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Do European Corn Borer Females Detect and Avoid Laying Eggs in the Presence of 20-Hydroxyecdysone?

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Abstract European corn borer larvae detect and avoid feeding in the presence of phytoecdysteroids (PEs) such as 20-hydroxyecdysone (20E). Therefore, we hypothesized that females would have taste receptors similar to larvae and avoid laying eggs in the presence of 20E. We found female-specific taste sensilla on the tarsi that respond to 20E at concentrations as low as 10^{-6} M, a threshold comparable to that of larvae. However, in choice tests, females laid a similar number of eggs on 20E-treated and on nontreated artificial substrates (filter paper, glass, and nylon), although they spent significantly more time in behavioral sequences related to substrate assessment when 20E was present. In contrast, when given a choice between maize plants (eight leaves) sprayed with 20E or only the solvent, females laid 70% fewer eggs on the treated than on control plants. These observations suggest that other chemical cues of plant origin must be present at the same time as 20E for females to modify their oviposition behavior.

Keywords *Ostrinia nubilalis* · 20-hydroxyecdysone · Taste · Electrophysiology · Oviposition behavior · Host plant · Maize

Introduction

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera, Crambidae), is a highly polyphagous insect that has been reported on 224 plant species (Hodgson 1928). It is considered a serious pest of several major crops, including maize, tomato, and cotton. As adults are much more mobile than larvae, host and habitat preferences of females are considered to play a decisive role in this polyphagy (Thompson and Pellmyr 1991; Kennedy and Storer 2000). ECB females' oviposition choices are influenced by many factors, including plant quality (phenology, developmental stage, variety, planting date), climate, and habitat (Kennedy and Storer 2000). There is also a

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probable genetic component associated with choice, as insects feeding on corn (*Zea mays* L.) are genetically different from those feeding on mugwort (*Artemisia vulgaris* L.) and hop (*Humulus lupulus* L.; Bethenod et al. 2005; Malausa et al. 2005). Females use plant odors to avoid damaged maize plants (Schurr and Holdaway 1970) or to select some maize hybrids over others (Lupoli et al. 1990), as well as to discriminate among plant species (Udayagiri and Mason 1995). Certain odors are even repellent to them (Binder et al. 1995; Binder and Robbins 1997). Some studies have also documented how contact chemicals, like sugars (Derridj et al. 1986) and surface waxes (Udayagiri and Mason 1997), affect host plant choices and oviposition. These observations suggest that the chemosensory system of ECB females is sensitive to a number of host and nonhost plant chemicals and that despite its large range of hosts, they monitor volatile and contact chemicals that allow them to select the proper plant and avoid "unsuitable" hosts.

In selecting or avoiding a potential host plant, do females use the same stimuli as their larvae? Although some studies have reported that adult females may use stimuli different from larvae to select their host (Roessingh et al. 2000), others suggest that larvae and adults use the same contact stimuli when accepting (Renwick 2002; Miles et al. 2005) or rejecting host plants (Qiu et al. 1998). We have recently shown that larval stages and adult females of the grapevine moth, *Lobesia botrana*, avoid 20-hydroxyecdysone (20E; Calas et al. 2006). 20E, the major molting hormone of insects, is the most common of the PEs, a structurally homogenous class of secondary substances produced by plants (Marion-Poll et al. 2005). PEs are considered to be plant defense compounds (Schmelz et al. 1999, 2000) that mimic the genuine insect hormone. They have been found in the seeds of 5–6% of 5,000 plant species sampled across different plant taxa (Dinan et al. 2001), ranging from primitive ferns, to the more evolved Angiosperms. Therefore, we propose that polyphagous insects like ECB will have been repeatedly exposed to PEs and consequently have evolved strategies to avoid plants using this family of defense compounds.

We have already shown that 20E is deterrent and toxic for ECB larvae (Darazy-Choubaya 2002) and that they have taste receptors that can detect 20E at a threshold of 10^{-6}–10^{-7} M (Marion-Poll and Descoins 2002). Therefore, we tested the hypothesis that ECB adult females would avoid laying eggs on 20E-containing substrates. We first determined if ECB females can detect 20E through neurons in taste sensilla located on the legs or on the ovipositor (Marion-Poll et al. 1992). We also monitored the number of eggs laid on different artificial substrates (filter paper, glass, and nylon) and plants treated with 20E, as well as timing the different sequences of behavior leading to egg laying when females were provided on treated or untreated glass oviposition sites. Our results indicate that ECB adult females detect 20E with their taste receptors and change the duration of certain oviposition behaviors. However, the number of eggs laid only declined when natural rather than artificial substrates were used. Several hypotheses are proposed to explain the differences observed.

