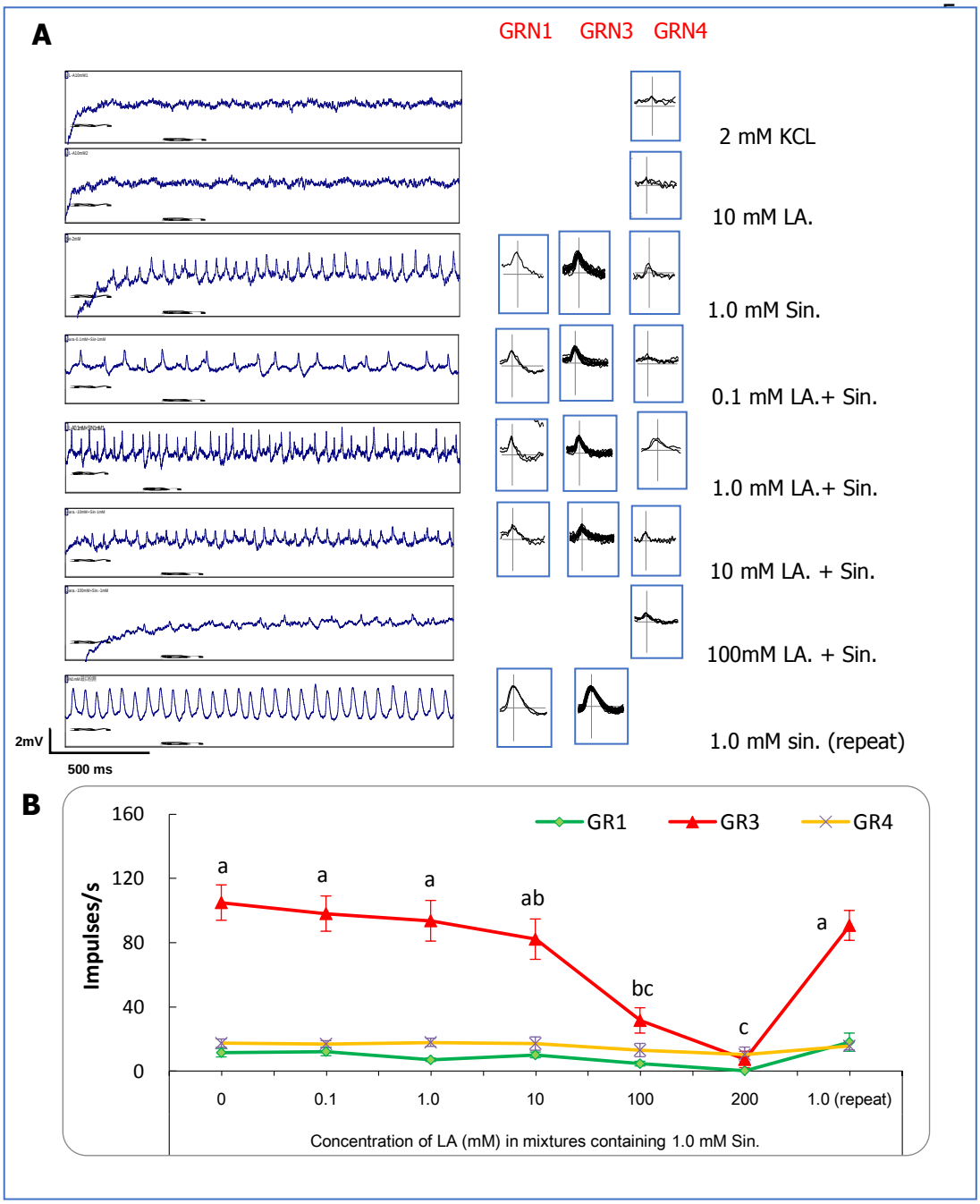

A
GRN1
GRN3
GRN4
2 mM KCL
10 mM LA.
1.0 mM Sin.
0.1 mM LA.+ Sin.
1.0 mM LA.+ Sin.
10 mM LA. + Sin.
100mM LA. + Sin.
1.0 mM sin. (repeat)
2mV
500 ms
B
GR1
GR3
GR4
Impulses/s
160
120
80
40
0
a
a
a
ab
bc
c
a
0
0.1
1.0
10
100
200
1.0 (repeat)
Concentration of LA (mM) in mixtures containing 1.0 mM Sin.

