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ORIGINAL ARTICLE

Pharmacological analysis of the feeding response of codling moth (*Cydia pomonella*; Lepidoptera: Tortricidae) neonates to bitter compounds

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Abstract. Feeding in codling moth neonate caterpillars was inhibited by 0.67 mM and 2.24 mM concentrations of denatonium benzoate. This inhibitory effect was abolished by phospholipase C inhibitor, U-73122 and the phosphodiesterase inhibitor, Rolipram. Quinine and quinidine did not have inhibitory effects at concentrations as high as 1.64 mM and 0.43 mM, respectively. The inhibitory effect of denatonium was partially reversed in the presence of the calcium ion chelator, EGTA, at concentrations ranging from 2.5 μM to 250 μM. These results indicate that transduction of the taste of denatonium in codling moth neonates relies on signalling pathways that involve phospholipase C, phosphodiesterase and calcium ion influx into cells.

INTRODUCTION

A lepidopteran, the codling moth (*Cydia pomonella* L.) is a major, cosmopolitan pest of apples. Neonate larvae of this insect burrow into the fruit and feed inside until their development is complete. Codling moth neonates are currently controlled by an application of insecticide shortly after egg hatch. An alternative strategy proposes using botanical feeding deterrents to reduce fruit infestation by codling moth neonates. Some of these deterrents are present in the foliage of plants that taste bitter to humans; *Ginkgo biloba* (Pszczolkowski et al., 2011), *Artemisia absinthium* (Durden et al., 2009), *Artemisia annua* (Durden et al., 2011) and *Artemisia arborescens* "Powis castle" (Creed et al., 2015). These findings indicate that codling moth neonates can sense bitter chemicals. More basic research on this topic is needed.

Both the molecular and cellular basis of the bitter taste in insects have been extensively studied in *Drosophila melanogaster*, in which bitter substances are mostly sensed by 68 receptors (heptahelical transmembrane proteins) (Clyne et al., 2000; Dunipace et al., 2001; Scott et al., 2001). Although, originally, these receptors were anticipated to function as G-protein coupled receptors, it is still not clear whether they signal through G-protein-dependent second messenger cascades or operate as ligand-gated ion channels (Yarmolinsky et al., 2009; Apostolopoulou et al., 2014; Liman et al., 2014; Choi et al., 2016). Less is known about bitter-taste perception by lepidopterous larvae. Most caterpillars have eight pairs of taste sensillae and in each sensillum there is one cell capable of detecting deterrents (Bernays & Chapman, 1994). In caterpillars of *Manduca sexta*, these cells respond to substances that are perceived as bitter by humans: aristolochic acid, caffeine and salicin (Glendinning et al., 2006) and as a consequence, caterpillars reduce their feeding activity (Glendinning et al., 1999). Similar results are reported for caterpillars of *Helicoverpa armigera* and *Bombyx mori* (Zhang et al., 2013). Not much is known about the signal transduction involved in perceiving bitter substances by caterpillars, but it is postulated that *Manduca sexta* larvae have more than one transduction and one signalling pathway for perceiving bitter chemicals (Glendinning & Hills, 1997; Glendinning et al., 2002).

Basic research on taste perception by codling moth neonates was done in the past. Two taste modalities were examined using behavioural assays: umami and sweet. Monosodium glutamate (MSG) increases food consumption by codling moth neonates (Pszczolkowski et al., 2002b) and it is likely that two classes of glutamate receptors are involved in this process (Pszczolkowski et al., 2003, 2005). Saccharin hemicalcium also increases food consumption by codling moth neonates (Pszczolkowski & Brown, 2003) and it is likely that phospholipase C has a role in the perception of this compound (Pszczolkowski et al., 2009). There are no studies on the effects of bitter compounds on feeding by the neonate larvae of codling moth.