Methods and Materials

Chemicals 20E, purified from *Leuzea carthamoides* (Willd.) DC. (Asteraceae; 95% minimum purity with minor ecdysteroids mixtures of polypodine B, ajugasterone C, and inokosterone), was obtained from SciTech (Prague, Czech Republic) and from Lafont (University of Paris VI). It was dissolved in ultrapure water or in ethanol depending on the bioassays. For electrophysiology, 20E was dissolved in 10^{-4} M potassium chloride (KCl, Prolabo) and used at concentrations ranging from 10^{-8} to 10^{-2} M.

Plants Individual maize *Centena* (Pioneer, Toulouse) plants were grown in plastic pots in a greenhouse at INRA Versailles and were used in assays when they had developed eight leaves.

Insects ECB pupae were obtained from INRA Le Magneraud. This strain is reared on an artificial medium and regularly renewed with wild insects. Pupae were maintained in plastic boxes at 25°C under a 16-hr light/8-hr dark photoperiod and 70% relative humidity until adults emerged. We used 24-hr-old virgin females for the electrophysiological studies, to ensure that good recordings were obtained.

Oviposition-choice tests and the observation of oviposition-related behaviors were performed in an environmental chamber (25°C; 70–80% relative humidity) under a 16-hr light/8-hr dark photoperiod. Couples of mature females and males (48 hr old) were placed in cages to mate, and females started to lay eggs at the beginning of the next night. We used this time as a pretest period to check if adults were mated, and the actual behavioral tests were performed at the beginning of the following scotophase.

Electrophysiology Before dissection, females were briefly anesthetized with carbon dioxide. For the recording from tarsal sensilla, females were decapitated by using fine scissors and then fixed on a polystyrene block with fine strips of tape. A glass capillary electrode, filled with Ringer solution, was inserted into the abdomen and connected to the ground. One of the prothoracic legs was taped to the support to expose the ventral side of the fifth tarsomere. Two types of taste sensilla were found on the tarsi of ECB adults, CRa and CRb (Marion-Poll et al. 1992), but as CRa sensilla did not respond to 20E (data not shown), measurements were only recorded from the CRb. For the recording from the ovipositor, the distal part of the abdomen was excised with a scalpel and placed on a small silicone dish filled with Ringer solution. Previous observations showed that there are about 12 taste sensilla of type CRa (30–40 μm) on the outer rim of each papilla, within a field of about 200 bristles (80–160 μm; Marion-Poll et al. 1992). These taste sensilla are identifiable under the microscope by their dark, blunt tip. Recordings were made from randomly selected sensilla.

For stimulation, we used glass capillary electrodes (borosilicate glass, outer diameter = 1.0 mm, inner diameter = 0.78 mm) pulled to a 10-μm-diameter tip (Narishige PC-10) filled just before the recording. The electrode was placed on the tip of a sensillum chaeticum by using a Leica MZ12 (×350). The electrical signal was preamplified (TastePROBE DTP-02, Syntech, The Netherlands), amplified (×1,000), and filtered (8-poles Bessel filter: 0.1–30 to 2,800 Hz; CyberAmp, 320, Axon Instruments). The electrical artifact generated at the moment of the contact was detected by the TastePROBE amplifier, which triggered a 2-sec data acquisition. Data were recorded and stored on a computer with a 16-bit A/D conversion card (DT9803 USB A/D; Data Translation, USA) under the control of a custom software, dbWave. Recordings were then analyzed with dbWave, to detect and sort spikes according to their amplitude and shape using interactive procedures.

Responses to the different stimuli were evaluated by counting the total number of spikes of the recording. Series of recordings were done starting with 10^{-4} M KCl (also used as electrolyte of nonconductive solutions) and followed by ascending concentrations of 20E (tarsal sensilla: decadic steps from 10^{-8} to 10^{-2} M; ovipositor: decadic steps from 10^{-6} to 10^{-3} M). Consecutive stimuli on a sensillum were applied at an interval of greater than 2 min to avoid adaptation. Data from the tarsi were collected from 16 insects ($N = 9$–37 recordings × 8 doses) while from ovipositors were collected from ten insects ($N = 12$–26 recordings × 5 doses).

