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Laboratory tests on the ability of *Oryzaephilus surinamensis* adults to locate different types of chocolate varying in quantity of cocoa

Pasquale TREMATERRA[1], Nickolas G. KAVALLIERATOS[2], Christos G. ATHANASSIOU[3]
[1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*
[2]*Department of Crop Science, Agricultural University of Athens, Greece*
[3]*Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Nea Ionia, Greece*

Abstract

Results obtained by olfactometric trials on chocolate preferences of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera Silvanidae), adults testing unpackaged commercial chocolate realized with different quantity of cocoa are reported. The following five chocolate types of the same brand were tested: chocolate with 30% cocoa; chocolate with 50% cocoa; chocolate with 70% cocoa; chocolate with 85% cocoa; chocolate with 90% cocoa. The present study has shown that adults of *O. surinamensis* could establish different levels of infestation on the five tested types of chocolates. The chocolate type with the less percentage of cocoa (30%) was found as the most preferable one by *O. surinamansis* compared with the other types tested.

Key words: *Oryzaephilus surinamensis*, chocolate types, cocoa, preferences.

Introduction

The cocoa bean, from which cocoa solids and cocoa butter are extracted, is the basis of chocolate which is commercially known as plain, milk, white, dark, blended, bitter or composite chocolate. Cocoa, the basic ingredient of these chocolates varies from 20% to 90% (Begum *et al*., 2007). The study has shown that milk, nut, dried fruit and nut, and wafer chocolates can support insect infestation and therefore, insect-proof packing of the chocolates and storage under hygienic conditions are important to avoid customers' complaints.

Several packaging materials are used for chocolates, i.e., aluminium foil, composite films, paper or plastic trays. The packaged commercial chocolates keep their quality up to 5 months when stored at 10-18 °C and 60-70% RH. The actual storage period, however, may be prolonged during the distributional process to the retail market where the hygiene varies and therefore products are prone to insects infestation (Bowditch and Madden, 1997). When insects are found in chocolates, consumers often direct their complaints to the manufacturer and the image of the company is negatively affected (Locatelli and Garavaglia, 1995; Bowditch and Madden, 1997; ICCO, 2000; Trematerra, 2013).

The chocolate industry uses several different materials that exhibit variable susceptibility to the attack of *Ephestia cautella* (Walker) (Lepidoptera Pyralidae) and *Plodia interpunctella* (Hubner) (Lepidoptera Pyralidae). From Italy, Locatelli and Garavaglia (1995) reported that the most suitable substrates for both insect pests are raw and toasted hazelnuts, as well as chocolate with hazelnuts. However, the highest index of susceptibility was recorded on raw hazelnuts; the least suitable substrates were puffed rice and chocolate with rice dough. The authors also noted that raw hazel nuts used in the manufacture of chocolates were more susceptible than the basic ingredients of the chocolate. In Australia, pyralid moths, i.e., *E. cautella*, *P. interpunctella*, *Ephestia elutella* (Hubner), *Ephestia figulilella* Gregson, *Ephestia kuehniella* (Hubner), and *Paralipsa gularis* (Zeller) caused almost exclusively infestations to chocolate related products, mostly after their package (Bowditch and Madden, 1997). From India, Begum *et al*. (2006) reported that different types of unpackaged chocolates, such as milk, nut, dried fruit and nut or wafer chocolates, are susceptible to different level of infestations by *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Coleoptera Silvanidae), *Lasioderma serricorne* (F.) (Coleoptera Anobiidae) and *E. cautella* over a storage period of 45 days.

In the Italian factories raw cocoa is not stored but is processed in a few days, while raw dried fruit and spices are generally kept in flat stores or in refrigerated chambers for a period of 2-3 months (Trematerra, 2004). Pest infections on cocoa occur in imported cocoa seeds, chocolate factories and confectionary products. The insect pest species that infest imported cocoa seeds are *E. cautella*, *E. elutella*, *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae), *Trogoderma granarium* Everts (Coleoptera Dermestidae); chocolate factories are visited by *Attagenus* spp. (Coleoptera Dermestidae), *Carpophilus* spp. (Coleoptera Nitidulidae), *Cryptolestes* spp. (Coleoptera Laemophloeidae), *L. serricorne*, *Necrobia rufipes* (F.) (Coleoptera Cleridae), *O. surinamensis*, *Tribolium* spp.; confectionary products containing cocoa are infested by *E. cautella*, *O. surinamensis*, *P. interpunctella* and *Stegobium paniceum* (L.) (Coleoptera Anobiidae). Furthermore, chocolate and confectionary factories could be infested by rodents and birds (Trematerra, 2004).

