Assessment of Potential Fumigants to Control *Chaetodactylus krombeini* (Acari: Chaetodactylidae) Associated With *Osmia cornifrons* (Hymenoptera: Megachilidae)

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ABSTRACT With the recent decline of honey bees, *Apis mellifera* (L.) (Hymenoptera: Apidae), there is a need for alternative or supplemental crop pollinators, such as *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae). However, *O. cornifrons* propagation could be impeded by a cleptoparasitic mite, *Chaetodactylus krombeini* Baker. We investigated the effects of formic acid and wintergreen oil on mortality of *C. krombeini* hypopi and *O. cornifrons* adults by determining the lethal concentration of each compound on each species. On average, >4.8 and >1.8 h were required to cause mortality in *O. cornifrons* adults when <2,473.5 ppm of formic acid and wintergreen oil was applied as a fumigant, respectively. When the two chemicals were directly applied to the exoskeleton of *O. cornifrons* adults, 353.4 ppm of wintergreen oil caused bee mortality within 10 min; however, no mortality was found with any formic acid application attempted. Mortality of *C. krombeini* hypopi occurred 5 and 10 min after application of >176.7 ppm of formic acid and wintergreen oil, respectively. Estimates of LC_{50} for *C. krombeini* hypopi treated with formic acid and wintergreen oil were 54.3 and 271.3 ppm, respectively. This study showed that *C. krombeini* could be controlled effectively without inducing *O. cornifrons* adult mortality based on concentration and duration of fumigation.

KEY WORDS formic acid, essential oil, wintergreen oil, *Chaetodactylus krombeini*, *Osmia cornifrons*

Crop and fruit tree pollination is a $15 billion industry each year in the United States (Morse and Calderone 2000). With the recent decline of honey bees associated with parasitic mites, Africanized honey bees (Sammataro and Avitable 1998), and colony collapse disorder (Stokstad 2007), there is a need for alternate pollinators such as *Osmia cornifrons* (Radoszkowski) (Hymenoptera: Megachilidae) (Adams 2001). *O. cornifrons* has been used successfully for the pollination of apple trees in Japan (Sekita and Yamada 1993), whereas a native species *O. lignaria* has been widely used for the pollination of fruit trees in the United States (Bosch et al. 2006, Gardner and Ascher 2006). Since the first introduction of *O. cornifrons* to the United States in 1976 (Batra 1997), *O. cornifrons* has been used for the pollination of spring blooming fruit trees (Batra 1997, Adams 2001) and vegetable crops (Wilson et al. 1999). Because the life cycle of the bee coincides well with blueberry flowering, it is particularly useful in blueberry pollination (West and McCutcheon 2009). *O. cornifrons* is also timid and rarely stings, which makes using the bee in commercial orchards attractive to growers (Batra 1997, Bosch and Kemp 2001).

Osmia spp. (e.g., *O. cornifrons* and *Osmia lignaria* Cresson) are solitary bees nesting in preexisting cavities, such as reeds under natural conditions. In commercial settings, however, are commonly managed in small cardboard tubes used as nests (Batra 1997, Bosch and Kemp 2001). *O. cornifrons* constructs cells with mud partitions within the nesting tubes that are generally grouped together in bundles. However, using bundles of nesting tubes not only makes *O. cornifrons* management and propagation easier but also provides an attractive habitat for pests such as parasitic wasps and mites (Krunić et al. 2005, Park et al. 2009). One major pest associated with *O. cornifrons* in the eastern United States is *Chaetodactylus krombeini* Baker, a pollen mite that infests the tubes in which *Osmia* spp. nest (Bosch and Kemp 2001). *C. krombeini* is of particular importance with respect to *O. cornifrons* propagation and management (Park et al. 2009).