Oviposition Tests on Artificial Substrates The oviposition cages were metal cylinders (height = 20 cm, diameter = 19 cm, 2.5-mm mesh). The top of each cage was closed with a paper, nylon, or glass (19 cm diameter) disk supported by a larger cloth (6-mm mesh). This cloth allowed females to hang on and touch the disk. The two opposing quarters of the disk received 200 μl of a solvent or 10^{-4} to 10^{-2} M of 20E. Ultrapure water was used as the solvent for nylon disks, while ethanol was used for glass and filter paper substrates. After letting the solvent evaporate for 20 min, a disk was placed in a cage containing five females and eight males. We performed ten replicates, each lasting an hour, for each substrate and each concentration.

Oviposition-related Behaviors on Glass Substrates At the beginning of the scotophase, mated females were introduced into small individual nylon cages (height = 12 cm; diameter = 10 cm), topped with a metal cloth (mesh size = 6 mm) and covered by a glass Petri dish (diameter = 11 cm). The surface of the Petri dish facing the inside of the cage was treated with 240 μl of either ethanol or 10^{-4} M 20E in ethanol. We recorded the oviposition behavior from above the cage, under red light, with a digital video camera recorder (Sony DCR-HC20). The behavioral sequences recorded (flying, walking, sweeping, and egg laying) correspond to those previously described for the ECB (Binder and Robbins 1996; Garnier-Geoffroy et al. 1996). Each experimental session lasted 2–3 hr. We monitored the behavior of ten females for each condition (control and 10^{-4} M 20E).

Oviposition Tests on Plants Maize plants were sprayed (Ecospray, VWR, France) with 15 ml of 10^{-4} M 20E or pure water. Half an hour later, when plants were dry, two plants (20E+control or control+control) were placed together in a plastic pot (diameter = 30 cm) filled with vermiculite. The plants were covered with a nylon cage (a conic section: top = 25 cm diameter, bottom = 40 cm diameter, height = 70 cm), and five females were introduced. Experiments were run for 2 hr at the beginning of the scotophase. The number of eggs deposited on each leaf of both plants was noted. The leaves were numbered 1 to 8, from the bottom to the top. We performed ten replicates for choice tests using 20E and five replicates for control tests (control vs. control).

Statistics Statistical tests were done with Minitab 12 software (Minitab, France). Electrophysiological responses were compared with the response to the electrolyte (0.1 mM KCl) using Mann–Whitney (MW)'s *U* test. Oviposition scores were compared by using nonparametric statistics with the Wilcoxon matched-pair signed rank test for choice experiments and the MW test for nonchoice ones.

Results

Taste Sensilla on the Tarsi In CRb sensilla, we found at least two classes of spikes in response to KCl and to 20E, which we called class 0 and class 1. Class 0 is characterized by medium amplitude spikes (1.4–1.8 mV, Fig. 1). Its firing activity was quite high, except at the highest concentration of 20E where it decreased by almost half (average rate: 40 vs. 23 spikes/s; P=0.005; Fig. 2). Although this class of spikes could originate from a "water" cell, its activity did not decrease in the presence of higher concentrations of salt (data not shown).

Class 1 has smaller amplitude spikes (0.5–0.8 mV) when activated by 20E (Figs. 1 and 2). When near the detection threshold, between 10^{-7} and 10^{-6} M, there was a response delay of 200–300 msec, which decreased at higher concentrations. The activity of this class of spikes

