Novel use of aliphatic \(n\)-methyl ketones as a fumigant and alternative to methyl bromide for insect control

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Abstract

BACKGROUND: Fumigants like phosphine, methyl bromide and sulfuryl fluoride are highly effective for the control of structural, storage and agricultural arthropod pests. Unfortunately, many of these synthetic compounds are highly toxic to people, many pests have developed resistance to these compounds and methyl bromide, the ‘gold standard’ for fumigants, was de-registered because of its contribution to depletion of the stratospheric ozone layer. Alternative fumigant chemistry is needed.

RESULTS: Several plant species produce \(n\)-aliphatic methyl ketones to prevent plant herbivory. To examine the use of methyl ketones as a fumigant, structure–mortality studies were conducted using the red imported fire ant, *Solenopsis invicta* Buren, as a model. A new easy-to-use, inexpensive and disposable bioassay system was developed for this study. The LC\(_{50}\) values for heptanone, octanone, nonanone and undecanone were 4.27, 5.11, 5.26 and 8.21 \(\mu\)g/cm\(^3\) of ambient air, respectively. Although heptanone, octanone and nonanone were more effective than undecanone, subsequent research was conducted with 2-undecanone because this compound already has US Environmental Protection Agency (EPA) registration as a biopesticide. In dose–response field studies, 12.4 mL of undecanone injected into mounds was the lowest application rate that produced no ant activity in the mound with no re-establishment of ants. Reagent grade undecanone was more cost-effective than methyl bromide for fire ants, adult German cockroaches and tobacco budworm eggs, but slightly more expensive for adult flour beetles.

CONCLUSION: The naturally occurring methyl ketone undecanone has the potential to be an alternative to current fumigants for a variety of pest applications.

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Keywords: fumigant; methyl bromide; methyl ketone; 2-undecanone; *Solenopsis invicta*

1 INTRODUCTION

Fumigation is an effective and efficient method for controlling structural, storage and agricultural pests, and methyl bromide (MeBr) has been the fumigant of choice for many years. MeBr has broad spectrum activity against insects and other pest species including nematodes, plants and bacteria, and has been used in many different applications including soil, storage and transport of goods, for structural and agriculture pests prior to planting, and for post-harvest products. One important attribute of MeBr is its low absorption rate; this allows for rapid and efficient penetration into the substrate being treated, but at the same time, rapid dissipation after ventilation with MeBr-free air. There are also some negative aspects to this technology. For example, applicators must be thoroughly trained in the safe use of the product, and permits are required for applications. Because MeBr is an odorless and colorless gas, it cannot be detected readily. Consequently, safe handling is critical to avoid accidental exposure. Accidental injury from the use of MeBr continues to occur, regardless of safety precautions. Tracer (warning) compounds such as chloropiridrin that are easily detected by smell or tearing at low levels are required as an addition to MeBr to treat some commodities or fields of use. However, additives produce transient symptoms that can be quite uncomfortable. The major shortcoming of MeBr is its contribution to depletion of the stratospheric ozone layer. Under the Montreal Protocol and the Clean Air Act, the production and use of MeBr was to be phased out after 1 January 2005. There are limited MeBr alternatives available, i.e., sulfuryl fluoride and phosphine, but these too are US Environmental Protection Agency (EPA)-restricted use compounds because of safety concerns. All these fumigants are synthetic chemistries. The concerned public is looking for solutions that come from natural sources, which are potentially safer, but are at least as efficient and cost-effective as current synthetic technologies for fumigation.

The red imported fire ant, *Solenopsis invicta* Buren, the model insect pest used in this study to evaluate our new potential fumigants, was introduced into the USA in Mobile, Alabama in the 1930s. Since then, *S. invicta* has spread and currently occupies a much greater area, now occurring in the southern USA from the Carolinas to Texas and in California. Fire ants are known...
for their aggressive behavior and are notorious for their potent sting, inflicting pain and inducing hypersensitive reactions in humans. They also form large colonies at high densities, are capable of damaging agricultural machinery and interfering with crop production, and can displace native ant species, affecting the ecology of new areas where they become established.3,4 The economic impact of the red imported fire ant was estimated to be US $5 billion in the USA.5 The warm, wet weather of the southern USA is ideal for fire ants, and they flourish in crop lands, roadsides, parks and residential lawns.6 Fire ants in hay pastures can cause equipment breakdowns and in hay are a hazard to workers and harmful to livestock. Infested hay is also regulated and cannot be shipped to areas of the USA where fire ants are not yet established. Shipment of regulated items from infested areas under federal quarantine must be fumigated before shipping, but as discussed earlier, desirable fumigants for this application are not available.