The life cycle of *C. krombeini* includes egg, larval, protonymph, deutonymph, tritonymph, and adult stages. There are two additional forms of *C. krombeini* deutonymphs: hypopus as migratory deutonymph and cyst as resting deutonymph. *C. krombeini* hypopi generally infest and migrate from nest to nest by hitchhiking on adult *Osmia* spp. When *C. krombeini* hypopi hitchhike on *O. cornifrons* adults, they become entangled in the setae of adults and detach themselves in new nests when females construct brooding cells. In addition to hitchhiking on adult bees, *C. krombeini*

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hypopi can disperse from nest to nest by walking from adjacent tube openings and by entering through holes in tubes created by parasitic wasps (Park et al. 2009). Once hypopi enter the tubes, their population can increase up to a range of 170–5,000 mites per cell (Park et al. 2009). *C. krombeini* populations compete with *O. cornifrons* larvae for pollen provision in the tubes and can kill developing larvae (Bosch and Kemp 2001). Bee larvae that survive competition with *C. krombeini* inside nests could experience a reduction in size due to a decreased food supply during development (Bosch and Kemp 2001). A reduction in larval size may also cause a corresponding reduction in pollinating efficiency and decreased fitness as adults. Heavy infestation of *C. krombeini* hypopi also directly reduces pollination efficiency of *O. cornifrons* adults, as we observed that *O. cornifrons* adults are visibly irritated when infested by *C. krombeini* hypopi and that some adults had difficulty flying because of the number of mites on their body.

Currently, *C. krombeini* is controlled by cultural methods such as physically removing *O. cornifrons* cocoons (Krunić et al. 2005) and cleaning them with bleach (Bosch and Kemp 2001). However, physically removing cocoons disturbs the bees' natural nesting behavior, and disturbances to the bees may cause a reduction in the number of females returning to nesting tubes to lay eggs (Bosch and Kemp 2001). Cleaning with bleach requires the removal of immature bees from the nesting tube and presents further complications when the cocoons are exposed to bleach treatment. Thus, less invasive control methods such as fumigation that allow *O. cornifrons* to remain undisturbed would benefit their use as a supplemental pollinator (Park et al. 2009). Because all stages of *C. krombeini* except the hypopus stage inhabit interior nest cells, the hypopus stage could be targeted for less invasive control when it disperses from nest to nest.

Mites associated with honey bees have been effectively controlled by both formic acid and essential oils (Amrine 2006). In our preliminary studies, we tested formic acid and three essential oils (wintergreen oil, spearmint oil, and lemon grass) as potential fumigants. We found that formic acid and wintergreen oil effectively killed *C. krombeini* hypopi but did not affect survivorship and behavior of *O. cornifrons* adults. The objective of this study was to investigate the effects of formic acid and wintergreen oil on mortality of *C. krombeini* hypopi and *O. cornifrons* adults. We sought to determine the LC_{50} and LC_{90} of formic acid and wintergreen oil for *C. krombeini* hypopi and to investigate tolerance and survivorship of *O. cornifrons* adults when exposed to these compounds at various volumes and exposure times.

Materials and Methods

Experimental Animals. *O. cornifrons* and *C. krombeini* hypopi were obtained from a local grower in Grafton, WV, who has been using *O. cornifrons* to pollinate blueberry bushes for the past 10 yr. The grower uses cardboard tubes (7.9 mm in diameter, 0.8 mm in thickness, and 152 mm in length; Jonesville Paper Tube Co., Jonesville, MI) as artificial nests for *O. cornifrons.* All *O. cornifrons* used in this study were adult females actively making nests at the time of capture for experiments.

Effects of Formic Acid and Wintergreen Oil Treatments on *O. cornifrons* Adults. Mortality of *O. cornifrons* adults was determined through exposure to various volumes of formic acid and wintergreen oil. Both formic acid and wintergreen oil have been shown to kill mites associated with honey bees while not causing side effects to the bees (Amrine 2006). Two laboratory bioassays were conducted under ambient environment (23 ± 2.1°C and 58 ± 6.2% RH). In the first bioassay, we directly applied wintergreen oil (Lorann Oils, Lansing, MI) or 50% formic acid (Chemical and Solvents Inc., Roanoke, VA) to the body of *O. cornifrons* adults to investigate the effect of the chemicals as a direct contact toxin. Four different amounts (0, 5, 10, and 20 μl) of 50% formic acid or artificial wintergreen oil were applied directly to the body by using micropipettes, and ten adults were used for each treatment-amount combination. Time to death among the four chemical treatments was recorded. Scheffé's multiple-comparison procedure at a 0.05% error rate (PROC GLM, SAS Institute 2008) was used to compare between the treatments and among times to death.