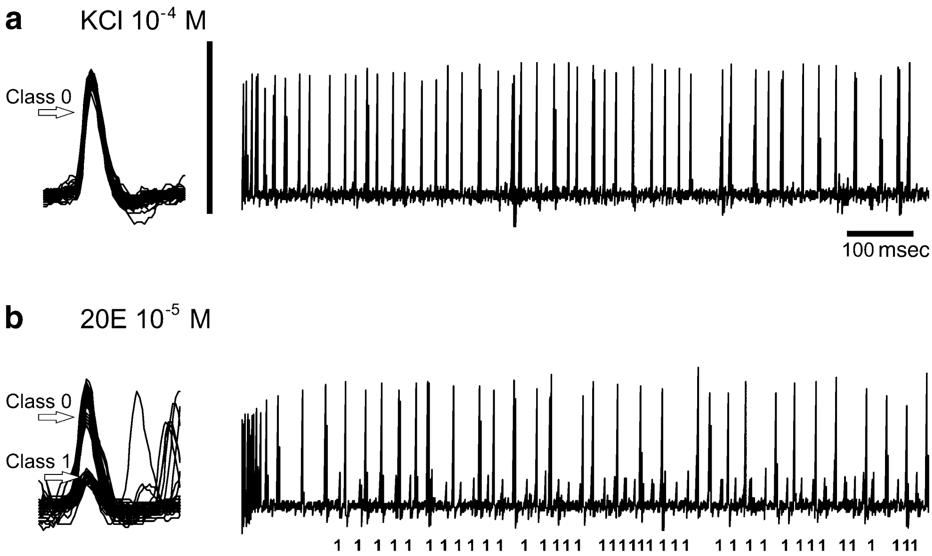
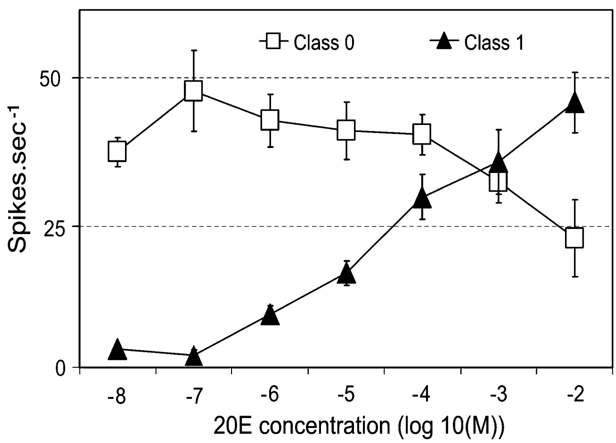


Fig. 1 Electrophysiological recordings from sensilla chaetica type CRb of tarsi of *Ostrinia nubilalis* showing that taste receptor neurons are activated by 20E. Each *horizontal trace* represents a 1-sec recording. *On the left*, the spikes detected in these recordings are superimposed. **a** KCl 10^{-4} M and **b** 20E 10^{-5} M. *1* Spikes of class 1. Vertical bar=2 mV; spike window length: 6 ms

was tonic and increased with concentration (Fig. 2), similar to those of deterrent cells previously described in larvae (Marion-Poll and Descoins 2002).

Taste Sensilla on the Ovipositor At least two cells (rarely three) were active in recordings from these sensilla (Fig. 3), for although the spikes were of similar amplitudes (0.5–0.8 mV), we consistently observed spike superpositions or doublets (i.e., spikes separated by 10–15 msec), indicating that two neurons were active (Meunier et al. 2003). We did not attempt to sort spikes into separated classes and analyzed only the total firing rate. Overall, the responses to 10^{-4} M KCl and 20E were similar over the range of 20E tested (Fig. 4), although there was a marginally significant decrease in the number of spikes to 20E 10^{-5} M compared with the KCl control (25±2.7 vs. 31.8±2.8 spikes/s, P=0.047).

Fig. 2 Electrophysiological responses of sensilla chaetica type CRb of tarsi of *Ostrinia nubilalis* to ascending concentrations of 20E (from 10^{-8} to 10^{-2} M). *White squares*: Responses from class 0 spikes (medium amplitude); *black triangles*: responses from class 1 spikes (small amplitude). Each *data point* represents the average number of action potentials for 2 s (mean ± s.e.m.; N=10–30 recordings)



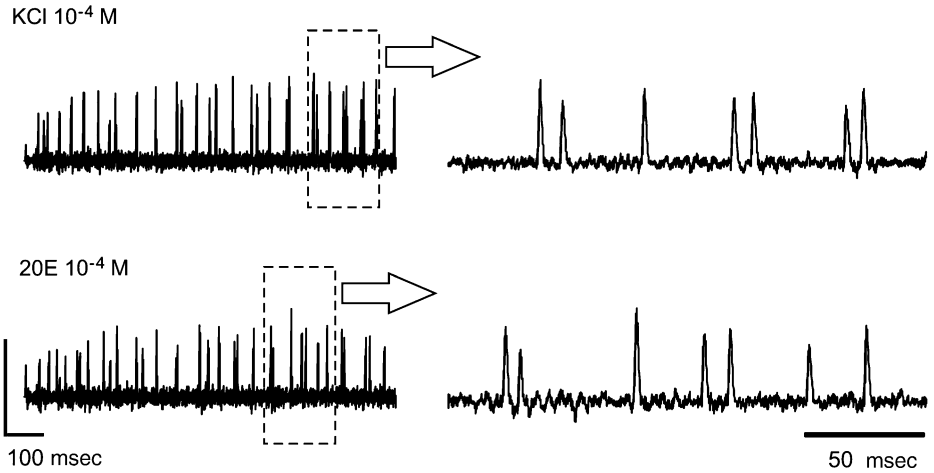


Fig. 3 Electrophysiological recordings from sensilla chaetica type CRa of the ovipositor of *Ostrinia nubilalis*. *On the left*, a 1-sec recording sample to KCl 10^{-4} M and 20E 10^{-4} M. *On the right*, an enlarged view of 200 msec, showing that several cells are active. The recordings look very similar at all concentration of 20E. Vertical bar=1 mV