Plant-based insecticides have been used for centuries.7,8 Many of them are secondary plant substances such as quinones and essential oils.9 Surprisingly, many of the same compounds are found in cosmetics, foods and pharmacological additives where they provide flavor and fragrance. The glandular trichomes of the wild tomato plant, Lycopersicon hirsutum Dunal f. glabratum C. H. Müll, produce an aliphatic methyl ketone, 2-undecanone, that protects the plants against herbivorous caterpillars.10 A variety of methyl ketones are produced by other plants. For example, 2-nonanone, the main constituent of Algerian oil of rue can also be found in the essential oil of cloves, Eugenia coryophyllata, and 2-heptanone is found in cinnamon oil and in oil of cloves. Nonanone, undecanone and tridecanone have been identified in the essence of coconut oil. Many plant species produce volatile essential oils in which 2-undecanone or a combination of methyl ketones occur mixed with other organics to protect the plant from herbivory by arthropod pests.11–14

The compound 2-undecanone has strong repellent activity against mosquitoes and ticks, and has been developed into a US EPA-registered commercial arthropod repellent, BioUD® (HOMS LLC, Clayton, NC, USA), for use on skin and clothing to control mosquitos and ticks. BioUD® was two to four times more repellent than N,N-diethyl-meta-toluamide (DEET) against three species of ixodid ticks, Amblyomma americanum, Dermacentor variabilis and Ixodes scapularis, in in vitro two-choice assays using filter paper as a substrate15 and showed >90% repellency for 5 weeks on cotton cheesecloth against A. americanum.16 When applied to human skin under field conditions, BioUD also provided excellent repellency against mosquitoes, more efficacious than 30% DEET for 6 h.10 Like many spatial repellents, 2-undecanone also demonstrated insecticidal activity as a vapor which has not been previously investigated. Because 2-undecanone is registered by the US EPA as a biopesticide, it has both repellent and insecticidal activity as a vapor, it has a safe toxicity profile including being approved by the US EPA for use on human skin, and undecanone is found in nature (produced by plants), we examined the potential use of this compound, and its related chemistry as a fumigant and possible alternative to MeBr using the red imported fire ant as a model insect system (with selected comparisons with other insect pests).

2 MATERIALS AND METHODS

2.1 Insects

Red imported fire ants, Solenopsis invicta, were collected using an insect aspirator (model number 1135A, BioQuip Products Inc., Compton, CA, USA) from colonies within a 50-m radius of the Dearstyne Entomology Building (35°47’18.9”N 78°41’56.7”W; Fig. 1) on the North Carolina State University Campus (Raleigh, NC, USA). Approximately 500 fire ants were transferred to nest material (a plastic Petri dish 14 cm in diameter and 1.5 cm high, the bottom dish filled with dental plaster up to a height of 8 mm) and one or more collections placed into plastic trays (40 × 52 × 10 cm) painted along the top (inside) open edge of the container with a Teflon® emulsion (PTFE-30, Dupont, Wilmington, DE, USA) to prevent the ants from escaping. A 20% Teflon® emulsion was made of fluon (Catalog # 2871D, BioQuip) in distilled water (v/v) Only the top 6 cm of the tray sides was coated using a cotton ball. The Petri dish provided the ants with an artificial nesting site. Ants

Figure 1. (Left) Aerial view of the site (35°47’18.9”N, 78°41’56.7”W) where fire ants were collected for the laboratory methyl ketone structure–activity toxicity studies and the field evaluation of 2-undecanone for fire ant mound control. Red dots indicate location of the actual mounds. (Right) Close-up of flagged fire ant mounds used for field studies (in same area shown on left).

were held at 25 ± 3 °C and 60 ± 5% relative humidity (RH) with a photoperiod of 12: 12 h (L:D) and were supplied ad libitum a 10% (w/v) sucrose (99.7% purity; Acros Organics, Fisher Scientific, Pittsburgh, PA, USA) solution in distilled water (provided via a cotton wick). Tobacco budworm, Heliothis virescens, eggs were purchase from Benzon Research Inc. (Carlisle, PA, USA). At the start of the experiment, the eggs were 1 day old. A German cockroach, Blatella germanica, colony was established from insects provided by TyraTech Inc. (Morrisville, NC, USA). The colony was maintained in a plastic bucket (22 cm in diameter and 24 cm high). The top 6 cm from the opening on the inside of the container was treated with Teflon® emulsion as described before. The opening was covered with cheesecloth (26 × 26 cm; Uline, Hudson, WI, USA) and held in place with two rubber bands to prevent cockroaches escaping. The colony was held at 25 ± 3 °C and 60 ± 5% RH with a 12: 12 h (L:D) cycle and provided ad libitum Home 360® premium dog food (Hannaford, Portland, ME, USA) and distilled water (using cotton wicks). A red flour beetle, Tribolium castaneum, colony (North American GA-1) was a gift from M. Lorenzen (Department of Entomology, NCSU). This colony was originally collected in a farmer’s corn bin in Georgia, USA in 1980.17 The beetles are reared in a mason jar (8 cm in diameter and 13 cm high) at 25 ± 3 °C and 60 ± 5% RH with a 12: 12 h (L:D) cycle and fed Pillsbury wheat flour (The Pillsbury Co., Minneapolis, MN, USA)/brewer’s yeast (MP Biomedical, Salon, OH, USA (95: 5 w/w).

