INTRODUCTION

When plants are attacked by insects, volatile chemical signals can be released, not only from the damaged parts, but also systemically from other parts of the plant and this continues even after the cessation of feeding by the insect. These signals are perceived by olfactory sensory mechanisms in both herbivorous insects and their parasitoids. Evidence is mounting that such signals can also affect neighboring intact plants, which initiate defence by the induction of further signalling systems, such as those that increase parasitoid foraging. Recently, it was found that certain plants can release stress signals even when undamaged, which can cause defence responses in intact plants (Walling, 2000; Pickett et al., 2003). Stored grain can vary in quality due to biotic and abiotic factors, and volatiles that come from grain can be indicative of the current grain “status” quality (Christensen and Kaufman, 1969; Sinha et al., 1988; Sinha, 1990). Several studies demonstrate that grain volatiles may have important effects on host selection behavior in granivores and that these effects may differ substantially among species sharing the same resources (Levinson and Levinson, 1978; Walgenbach et al., 1983, 1987; Walgenbach and Burkholder, 1986; Trematerra and Girgenti, 1989; Phillips et al., 1993).

Olfactory attraction of storage insects can be induced by a blend of food volatiles such as the aroma of wheat or other cereals, while aggregation and feeding may be stimulated by less volatile food components including salts, sugars and lipids. Several saturated or unsaturated fatty acids with a chain length of 12 to 18 carbon atoms induce aggregation and/or feeding in adults and/or larvae of some Curculionidae, Cleridae, Dermestidae, Gelechiidae and Pyralidae species (Levinson and Levinson, 1978). Unprocessed oat stored for one year increased the levels of phenolic acids and aldehydes, and this increase of phenolic acids was...
most pronounced after storage at high relative humidity (Dimberg et al., 1996).

Early detection of infestation is one of the key elements in stored-product Integrated Pest Management. For this purpose, several trapping devices have been developed, which can be used with the addition of pheromones or other attractants (Trematerra and Girgenti, 1989; Dowdy et al., 1993; Phillips, 1997; Dowdy and Mullen, 1998; Likhayo and Hodges, 2000; Collins et al., 2004). Apart from these attractants, the behavioral trends of stored-product insect species are regulated by the characteristics of the commodity. Hence, although the characteristics of these attractants are generally well studied, very little is known about the cue role of the commodity itself (Landolt and Phillips, 1997). For instance, the condition of a given grain kernel may determine the behavior of a given insect individual. Trematerra et al. (2000) found that wheat kernels damaged by the rice weevil *Sitophilus oryzae* (L.) were more attractive than intact or mechanically damaged kernels to *Tribolium castaneum* (Herbst), *Tribolium confusum* Jacquelin du Val and *Oryzaephilus surinamensis* (L.); however, intact, mechanically and insect-damaged kernels may coexist in a storage facility. One of the main questions concerning this coexistence can be whether there are specific kernel-kernel interactions that can regulate insect behaviors, as reported in plant-plant interactions by Pickett et al. (2003). For instance, if insect-damaged kernels affected the “attractiveness” of intact kernels it would be very useful to know exactly how this happens.

The maize weevil, *Sitophilus zeamais* Motschulsky, is one of the most serious pests of stored cereals worldwide, especially for maize in tropical and temperate regions of the world (Aitken, 1975; Throne, 1994). As a primary pest, it can easily infest sound kernels, while it is particularly important in warm climates, where it can cause very serious losses in stored maize, as well as in maize before harvest (Crop Protection Compendium, 2002). Furthermore, it shows resistance to insecticides (Perez-Mendoza, 1999; Fragoso et al., 2003). In studies conducted in our laboratories recently we have made significant progress in clarifying some aspects of the behavior of *S. oryzae* and *T. confusum* (Athanassiou et al., 2006). In the present work, we examined the behavioral responses of adult maize weevil to semiochemicals from several kernel categories of maize, including the interactions between kernels of different status.

**MATERIALS AND METHODS**

**Insects.** Adults of *S. zeamais*, of mixed sex and age, were taken from cultures kept under laboratory conditions on maize kernels at 28±1°C and 70±5% relative humidity (rh), and continuous darkness.