Oviposition Tests on Artificial Substrates Females readily oviposit on all artificial substrates tested. In control vs. control assays, the total number of eggs (mean±s.e.m.) laid during the first hour was similar for glass (207.9±117.5, N=10), paper (201.6±116.4, N=14), and nylon (211.4±112.1, N=10). Similarly, in choice tests, there were no significant differences in the number of eggs laid on the treated and nontreated areas of each disk, for any given concentration and substrate (Fig. 5). We also compared the total number of eggs deposited during the experiment, irrespective of position on the disk. On glass, females laid slightly fewer eggs in the presence of 20E than on control disks (Fig. 5); on paper, there were more eggs on disks treated with high concentrations of 20E (MW: 20E 10^{-2} M/control vs. control/control, P=0.015) than with 10^{-4} M 20E. On nylon, the total number of eggs laid is lower in 20E than the control assays, especially at 10^{-4} M (MW: 20E 10^{-4} M/control vs. control/control: P=0.009). No significant difference in the number of egg masses was noted (data not shown).

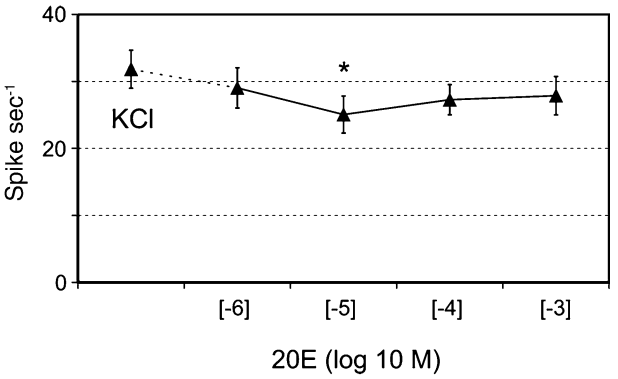
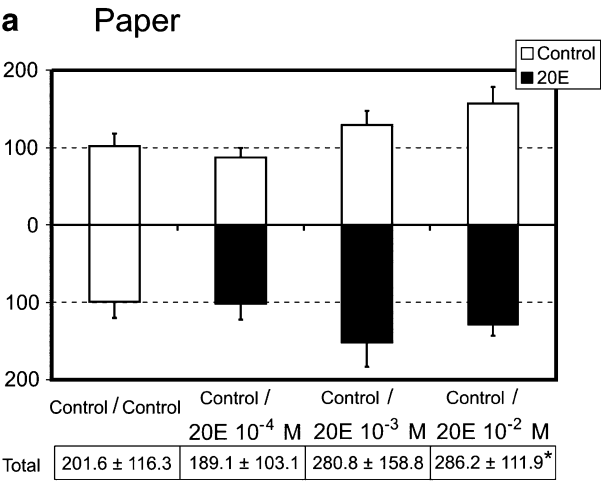
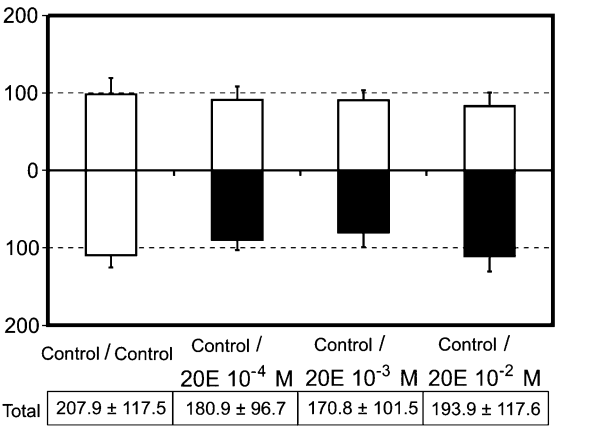