In the second bioassay, we used 56.6-cm^3 petri dishes (3-cm radius by with 2-cm depth; Lab-Tek 4036, LAB-TEK Division Miles Laboratories, Inc., Naperville IL) with 55-mm-diameter filter paper (Whatman 1001-055, Whatman International Ltd., Maidstone England) taped onto the lid of the petri dish. Seven different volumes (0, 5, 10, 20, 40, 60, and 80 μl per 56.6 cm^3) of 50% formic acid or artificial wintergreen oil were applied to the filter paper; such volumes of chemicals can be converted as 0, 88.3, 176.7, 353.4, 706.7, 1,060.1, and 1,413.4 ppm. One *O. cornifrons* adult female was placed inside a petri dish which was wrapped with Parafilm (American National Can, Neenah, WI). Time required to cause death in each *O. cornifrons* adult was recorded, and the bioassay was replicated five times for each chemical treatment. Mortality of *O. cornifrons* between treatments and among the seven different chemical treatments were statistically compared using Scheffé's multiple-comparison procedure at a 0.05% error rate (PROC GLM, SAS Institute 2008).

Effects of Formic Acid and Wintergreen Oil Fumigation on *C. krombeini* Hypopi. Mortality of *C. krombeini* hypopi exposed to various amounts of 50% formic acid and wintergreen oil was conducted using the similar design as mentioned for *O. cornifrons.* In total, 28 ± 11.7 *C. krombeini* hypopi were placed in a petri dish with a fine brush. Four different volumes (0, 176.7, 265.0, and 353.4 ppm) of 50% formic acid or wintergreen oil were applied to the filter paper by using micropipettes. The petri dish and lid were sealed with parafilm and mortality was recorded at intervals of 0, 5, 10, and 15 min. We set these time intervals and amounts based on our preliminary study that showed

that both formic acid and wintergreen oil were effective at <353.4 ppm when applied under 20 min. At each interval, the number of dead hypopi was recorded. However, because the size of *C. krombeini* hypopi is ≈0.3 mm in length (Park et al. 2009), and they are generally very active when they are out of the nest, accurate counting of dead and live mites can be very difficult and prone to observation error. Therefore, we developed an image analysis approach as a more practical and effective method to record mite mortality.

During the experiment, photographs of a bioassay arena were taken with a digital camera (Sony model DCS-W50, Sony Corporation of America, New York, NY) at each interval. Two successive interval photos were overlaid in Photoshop CS3 Extended (Adobe Systems, Mountain View, CA). Any movements of hypopi were detected with the "difference" blend mode function in Photoshop CS3. This function made it possible to align two images and look for differences in pixels. When movement of hypopi was detected between two images, the function detected the difference in pixels, and the hypopi were considered "moved"; otherwise, they were considered "not moved." These steps were repeated with successive images in the time series. In benchmarking studies, we confirmed under a microscope that all of not moved hypopi were actually dead with all of their legs curled toward the middle of sternum. When live hypopi were detected, the mites were digitally marked and counted to generate number of live mites. Based on this data, the number of dead hypopi for each chemical amount and time interval was determined.

Abbott's formula (Abbott 1925) was used to correct for control mortality with acceptable control mortality at 15%. Corrected data were subjected to logit analysis to determine the main effects of dose and treatment, and their interaction (PROC GENMOD, SAS Institute 2008). Overdispersion in data was corrected using the scaled deviance parameter in SAS. Significance of effects was based upon the likelihood ratio chi-squared and logit analysis was used to estimate LC_{50} and LC_{90} (Robertson et al. 1984, Stokes et al. 2000). In addition, the two chemical treatments were compared for treatment effects using survival analysis (PROC LIFE TEST, SAS Institute 2008) to determine interactions among treatment, volumes of chemicals and exposure time.