In recent years, *O. surinamensis* exhibits an increasing importance as a pest in food industries and confectionery factories at cool temperate areas (CABI, 2015). The small size and flattened shape of *O. surinamensis* adults makes it relatively easy to enter cracks in grains, nuts in shell and products in packets or boxes (for example biscuits, breakfast cereals, chocolate) in commercial and domestic premises (Rees, 1996). *O. surinamensis* is an important pest which infests barley, bird seed, breakfast food, candy, chocolate, cocoa beans, corn, cornmeal, cornstarch, dog food, dried fruits, dried meats, flour, herbs, milo, mixed feed, nutmeats, nuts, pasta, peas, rice, rye, seeds, sorghum, spices, sugar, stored oats, tea, tobacco, wheat, wheat bran, and yeast (Trematerra and Throne, 2012). In cereal grain commodities, *O. surinamenis* is a secondary pest that can follow infestations of primary pests such as *Sitophilus* spp. (Coleoptera Curculionidae) or *Rhyzopertha dominica* (F.) (Coleoptera Bostrychidae) (Trematerra and Throne, 2012).

Yet, there are not many data for the choice of *O. surinamensis*, towards variable types of chocolate. In this paper we report results obtained by olfactometric trials on chocolate preferences of *O. surinamensis* adults testing unpackaged commercial chocolate realized with different quantity of cocoa. Normally, cocoa is expressed as a percentage of weight to the final product, ranging from 20% up to 99% (Minifie, 1989).

Materials and methods

Chocolate types

The following five chocolate types of the same brand were tested (ingredients are listed according to product labels):

- chocolate with 30% cocoa (milk solids 20% min., sugar, milk ingredients, cocoa butter, cocoa mass, lactose, barley malt extract, soya lecithin, artificial flavour. It may contain peanuts, tree nuts and sesame);
- chocolate with 50% cocoa (sugar, cocoa mass, cocoa butter, butter fat, soya lecithin, glucose syrup, natural bourbon vanilla bean. It may contain traces of peanuts, hazelnuts and almonds);
- chocolate with 70% cocoa (cocoa mass, sugar, cocoa butter, natural bourbon vanilla beans. It may contain traces of peanuts, hazelnuts, almonds, milk and soya lecithin);
- chocolate with 85% cocoa (cocoa mass, cocoa powder, cocoa butter, demerara sugar, bourbon vanilla beans. It may contain peanuts, hazelnuts, almonds, milk and soy);
- chocolate with 90% cocoa (cocoa mass, fat-reduced cocoa, cocoa butter, sugar, natural bourbon vanilla bean. It may contain traces of peanuts, hazelnuts, almonds, milk and soya lecithin).

Insects

Adults of *O. surinamensis*, of mixed sex and age, were taken from cultures kept in laboratory conditions on barley kernels at 28 °C and 70% RH, and continuous darkness. The experiments were conducted in a cylindrical arena made of Plexiglas (45 cm in diameter, 30 cm in height) for olfactometer assays (Trematerra *et al*., 2007). All tests were conducted in controlled rooms set at 28 °C with 70% RH and continuous darkness.

Olfactometer assays

Six modified Flit-Track M^2 trap-devices (Trécé Inc., Adair, OK, USA) (Trematerra *et al*., 2000) were placed in the arena: 5 traps baited with different chocolate type each and one trap remained empty and served as control. In order to measure the different attractiveness of each chocolate type, 20 g of chocolate were used as bait for each trap device. In all experiments trap devises positions were randomized and trap-devices contents were renewed after each replication. In each trial, 120 adult beetles of *O. surinamensis* were released at the centre of the arena. The number of trapped insects was checked 15 h after their introduction in the arena. Teflon paint was used to prevent insects' escape from the traps and from the arena. Twenty four replicates were performed by changing the position of the traps in the arena. Totally, 2880 adult individuals of *O. surinamensis* were used in the trials.