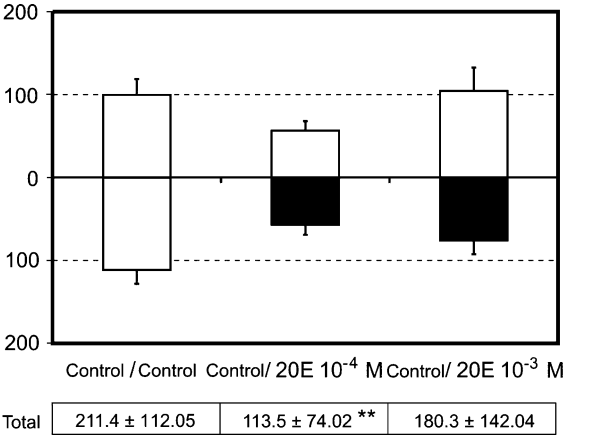
Fig. 4 Electrophysiological responses of sensilla chaetica type CRa on the ovipositor of *Ostrinia nubilalis* to ascending concentrations of 20E (from 10^{-6} to 10^{-3} M). Each *data point* represents the average of action potentials for 2 sec (mean± s.e.m.; N=16–26 recordings). Mann–Whitney test: KCl vs. 20E, *asterisk*, P<0.05



Total	201.6 ± 116.3	189.1 ± 103.1	280.8 ± 158.8	286.2 ± 111.9*



Total	207.9 ± 117.5	180.9 ± 96.7	170.8 ± 101.5	193.9 ± 117.6



Total	211.4 ± 112.05	113.5 ± 74.02 **	180.3 ± 142.04

Fig. 5 Oviposition tests on artificial substrates: In choice tests, no significant differences could be observed between the treated and the nontreated areas. Different type of substrates: **a** paper, **b** glass, and **c** nylon. *White bars*: Control (solvent alone); *black bars*: 20E treatment (from 10^{-4} to 10^{-2} M). *Bars* indicate the number of eggs (mean±s.e.m.) laid on treated and nontreated parts. Under each experiment, the total number of eggs laid on the disk (mean±s.e.m.); Mann–Whitney test: control/control vs. 20E/control, *asterisk*, $P<0.05$, *double asterisk*, $P<0.01$. $N=10$–14 (with five females per test). Test duration: 1 hr

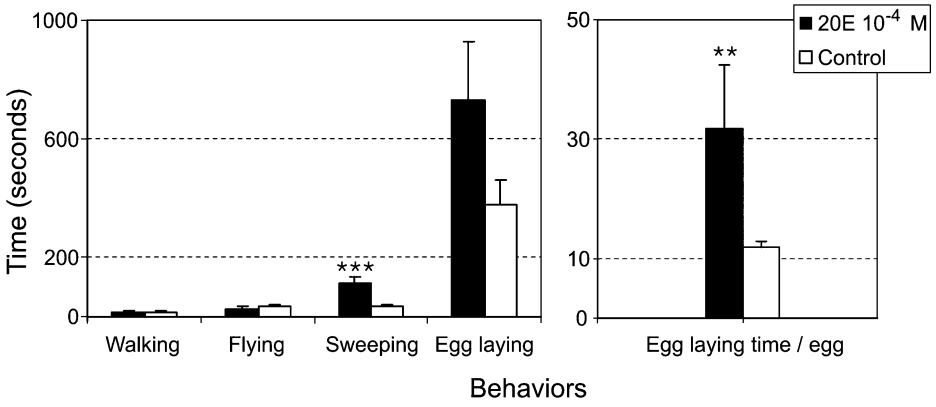


Fig. 6 Oviposition-related behaviors: Female *Ostrinia nubilalis* take longer to lay eggs on glass treated with 20E. *White bars*: Glass Petri dish treated with solvent (control); *black bars*: glass Petri dish treated with 20E 10^{-4} M (diluted in ethanol). *Bars* indicate the time in seconds (mean±s.e.m.) spent by a female for each behavior. N=10 for each treatment. Wilcoxon test: 20E vs. control, *double asterisk*, P<0.01, *triple asterisk*, P<0.001

Oviposition-related Behaviors Most females were initially resting on the side of the cage, before they started to walk or fly. There may be several flights, none exceeding a few seconds because of the size of the cage, before a female landed near the oviposition site. There were no significant difference in walking or flight between the treated and control assays (Fig. 6). Antennal movement occurred just before the flight, as well as during the