**Experiment 1.** Conditioning of kernels was realized in plastic boxes (9 cm×12.5 cm×4.5 cm) containing two caps (diam. 3 cm) a cap with 2 g of a certain “kernel status” (mechanically damaged where the kernels were broken with a hammer, insect damaged where culture kernels were left for 30 d with adults of *S. zeamais* and intact kernels) and the other cap with 2 g of whole kernels to be contaminated by the volatile semiochemicals released from time to time from the different “kernel status”. Depending on the test, kernel conditioning was realized for different periods of time (1, 2, 4 and 14 d). All conditioning tests were conducted in controlled rooms set at 27±1°C, 70±5% rh, and continuous darkness.

The tests were carried out in a cylindrical arena of plexi-glass (45 cm diam.×30 cm high) for olfactometer assays. Three modified Flit-Track M² trap devices (Trécé Inc, USA) were placed in the arena. In each trial, 50 adult beetles of mixed sex and age were released in the center of the arena (Fig. 1). The number of trapped insects was checked 15 h after their introduction into the arena; teflon paint was used to prevent maize weevil escape from the

Fig. 1. Plastic box for kernel conditioning (MD=mechanically damaged kernels; ID=insect-damaged kernels; I=intact kernels; WK=whole kernels) and circular area used in the experiments (R=insect-releasing point; T-D=trap device).
traps and from the arena.

Nine replicates were performed for each “case study”, using 450 insects. In order to measure the different attractiveness of each kernel status, 2 g of whole conditioned or not conditioned kernels were used as bait for each trap device.

Three traps (three-choice tests) for each case study were baited with kernels under the following conditions. Case study 1, without kernel-kernel conditioning (0 d): mechanically damaged vs insect-damaged vs intact kernels. Cases studies 2-3-4-5, with kernel-kernel conditioning (after 1-2-7-14 d of conditioning, respectively) semiochemically: mechanically damaged vs insect-damaged vs intact kernels.

Data were submitted to two-way ANOVA (for the main effects of kernel status and conditioning duration as well as the interaction). Means were separated using the Tukey-Kramer HSD test at p=0.05.

**Experiment 2.** The conditions were as above, but in this case, two-choice tests were carried out in the arena. Six replicates were performed for all case studies, using 300 insects for each case. The conditioning periods were as follows: 1, 7 and 14 d.

Case studies 1A-2A-3A, with kernel-kernel conditioning (after 1-7-14 d of conditioning, respectively): mechanically damaged and insect-damaged kernels were compared. Case studies 1B-2B-3B, with kernel-kernel conditioning (after 1-7-14 d of conditioning): fresh mechanically damaged and stored mechanically damaged kernels were compared. Case studies 1C-2C-3C, with kernel-kernel conditioning (after 1-7-14 d of conditioning): fresh insect-damaged and stored insect-damaged kernels were compared. For each case the data were analysed as in Experiment 2.

For all experiments, all capture counts were transformed prior to analysis as suggested by Trematerra et al. (2000).

**RESULTS**

**Experiment 1 (Fig. 2)**

Concerning the overall data, “kernel status” was significant ($F=30.67$, df=$2.120$, $p<0.0001$). In contrast, no significant differences were noted among conditioning periods ($F=0.12$, df=$4.120$, $p=0.9743$); interaction was similarly not significant ($F=0.67$, df=$8.120$, $p=0.7106$).

Without kernel conditioning (case study 1), significantly more adults were found in trap devices containing insect-damaged kernels, than in those containing mechanically damaged and intact kernels (0 d, $F=8.65$, df=$2.24$, $p=0.0015$). In this case, almost 45% of the total *S. zeamais* individuals were found in traps with insect-damaged kernels.

This trend was also evident in the conditioning kernel categories, where a similar proportion of released adult weevils was found in traps containing semiochemically conditioned kernels coming from insect-damaged kernels (for 1 d: $F=6.26$, $p=0.0065$; for 2 d: $F=4.98$, $p=0.0155$; for 7 d: $F=4.29$, $p=0.0254$; for 14 d: $F=13.91$, $p<0.0001$; $df=n−2$ (Snedecor and Cochran, 1980).

**Experiment 3.** These tests were two-choice tests, set as in Experiment 2 and under the same conditions. Conditioned tests were carried out in plastic boxes (as above) containing plastic capsules in which 2 g of fresh maize (~8 months after harvest) or 2 g of stored maize (~20 months after harvest) were placed. Three conditioning periods were used: 1, 7 and 14 d.

