JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2005, 53, 4633–4636 4633

Citronella as an Insect Repellent in Food Packaging

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Of five commercial plant extracts (citronella, garlic oil, neem extract, pine oil, and pyrethrum), citronella was found to be effective in deterring the infestation of cartons containing muesli and wheat germ by red flour beetles. The chemical components were applied as part of a coating on the carton board. In an experimental set up that accelerates infestation over a 2 week period, citronella-treated cartons (0.2 g/m^2 of carton board) reduced beetle infestation to approximately 50% of the level observed in control cartons. Evidence was provided to indicate that an insect repellent effect persists for at least 16 weeks. Additional work on the controlled release of the insect repellent would be required to prolong the effect.

KEYWORDS: **Essential oils; insect repellent; citronella; packaging; stored-products pests; infestation**

INTRODUCTION

The increasing transportation of goods and travel by people has made it more difficult to manage biosecurity hazards worldwide. For foods, produce, and food products, insect infestation leads to the additional problems of spoilage and loss. Further difficulties also occur for fresh produce because it may be carrying insects as it is being packed. In recent times, the control of insect infestation is generally handled using fumigation with methyl bromide. However, there is an urgent desire to phase out the use of this chemical as a fumigant because it contributes to the depletion of the Earth's ozone layer (*1*). Another disadvantage of using insecticidal fumigants and insecticides in general is that dead insect carcasses remain in the package, thus reducing its general appeal. There are therefore a number of opportunities for insect repellent packaging that effectively reduces the presence of insects and the requirement for chemical fumigants.

There have been many studies on the controlled release of insect repellent (*2*−*4*) and the incorporation of insecticides into packaging materials (*5*−*8*). Two patents are known to describe the incorporation of insect repellents into paper-based packaging (*9*, *10*). To our knowledge, there have only been two paperboard products marketed for their insect repellent properties. RepelKote is a product of Tenneco Packaging (now Pactiv Corp., United States) that incorporates methyl salicylate into a coating for paperboard (*9*). More recently, newspaper articles in 2003 (e.g., Nikkei, Tokyo, 2 September 2003) reported that Oji Paper (Japan) has started manufacturing papers and adhesive tapes that are surface-treated with a combination of plant substances, mainly hinokitiol, which act as insect repellents. The methyl salicylate product has not yet gained widespread use, while the success of the hinokitiol product remains to be seen.

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For paper-based packaging, insect repellents could be applied on carton board (for breakfast cereal, confectionery, and pet food), bags/sacks (grains, stock feed, milk powder), and container board (produce, secondary packaging). The use of natural plant extracts in this application could facilitate acceptance by food regulators as well as the general public. Many of the repellents are highly volatile and are readily lost from carton board unless they are partially immobilized. The present report discusses the development of a bioassay for the evaluation of insect repellent packaging, the use of paper board coatings as carriers of insect repellents, and the persistence with which citronella-treated cartons deter beetle infestation.

MATERIALS AND METHODS

Insects and Feed. A small colony of red flour beetle (*Tribolium castaneum* Hbst.) was reared on wheat germ obtained in bulk from a local supermarket. In the cartons used for bioassays to assess infestation levels, toasted muesli (First Choice Ltd, Manukau, New Zealand) was added to the wheat germ at 1:1 by weight to increase the level of attractants.

Candidate Insect Repellent. The selection of plant-derived essential oils and extracts included the following: (i) citronella (leaf, 100% v/v pure essential oil) from *Cymbopogon nardus*, distributed by Sunspirit Oil Pty Ltd. (Byron Bay, NSW, Australia); (ii) garlic oil from Red Seal Natural Health (Auckland, New Zealand); (iii) neem 900 EC distributed by Suntec NZ Ltd. (Tokomaru, New Zealand); (iv) pine oil (needle, 100% v/v pure essential oil) from *Pinus sylvestris*, distributed by Sunspirit Oil Pty Ltd; and (v) pyrethrum product (Coopers; distributed by Yates NZ Ltd.) containing 14 g/L of pyrethrum and 56.5 g/L of piperonyl butoxide. Dilution of these liquid products was generally based on their weight, except that the pyrethrum product was diluted according to the reported amount of pyrethrum in this liquid formulation.