Data analysis

The data were submitted to one-way analysis of variance (ANOVA), for chocolate type, by using the statistical package JMP 10 (SAS Institute Inc., 2012). Means were separated by the Tukey-Kramer honestly significant difference (HSD) test at the 0.05 significance level (Sokal and Rohlf, 1995).

Results

According to our results, all five types of chocolate could be infested by *O. surinamensis* adults, but the degree of infestation varied. Significant differences were recorded in captures among traps that contained the tested chocolate types and control ($F = 137.5$; $df = 5, 138$; $P < 0.01$).

Traps that contained chocolate with 30% cocoa captured significantly more adults than all other traps with different chocolate types or control (figure 1). Captures in traps with 50, 70 or 85% of cocoa did not differ significantly. Finally, traps that contained 90% cocoa captured significantly more *O. surinamensis* adults than traps with chocolate containing 50, 70, 85% cocoa or control.

From the actual numbers point of view, in trap devices that baited with chocolate realized with 30% of cocoa were found 855 adults of *O. surinamensis*, 385 adults were trapped in trap devices baited with chocolate realized with 50% of cocoa, 356 adults were trapped in devices baited with chocolate realized with 70% of cocoa, 314 were in traps baited with chocolate with 85% cocoa, 488 in traps containing chocolate with 90% of cocoa, and 242 adults were found in control unbaited traps.

On the 2880 adults of *O. surinamensis* used in our tests, 2640 were found in different trap devices (91.6%) whereas 240 were adults (8.3%) that did not make any choice and remained free in the alfactometer arena.

Discussion

O. surinamensis, a common pest on processed foods and tree nuts, also breeds easily upon chocolates (Hill, 1990; Finkelman *et al*., 2003). The storage period of manufactured chocolates depends on a variety of factors in the retail market. During longer storage, more insects are

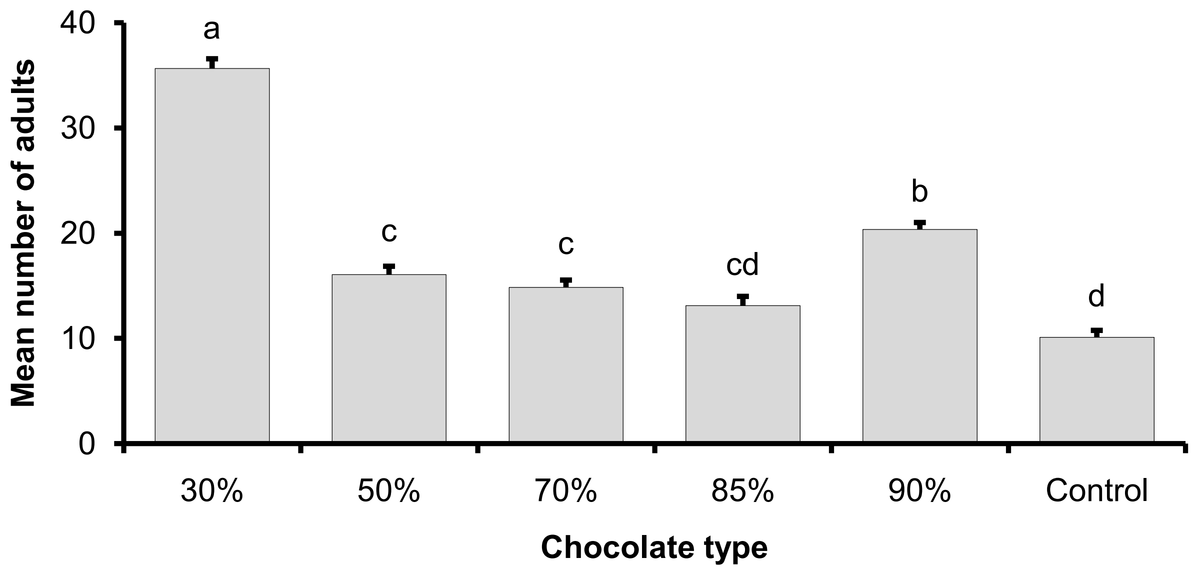

Figure 1. Mean number (+ SE) of *O. surinemensis* adults captured in trap devices baited with different chocolate types.

likely to invade or penetrate the packaging material causing more damage to the chocolates. In temperate conditions shelf-life recommendations of chocolate vary from 12-24 months whereas in tropical conditions vary from 9-24 months (Stauffer, 2007).