Case studies 1A-2A-3A, with kernel-kernel conditioning (after 1-7-14 d of conditioning, respectively): fresh intact and stored intact kernels were compared. Cases studies 1B-2B-3B, with kernel-kernel conditioning (after 1-7-14 d of conditioning): fresh mechanically damaged and stored mechanically damaged kernels were compared. Case studies 1C-2C-3C, with kernel-kernel conditioning (after 1-7-14 d of conditioning): fresh insect-damaged and stored insect-damaged kernels were compared. For each case the data were analysed as in Experiment 2.

![Fig. 2. Experiment 1, comparison of mechanically damaged (MD), insect-damaged (ID) and intact (I) kernels (0 d =case study 1, without kernel-kernel conditioning; 1-2-7-14 d =case studies 2-3-4-5, with kernel-kernel conditioning). Data with asterisk are significantly different.](image-url)
in all cases df = 2.24).

**Experiment 2** (Fig. 3)

After 1, 7 and 14 d of conditioning, significantly more adults were captured in the trap devices that contained conditioned insect-damaged kernels, than in those that contained conditioned mechanically damaged kernels (for case study 1A: \( t = -2.729, p = 0.0212 \); for case study 2A: \( t = -3.664, p = 0.0043 \); for case study 3A: \( t = -4.980, p = 0.0005 \); in all cases df = 10).

No significant differences were noted between traps containing conditioned intact and mechanically damaged kernels, either at the 1 d or at the 7 d conditioning level (for case study 1B: \( t = 1.686, p = 0.1228 \); for case study 2B: \( t = 1.344, p = 0.2086 \); in both cases, df = 10). In contrast, significant differences were noted between the same traps after 14 d of conditioning where more adults were captured in the trap devices that contained conditioned intact kernels than in those that contained conditioned mechanically damaged kernels (case study 3B: \( t = 3.543, p = 0.0053 \), df = 10).

Traps baited with conditioned insect-damaged kernels were significantly more attractive than those baited with conditioned intact kernels (case studies 1C–3C), in all semiochemically conditioning periods examined (for 1 d: \( t = -4.406, p = 0.0013 \); for 7 d: \( t = -2.457, p = 0.0339 \); for 14 d: \( t = -2.323, p = 0.0424 \); in all cases df = 10).

**Experiment 3** (Fig. 4)

After 1 d of conditioning, no significant differences were noted in capture rates among trap devices in all cases examined (for case study 1A: \( t = 0.518, p = 0.6157 \); for case study 1B: \( t = -0.254, p = 0.8049 \); for case study 1C: \( t = 0.668, p = 0.5192 \); in all cases df = 10).

After 7 d of semiochemical conditioning, no significant differences were noted in case study 2A (\( t = -0.414, df = 10, p = 0.6875 \)) and 2C (\( t = -0.535, df = 10, p = 0.6042 \)). In contrast, in case study 2B, significantly more weevils were found in traps that contained semiochemically conditioned kernels coming from stored mechanically damaged kernels, than in traps that contained fresh contaminated mechanically damaged kernels (\( t = 3.194, p = 0.0096, df = 10 \)).

After 14 d no significant differences were noted in case study 3A (\( t = 0.785, p = 0.4505, df = 10 \)). In case study 3B (\( t = -2.499, p = 0.0315, df = 10 \)) significantly more weevils were found in traps that contained semiochemically conditioned kernels coming from fresh mechanically damaged kernels; in contrast, in case study 3C (\( t = 4.366, p = 0.0014, df = 10 \)), significantly more weevils were found in traps that contained allelochemically conditioned kernels coming from stored insect-damaged kernels.

**DISCUSSION**

Several plant-derived volatiles have been proved to be determinative of stored-product beetle behavior (Phillips et al., 1993; Landolt and Phillips, 1997; Trematerra et al., 1999, 2000; Bashir et al., 2002; Athanassiou et al., 2003); however, there is still inadequate information on kernel-kernel inter-
actions and behavioral responses of stored-product insects.