### 2.2 Methyl ketones

Technical methyl ketones were purchased from Sigma-Aldrich (St Louis, MO, USA). The methyl ketones were 2-undecanone, 2-nonanone, 2-octanone and 2-heptanone (Table 1). All four methyl ketones were a liquid at room temperature. The 2-undecanone (CAS number 112-12-9) was 99% pure and appeared colorless to light yellow with a density of 0.825 g/mL at 25 °C; 2-nonanone (CAS number 821-55-6) was ≥99% pure and colorless to faint yellow with a density of 0.82 g/mL at 25 °C; 2-octanone (CAS number 111-13-7) was ≥98% pure and colorless to pale yellow with a density of 0.819 g/mL at 25 °C; and 2-heptanone (CAS number 110-43-0) was ≥98% pure and colorless with a density of 0.82 g/mL at 25 °C. These methyl ketones are volatile compounds at room temperature; the bottles in which they were received were wrapped (several times) between the plastic cap and bottle with Parafilm®. These methyl ketones were stored in plastic cap and bottle with Parafilm® and kept in a fume hood at room temperature until needed. All methyl ketones were used within 6 months of receipt from Sigma-Aldrich.

### 2.3 Fire ant fumigation bioassay and dose response

A fumigation chamber was constructed with two plastic Petri dish bottoms (each 8.6 cm in diameter and 1.8 cm high), stacked lip-to-lip to create a total inside volume of 210 cm³ (Fig. 2). The two Petri dish halves were separated by a 10 × 10 cm section of cheesecloth. The technical methyl ketone to be tested is applied to the lower chamber on a glass cover slip. The chamber above the cheesecloth contains a 2 cm cotton wick saturated with 10% sucrose; 25 fire ants of random size and caste were used for each test.

![Figure 2. Fumigation chamber consisting of two plastic Petri dish bottoms which, as shown, create an inside space 8.6 cm in diameter and 3.6 cm high. The total volume is 210 cm³ (test arena). The two Petri dish halves were separated by a 10 × 10 cm section of cheesecloth. The technical methyl ketone to be tested is applied to the lower chamber on a glass cover slip. The chamber above the cheesecloth contains a 2 cm cotton wick saturated with 10% sucrose; 25 fire ants of random size and caste were used for each test.](image-url)

| Table 1. Fumigation LC₅₀, LC₈₀, 95% confidence intervals, \( \chi^2 \), slope and \( R^2 \) values for heptanone, octanone, nonanone and undecanone against fire ants |
|-----------------|--------|------------------|------------------|------------------|------------------|------------------|------------------|
| Methyl ketone   | n      | LC₅₀ (µg/cm³)   | 95% CI           | LC₈₀ (µg/cm³)   | 95% CI           | \( \chi^2 \)    | Slope ± SE       | \( R^2 \) |
| Heptanone       | 75     | 4.27<sup>AB</sup> | 2.14–7.88       | 5.37<sup>AB</sup> | 3.50–12.90       | 6.47            | 4.66 ± 0.66      | 0.94          |
| Octanone        | 75     | 5.11<sup>A</sup> | 3.66–6.64       | 6.38<sup>A</sup> | 4.86–8.62       | 4.46            | 8.74 ± 1.36      | 0.93          |
| Nonanone        | 75     | 5.26<sup>A</sup> | 4.24–6.39       | 7.58<sup>AB</sup> | 6.39–9.47       | 2.05            | 7.16 ± 0.85      | 0.96          |
| Undecanone      | 75     | 8.21<sup>B</sup> | 7.24–9.12       | 9.85<sup>AB</sup> | 8.85–11.15      | 1.47            | 10.65 ± 1.17     | 0.96          |