Results

Effects of Formic Acid and Wintergreen Oil Treatments on *O. cornifrons* Adults. When formic acid was directly applied to the adult *O. cornifrons*, the dense body hairs seemed to prevent formic acid from coming into contact with the cuticle. Consequently, formic acid caused no direct contact mortality to *O. cornifrons* adults. In contrast, wintergreen oil quickly soaked into the dense hairs on adults, resulting in significantly high mortality (Fig. 1). *O. cornifrons* adults exposed to wintergreen oil through direct contact became highly irritated suggested by shaking and twitching their bodies.

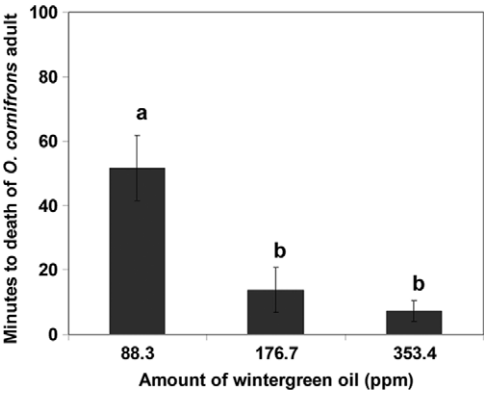

Fig. 1. Time (min) to death of *O. cornifrons* adults after direct application of wintergreen oil to *O. cornifrons* adults. Abbott's formula (Abbott 1925) was used to correct for natural mortality. Bars indicate standard deviations of the means and the same letter indicates no significant difference in mortality ($P > 0.05$; Scheffé's multiple-comparison procedure).

When formic acid was applied as a fumigant, there was no difference in mortality of *O. cornifrons* adults between the untreated control and a dose of 88.3 ppm, whereas all doses between 176.7 and 2,473.5 ppm were statistically the same (Table 1). Adults survived at least 1.8 h at the highest dose of formic acid. In contrast, mortality at the lowest dose of wintergreen oil (i.e., 88.3 ppm) was significantly different than the untreated control (Table 1). *O. cornifrons* adults could survive ≈4.8–10.1 h after exposure to various doses of wintergreen oil, each of which was not significantly different from each other. We observed from our preliminary study that adult bees fumigated with <1,413.4 ppm of formic acid and wintergreen oil became active to visit flowers within 30 s after opening the petri dish.

Effects of Formic Acid and Wintergreen Oil Fumigation on *C. krombeini* Hypopi. We observed significant effects in the dose of chemicals ($\chi^2 = 14.31$, df = 1, $P < 0.01$), types of chemicals (i.e., formic acid

Table 1. Mean time to death in *O. cornifrons* adults when exposed to different doses of formic acid and wintergreen oil as a fumigant

Dose (ppm)	Time to death (h)[a]	
	Wintergreen oil	Formic acid
0	170.9 ± 81.64a	132.5 ± 42.66a
88.3	10.1 ± 2.07b	114.8 ± 81.1a
176.7	8.3 ± 2.89b	18.5 ± 4.81b
265.0	8.0 ± 3.29b	9.9 ± 5.32b
353.4	6.2 ± 2.38b	5.5 ± 2.51b
706.7	5.2 ± 2.75b	3.5 ± 0.68b
1,060.1	3.7 ± 0.71b	3.1 ± 0.69b
1,413.4	5.7 ± 2.60b	2.6 ± 0.48b
1,766.8	6.2 ± 2.19b	2.7 ± 1.04b
2,120.1	4.8 ± 0.53b	1.8 ± 0.94b

[a] Means within a column followed by the same letter are not significantly different ($P > 0.05$; Scheffé's multiple-comparison procedure).