Although insect taste receptors are often studied using electrophysiological techniques (Hodgson et al., 1955), the small size (2 mm in length, 60 µg of weight) of the codling moth neonate precludes this approach. Behavioral studies, however, have been shown to be effective in understanding the chemoreceptive capabilities of codling moth larvae. Because codling moth larvae can feed on apple foliage (Pszczolkowski et al., 2002a) such assays can be used for investigating effects of various substances (including signal transduction modulators or calcium ion chelators) on codling moth neonate feeding and delineating possible pathways of taste signal transduction (Pszczolkowski et al., 2002b, 2005, 2009). Briefly, in these assays, neonate larvae are individually exposed to residues of tested substances on apple foliage and feeding commencement time is recorded for each neonate. In the present paper, the effects of three substances that taste bitter to humans on codling moth neonate feeding are recorded. Quinine, quinidine and denatonium benzoate were tested alone or in combination with signal transduction modulators or the calcium ion chelator, EGTA. Time to feeding commencement was used to evaluate the effects of the aforementioned chemicals on feeding by codling moth neonates.

MATERIALS AND METHODS

Insects and feeding substrate

Codling moths, C*ydia pomonella* (L.), originating from Yakima, WA, were reared at 25°C, 70–80% RH, under a 16L : 8D photoperiod. The moths were given saturated sucrose solution as food and wax paper as an oviposition surface. Neonates were collected 0.5–1.0 h posthatch and immediately tested on apple leaves of the Honeycrisp variety, provided by Willow Green Gardens & Tree Farm, Rogersville, MO, USA. New foliage (up to three days old) was used in all assays.

Chemicals

Quinine, quinidine, denatonium benzoate, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and Triton X-100 were obtained from Sigma (St Louis, MO, USA). Phospholipase C inhibitor, 1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1H-pyrrole-2,5-dione (U-73122) and phosphodiesterase-4 inhibitor, (*R*,*S*)-4-(3-cyclopentyloxy-4-methoxy-phenyl)pyrrolidin-2-one (Rolipram) were obtained from Tocris Cookson (Ballwin, MO, USA). All chemicals were dissolved in double-distilled water, containing 0.02% Triton X-100.

Preparation of feeding bioassay stations

Discs of uniform size were removed from the midrib area of apple leaves using a 12-mm diameter punch. Test solutions (10 µl) were applied to the upper surface of disks, distributed evenly, and the disks allowed to air dry. Afterwards, the lower surfaces were treated with an additional 10 µl of the test solution and the drying procedure repeated. Then, feeding stations were made by mounting each circular section on a glass microscope slide, in a sandwich configuration, between 400 Crepe Liner Double-coated Tape (3M Industrial Tape and Specialties Division, St. Paul, MN, USA) and self-adhesive binder reinforcement labels (05721 Avery Dennison Office Products, Brea, CA, USA) with a circular opening of 6 mm. A single neonate larva was placed in each station, containing one section of leaf, covered by a glass coverslip and observed to record feeding activity. To prevent dehydration, the microscope slides were kept in Petri dishes with wet filter paper placed on the bottom of each dish. Additional details of this procedure are given elsewhere (Pszczolkowski & Brown, 2002).

Experimental design

To examine the effects of bitter substances on feeding commencement, codling moth larvae were individually exposed in feeding bioassay stations to various concentrations of bitter substances, or to the solvent alone. Twenty larvae were individually exposed to each concentration of quinine, quinidine or denatonium benzoate, and 20 control larvae only to aqueous 0.02% Triton X-100. Effects of quinine were tested at concentrations ranging from 0.003 mM to 1.64 mM, those of quinidine from 0.003 mM to 0.43 mM and those of denatonium from 0.002 mM to 2.24 mM. The larvae were monitored for 3 h at 15 min intervals. This procedure was repeated three times. The results were expressed as the average time (mean ± S.E.M.) to feeding commencement.