<sup>a</sup> LC, lethal dose. Results were corrected for control mortality using Abbott’s correction. LD₅₀ and LD₈₀ values followed by capital letter are not significantly different based on the 95% confidence interval (CI).
held in place by a 1.5 × 2.5 cm strip of Scotch® tape (3 M, Maplewood, MA, USA). The two Petri dish bottoms were held together and sealed by wrapping with several layers of Parafilm® (Fisher Scientific), and the insects were held at 25 ± 3 °C and 60 ± 5% RH for 24 h. After the exposure period, mortality in the treatment and control was recorded, where death was defined as no movement of the insect when touched with a blunt probe. Only the two Petri dish bottoms of the fumigation chamber were re-used; the inside was rinsed with 95% ethanol to remove any residual chemicals, then washed with dish soap (Dawn, P&G, Browns Summit, NC, USA), rinsed in distilled water and air dried in a fume hood for at least 12 h. Range finding to determine an approximate dose (= volume) of the methyl ketones to add to the fumigation chamber to obtain 50% mortality in 24 h was conducted first with 10 ants per test. Once the dose range was obtained, more detailed dose–response studies were conducted with 25 ants per assay and replicated at least three times at each dose. All the methyl ketones tested were liquid when applied but had fully evaporated by the end of the 24-h incubation period. The fumigant concentration (μg/cm³) was calculated based on the weight applied to the cover slip (calculated from the volume applied and density of the methyl ketone) per unit volume of the fumigation chamber; assuming that no methyl ketone escaped from the sealed chamber during the 24-h incubation. This assumption of no methyl ketone escape was deemed reasonable because the compounds all had a distinct smell and even when the assays were conducted in as many as nine tests at once, no methyl ketone odor was ever detected. Compounds (concentrations) tested were as follows: 2-undecanone (7.86, 8.25, 8.64, 9.04 and 11.79 μg/cm³), 2-nonanone (3.90, 5.00, 5.86, 6.64 and 7.81 μg/cm³), 2-octanone (3.90, 5.56, 6.64, 7.03 and 7.81 μg/cm³) and 2-heptanone (1.95, 3.90, 5.86, 7.81 and 11.71 μg/cm³).

2.4 Field tests
Red imported fire ant colonies (mounds) within a 50-m radius of the Dearstynge Entomology Building (35°47′18.9″N 78°41′56.7″W) were flagged on 16 April 2014 (Fig. 1). Mounds ranged from 10 cm in length by 12 cm in width by 3 cm in height to 45 cm in length by 38 cm in width by 9 cm in height. Each fire ant mound was disturbed using a wooden dowel and classified as described in Table 2 according to the rating system of Harlan.19 An active mound contains worker brood during the warm season, which is strong evidence that the colony contains an egg-laying queen. By contrast, the absence of worker brood would indicate that the colony lacked a queen. A rating index of 0 to 10 (Table 2) was assigned depending on the estimated number of worker ants and brood. A mound with an activity rating < 5 was not used in our field tests. A mound-injection tool was developed for this study. It consisted of 20 cm of rubber tubing with a 10 mL glass pipette attached at one end and a 100 mL plastic syringe at the other. Different concentrations of 2-undecanone were emulsified in distilled water (100%, 50%, 25% and 15% v/v) with a handheld milk frother (aerolatte®, model number 56AL3SAT, aerolatte® Ltd., Radlett, UK) for 60 s. A volume of 10 mL of test solution or a water control was drawn into the glass pipette, inserted into the mound to a depth of 10–15 cm, and then dispensed over 5 s while slowly pulling the pipette out of the mound. This application was repeated five times at different random positions over the surface of the mound approximately equal distance from each other, but also with injections that spanned the entire mound surface (for a total application of 50 mL). Mounds chosen for injection were a minimum distance of 10 m apart to limit inter-mound treatment effects. In addition, a distance of 50 m was maintained between control mounds and 2-undecanone treated mounds. Three replicate mounds were treated for each concentration and control. The activity of each mound was evaluated daily after treatment according to the rating system of Harlan19 (Table 2). We categorized the fire ant activity in the mounds for 4 days post treatment.

