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doi: 10.1007/s10886-014-0429-x

Published in Journal of Chemical Ecology (2014) 40: 452–457
Postprint; The final publication is available at Springer via
http://dx.doi.org/10.1007/s10886-0414-0429-x

Catechol - an Oviposition Stimulant for Cigarette Beetle in Roasted Coffee Beans

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Abstract

The cigarette beetle, *Lasioderma serricorne*, is a serious global pest that preys on stored food products. Larvae of the beetle cannot grow on roasted coffee beans or dried black or green tea leaves, although they oviposit on such products. We investigated oviposition by the beetles on MeOH extracts of the above products. The number of eggs laid increased with an increase in dose of each extract, indicating that chemical factors stimulate oviposition by the beetles. This was especially true for coffee bean extracts, which elicited high numbers of eggs even at a low dose (0.1 g bean equivalent/ml) compared to other extracts. Coffee beans were extracted in hexane, chloroform, 1-butanol, MeOH, and 20% MeOH in water. The number of eggs laid was higher on filter papers treated with chloroform, 1-butanol, MeOH, and 20% MeOH in water extracts than on control (solvent alone) papers. The chloroform extract was fractionated by silica-gel column chromatography. Nine compounds were identified by gas chromatography/mass spectrometry from an active fraction. Of these compounds, only a significant ovipositional response to catechol was observed.

Keywords: Cigarette beetle, oviposition stimulant, coffee, catechol

INTRODUCTION

Larvae of the cigarette beetle, *Lasioderma serricorne* (Fabricius), damage a wide range of dried plant and animal matter (Ashworth 1993; Hill 2002). In contrast to the damage by larvae, adult feeding is limited (Ashworth 1993). However, adult beetles fly and oviposit onto dried food material leading to damage by the resulting larvae (Ashworth 1993).

Adult beetles are attracted to cured tobacco leaves, coffee beans, black tea leaves, cocoa powder, wheat flour, soybean flour, corn, chili, cayenne pepper, paprika, and coriander seeds (Hori et al. 2011; Kohno et al. 1983; Mahroof and Phillips 2007), and oviposit on roasted coffee beans, cocoa powder, black and green tea leaves, and unpolished rice (Hori et al. 2011). In spite of this, the larvae are not able to grow on roasted coffee beans or black or green tea leaves (Hori et al. 2011). This indicates that the factors that affect oviposition selection and larval growth are likely different. It is not known why larvae cannot grow on roasted coffee beans or black or green tea leaves. However, this phenomenon of beetles ovipositing on unsuitable food suggests a possibility for controlling this pest: if beetles oviposit on unsuitable food, it may preclude them from ovipositing on other food products upon which they can feed and develop.

The cigarette beetle oviposits on ethanol extracts of tobacco (Fletcher and Long 1971; Kohno and Ohnishi 1986), and methanol (MeOH) extracts of roasted coffee beans (Fletcher and Long 1971; Hori et al. 2011; Kohno and Ohnishi 1986). These results indicate that chemical factors affect the choice of oviposition sites by beetles. Other stored food pests are known to be stimulated to oviposit by chemicals; e.g., the adzuki bean beetle *Callosobruchus chinensis* (L.) is stimulated by D-catechin (Ueno et al. 1990). Oviposition stimulants for the cigarette beetle have yet to have been identified. In this study, we compared oviposition responses of the beetles to different extracts of food products (roasted coffee beans and black and green tea leaves) that are unsuitable for larval growth, and we identified an oviposition stimulant from roasted coffee beans that elicited the greatest oviposition.

METHODS AND MATERIALS

Insects. Lasioderma serricorne were obtained from cultures at the Leaf Tobacco Research Center of Japan Tobacco Inc. (Tochigi, Japan) in 2007 and maintained in our laboratory. They were reared on corn flour (Nippon Flour Mills Co., Tokyo, Japan) containing 10% dry brewer's yeast (Ebios®; Asahi Food & Healthcare Co., Tokyo, Japan), and maintained at 25±1°C and ca. 70% RH under a photoperiod of 16:8 L:D.