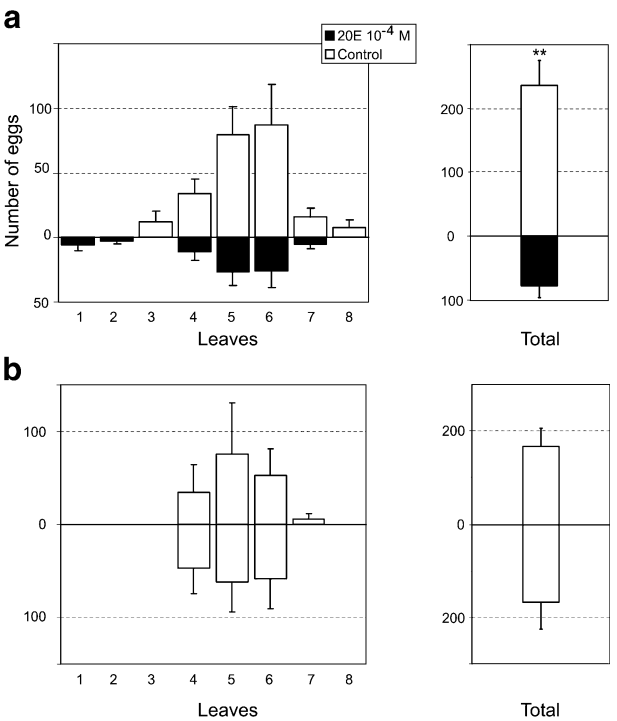


Fig. 7 Oviposition tests on plants: Female *Ostrinia nubilalis* laid significantly fewer eggs on plants treated with 20E. **a** 20E vs. control; **b** control vs. control. *White bars*: Plant treated with ultra pure water; *black bars*: plant treated with 20E 10^{-4} M (diluted in water). *Bars* indicate the number of eggs (mean±s.e.m.) laid on each leaf (1 to 8). Wilcoxon test: control vs. control and 20E vs. control, *double asterisk*, P<0.01. N=10 (20E/control), N=5 (control/control) with five females per test. Test duration: 2 hr

exploration of the substrate, but not during oviposition. The female curved her abdomen and made lateral sweeps of the substrate surface with the expanded lobes of her extruded ovipositor, which lasted significantly longer on treated than control sites (113±22.7 vs. 36.5±3.5 sec; $N=10$; $P<0.001$; Fig. 6). The female immobilized her ovipositor when she started laying eggs, one after another, in consecutive rows. While the total time to lay an egg mass took longer on the 20E substrate than on the control, the difference was not significant, although the time taken to lay each egg was (31.8±10.5 vs. 11.8±1.0 sec; $P<0.01$; Fig. 6).

Oviposition Tests on Plants The total number of eggs deposited in both experiments was comparable (20E vs. control: 315.3±55.8; control vs. control: 333.6±96.0). However, females laid significantly fewer eggs on the treated than on control plants (78.8±17.0 vs. 236.5±38.8; $P<0.01$, $N=10$) and laid more eggs on all nontreated leaves except for positions 1 and 2 (Fig. 7a). For both treatments, females laid more eggs on leaves 5 and 6. For control tests (including two plants sprayed with ultrapure water), females laid similar numbers of eggs on each plant (plant a=167±38.3; plant b=166.6±57.6 eggs), and they only laid eggs on leaves 4 to 7 with a higher number of eggs on leaves 4, 5, and 6 (Fig. 7b). This is in contrast with the distribution in the 20E vs. control, where eggs were laid on leaves 3–8 of the control plant.

Discussion

Females possess taste receptor neurons in CRb sensilla on the ventral part of the distal tarsomere, which respond to 20E, at a threshold similar to that observed in larval receptor neurons (Darazy-Choubaya 2002; Marion-Poll and Descoins 2002). One noticeable difference is that the adult female CRb sensilla have a maximal firing rate at about 50 spikes/s at 10^{-2} M 20E compared with 100–150 spikes/s for the sensilla styloconica of larvae (Marion-Poll and Descoins 2002). Such a difference was already reported between adults and larvae of *L. botrana* (Calas et al. 2006).

We did not detect any sensilla that respond to 20E on the ovipositor, but these findings should be viewed with caution, especially given the increased ovipositor-sweeping times that we observed on 20E-treated glass. We could not sample all sensilla on the ovipositor of the same insect and thus may have missed some sensilla that are responsive to 20E. Furthermore, the spikes obtained from the ovipositor sensilla were too similar in amplitude and shape to sort them accurately.