Preliminary experiments indicated that U-73122 and Rolipram reversed the inhibitory effects of 2 mM denatonium. Therefore, we used this concentration in further experiments in which we tested whether the addition of phospholipase C inhibitor, (U-73122), phosphodiesterase-4 inhibitor (Rolipram) or calcium ion chelator, EGTA, affected the influence of denatonium on commencement of leaf consumption. U-73122 was tested at concentrations of 0.000215, 0.00215, 0.0215, 0.215 and 2.15 mM. Rolipram was tested at concentrations of 0.000363, 0.00363, 0.0363, 0.363 and 3.63 mM. EGTA was tested at concentrations of 2.5, 25 and 250 µM. EGTA, U-73122 and Rolipram were mixed with denatonium dissolved in aqueous 0.02% Triton X-100. Control larvae were exposed to respective signal transduction modulators or EGTA alone, or to 0.02% Triton X-100. Each solution was tested on 60 larvae. The results were expressed as the average time (mean ± S.E.M.) to feeding commencement.

Statistics

All data sets were analyzed with GraphPad InStat, (GraphPad Software, San Diego, CA, USA). All data sets passed tests for normality with $P < 0.05$. Therefore, mean times of feeding initiation ± S.E.M. were compared among control and experimental groups using ANOVA followed by Bonferroni comparison.

RESULTS

Effects of bitter substances on feeding commencement by codling moth neonates

Only denatonium benzoate altered the time of feeding commencement in comparison to controls (Fig. 1). Larvae started to feed 40–50 min later in the presence of 0.67 mM and 2.24 mM denatonium ($P < 0.05$, ANOVA, Fig. 1). We did observe a slight delay (about 20 min) in feeding commencement on foliage treated with 1.64 mM quinine (saturated solution) but this delay was not statistically significant ($P > 0.05$, ANOVA, Fig. 1). Quinidine did not have any effects on feeding even at a concentration as high as 0.43 mM (saturated solution).

Effects of signal transduction modulators on feeding commencement delayed by denatonium benzoate

In response to denatonium benzoate alone, the neonates started to feed about 130 min after the initial exposure to this chemical. The addition of the phospholipase C inhibitor, U-73122, abolished feeding inhibitory effects of denatonium and reduced feeding commencement time to

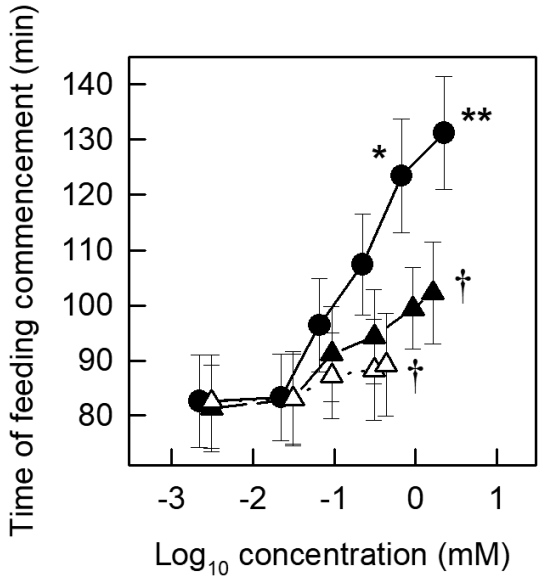

Fig. 1. Effects of substances bitter to humans on the time to the commencement of feeding by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves treated with either quinine (solid triangles), quinidine (open triangles) or denatonium (solid circles). Daggers (†) indicate that the highest concentration of quinine and quinidine shown on the graph that corresponds to saturated solutions. The average time of commencement of the feeding on leaves by the controls (0.02% aqueous Triton X-100 only) was 84.3 ± 7.2 min. Each datum point shows mean ± S.E.M. for 58–60 larvae. Asterisks indicate averages significantly different than those at lower concentrations of substances tested or those of the controls (**P* < 0.05 and ***P* < 0.01, ANOVA).