Standard Chemicals. Furfuryl alcohol, palmitic acid, linoleic acid, 2-pyrrolecarbaldehyde and 2-acetylpyrrole were obtained from Wako Chemical Ltd (Miyazaki, Japan), catechol from Tokyo Chemical Industry Co. Ltd, (Tokyo, Japan), 4-ethylcatechol from Alfa Aesar Avocado Organics (Heysham, Lancashire, UK), stearic acid from Sigma-Aldrich (St. Louis, Missouri, USA), and *β*-sitosterol from Acros Organics (Geel, Antwerpen, Belgium).

Methanol Extraction. Because MeOH can dissolve a relatively wide range of organic chemicals, MeOH extracts were used initially to extract oviposition stimuli. Roasted coffee beans, as well as black and green tea leaves were powdered using a mill. The powders (50 g each) were soaked in MeOH (750 ml) for 24 h and then filtered. Extractions were conducted three times. The MeOH extracts were evaporated to dryness under reduced pressure at <40°C.

Extraction and Fractionation of Coffee Beans. Roasted coffee beans (500 g) were powdered by a mill. The coffee powder (497.30 g) was soaked in hexane (2 l) for 24 h and then filtered.

Extractions were conducted 10 times. The residue was then soaked in chloroform 10 times in the same manner as for hexane. Extractions were subsequently conducted using 1-butanol, MeOH, and 20% MeOH in water. Each extract was evaporated to dryness at reduced pressure at <40°C.

The chloroform extract was fractionated by normal-phase silica-gel column chromatography (Wako-gel C-200, Wako Pure Chemical Industries, Osaka, Japan, 200 g). Fractions were eluted with 10, 20, and 100% ethyl acetate in hexane and finally MeOH (1500ml each). Each fraction (Fr.) was evaporated to dryness at reduced pressure at <40°C.

Chemical Analysis by Coupled Gas Chromatography-Mass Spectrometry. Fraction 2 (eluted using 20% ethyl acetate in hexane) of the chloroform extract was analyzed by coupled gas chromatography/mass spectrometry (GC/MS; Shimadzu GCMS-QP2010 Ultra, equipped with a DB-5MS column, 30 m × 0.25 mm i.d., 0.25 µm film thickness, J&W, Santa Clara, CA). Samples were diluted to 1% (w/w) in chloroform, and 1 µl injected into the GC/MS. Chemicals in the fraction were identified by comparing retention times and mass spectra with those of authentic standards. Concentrations of chemicals in fraction 2 were determined by the external standard method. The calibration curve for each chemical was obtained from the peak area of the standard chemicals in the total ion chromatogram. Concentrations were measured five times and values averaged. Helium was used as the carrier gas at a column head pressure of 100 kPa. The GC was set for split injection (split ratio, 100:1). The temperature program of the column oven was as follows: initial temperature 60°C, 3°C.min^{-1} to 200°C, 10°C.min^{-1} to 300°C, and then held for 10 min. The injector, detector, and interface temperatures were 220°C, 200°C, and 240°C, respectively. Mass spectral data were analyzed using Shimadzu GCMS Solution with the Wiley Registry (9th ed.), National Institute of Standards and Technology (NIST05), and Flavor and Fragrance Natural and Synthetic Compounds (FFNSC ver. 1.3) mass spectral databases.

Oviposition Bioassay using Methanol Extracts. The bioassay methods of Hori et al. (2011) were modified slightly. MeOH extracts of coffee bean and black and green tea leaves were tested at 0.01, 0.1, and 1 g dry-leaf (or roasted-bean) equivalent per ml. One milliliter of each solution was applied to two sheets of filter paper (55 mm diam.). Control filter paper was prepared similarly, using solvent alone. After air-drying, the two sheets of filter paper were placed on the bottom of a glass Petri dish (70 mm diam., 20 mm height). As females begin to oviposit within 24 h after emergence, a pair of male and female beetles, within 24 h of emergence from the corn flour diet, was released into the Petri dish after confirmation of copulation. Eight replicates (eight separate Petri dishes) were used for each treatment. The tests were conducted over 6 days under the same conditions as used for rearing. This duration was used because females lay almost all their eggs by seven days after emergence (personal observation). Because adults eat little food, we did not provide adults with food after emergence.