2.5 Fumigation bioassay for other insect pests
The bioassay system described earlier for fire ants was also used to evaluate the fumigant efficacy of 2-undecanone against adult males of the German cockroach, B. germanica, adults of mixed sex red flour beetles, T. castaneum, and tobacco budworm, H. virescens eggs (1 day old), and to determine what level of undecanone was needed to obtain 100% mortality for these insects and fire ants. Three German cockroaches, 10 red flour beetles and 10 fire ants were assayed at each dose. Tobacco budworm eggs were obtained from Benzon Research deposited on a 36 × 36 cm piece of cloth. The cloth was cut with scissors to obtain 250 budworm eggs per unit cloth, and this used per test. The minimum 2-undecanone concentration achieving 100% mortality of the German cockroach, red flour beetle and fire ant, and 0% egg hatch for budworms were determined using a range-finding approach similar to that for the fire ant dose–response studies (described earlier). The volume of 2-undecanone applied to the fumigation chamber was increased until all insects were dead or did not hatch at 24 h post treatment. Once a threshold volume was obtained that produced 100% mortality, the results at this dose were replicated two more times, again showing 100% mortality for both replicates for all species examined. The same number of insects per replicate was used, except for cockroaches, where the number was increased from three to five insects per replicate. After exposure to the fumigant for 24 h, the hatch rate of the tobacco budworm eggs was monitored daily for 5 days; otherwise, the exposure period (fumigation period) was 24 h and percent mortality was determined at 24 h for the other insects. The concentrations obtained for 100% mortality were 4.1 g/m³ for the fire ant, 41 g/m³ for the German cockroach, 101.2 g/m³ for tobacco budworm eggs and 165.8 g/m³ for the red flour beetle (Table 3). For the former two german cockroach and red flour beetle, mortality was defined as described earlier in this paper for fire ants. Egg hatch was defined as the appearance of a

| Table 2. Red imported fire ant, Solenopsis invicta, mound activity rating system used in the current study adapted from Harlan et al.19 |
|-----------------------------|------------------------|------------------------|
| Colony activity rating | Number of worker ants | Brood presence |
| 0 | No activity | NA |
| 1 | < 100 | – |
| 2 | 100–1 000 | – |
| 3 | 1 000–10 000 | – |
| 4 | 10 000–50 000 | – |
| 5 | > 50 000 | – |
| 6 | < 100 | + |
| 7 | 100–1 000 | + |
| 8 | 1 000–10 000 | + |
| 9 | 10 000–50 000 | + |
| 10 | > 50 000 | + |

NA, not applicable.
budworm larva separate from the egg shell regardless of whether the larva was alive or dead at the time of the observation.

2.6 Data analysis

Abbott’s correction was applied to all data in the dose–response experiments. Median lethal doses were calculated by plots of probit mortality versus log dose. The variance–covariance matrix and 95% confidence intervals (CI) for the toxicity ratios were estimated by the methods of Steel and Torrie and Robertson and Preisler. Formulas and calculations were made in Microsoft Excel (Microsoft, Redmond, WA, USA) developed by Young et al.

One-way analysis of variance (ANOVA) was carried out using JMP 12 (SAS Institute, Cary, NC, USA).

3 RESULTS AND DISCUSSION

3.1 New bioassay system for evaluating fumigants

An easy to use and inexpensive bioassay system was developed to evaluate fumigants against a variety of pest organisms and with these organisms in or on a variety of substrates (Fig. 2). The assay was designed for fumigants that are liquid or solid at room temperature with volatility low enough to allow time for compound transfer to the glass cover slip in the bottom chamber (Fig. 2), transferring of insects to the top chamber, and sealing of the container with Parafilm (Fisher Scientific) before a significant amount of the fumigant has evaporated. Because the assay device is constructed from plastic Petri dishes, the operator has the option of using the device only once at a minimal cost per assay. Because the plates are transparent and the surface area to depth ratio is high, observations on the test organism can be viewed easily by eye or under a dissecting microscope. Insects can be added to the bioassay in or on different materials.

In the studies described, we examined a variety of insects and different insect stages using this method. In dose–response studies on fire ants, we demonstrated that probit models could be established for different aliphatic methyl ketones. Examples of other test materials that could be used in this bioassay system include: seeds, separate or in soil; nematodes on artificial media or in soil; bacteria and other microorganisms in a variety of media including soil; and other insects in and on a variety of media. Because of the small size of the bioassay system, the amount of fumigant needed is minimal; this is especially important in screening programs where the amount and/or cost of the test compounds might prohibit larger scale testing.