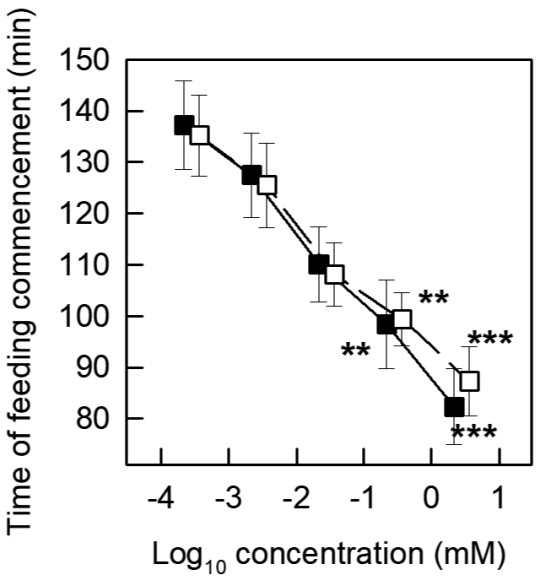

Fig. 2. Effects of signal transduction modulators on time to the commencement of feeding by codling moth neonate larvae inhibited with 2 mM denatonium. Newly hatched neonates were allowed to feed on apple leaves concurrently treated with denatonium and either phospholipase C inhibitor, U-73122 (solid squares) or phosphodiesterase inhibitor, Rolipram (open squares). The average time to the commencement of the feeding by larvae on leaves treated with denatonium was only 139.2 ± 10.3 min. Each datum point is the mean ± S.E.M. for 57–60 larvae. Asterisks indicate averages significantly different than those recorded at lower concentrations of the substances tested or the controls (***P* < 0.01 and ****P* < 0.001, in ANOVA).

the levels of the solvent-treated control, which averaged at 84.3 ± 7.2 min. The average time for commencement decreased with increasing concentrations of U-73122 with a threshold of 0.215 mM (Fig. 2, $P < 0.01$ or better, ANOVA). Here, the time of feeding commencement was reduced to 98.4 ± 8.6 min. The maximum inhibitory response to U-73122 (reduction of feeding commencement time to 82.3 ± 7.4 min) was recorded at 2.15 mM ($P < 0.001$, ANOVA). Similar results were obtained if the phosphodiesterase inhibitor, Rolipram, was combined with denatonium. Here, the threshold was 0.363 mM ($P < 0.01$, ANOVA) and corresponded to a reduction in feeding commencement time to 99.4 ± 5.24 min. The maximum inhibitory response (reduction of feeding commencement time to 87.3 ± 6.72 min) was recorded at 3.63 mM ($P < 0.001$, ANOVA).

Neither U-73122 nor Rolipram alone affected feeding by codling moth neonates. Times of feeding commencement ranged from 83.31 ± 3.32 min to 87.45 ± 2.29 min for U-73122 and from 81.67 ± 3.52 min to 85.81 ± 2.73 min for Rolipram ($P > 0.05$, ANOVA). These results were similar to the time of feeding commencement of control neonates exposed to solvent only ($P > 0.05$, ANOVA).

Table 1. Effects of concurrent exposure to EGTA and denatonium benzoate on the time to the commencement of feeding (min) by codling moth neonates. *N* = 59–60 for each data point (mean ± S.E.M.).

EGTA concentration (μM)	Concurrent treatment	
	None	2 mM denatonium
0	78.32 ± 9.32	137.24 ± 9.12
2.5	75.62 ± 9.78	102.70 ± 10.11*
25	80.01 ± 10.14	100.34 ± 8.29*
250	74.34 ± 9.21	99.92 ± 7.46*

* $P < 0.05$ (ANOVA comparison of the values in column 3).

Effects of calcium ion chelator on feeding commencement delayed by denatonium benzoate

Interestingly, the delaying effects of denatonium on feeding commencement were partially reversed by exposure to 2.5, 25 or 250 μM EGTA ($P < 0.05$, ANOVA, Table 1). EGTA alone, at the concentrations tested, did not influence feeding commencement ($P > 0.05$, ANOVA, Table 1).