Oviposition Stimulant Bioassay. Each extract from the roasted coffee beans was dissolved in the same solvent as used for extraction. Fractions of the chloroform extract and identified chemicals (standards) were dissolved in chloroform. The concentrations of the solutions were 0.5 or 1 g bean equivalent/ml. Each sample (sample amount was equivalent to 1 g roasted bean) was applied to two sheets of filter paper (55 mm diam.). Oviposition by beetles was assayed by the same method as that for the oviposition bioassay using MeOH extracts.

Statistical Analyses. We performed statistical analyses using R v. 3.0.2 for Mac OS X (R Core Team 2013). In the oviposition bioassay using MeOH extracts of roasted coffee beans, black tea and green tea leaves, egg numbers on an extract were compared across doses by Tukey HSD tests (Hsu 1996), after log transformation [log (x+0.5)]. In the oviposition stimulant assay using coffee extract, the numbers of eggs on each treatment were compared to that of the control using Dunnett's test (Hsu 1996) after log transformation.

RESULTS

Oviposition Response to Methanol Extracts of Coffee Beans and Black Tea and Green Tea Leaves. The number of eggs laid on filter paper treated with MeOH extracts of coffee beans, and black and green tea leaves were greater than that on the control, although this varied with dose (Fig. 1). For coffee extract, the number of eggs laid increased (Tukey HSD test, df = 28, P < 0.001) as dose increased from 0.01 to 0.1 g food equivalent (GFE). However, for black tea and green tea, the number of eggs laid only increased as dose was increased from 0.1 to 1 GFE (Tukey HSD test, df = 28, P = 0.023) and from 0.01 to 1 GFE (Tukey HSD test, df = 28, P = 0.005), respectively. Similar numbers of eggs were laid on each MeOH extract treatment at 1 GFE (Tukey HSD test, df = 21, P > 0.05) (Fig. 1).

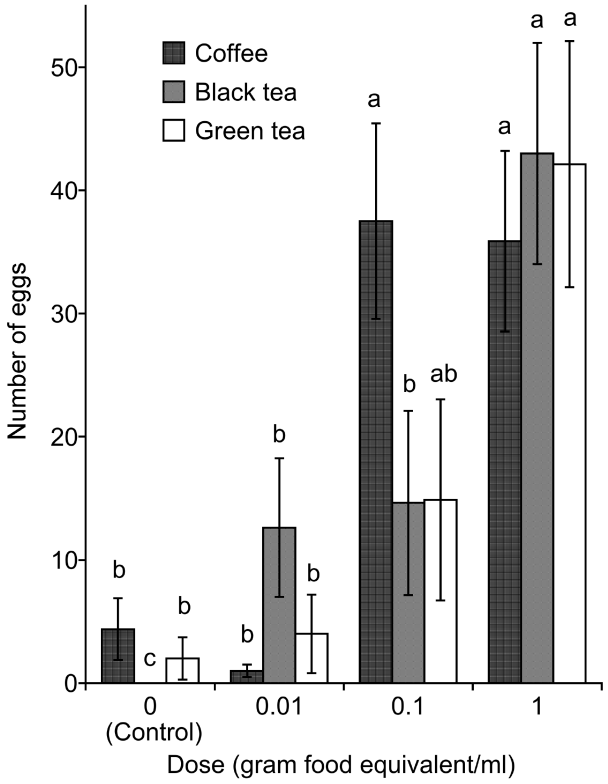

Fig. 1 Numbers of eggs laid by cigarette beetles on methanol extracts of roasted coffee beans and black and green tea leaves. Each extract was provided on filter paper to a pair (male and female) of beetles for six days. Bars with the same letter in the same extract are not different [Tukey HSD test after a log (x + 0.5) transformation, P > 0.05].