3.2 Fire ant fumigation structure–activity studies with methyl ketones

This is the first study to determine the structure–activity relationship for n-aliphatic methyl ketones as an insect fumigant. In these experiments, we used the red imported fire ants as a model insect and the bioassay system described earlier (Fig. 2). Four methyl ketones were studied, i.e., 2-heptanone, 2-octanone, 2-nonanone and 2-undecanone (Table 1); these were chosen over other methyl ketones because they are found naturally in different plant species and were available from commercial sources. Tridecanone (an aliphatic methyl ketone produced by plants) is a solid at room temperature with a low volatility rate and could not be tested as a fumigant under the standard conditions of our bioassay. In the development of the environmental and incubation parameters for the bioassay, we examined the relative rate of evaporation of the C-7 to C-11 methyl ketones at room temperature. In different experiments, different amounts of each methyl ketone were transferred separately to the surface of the cover slip (Fig. 2), the fumigation chamber assembled as described earlier, and then the device incubated at room temperature (25 ± 3 °C). In these experiments, all the methyl ketones examined were completely evaporated by 24 h (2-heptanone, 0.5 h; 2-octanone, 4 h; 2-nonanone, 10 h; and 2-undecanone, 24 h). For the fire ant structure–activity studies, a total of 25 worker fire ants were randomly selected and placed in the fumigation chamber (Fig. 2). Mortality was recorded 24 h after this transfer. It was assumed in these assays and in development of the probit models that there was minimal or no escape of the methyl ketone from the fumigation chamber, no absorption of the compound from either the liquid or vapor phase to any of the inner surfaces of the chamber, and an even distribution of the fumigant in the device at 24 h. No visible changes in the plastic Petri plates or materials used in the bioassay chamber were observed during any of the assays.

The probit models for each methyl ketone are shown in Fig. 3; LC50, LC90 and statistics are provided in Table 1. For all the methyl ketones examined, there was a significant increase in mortality with an increase in concentration (2-undecanone, $F = 8.14, df = 4, P < 0.05$; 2-nonanone, $F = 71.29, df = 4, P < 0.05$; 2-octanone, $F = 83, df = 4, P < 0.05$; and 2-heptanone, $F = 88.47, df = 4, P < 0.05$). The best estimate of a population response to a toxicant is expected at the LC90. The trend was for the LC90 to decrease with carbon chain length, from 8.21 μg/cm² for 2-undecanone (C-11) to 5.26 μg/cm² for 2-nonanone (C-9) to 5.11 μg/cm² for 2-octanone (C-8) to 4.27 μg/cm² for 2-heptanone (C-7) (Table 1). Based on 95%
The horizontal line represents 50% mortality.

As discussed earlier, there was an increase in the volatility of the methyl ketones from C-11 to C-7, as measured by reduced time to total evaporation with carbon chain length. Fire ant increased susceptibility (lower LC50) to these methyl ketones was negatively correlated with carbon chain length and positively correlated with increased volatility. Because we only examined mortality at 24 h, and the concentration of fumigant by weight in the vapor phase was higher earlier during the incubation period for the shorter methyl ketones, we are unable to determine what impact volatility alone had on toxicity of the compounds tested. It is possible that the lower molecular mass methyl ketones are more toxic to fire ants. However, it is also possible that because of the more rapid evaporation rate, the time of exposure of the full dose of vapor is longer as the carbon chain is reduced, and this is affecting the measure of susceptibility. There also could be a combination of effects based on toxicity differences between compounds and time to full evaporation. As we observed previously, 1 μL of 2-heptanone evaporates in 30 min, whereas 1 μL of 2-undecanone required much longer, up to 24 h. An additional factor to consider is the difference in molecular mass for the compounds tested. For example, there is a 1.6-fold statistically significant difference in the LC50 between 2-undecanone and 2-octanone, correlated with a 1.3-fold difference in molecular mass between these two compounds. The latter results in a higher molar concentration of 2-octanone versus 2-undecanone in the vapor phase in our assay, which could also be a factor contributing to differences in fire ant susceptibility to these compounds.

Additional studies are needed to fully understand the mode of action of methyl ketones as a fumigant on the red imported fire ant. The current hypothesis for the toxic action of these compounds is physical disruption of cuticular lipids resulting in water loss (a non-toxic mode of action). This hypothesis is partly derived from their apparent mode of action as an herbicide. When plants are treated topically with 2-undecanone in ethanol or exposed to 2-undecanone vapor, after a 30 min treatment, the plant wilts and dies, presumably because of disruption of the plant cuticle and subsequent water loss.25

### 3.3 Field tests

Although 2-heptanone, 2-nonanone and 2-octanone were more effective than 2-undecanone based on LC50 values (Table 1), field tests against fire ant mounds were conducted using 2-undecanone. There were several reasons for this. Undecanone already has US EPA registration as a biopesticide for use on human skin as an arthropod repellent at concentrations as high as 8%,10,15,16,26,27 Therefore, animal and human toxicology data are already established showing that 2-undecanone is safe. In addition, there are commercial efforts to develop 2-undecanone as a herbicide, and herbicide research suggests that this compound could have broad activity as a fumigant for weed, microbial plant disease and nematode control in soil. The current work is to investigate its use on soil insects as well, in this case ants.