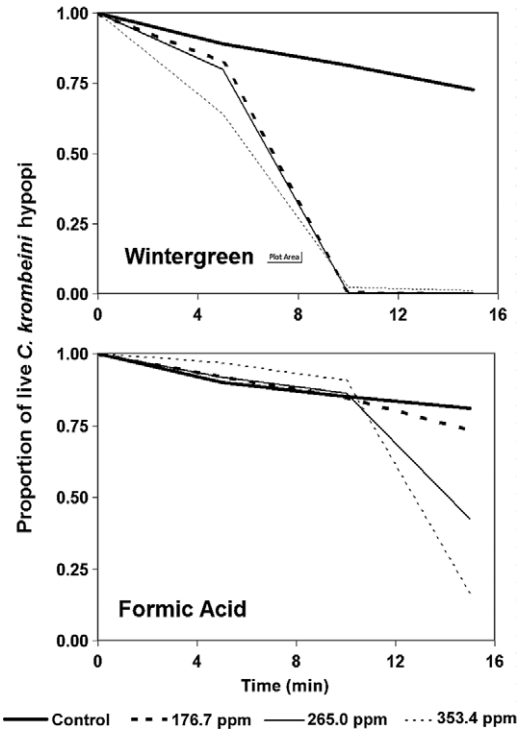

Fig. 2. Survivor analysis of *C. krombeini* hypopi fumigated with wintergreen oil and formic acid at three different doses (ppm) and an untreated control.

and wintergreen oil) ($\chi^2 = 19.46$, df = 1, $P < 0.01$), and their interaction ($\chi^2 = 17.28$, df = 1, $P < 0.01$). Highly significant differences between formic acid and wintergreen oil was also confirmed by survival analysis ($\chi^2 = 1{,}355.7$; df = 1; $P < 0.01$) (Fig. 2). When homogeneity among amounts and exposure duration was tested for wintergreen oil only, the survival of *C. krombeini* hypopi at 176.7, 353.4, and 706.7 ppm was statistically the same across all time intervals ($\chi^2 = 2.6$, df = 2, $P > 0.05$); however, when these amounts were grouped together, there was a significant difference from the control ($\chi^2 = 465.4$, df = 1, $P < 0.01$). In the formic acid treatment, *O. cornifrons* adults treated with 176.7 ppm of formic acid was not significantly different from the control ($\chi^2 = 3.4$, df = 1, $P > 0.05$). However, we found significantly higher mortality in treatment with 265.0 ppm ($\chi^2 = 63.9$, df = 1, $P < 0.01$) and 353.4 ppm ($\chi^2 = 10.5$, df = 1, $P < 0.01$) (Fig. 2). These results indicate that 176.7 ppm of formic acid and wintergreen oil would be enough to kill *C. krombeini* hypopi. Estimates of the LC_{50} and LC_{90} for *C. krombeini* hypopi treated with wintergreen oil were ≈5 times higher than those with formic acid (Table 2).

Survival analysis also showed that *C. krombeini* survivorship subjected to wintergreen oil decreased rapidly at 5 min, whereas survival was sustained twice as long when subjected to formic acid (Fig. 2).

By combining the results from both bioassays, we conclude that wintergreen oil and formic acid as fumigants can effectively kill *C. krombeini* hypopi at 88.3 ppm within 5 min and at 176.7 ppm within 5 min, respectively, without killing *O. cornifrons* adults. Also, > 1.8 h of exposure would be needed to cause mortality of *O. cornifrons* adults with <2,473.5 ppm of formic acid or wintergreen oil fumigation.

Discussion

All stages of *C. krombeini* except the hypopus stage develop and reproduce inside the nest partitioned by mud, so it is hard to monitor and control them without opening the nests. The use of binder board (Pollinator Paradise, Parma, ID) (i.e., boards with the appropriate sized hole drilled through and then cut in half) allows the user to pry the two pieces of the board apart, gaining access to the cocoon inside (Strickler and Mills 2002). Binder boards can be used for *C. krombeini* control by opening the boards and removing the cocoons to separate those infested with *C. krombeini.* Cocoons that have been removed from binder boards also can be washed with a bleach solution (Strickler 2009). However, the physical removal of *O. cornifrons* from the binder boards generally results in releasing loose cocoons and could increase unwanted dispersal of prenesting female bees (Torchio 1984, Bosch and Kemp 2001, Torchio 2003).