Gas Chromatography−Mass Spectrometry (GS-MS) Analysis. The citronella sample was diluted to 5 mg/mL in dichloromethane. An aliquot of 1 μL was injected into the GC-MS system (HP5890 Series II Gas Chromatograph interfaced with HP 5971A Mass Spectrometer)

10.1021/jf050096m CCC: $30.25
Published on Web 04/27/2005

equipped with an Ultra 2 (cross-linked (5%-phenyl)-methylpolysiloxane) capillary column. The GC conditions were as follows: helium carrier gas; injector temperature, 280 °C; initial oven temperature at 60 °C for 1 min, increased at 10 °C/min to 205 °C, then at 3 °C/min to 270 °C, and finally 6 °C/min to 300 °C; total run time, 85 min; MSD detection.

Carton. The carton board (19 × 24 cm^2) was obtained from a carton board mill, and it was 550 μm in thickness and coated with clay latex. After it was coated again in the laboratory with a coating containing insect repellent, it was left to dry overnight under ambient conditions. The coated board was taken to a converter where it was cut and scored with a die, partly folded, and glued along the spine with polyvinyl acetate. The trial was started when the partly assembled cartons were brought back to the laboratory on the same day. The length:width: height dimensions of the assembled cartons were 8.0 × 7.5 × 3.0 cm^3, with tucked in ends. A single unsealed greaseproof paper bag (14 × 18 cm^2; York, Auckland, New Zealand) containing feed (5 g each of wheat germ and toasted muesli) was placed in each carton being tested.

Coating. A commercial coating formulation used as a precoat was collected from a carton board mill. This precoat formulation was based on Georgia Kaolin clay with 14% latex binder and contained calcium stearate and polyacrylate.

The candidate repellent was generally dissolved in 70% ethanol (exceptions: water was used for pyrethrum; 95% ethanol was required to apply citronella at the high concentration of 0.5 g/m^2 on carton board) and mixed 1:1 (v:v) with the coating formulation. This coating mix was then applied on to the carton board with a K Control Coater (RK Print Coat Manufacturer Ltd., Litlington, United Kingdom), using a size 40 wired steel rod (wire diameter of 0.02 in. that typically forms wet film deposit of 40 μm). This rod was chosen because it yielded a coat of 10 g/m^2 solids. The stock solutions of candidate repellents were varied to yield coatings with the selected application level.

Citronella was also added as an ingredient in zeolite (Molecular Sieve M3010, sodium-alumino-silicate, particle size ≤ 10 μm, pore size diameter of 1 nm; Sigma, United States). The zeolite was first activated by heating at 250 °C for 18 h and stored over phosphorus pentoxide. The amount of citronella required for each application level was added to activated zeolite (2 g for every mL of citronella), before mixing with the commercial coating formation (diluted with water) using a Ultra-Turrax T25 basic disperser (S 25 KV-25 G-IL dispersing element, turbine rotor with eight teeth; IKA, Staufen, Germany). After zeolite was blended into the coating formulation, the coating of carton board was carried out using the K Control Coater. Only a few streaks formed in the coating, indicating that only a few of the particles were too large for the production of a uniform coating (typically requires particle size <3 μm).

Growth Room and Chambers. Insect rearing and trials were carried out in a growth room kept at 25 °C and ambient relative humidity. The red flour beetles were reared in cans containing wheat germ. Trials were carried out in clear poly(methyl methacrylate) boxes (71 × 51 × 30 cm^3) with a screened lid. The bottom of each box was lined with carton board to provide grip for the beetles and to mark sectors along a circumference for placement of cartons for the trials (*11*). The central circle was where the beetles were placed to start a trial. In general, the number of beetles introduced to each box was equivalent to 20 beetles for each carton in the box.

Beetle Counts and Subsequent Analyses. In general, most of the beetles were found in cartons rather than on or around cartons. Therefore, beetles on or in individual cartons in each box were counted for each trial, by removing the cartons from the box and spreading out their contents to find the beetles. The initial experiments had a duration of less than 2 weeks, and the beetles and feed were returned to their respective cartons after each count. Between counts in long-term experiments, the beetles were left to roam in the boxes, among cartons containing feed. These beetles and feed were removed from the boxes before "fresh" feed was added to each carton and "fresh" beetles from rearing colonies were added to the central circle the day before the next count.