Oviposition Response to Fractions of Coffee Bean Extract. The numbers of eggs laid on all extracts (Dunnett's test, df = 47, P < 0.05)

(Fig. 2), with the exception of the hexane one (Dunnett's test test, *df* = 47, *P* = 1.000), were greater than the number laid on the control. The chloroform extract was fractionated into four fractions by silica-gel column chromatography. The number of eggs laid on Fr. 2 was greater than that laid on the control (Dunnett's test, *df* = 112, *P* = 0.022) (Fig. 3). When assays were conducted with mixtures of the fractions, the numbers of eggs laid on Fr. 2 + 3, 2 + 4, 1 + 2 + 4, and 2 + 3 + 4 were greater (Dunnett's test, *df* = 112, *P* < 0.05) than that laid on the control. The numbers of eggs laid on Fr. 1 + 2 and 1 + 2 + 3 were not different (Dunnett's test, *df* = 112, *P* > 0.05) from that laid on the control, despite Fr. 2 being in the mixtures. The numbers of eggs laid on all mixtures without Fr. 2 (Fr. 1 + 3, 1 + 4, 3 + 4, and 1 + 3 + 4) were not different (Dunnett's test, *df* = 112, *P* > 0.05) from the number laid on the control.

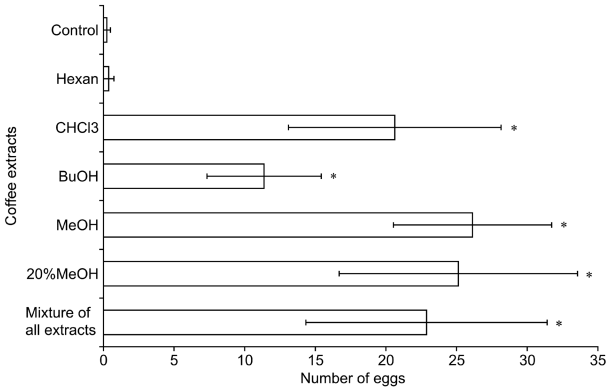

Fig. 2 Numbers of eggs laid by cigarette beetles on various extracts of roasted coffee beans. Each extract was provided on filter paper to a pair (male and female) of beetles for six days. Bars with asterisks are different from the control [Dunnett's test after a log (*x* + 0.5) transformation, *P* < 0.05].

Chemical Analysis of Fraction 2 from Chloroform Extract. Components that made up more than 1% of Fr. 2 were identified by GC/MS, and included (confirmed by comparison with standards) furfuryl alcohol (3.30 min), 2-pyrrolecarbaldehyde (7.03 min), 2-acetylpyrrole (8.78 min), catechol (14.04 min), 4-ethylcatechol (22.32 min), palmitic acid (45.11 min), linoleic acid (49.56 min), stearic acid (50.11 min), and *β*-sitosterol (63.61 min) (Fig. 4A). The mass spectra of the peak at 14.04 min in Fr. 2 (Fig. 4B) and the authentic sample of catechol (Fig. 4C) are given for comparison. Peaks that eluted between 52 and 63 min could not be identified. We determined the amounts of the identified nine components in 1 g of roasted coffee beans as: furfuryl alcohol, 0.0108 mg; 2-pyrrolecarbaldehyde, 0.0082 mg; 2-acetylpyrrole, 0.0056 mg; catechol, 0.0156 mg; 4-ethylcatechol, 0.0115 mg; palmitic acid, 0.1031 mg; linoleic acid, 0.1413 mg; stearic acid, 0.0438 mg and *β*-sitosterol, 0.0262 mg.