DISCUSSION

Insect feeding behaviour in the presence of bitter substances

There are studies on the effects of quinine, quinidine and denatonium on the feeding behaviour of several species of insects. The following paragraphs provide a short review of the existing literature.

The gustatory nerves of adults of *Drosophila melanogaster* can detect quinine at a concentration of 1 mM (Moon et al., 2009) and the same concentration inhibits intake of 35 mM fructose solutions by *Drosophila* adults in choice and no-choice assays (Sellier et al., 2011). At a concentration of 0.2 mM, quinine is tolerated by *Drosophila* adults, which exhibit the proboscis extension reflex to three subsequent exposures (Masek & Scott, 2010). In the blow-

fly, *Photophormia terranova*, 0.1 mM quinine abolishes the feeding stimulatory effects of sucrose (Liscia & Solari, 2000). Sensillae of Colorado potato beetles, *Leptinotarsa decemlineata*, detect 1 mM quinine in a 10 mM sucrose solution (Mitchell, 1987). Honeybee adults are slightly more sensitive to quinine: sensillae of this species detect 0.1 mM quinine in 15 mM sucrose (de Brito Sanchez et al., 2005) and the same concentration of quinine is sufficient for rejection of 1 mM sucrose solutions offered to the bees as food (Wright et al., 2010).

Less is known about the effects of quinidine on insect feeding. This compound inhibits sucrose stimulated feeding in the mosquito, *Anopheles gambiae*, at concentrations as low as 0.1 mM with dynamics similar to that of quinine (Kessler et al., 2013). Quinidine deters honeybees in feeding choice assays with an ED_{50} of 0.076 mM (Detzel & Wink, 1993).

Drosophila's sensillae detect denatonium at a concentration of 1 mM (Moon et al., 2009). In behavioural assays, 0.5 mM denatonium had no effect on adults of *Drosophila* (Masek & Scott, 2010) but 0.02 mM denatonium reduces feeding in potato aphids (Perera et al., 1995). On the other hand, in behavioural assays *Drosophila* adults perceive denatonium at concentrations of 0.02 mM and 0.05 mM as their sensillae are activated by these concentrations in electrophysiological experiments (Meunier et al., 2003).

Caterpillars seem to perceive bitter substances at concentrations similar to those perceived by adult insects. Larvae of *Trichoplusia ni* tolerate 0.8 mM quinine in an artificial diet: no effects on survival or development are recorded in long-term experiments (Carloye et al., 1998). More than 50% of the larvae of *Syntomis mogadorensis* are deterred from eating the foliage of host plants by 0.27 mM quinine (Wink et al., 1998). Ma (1969) reports that 0.01 mM quinine deters feeding induced by 0.1 M sucrose in larvae of *Pieris brassicae*.

A similar tendency was recorded during this study of the effects of quinidine and denatonium on the feeding of caterpillars. More than 50% of the larvae of *Syntomis mogadorensis* are deterred by 0.03 mM quinidine from feeding on the foliage of their host plant (Wink et al., 1998). In the same species, 0.03 mM quinidine reduces foliage consumption by 50% (Wink & Schneider, 1990). Denatonium at a concentration of 0.11 mM reduces the feeding of larvae of both *Plutella xylostella* and *Chrysodeixis eriosoma,* whereas concentrations of 0.011 mM and 0.0011 mM had no effect (Perera et al., 2000).

Summarizing, in most of the insects studied, quinine, quinidine and denatonium at concentrations as low as 0.1–0.5 mM inhibit feeding behaviour.