Formic acid and wintergreen oil have been used to control parasitic mites associated with honey bees such as *Varroa destructor* (Anderson & Trueman) (Mesostigmata: Varroidae), an external parasite and *Acarapis woodi* (Rennie) (Prostigmata: Tarsonemidae), an internal parasite of the respiratory tubules or tracheae. Amrine (2006) suggested that formic acid and essential oils killed the mites but did not affect honey bees. Also, formic acid could be applied as a fumigant for an economic method that achieves 90–95% mortality of the mites (Amrine and Noel 2006) depending on colony strength and environmental conditions. In parasitic mite control in honey bee hives, formic acid volatiles are circulated throughout the bee hive by wing fanning, penetrating the brood cells, and thereby killing varroa mites (Amrine and Noel 2006). Wintergreen oil for mite control in honey bee hives is generally used as an ingredient to grease patties, on which the honey bees feed, and slowly emit volatiles to kill mites. Honey bees will feed on grease patties and as they do, the wintergreen oil acts as an irritant to the varroa mite causing the mite fall off the

Table 2. Estimates of LC_{50} and LC_{90} (ppm) of wintergreen oil and formic acid toxicity to *C. krombeini* hypopi

Chemical	χ^2 (*P*)	Slope (95% CL)	LC_{50} (95% CL)	LC_{90} (95% CL)
Wintergreen oil	5.5 (0.0193)	3.6 (0.5–7.6)	54.3 (15.3–192.11)	100.6 (28.4–356.3)
Formic acid	319.1 (<0.001)	5.2 (4.5–5.9)	271.3 (262.9–271.3)	413.5 (400.8–426.5)

bee through the frames of the hive to the sticky bottom board (Amrine 2006). However, these methods may not be directly applicable to *O. cornifrons* because the adults do not ventilate air inside the nest and the volatiles of the chemicals are heavier than air so volatiles move down. Therefore, effective control of *C. krombeini* with formic acid and wintergreen oil needs to target the hypopus stage when they come out of the nest and disperse from nest to nest. Park et al. (2009) showed that *C. krombeini* hypopi could disperse from nest to nest in three ways: hitchhiking on female bees, walking from and to the entrance of nests, and through holes on nests made by parasitic wasps. Because *C. krombeini* hypopi are physically exposed when outside of nests, fumigation could be a very effective way to control the mites.

The results of this study suggest two considerations on the use of formic acid and wintergreen oil to control *C. krombeini* hypopi. First, formic acid and wintergreen oil can be used as fumigants for curative management of *C. krombeini* (Table 1; Fig. 2). We observed that formic acid and wintergreen oil were very effective in killing *C. krombeini* hypopi with a short exposure time and could be used to reduce the side effects associated with current *C. krombeini* management tactics. Due to the short exposure time required, active bees may be treated with formic acid and wintergreen (fumigate <1.8 h with application of <2,473.5 ppm of formic acid or wintergreen oil) outside of their nests without harming adult bees. In addition, formic acid and wintergreen oil can be used as a volatile, which allows the bees to remain undisturbed in the binder board during application. Therefore, fumigation can reduce prenesting dispersal by females and potentially increase *O. cornifrons* populations in the subsequent year (Bosch and Kemp 2001). However, physical contact of wintergreen oil on *O. cornifrons* adults needs to be avoided because it can induce mortality. Second, wintergreen oil and formic acid can be used for preventative control of *C. krombeini.* Growers intend to reuse nests which could be fumigated with either compound to manage *C. krombeini* when *O. cornifrons* does not inhabit the nests. When binder boards are used, the boards could be spread open allowing the fumigant to contact the mites. Further studies are needed to investigate practical and effective methods of applying both formic acid and wintergreen oil as fumigants for control of other stages of *C. krombeini* In addition, sublethal affects of the fumigants, such as effects on adult bee nest orientation, foraging ability, reproduction, and behavior need to be investigated.

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