Host selection of stored-product beetles is different among primary and secondary colonizers (Phillips et al., 1993; Landolt and Phillips, 1997; Trematerra et al., 1996, 1999, 2000). Trematerra et al. (1999) reported that, in pheromone traps, damaged kernels might have an additional capacity in attracting rice weevils when compared to intact kernels. The combination of a pheromone and a food attractant may extend the potential for detection of a mixed age population likely to be encountered in a practical situation (Wakefield et al., 2005). The same happens in the case of the maize weevil; adults seek a spot that is suitable for infestation, and then the emission of an aggregation pheromone calls additional individuals of the same species (Levinson et al., 1990). Furthermore, the presence of saliva or frass may increase the emission of semiochemicals from specific parts of the seed, such as the germ or the kernel endosperm, which are highly attractive to adult S. oryzae. In addition, damaged seeds offer easier access to weevil feeding (Trematerra et al., 1999).

Trematerra et al. (2000) reported that wheat kernels damaged by the rice weevil S. oryzae were more attractive to secondary colonizers than intact or mechanically damaged kernels. According to our findings, this is also true in the case of the primary maize colonizer S. zeamais. Hence, adult maize weevils seek kernels that have been contaminated by semiochemicals coming from adult-infested kernels of the same species. As a result, high populations may build up quickly around small sources of infestation. In addition, this behavior was not affected by the conditioning period. This suggests that the volatile semiochemicals derived from insect-damaged kernels are considerably different from those derived from intact or mechanically damaged kernels.

The weevils “contaminate and stimulate” the kernels they infest, and this “contamination and stimulus” is permanent and long lasting. From a practical point of view, this means that stock maize that contains some infested kernels is more likely to be heavily infested by S. zeamais adults than maize with intact or broken kernels.

In our experiments, the attractiveness of the kernel was affected by time, since the behavioral trends were different in fresh and stored kernels. Hence, the duration of conditioning may play a key role in the response of weevils to mechanically damaged kernels. However, in storage facilities where newly-harvested and aged maize is present at the same time, all kernel categories examined here are likely to coexist and, based on our results, fresh and stored maize has the same possibility of being infested by S. zeamais. Newly harvested grains are less likely to be heavily infested (Hagstrum, 1987; Athanassiou and Buchelos, 2001), but this is also because insect populations at that time are low.

One other important aspect we would like to point out, based on the results of the present work, is that mechanically damaged kernels do not add to the “attractiveness” of the kernel, and that such kernels are equally “vulnerable” as intact kernels to infestation by S. zeamais.

Apparently, this could be attributed to the fact that, in Experiment 1, the presence of semiochemicals coming from infested kernels “dominated” the other two kernel categories. Thus, weevils exhibited a strong preference to infested kernels, which may not allow the expression of a behavioral preference between the other two kernel categories. In contrast, the results of the two-choice tests in Experiment 2 indicated that intact kernels were more attractive, but not significantly, than conditioned mechanically damaged kernels. Hence, both sets of tests revealed that for S. zeamais adults, the “attractiveness” order in the kernel categories tested here (by decreasing order) is: insect damaged > intact > mechanically damaged.

Insect-kernel interactions have been examined for various species, especially primary grain colonizers (e.g. Honda et al., 1969; Tamaki et al., 1971; Levinson and Levinson, 1978; Kanaujia and Levinson, 1981; Levinson and Kanaujia, 1982; Dowdy et al., 1993; Phillips et al., 1993; Kucerova and Stejskal, 1994; Stejskal and Kucerova, 1996; Landolt and Phillips, 1997; Dowdy and Mullen, 1998; Trematerra et al., 1999, 2000; Ignacimuthu et al., 2000; Cox, 2004; Steidle et al., 2005). Our olfactometer assays suggest that these interactions have a certain impact on the regulation of stored-product insect behavioral trends (e.g. Sinha, 1990; Estabrook and Yoder, 1998; Dicke, 1999; Paré and Tumlinson, 1999; Walling, 2000; Pickett et al., 2003; Theis and Lerdau, 2003). Insect-damaged and mechanically damaged kernels seem to affect
other whole seeds, which has a direct effect on how an infestation is manifested in the stored-grain ecosystem. It is possible that the volatiles received during conditioning are able to activate a response in the conditioned kernels that change the chemicals produced as a defence response. Further work is necessary to study the signaling pathways activated in response to insects that feed on maize kernels and clarify other aspects regarding kernel-kernel interaction.

REFERENCES


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