Flagged fire ant mounds (Fig. 1) were examined for ant activity and rated according to the criteria shown in Table 2 using the system developed by Harlan et al.19 An active mound contains a worker brood during the warm season, and the presence of brood is strong evidence that the colony contains an egg-laying queen. A rating number of 0 to 10 is given based on the estimated number of workers and the presence/absence of worker brood. Any mound containing >100 live ants was considered active, but only mounds with an activity rating ≥5 were used in this study. Five different concentrations of 2-undecanone in water emulsions (100%, 50%, 25% and 10% v/v) were tested. The treatments and control (distilled water only) were injected in a total volume of 50 mL per mound as described earlier using the injection tool shown in Fig. 4. We repeated the field tests three times on different mounds for each concentration of 2-undecanone, as well as for the control. A different mound for each replicate but with the same colony activity rating system for all concentrations.

One day after treatment, mounds injected with 100% 2-undecanone were reassessed for activity and received a rating of 2 (no brood on the mound surface and the number of fire ants was reduced); this represented a three-step decrease in the colony activity rating (Fig. 5). Mounds treated with 50% and 25% 2-undecanone also showed signs of a decreased population. The activity of the 50% 2-undecanone treated mounds decreased from a mean rating of 7 to 4, and the 25% 2-undecanone treated mounds decreased from an average activity rating of 6 to 3. There was no ant activity (rating of 0) in mounds injected with 100%
that if 2-undecanone can be retained in the soil for longer by a formulation, a lower effective dose can be achieved and/or ants will not be able to re-inhabit the treated location for longer. It is clear, based on odor alone, that 2-undecanone is released into the atmosphere from the soil just above the treated area for at least 3 days.

3.4 Fumigation effect on other insect species and life stages

To better understand the utility of our fumigation bioassay and to determine the use of 2-undecanone as a fumigant for insects in general, we conducted additional studies with red flour beetle adults, adult male German cockroaches and tobacco budworm eggs. Range-finding experiments were conducted to determine the lowest 2-undecanone concentrations required for 100% mortality for each species and for fire ants. The studies were then repeated twice for concentrations that produced 100% mortality to validate the 100% kill. For 10 fire ants per replicate, 4.1 g/m³ was sufficient to produce 100% mortality in 24 h (Table 3 and Fig. 8). For five German cockroaches per replicate, an undecanone treatment of 41 g/m³ resulted in 100% mortality; for tobacco budworms (n = 250 for each replicate) 101.6 g/m³ resulted in 0% egg hatching; and for the red flour beetle (n = 10 for each replicate) 165.8 g/m³ produced 100% mortality (Table 3 and Fig. 9). Compared with the MeBr minimum and maximum recommended treatment ranges for most insects (48.08–112.2 g/m³) on a weight basis per unit volume basis, 2-undecanone had similar insect activity for a variety of insects and different life stages (egg versus adults); the value was slightly above the MeBr range for the flour beetle (Figs 8 and 9).

3.5 Cost comparisons of undecanone versus MeBr for insect fumigation

MeBr has been the ‘gold standard’ for fumigants because of its broad activity against insects and other pests for many different types of applications and its low cost. For this reason, comparisons were conducted between undecanone and MeBr. For common insect pests or commodities, the minimum dosage for MeBr is 48.08 g/m³ and the maximum is 112.2 g/m³. The cost of MeBr for field use in 2012 was US $0.013/g. A bulk price for undecanone in 2017 was US $0.014/g (https://www.alibaba.com/product-detail/2-Undecanone-cas-no-112-12_60431528103.html); the purity of undecanone is 99.9%. Table 3 shows the cost per gram for the control of different insects with 2-undecanone versus MeBr based on the minimum concentration needed for 100% mortality. In the cost comparison in Table 3, MeBr at its minimum recommended concentration was 10.9 times more expensive than 2-undecanone for 100% fire ant control, and at its maximum recommended concentration, it was 25.6 times more expensive. Note that the effective dose for 100% control of fire ants with 2-undecanone might change depending on the substrate in which the ants are located. To date, studies suggest that 2-undecanone is a cost-effective alternative to MeBr. Findings were similar for the other insects and insect stages examined (Fig. 9 and Table 3), i.e., 2-undecanone is a reasonable alternative to MeBr based on treatment cost for 100% insect control. The worst case was for red flour beetles, where 2-undecanone was 3.7 times more expensive than the maximum dose of MeBr and 1.6 times the cost of the minimum dose (Table 3).