Females not only have receptors that detect 20E, they also modify their egg laying when the PE is present. However, although ECB females spent more time sweeping and took longer to lay each egg on artificial substrates treated with 20E, the total number of eggs laid on treated and control sites did not differ. In contrast, when the choice assays were carried out by using corn, females clearly avoided ovipositing on treated plants. Furthermore, the actual distribution of eggs on the leaves of treated and untreated plants varied. Preferences for different leaves have been noted in the field, largely related to the available leaf surface area (Spangler and Calvin 2000, 2001). Females laid eggs on leaves 1 and 2 of treated plants, which may be due to difficulties effectively spraying such small leaves. This possibility is supported by the fact that egg masses on treated plants were often at the tips of leaves, which are also difficult to spray. However, the overall results of this study are consistent with our initial hypothesis that ECB females, like larvae, detect and avoid sites with PEs.

It is not clear why ECB females did not avoid ovipositing on artificial substrates treated with 20E. It is possible that the quantities of 20E we used (0.014–14.40 μg/cm^2) were too low to reduce oviposition in the ECB. However, we do not think this is the case. A leaf from a plant like spinach may contain 10–40 μg/cm^2 of 20E (Lafont, personal communication). It is generally believed that much lower concentrations of 20E occur on the surface relative to the total leaf content, given the hydrophobic nature of the cuticle and the absence of an active transport system (Vrieling and Derridj 2003; Müller and Riederer 2005).

Several alternate, not mutually exclusive, explanations exist. The plant cuticle plays a major role in many plant–insect interactions (Espelie et al. 1991; Eigenbrode and Espelie 1995; Eigenbrode 2004; Müller and Riederer 2005), and epicuticular waxes may provide important cues for herbivorous insects, including ECB (Udayagiri and Mason 1997) and other Crambidae (Li and Ishikawa 2006). Some nonvolatile secondary plant compounds such as the cucurbitacins that prevent oviposition by ECB and the beet armyworm, *Spodoptera exigua* (Tallamy et al. 1997), or toosendanin that is avoided by the cabbage looper, *Trichoplusia ni* (Akhtar and Isman 2003) diffuse onto the plant surface and become associated with the waxes (Müller and Riederer 2005). In some cases, synergy of secondary compounds with the epicuticular waxes may occur, and such synergistic interactions enhance the oviposition behavior of the diamond back moth, *Plutella xylostella* (Spencer 1996; Spencer et al. 1999). Therefore, it is possible that plant epicuticular waxes could play a role in the detection of 20E.

Many insects obtain information about plants by probing the leaf surface with their antennae or tarsi before ovipositing (Chapman and Bernays 1989), so a second possibility is that ECB females only integrate information about 20E when it is detected simultaneously with other contact or volatile host plant chemicals (Bruce et al. 2005). For example, *Papilio polyxenes* females laid more eggs on artificial plants treated with a combination of contact stimulants and volatiles than on plants treated only with contact stimulants (Feeny et al. 1989). Similarly, host acceptance in the cabbage root fly, *Delia radicum*, appears to result from a synergistic response to simultaneous olfactory and contact chemo-stimulation (de Jong and Städler 1999). Females might also need to detect primary metabolites, such as soluble sugars or sugar-alcohols on the plant surface, that stimulate oviposition in the ECB and other moths species (Derridj et al. 1986; Lombarkia and Derridj 2002).

Finally, we cannot rule out that the application of 20E affects the physiology of the plant. However, if this were the case, then the effect would have to be rapid because our experiments were completed within 2 hr after treatment. 20E could be taken up and transformed by the plant into a new, more potent molecule. Maize does not produce detectable nonconjugated PEs but is capable of forming polyphosphate ecdysteroids conjugates from precursors like mevalonic acid and cholesterol (Devarenne et al. 1995; Dinan 1998). These conjugates of 20E might be more potent on the sensory system than 20E or could interact with the plant brassinosteroid receptors, which are involved in the physiological reactions of plants to phyto-aggressors or stress (Krishna 2003; Nakashita et al. 2003; Wang and He 2004).

Additional experiments must be carried out to test the hypotheses presented to explain why the presence of 20E on artificial substrates does not deter oviposition by the ECB but does on intact plants. These will include assays that look at oviposition on artificial substrates to which primary metabolites or maize odors have been added. In addition, it will require the analysis of the leaf surface compounds before and after applying 20E, together with an analysis of volatile compounds released to check if the chemicals accessible to female ECB are modified by 20E. It would also be interesting to investigate why 20E on artificial substrates affects oviposition by grape berry moth, *L. botrana*, females (Calas et al.

2006) but not in the case of the ECB. Is this related to the greater variability of cues that a polyphagous species encounters when selecting suitable host plants compared with a monophagous one?

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