At least two replicate trials were carried out for each experiment. Each trial involved two boxes each containing two cartons for each treatment. The placement of cartons was randomized, except cartons with the same treatments were placed across each other in the circle. Proportional infestation was the ratio of beetles in a carton and the total number added to the box. Both the mean results (shown in the figures) and the median results (data not shown) provided similar patterns.

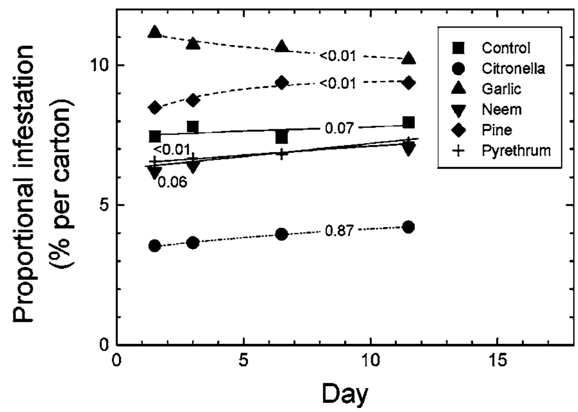

Figure 1. Infestation of cartons ($n = 8 = 2$ cartons × 2 boxes × 2 trials) coated with different candidate repellents at an application level of 0.2 g/m^2. Evidence for effect of treatment: $Pr > F = 0.15$; each curve is labeled with posterior probability that the corresponding treatment is the least preferred. The average of the standard errors for the four data points of each treatment was 1.9% for the control, 1.2% for citronella, 1.8% for garlic oil, 1.9% for neem extract, 2.0% for pine oil, and 1.4% for pyrethrum.

Statistical Analyses. Calculations and model fitting were carried out on a logit scale, using R System for Statistical Computation and Graphics (www.R-project.org) (*12*). The generalized linear model was used to estimate the "adjusted dependent variable" (*13*) that was then analyzed using a linear mixed model (*14*), weighted inversely proportional to variance. Where appropriate, the selected model included a Compound Symmetry structure for random treatment effects at the trial (at least two trials for each experiment) and box (two boxes for each trial) levels, allowing for negative correlations between cartons within boxes.

RESULTS

Initial Screening of Different Candidates. In the first series of trials, beetles were placed in boxes containing cartons treated with different chemicals at 0.2 g/m^2 application levels, and their location was monitored periodically. As shown in **Figure 1**, the mean population of beetles in each type of carton was relatively stable over a 2 week period, despite the large variation among cartons in different boxes. Only the cartons treated with citronella showed substantially lower infestation levels than the control cartons, reducing infestation by approximately 50%. Although this decrease was not statistically significant ($Pr > F = 0.15$), citronella was selected for further evaluation because this treatment was least preferred by the beetles (posterior probability of 0.87). GC-MS analysis of the commercial citronella product indicated that it was composed of 27.9% limonene, 14.2% α-terpineol, 8.7% citronella, and 7.8% geraniol (**Figure 2**).

Dose Response. The response of beetles was examined using different application levels of citronella on carton board. **Figure 3** shows the evidence for an effect of dose ($Pr > F = 0.003$). The application level of 0.2 g/m^2 yielded slightly better results than that of 0.05 g/m^2, reducing infestation to 40−50% of that found for control cartons. At the application level of 0.01 g/m^2, the increase in infestation with time suggested that beetles delayed their migration into these cartons and thus were moving among cartons during the trials.