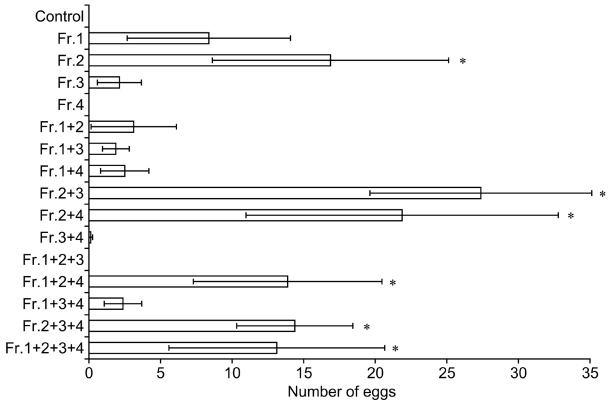

Fig. 3 Numbers of eggs laid by cigarette beetles on fractions of a chloroform extract of roasted coffee beans. was provided on filter paper to a pair (male and female) of beetles for six days. Bars with asterisks are different from the control [Dunnett's test after a log (*x* + 0.5) transformation, *P* < 0.05].

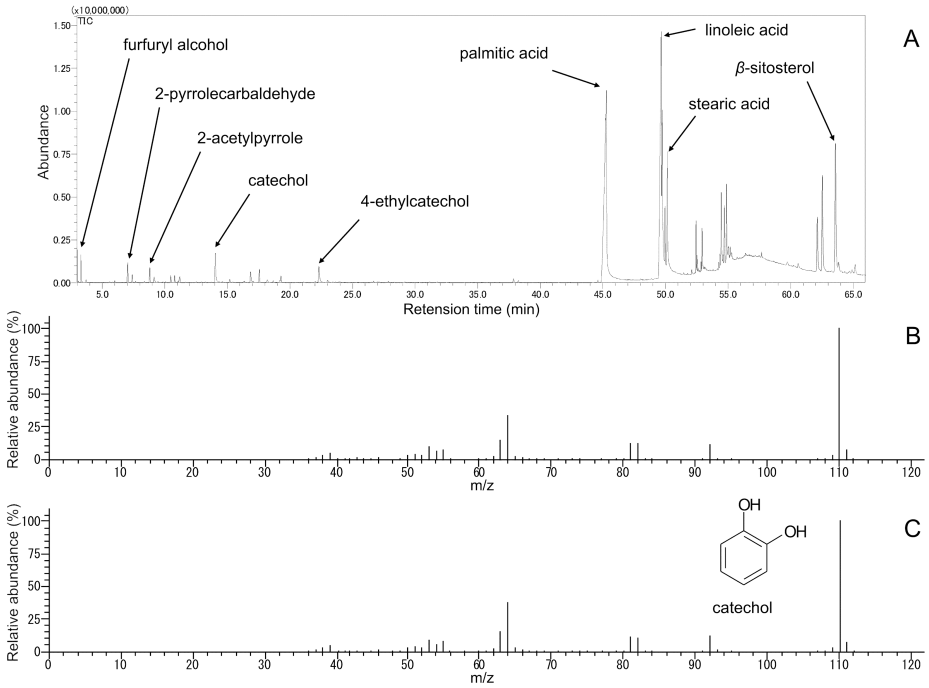

Fig. 4 Gas chromatography/mass spectrometry analyses. A) Total ion chromatogram (with identities of peaks) of fraction 2 of a chloroform extract of roasted coffee beans. Peaks eluting between 52 and 63 min. could not be identified. B) Mass spectrum of the peak detected at 14.04 min. C) Mass spectrum of the authentic sample of catechol.

Oviposition Response to Identified Chemicals. Of the nine identified chemicals in Fr. 2 of the chloroform extract, beetles only laid significant numbers of eggs on filter papers treated with catechol (Dunnett's test, $df = 77$, $P < 0.001$) compared to the control (Fig. 5). Some eggs were laid on filter papers treated with 4-ethylcatechol, although the number was not different (Dunnett's test, $df = 77$, $P = 0.084$) from that on the control. The beetles deposited few eggs on the other treatments.