Figure 7

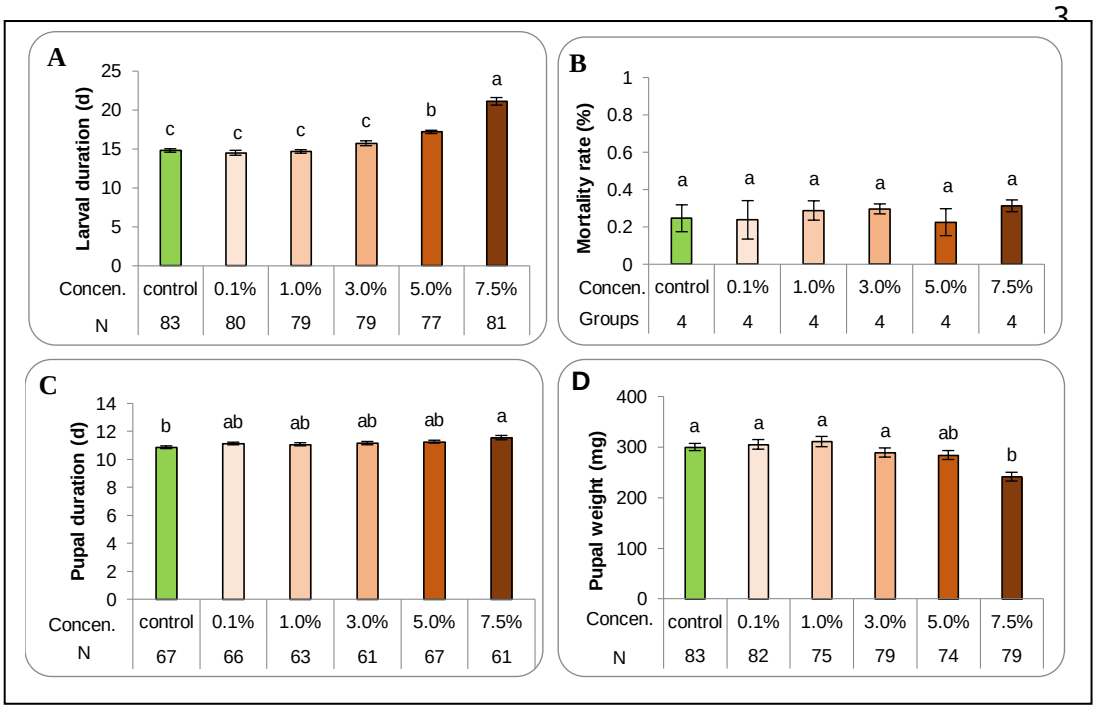

A
Larval duration (d)
25
20
15
10
5
0
c
c
c
c
b
a
Concen.
control
0.1%
1.0%
3.0%
5.0%
7.5%
N
83
80
79
79
77
81
B
Mortality rate (%)
1
0.8
0.6
0.4
0.2
0
a
a
a
a
a
a
Concen.
control
0.1%
1.0%
3.0%
5.0%
7.5%
Groups
4
4
4
4
4
4
C
Pupal duration (d)
14
12
10
8
6
4
2
0
b
ab
ab
ab
ab
a
Concen.
control
0.1%
1.0%
3.0%
5.0%
7.5%
N
67
66
63
61
67
61
D
Pupal weight (mg)
400
300
200
100
0
a
a
a
a
ab
b
Concen.
control
0.1%
1.0%
3.0%
5.0%
7.5%
N
83
82
75
79
74
79