Despite modern food storage and distribution systems, unpackaged products but also packaged food products are subject to attack by insects (Kelly, 2004; Athanassiou *et al*., 2011; Trematerra and Savoldelli, 2014). Substances or mixtures of substances that emanate from food and disperse easily through air, due to high volatility, are commonly denoted as food volatiles. These compounds consist of a chain of 5-20 carbon atoms and a variety of functional groups, e.g. ketones, aldehydes, esters and alcohols. The volatile compounds are working in long-distance attraction due to the high and fast dispersing ability, and can be differentiated by their effect on the receiving stored product insects; attractants, causing oriented movement towards the source of the chemical (Olsson, 2001; Wyatt, 2003). The food volatiles may also escape from defective sealing or damaged packaging material may attract insects for oviposition or feeding (Mowery *et al*., 2002). In the short term storage oxidative rancidity is unlikely to occur in appreciable concentration with plain or milk chocolate but it can rapidly affect other ingredients such as high-fat centres or roasted nuts which do not have a full covering of chocolate (Essien *et al*., 2006). Previous studies have shown that female *O. surinamensis* do respond to food odours (White, 1989; Trematerra *et al*., 2000). In this context, food attractiveness may not be the result of just one substance but due to a complex of constituents. For example, Mikolajczak *et al*. (1984) found that *O. surinamensis* responded to certain volatiles that exist in rolled oats, chiefly by (*E*)-2-nonenal and (*E*,*E*)-2,4-nonadeinal. Furthermore, food volatiles are affective to *Oryzaephilus* spp. according to the length of their chain. The short chain aldehydes (i.e., 2-methylpropanal, pentanal) are functional over long distances contrary to long chain acids (i.e., heptanoic, octanoic, nonanoic) which are effective in short distances (Pierce *et al*., 1990). In some cases volatile cues alone are sufficient stimulation to elicit oviposition, but results presented by Mowery *et al*. (2002) indicate that contact with food is an important oviposition stimulant. This indicates that a combination of tactile, gustatory, and olfactory cues may be involved in *O. surinamensis* oviposition.

The present study illustrates that adults of *O. surinamensis* could establish different levels of infestation on the five tested types of chocolates. The chocolate type with the less percentage of cocoa (30%) was found as the most preferable one by *O. surinamansis* compared with the other types tested. In fact, an important factor which determines the intensity of a chocolate's taste is the cocoa content and the cocoa butter (Essien, 2006). We noticed that the chocolate type that contained 30% cocoa was much softer than the other types tested. Running to the biology of this species, it is known that it prefers soft commodities rather than hard ones (Back, 1926). Commercial chocolate products, as the ones that were tested in the present study, have ingredients such as cocoa, tree nuts, dried fruits, wheat flour and therefore are susceptible to insect infestation growth and multiplication during storage (Begum *et al*., 2007). Insect infestation in chocolates may depend on storage conditions, storage temperature, type and thickness of the packaging materials, and storage period (Collins, 2003). The extent of infestation also depends on the insect pest complex occurring in the premises (Trematerra and Fleurat-Lessard, 2015).

The present study has shown that *O. surinamensis* tested could establish different levels of infestation on five types of chocolates. This research provides insight into the infestation behavior of *O. surinamensis*, that one of the most destructive stored-product pests. Further experimentation into the infestation behaviour of this and other stored-product insect species will be beneficial in demonstrating to food manufacturers the importance of developing chocolate types that can be less attracted to these pests.

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Authors' addresses: Pasquale TREMATERRA (corresponding author, trema@unimol.it), Department of Agricultural, Environmental and Food Sciences, University of Molise, via de Sanctis, I-86100 Campobasso, Italy; Nickolas G. KAVALLIERATOS, Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, Athens, Attica, Greece; Christos G. ATHANASSIOU, Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Nea Ionia, Magnissia, Greece.

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