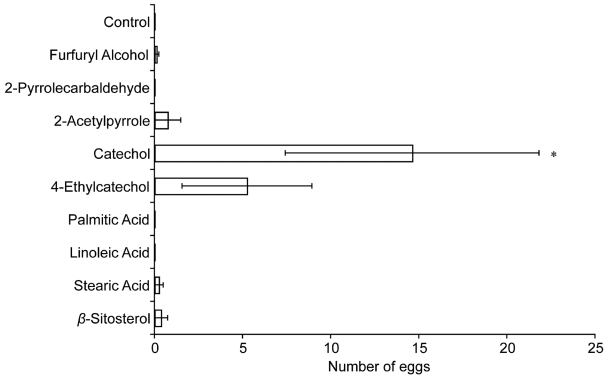

Fig. 5 Numbers of eggs laid by cigarette beetles on filter papers treated with each of the nine chemicals identified in fraction 2 of a chloroform extract of roasted coffee beans. The bar with the asterisk is different from the control [Dunnett's test after a log $(x + 0.5)$ transformation, $P < 0.05$].

DISCUSSION

The number of eggs laid by cigarette beetles generally increased with an increase in dose of MeOH extracts of roasted coffee beans or dry leaves of black or green teas, demonstrating that chemicals stimulate cigarette beetles to oviposit. Because greater numbers of eggs were laid on roasted coffee bean extract than on the other two treatments at 0.1 GFE, we investigated oviposition stimulants in roasted coffee beans.

Chloroform, 1-butanol, MeOH, and 20% MeOH extracts all stimulated oviposition by beetles. Reasoning that a narrower range of chemicals was likely to be extracted in choloform, we focused on this extract. Among the four fractions from the chloroform extract, only Fr. 2 alone and mixtures containing Fr.2 elicited significant oviposition. Of the nine compounds identified by GC/MS analysis in Fr. 2, beetles only laid significant numbers of egg (relative to the control) on filter papers treated with catechol. Therefore, catechol is an oviposition stimulant for cigarette beetles.

It is known that catechol content in coffee beans increases with increased roasting (Moon and Shibamoto 2009). Catechol content is also increased by roasting cocoa beans (Ziegleder 1991). This suggests that roasting causes beetles to oviposit on coffee and cocoa beans. Catechol is known to be produced by the decomposition of *O*-caffeoylquinic acids (Clifford 1979; Haffenden and Yaylayan 2005; Lang et al. 2006; Müller et al. 2006). As the larvae of cigarette beetles feed on stored foods, the adults may use degradation compounds that increase after harvest as oviposition stimulants. However, catechol has not been found in black or green tea (Hida et al. 1998), although the beetles lay many eggs on these materials (Hori et al. 2011). This indicates substances other than catechol must also stimulate cigarette beetle oviposition. Roasted coffee beans likely contain oviposition stimulants, other than catechol, because other, more polar, extracts also stimulated oviposition.

We believe that the use of oviposition deterrents to control insect pests is promising. However, it may not be appropriate to use these chemicals directly on food products. As an alternative, if cigarette beetles could be stimulated to oviposit, using oviposition stimulants, on materials other than foods that they can develop on, then we may be able to control their reproduction. Although egg fertility of the beetles varies with conditions, the average number of eggs produced by females is around 100. Moreover, most eggs are laid relatively soon after eclosion (Howe 1957). Therefore, we may be able to regulate the cigarette beetle populations by inducing them to lay most of their eggs on substrates unsuitable for development. Further work is needed to determine this.

Adults of many species of Coleoptera are attracted to volatile chemicals. For example, many longhorn, bark and ambrosia beetles can be caught in traps baited with ethanol and α-pinene (Miller 2006; Miller and Rabaglia 2009). The dock leaf beetle, *Gastrophysa polygoni*, is attracted to a mixture of (*Z*)-3-hexenal and (*Z*)-3-hexenyl acetate, which are emitted from leaves of the host *Rumex confertus* infested by beetles (Piesik et al. 2011). The cigarette beetle is also known to be attracted to food odors (Mahroof and Phillips 2007) and its sex pheromone (Chuman et al. 1979; Levinson et al. 1981). Combination of these attractants with oviposition stimulants may prove to be a more effective control method than use of these compounds alone.

Acknowledgments—We wish to thank the Leaf Tobacco Research Center of Japan Tobacco, Inc., for kindly supplying the insects. This work was supported by The Tojuro Iijima Foundation for Food Science and Technology Research Grant 2013.

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