The current study indicates that codling moth neonates are more tolerant of bitter substances than those of other species; no deterrent or feeding inhibition were recorded at 1.64 mM quinine and 0.43 mM quinidine (in both cases saturated solutions). Denatonium had a deterrent effect at a concentration of 0.215 mM. The tolerance of bitter substances by codling moth neonates correlates with the fact that gravid females of this species are not discriminative in their selection of foliage as an oviposition substrate. The adults oviposit on foliage and the larvae feed on fruits or seeds of at least eight hosts, walnuts among them (Hagley et al., 1980; Curtis et al., 1990; Barnes, 1991). Foliage, husks and seeds of walnuts taste bitter to humans (Willis, 2007) and contain large amounts of juglone, a secondary metabolite that is a potent insect feeding inhibitor (Chapman, 1974). Codling moth larvae are, however, tolerant of juglone and have very effective digestive mechanisms for metabolizing juglone (Piskorski & Dorn, 2011). The finding that codling moth neonates tolerate higher concentrations of substances that taste bitter to humans (current paper) are consistent with the findings of Piskorski & Dorn (2011).

Effects of signal transduction modulators on feeding commencement delayed by denatonium benzoate

It is generally accepted that in most cases transduction of denatonium taste in vertebrates is mediated by G-protein-coupled receptors that activate the phospholipase C-signalling cascade, which liberates calcium ions from internal calcium ion stores and, consecutively, opens the membrane-located sodium ion channel, TRPM5 (Zhang et al., 2003; Liman et al., 2014). However, there is also evidence that in some vertebrate systems, denatonium taste may be transduced via a signalling pathway independent of the phospholipase C-signalling cascade (Ruiz-Avila et al., 1995). Here, a protein α-gustducin is activated by denatonium membrane receptors, stimulates phosphodiesterase to hydrolyze cyclic adenosine monophosphate (cAMP), and consequently causes a transient decrease in cAMP level, a process that liberates calcium ions from internal calcium ion stores (Gilbertson et al., 2000; Margolskee, 2002). In mice, there are two classes of taste cells: those in which denatonium signals via a transduction pathway dependent on a G-protein, and others that respond to denatonium via a G-protein-independent pathway (Sawano et al., 2005). The same taste cell in the mudpuppy, *Necturus maculosus*, responds to bitter substances using two different signalling pathways: PLC-dependent in response to denatonium and PLC-independent in response to dextromethorphan (Ogura & Kinnamon, 1999).

The bitter-taste related signal transduction pathways in insect cells are poorly characterized. As mentioned in the Introduction, it is not clear whether insect bitter-taste receptors signal through G-protein-dependent second messenger cascades or operate as ligand-gated ion channels (Yarmolinsky et al., 2009; Apostolopoulou et al., 2014; Liman et al., 2014; Choi et al., 2016). Noteworthy, the putative bitter-taste receptors of *Drosophila* flies share no sequence relationship with G-protein receptors. Instead, they are relatives of insect odorant receptors, which it is proposed function as ion channels (Liman et al., 2014). In *Drosophila*, at least one transient receptor potential channel is involved in the perception of the bitter compound, aristolochic acid (Kim et al., 2010). Several reports indicate that a similar transduction mechanism may be involved in

the perception of aristolochic acid by other species of insects (see Liman et al., 2014).

Phospholipase C inhibitor, U-73122, inhibits the signalling pathways for denatonium in vertebrate taste cells. This chemical prevents the taste cells of rats (Rössler et al., 1998), mudpuppies (Ogura & Kinnamon, 1999) and mice (Sawano et al., 2005; Hacker et al., 2008) from perceiving denatonium. Effective concentrations of U-73122 ranged from 1 μM to 10 μM.

The author is aware of only one report on the effects of U-73122 on the response of insect taste cells to bitter substances. It indicates that the responses of tarsal sensillae in the blowfly, *Phormia regina*, to quinine and strychnine are mediated by an inositol 1,4,5-trisphosphate (IP3)-dependent transduction cascade characteristic of G-protein-coupled receptors (Ouyang et al., 2009). In their experiments, 10 μM U-73122 was sufficient to inhibit the action potentials evoked by 1 mM quinine. In the experiments on the effects of U-73122 on codling moth neonates, concentrations higher than 10 μM were needed to reverse the effects of denatonium. It should be noted, however, that these experiments were in vivo experiments and are different from the in vitro experiments with *Phormia regina* and vertebrates discussed above. Drug concentrations effective in vivo are higher than those effective in vitro (Blaauboer, 2010).