Long-Term Experiments. Three sets of long-term experiments of up to 35 weeks were conducted to evaluate the

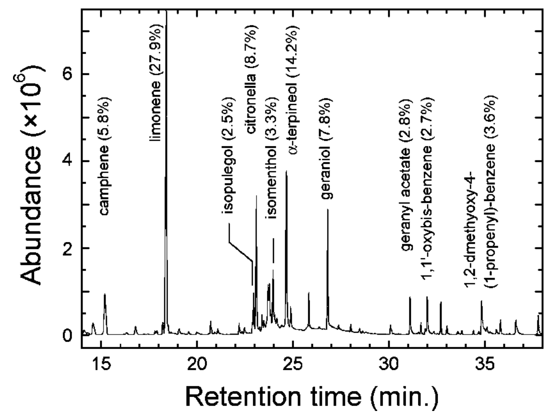

Figure 2. GC-MS analysis of citronella sample showing components constituting at least 2% of the commercial product (unidentified component at 23.7 min; constituted 2.7%).

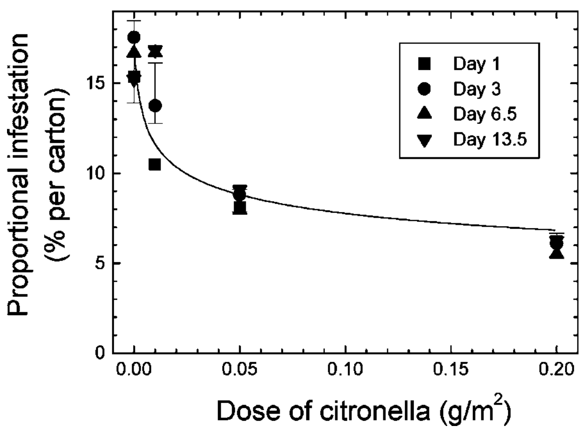

Figure 3. Infestation of cartons ($n = 8$) coated with different application levels of citronella. Evidence for effect of treatment: $Pr > F = 0.003$; error bar = standard error about the overall mean for each dose; line = statistical model fitted to raw data on logit scale.

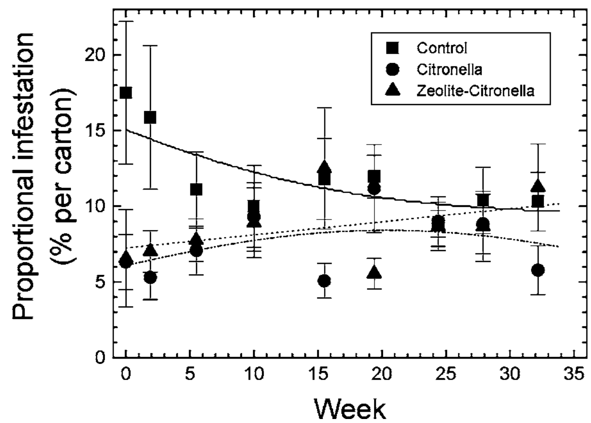

Figure 4. Infestation of cartons ($n = 8$) coated with citronella (application level of 0.2 g/m^2) by fresh beetles. Citronella was added to the coating as a solution in ethanol (citronella) or as an adsorbent on zeolite (zeolite-citronella). Evidence for effect of treatment: $Pr > F = 0.22$; error bars = standard error about data points; lines = statistical model fitted to raw data on logit scale.

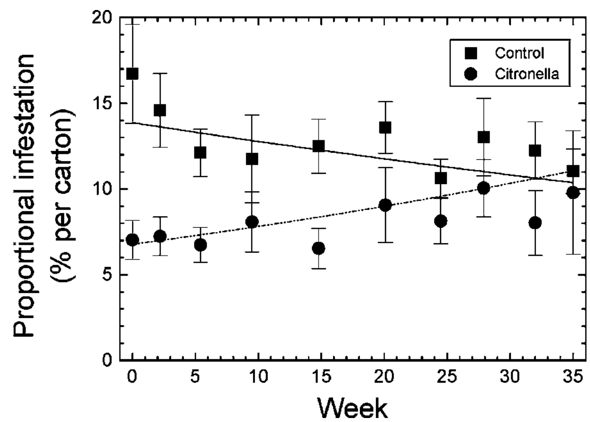

Figure 5. Infestation of cartons ($n = 6$–32) coated with citronella (application level of 0.2 g/m^2) by fresh beetles. The data from three sets of experiments were pooled, with each data point in the graph representing the mean infestation at the indicated week ± 1.6 weeks. Evidence for effect of treatment: $Pr > F = 0.01$; error bars = standard error about data points; lines = statistical model fitted to raw data on logit scale; lines estimated to cross at 32 weeks (95% C. I. = 16–68 weeks).