Considering that MeBr has been banned since 2005 because of its impact on the stratosphere ozone layer, the difficulty of its detection in treated areas (it has no odor or color) and that it represents a synthetic chemistry (not found in nature), 2-undecanone
Figure 6. Typical before and after comparison of the control mound (A, B; treated with 50 mL distilled water) with a mound treated with 50 mL of 100% 2-undecanone (C, D) and 50 mL of a 25% 2-undecanone in water emulsion (E, F) for the field results shown in Fig. 5. The field study was replicated three times for each concentration.

Figure 7. (Left) Swarm of fire ants rushed out of control mound with a colony activity rating of 5. (Right) Mound treated with 50% 2-undecanone at day 4, now with a colony activity rating of 0 because there was no activity when disturbed.

could be a reasonable substitute which needs further consideration. Some of the advantages of 2-undecanone are that it: 1 is found in nature; 2 is produced in plants that we eat; 3 has a known safety record; (iv) has a moderate odor allowing easy detection; 5 can be easily transported as a liquid at atmospheric pressure and ambient temperatures; 6 potentially has a non-biochemical or non-toxic (physical) mode of action with potential broad pest (arthropods, plant, nematode, and bacteria) and commodity uses;
and is currently safe enough to be used on human skin as an arthropod repellent without restrictions, approved by the US EPA. Also, public acceptance of a natural fumigant should be much easier than synthetic compounds or compounds with high off-target toxic effects.

3.6 Use of methyl ketones as a fumigant

The modes of action of fumigants vary greatly. Some fumigants kill rapidly, whereas others can be slow acting. Some fumigants may have a paralyzing effect on the pest at sublethal dosages, whereas others do not allow the pest to recover. Also in general, the toxicity of a fumigant depends on the respiration rate of the target pest, the lower the respiration rate of the organism, the lower the susceptibility. The mode of action of 2-undecanone is unknown, but one hypothesis is that it acts directly on the cuticular surface of insect and plants, suggesting that respiration is not a target in its action. Even under cold environmental conditions, 2-undecanone was found to be fast acting as an herbicide.

With the complete phasing out of MeBr and the reduction in the number of labeled fumigants overall, there are limited choices where fumigation is needed for pest control. Factors such as the type of commodity to be treated, type of pest and developmental stages present, type of structure to be treated, the cost of the fumigant, and the safety to the applicator and the environment are important considerations. Also, for any control chemistry, public acceptance is becoming critical, and the public are increasingly demanding ‘green technologies’. Many natural essential oils have been explored for pest control. For example, cinnamaldehyde, the main constituent of cinnamon oil, produced direct contact toxicity to both T. castaneum and the maize weevil, Sitophilus zeamais. Oil of clove is toxic to the rice weevil, Sarcocladium oryzae, and the lesser grain borer, Rhyzopertha dominica. Extracts of the flower buds of clove, Syzygium aromaticum, and star anise, Illicium uvrum, are insecticidal to T. castaneum and S. zeamais, and suppressed progeny production. Essential oil extract from the neem tree, Azadirachta indica, prevented adult insect emergence, reduced oviposition rates and prevented insect development.

The methyl ketone, 2-undecanone, is synthesized by the trichomes of the wild tomato plant, L. hirsutum Dunal f. glabratum C. H. Müll. It has been used as a food additive, in cosmetics and perfumes, and as a flavoring agent, and can be derived from natural plant extracts. This methyl ketone has also been used as an arthropod repellent. Undecanone has relatively low mammalian toxicity, and already has a US EPA registration as an ingredient in a biopesticide for application to human skin as an arthropod repellent.

In summary, we explored the insecticidal potential of methyl ketones. In fumigation bioassays, four different methyl ketones (2-undecanone, 2-nonanone, 2-octanone and 2-heptanone) were found to be effective in eliciting mortality of fire ants in a dose-dependent matter. Heptanone exhibited the lowest LC₅₀ (4.27 μg/cm³) in comparison with 2-octanone, 2-nonanone and 2-undecanone (Table 1). Undecanone also had fumigant effects against adult German cockroaches, adult red flour beetles and the eggs of the tobacco budworm. The concentration of 2-undecanone required to treat these pests fell within the lower to upper range for MeBr (48.08–112.2 μg/cm³); the value slightly higher for the red flour beetle (Table 3). In field tests, direct injection of 2-undecanone was 100% effective in eliminating ant activity in fire ant mounds at concentrations as low as 25%, representing a total treatment of technical 2-undecanone of 12.5 mL (Fig. 5). Direct injections for ant mound control has been suggested previously. An aerosol can with a 10–20 cm dispensing tip could be used by a homeowner to treat individual mounds. Methyl ketones also have potential uses as fumigants for the rapid control of insect pests in applications where current fumigants are not appropriate or preferred, including as a replacement for MeBr.

REFERENCES