The author is unaware of any reports on the effects of Rolipram on the perception of bitter substances by vertebrates or insects or any studies on the signal transduction pathways for denatonium in insects. Therefore, the results reported herein are novel.

The current study on codling moth neonates indicates that the addition of either phospholipase C inhibitor, U-73122, or phosphodiesterase inhibitor, Rolipram, abolished the inhibitory effects of denatonium on feeding and reduced feeding commencement time to the levels of the solvent-treated control. These findings indicate that two signal transduction pathways may be involved in the transduction of signals from denatonium: one PLC-dependent, perhaps signalling via liberation of calcium ion stores, and a PDE-dependent pathway, perhaps signalling via inhibition of cAMP. Interestingly, a similar dual signal transduction system underlying the response to denatonium is postulated by Gilbertson et al. (2000) for vertebrate cells. The suggestion that more than one transduction system and one signalling pathway are involved in the perception of bitter-tastes by codling moth larvae corroborates earlier work that postulate the same for the response of larvae of *Manduca sexta* to bitter compounds (Glendinning & Hills, 1997; Glendinning et al., 2002).

Effects of calcium ion chelator on feeding commencement delayed by denatonium benzoate

The addition of EGTA significantly reduced the inhibitory effects of denatonium on feeding by codling moth neonates, which indicates that external calcium ion stores are required for the perception of the bitter taste of denatonium. This finding corresponds well with earlier postulates that ligand-gated ion channels are needed for bitter-taste perception in insects (Yarmolinsky et al., 2009; Apostolopoulou et al., 2014; Liman et al., 2014; Choi et al., 2016).

However, treatment with EGTA did not completely reverse the inhibitory effects of denatonium on feeding. Concurrent exposure to EGTA had similar effects at concentrations of 2.5 μM, 25 μM, and 250 μM, and the inhibitory effect of denatonium was reversed by only about 25%. This finding indicates that the feeding inhibitory effects of denatonium are only partially dependent on external calcium ion influx and that alternative signalling pathways are needed for the full inhibitory effect of denatonium in codling moth neonates. Interestingly, the involvement of extracellular calcium ions in the perception of the taste of denatonium is confirmed for a vertebrate, *Necturus maculosus* (Ogura et al., 2002).

Concluding remarks

After hatching from eggs the neonate larvae of codling moths infest apples and stay inside the fruit until their development is complete. Consequently, growers have limited means of controlling this insect pest. Several insecticides, such as chlorantraniliprole, emamectin benzoate and Spinetoram are currently used to control codling moth in the United States and Europe, but the development of resistance to these insecticides is only a question of time. Strategies based on the behavioural manipulation of moths with pheromones or kairomones, efficient when used on large and well-managed apple plantations, do not resolve problems caused by dense codling moth populations, or migration of gravid moths from adjacent unmanaged areas in mosaic landscapes typical of small-scale apple production (Witzgall et al., 2008).

Recently, Pszczolkowski et al. (2011) suggested that feeding deterrents for codling moth neonates could be expressed in genetically modified apples, in amounts making the fruit unpalatable to codling moth larvae, but still acceptable to consumers. However, the results reported in the current paper show that codling moth neonates are far more tolerant of bitter tasting compounds than humans. Codling moth neonates perceived denatonium only at concentrations higher than 0.5 mM and did not sense quinine at a concentration as high as 1.64 mM. Studies of Keast et al. (2003) place human tasting thresholds for these compounds at 0.001 mM and 0.01 mM, respectively. Expressing any bitter compounds in the flesh of apples at concentrations that deter codling moth larvae would undoubtedly make the fruit unpalatable to humans. However, it is possible that bitter deterrents could be expressed in the waxy layer covering apples. The wax layer could be washed off together with bitter chemicals after harvest and the fruit rewaxed. Such a practice is a standard measure in the USA and ensures removal of pesticide residues before the fruit reaches the consumer.

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