persistence of various doses of citronella and the use of surfactants or zeolite for convenient application of citronella. The replacement of beetles with "fresh" beetles, before each count in long-term trials, increased the variability of the counts among different cartons and thus yielded statistically inconclusive results. Adsorption of citronella to zeolite did not appear to alter the effectiveness of citronella applied at 0.2 g/m^2 (**Figure 4**). The amount of citronella in these carton board samples could not be compared because it was below the detection limit of conventional GC in conjunction with solid phase microextraction. Preliminary results were also obtained with surfactants (sodium dodecyl sulfate, Triton, and Tween; data not shown), indicating that certain surfactants altered the response of the beetles (e.g., Triton attracted beetles), while others altered the quality of the coating (e.g., Tween yielded nonuniform coatings).

Each of the three sets of long-term experiments had controls and treatments prepared using carton board coated with citronella at an application level of 0.2 g/m^2, the latter designated as the "positive control". This large data set allowed statistical differentiation of the control from the positive control ($Pr > F = 0.01$). Infestation of the control cartons declined over time while that of the positive control cartons increased, with the estimated time for the two lines to cross being 32 weeks (**Figure 5**). The corresponding 95% confidence interval suggested that the insect repellent effect of carton board treated with citronella persisted for at least 16 weeks.

DISCUSSION

A bioassay was established for the evaluation of insect repellent packaging, based on accelerated infestation of unsealed cartons containing dry food. It could be used to compare different treatments or controlled release systems to identify effective agents. In long-term experiments where freshly added insects were used to determine infestation levels, the higher experimental variation suggested that a sample size of approximately 24 is required for the observation of statistically significant results. More stringent control of experimental conditions (such as lighting, air flow, relative humidity, insect rearing, and feed) may reduce the minimum sample size required. The effectiveness of any active ingredient identified by the bioassay would need to be confirmed using field trials that consider storage and transport conditions and the complete sealing of the carton and inner packaging.

The bioassay was successfully used to identify a potential for using a commercial citronella preparation as the active ingredient against red flour beetles and to demonstrate a dose-dependent response. The initial screening of several plant extracts was not exhaustive, as it assessed only one extract preparation, one application level, and one pest. Therefore, the results could not be used to invalidate previous reports that extracts from garlic (*15*), neem (*16*), and pine (*17*) have insect deterrence properties. Although citronella has previously been identified as a natural insect repellent (*9*, *18*), there has been little published results on its application to packaging materials. The use of citronella in food packaging is particularly attractive because it is already used for food flavoring (commonly known as lemon grass). The commercial product examined in the present study contained substantial amounts of limonene and α-terpineol and thus appeared to be mixed with lime juice. Further experimentation would be required to evaluate the contribution of the different components in the commercial citronella.

The results of the present study indicate that commercial citronella applied at 0.2 g/m^2 of carton board could reduce beetle infestation, initially by approximately 50%. Insect repellency appeared to endure for at least 16 weeks when the citronella was added directly into carton board coating as a solution in ethanol. To find ways to avoid using flammable alcohol in paper mills, zeolite was evaluated as a citronella carrier because it has been successfully used for the sustained release of volatiles (*19*). The results were, however, statistically inconclusive. Other controlled release mechanisms include microencapsulation in carbohydrates (*20*−*22*), proteins (*23*), and their composites (*24*, *25*).

ACKNOWLEDGMENT

We thank Bill Faulds for his assistance and advice on insect rearing and handling and Nathan Hamilton for his assistance in setting up trials and collecting data. We also thank Rod Ball for carrying out the statistical analyses. The initial colony of red flour beetles was provided by Genera Ltd. (Mt. Maunganui, New Zealand), and the carton board was scored, cut, and glued by Busy Bee Cartons Ltd. (Tauranga, New Zealand).

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Received for review January 15, 2005. Revised manuscript received March 30, 2005. Accepted March 31, 2005.

JF050096M