Figure 8

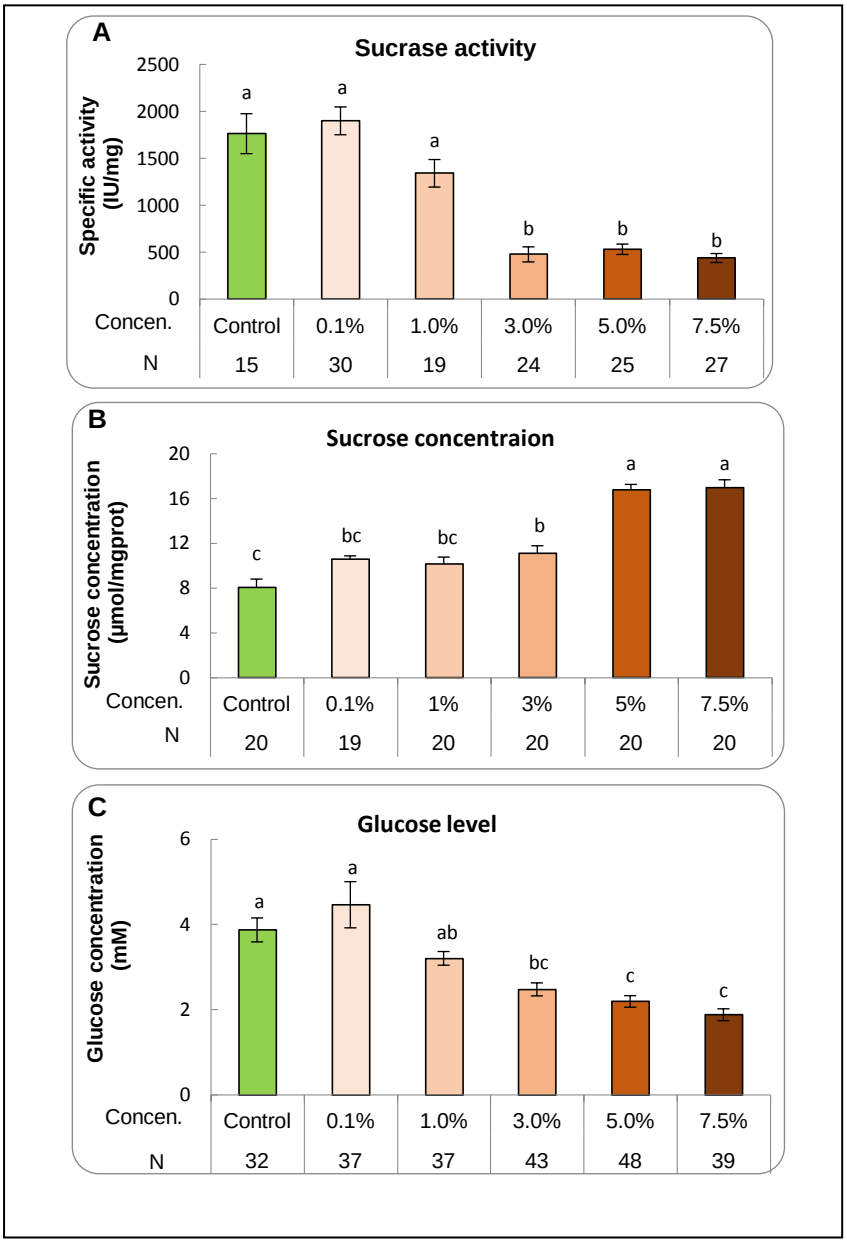
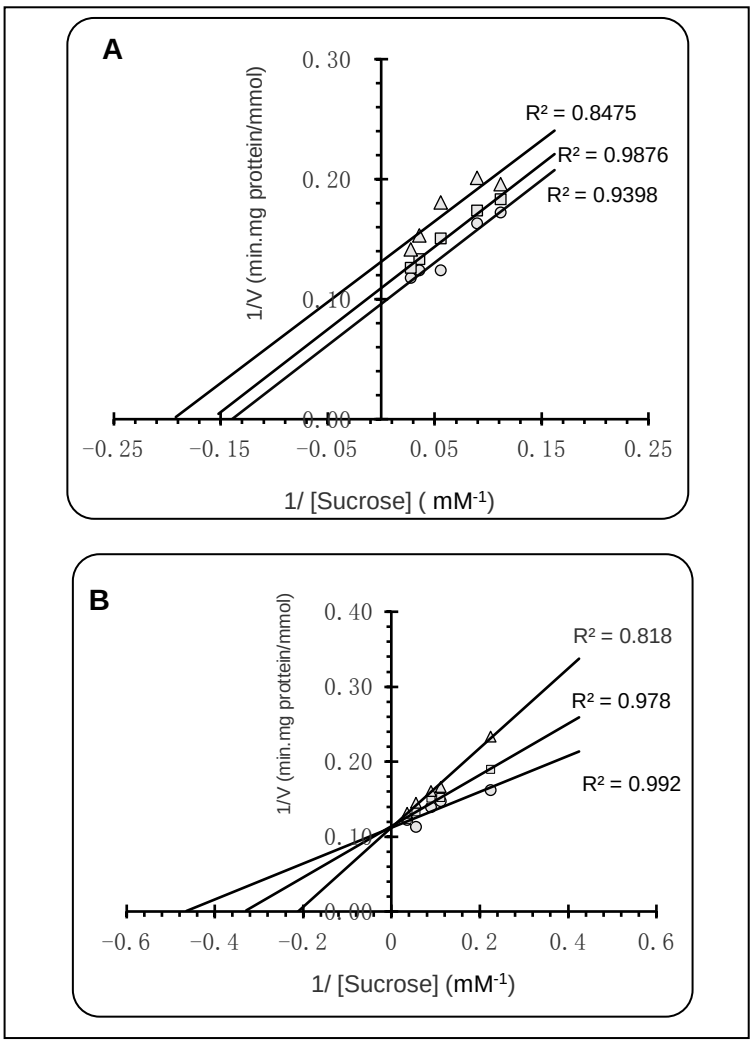

A
Sucrase activity
Specific activity (IU/mg)
2500
2000
1500
1000
500
0
a
a
a
b
b
b
Concen.
Control
0.1%
1.0%
3.0%
5.0%
7.5%
N
15
30
19
24
25
27
B
Sucrose concentraion
Sucrose concentration (µmol/mgprot)
20
16
12
8
4
0
c
bc
bc
b
a
a
Concen.
Control
0.1%
1%
3%
5%
7.5%
N
20
19
20
20
20
20
C
Glucose level
Glucose concentration (mM)
6
4
2
0
a
a
ab
bc
c
c
Concen.
Control
0.1%
1.0%
3.0%
5.0%
7.5%
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 = 0.8475
R² = 0.9876
R² = 0.9398
−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)