

# Larval pheromone disrupts pre-excavation aggregation of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) neonates precipitating colony collapse

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## Abstract

Newly enclosed larvae of *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) contain their activity to an arena formed at the base of their eggstick, marked with a mandibular gland pheromone. Laboratory and field studies were undertaken to determine if these pre-excavation aggregations, essential to their successful penetration of the host plant, could be disrupted with mandibular gland extract causing the incipient colonies to perish. Cladodes or whole plants were sprayed with the pheromone, obtained by extracting macerated caterpillars in hexane, hexane only, or left unsprayed, and the survivorship of caterpillars that eclosed from eggsticks attached to the cladodes recorded at a later date. In 4 separate experiments, the average survivorship of *C. cactorum* larvae from cohorts on cladodes sprayed with the extract (15%) differed markedly from survivorship of caterpillar cohorts on cladodes treated with the solvent only (84%) or left untreated (80%). This differential mortality was attributed to the elicitation of the independent dispersal of the caterpillars by the mandibular gland pheromone and their failure to reaggregate in numbers sufficient to mount a successful attack on the host plant. The potential for managing pest populations of caterpillars employing this target-specific alternative to conventional pesticides is discussed.

Key Words: Argentine cactus moth caterpillar; mandibular gland; pest control; prickly pear cactus; *Opuntia* spp.

## Resumen

Las larvas recién nacidas de *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) mantienen su actividad en una arena formada en la base del estipe del huevo, marcada con una feromona de la glándula mandibular. Se realizaron estudios de laboratorio y de campo para determinar si estas agregaciones antes de la excavación, que son esenciales para su penetración exitosa de la planta hospedera, podrían interrumpirse con el extracto de la glándula mandibular que hace que las colonias incipientes perezcan. Los cladodos o plantas enteras se rociaron con la feromona, obtenida mediante la extracción de orugas maceradas en hexano, solo hexano, o se dejaron sin pulverizar y la sobrevivencia de las orugas que nacieron de los huevos adheridos por el estipe a los cladodos registrados en una fecha posterior. En 4 experimentos separados, el promedio de la sobrevivencia de cohortes de larvas de *C. cactorum* sobre cladodos rociados con el extracto (15%) difirió notablemente de la sobrevivencia de los cohortes de orugas sobre cladodos tratados solo con solvente (84%) o sin tratar (80%). Esta mortalidad diferencial se atribuyó a la provocación de la dispersión independiente de las orugas por parte de la feromona de la glándula mandibular y su incapacidad de reagruparse en cantidades suficientes para organizar un ataque exitoso en la planta hospedera. Se discute el potencial para el manejo de poblaciones de plagas de orugas empleando esta alternativa específica a los pesticidas convencionales.

Palabras Clave: oruga de la polilla cactus argentina; glándula mandibular; control de plagas; nopal; *Opuntia* spp.

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The Argentine cactus moth, *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), an invasive insect that feeds on species of *Opuntia* (Cactaceae), was first found in the USA in 1989 in the Florida Keys (Habeck & Bennett 1990; Dickle 1991). Since then, the pest has extended its range northward along the coast of North Carolina (J. Driscoll, North Carolina Department of Agriculture and Consumer Services), and westward to coastal Louisiana (Hight & Carpenter 2009). While *C. cactorum* currently poses a threat to native species of cactus in the American south and Mexico, the cactus also is widely cultivated as an agricultural crop in Mexico, and there is concern that if the insect is successful in entering Mexico, the caterpillar will have a significant economic impact on the industry. To contain the insect in ecologically sensitive and agricultural areas, research has been directed at developing bio-rational

techniques that have proven successful in the mitigation of other pest species. These techniques include the use of sterile insect releases (Hight & Carpenter 2016), trapping with a synthetic sex pheromone to monitor populations in the field (Bloem et al. 2005; Heath et al. 2006; Cibrián-Tovar et al. 2017), and the identification and study of an Argentine larval parasitoid (*Apanteles opuntiarum* Martínez & Berta; Hymenoptera: Braconidae), a potential biological control agent (Varone et al. 2015). Here we report the results of a pilot study undertaken to determine the feasibility of managing *C. cactorum* using a relatively novel, eco-sensitive approach that employs the use of a larval pheromone.

*Cactoblastis cactorum* eclose from an eggstick and the gregarious larvae contain their activity to a circular, silken arena that they construct around the base of their eggstick (Fitzgerald et al. 2016). The

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cohort of caterpillars sharing a common eggstick, a colony, does not eclose in synchrony but over a period of hours, and the arena serves to keep the neonates from wandering away (Fitzgerald et al. 2016). This allows for the gradual buildup of numbers to a critical mass that facilitates the penetration of the host plant. Fitzgerald et al. (2016) also showed that, although aggregates of as few as 5 caterpillars can successfully penetrate a cladode, laboratory colonies did not begin to excavate the host plant until, on average, 25 caterpillars had aggregated at the base of the eggstick some 15 h after the first of the cohort had eclosed.

Larvae of *C. cactorum* mark and follow trails that serve to keep the cohort together as they move over the surface of the host plant (Fitzgerald et al. 2015). Systematic dissection and chemical extraction of body components revealed that 1 or more compounds found in the caterpillar's mandibular glands, hereafter referred to as the mandibular gland pheromone, are responsible for eliciting trail-following behavior (Fitzgerald et al. 2014, 2015, 2016). The glands are remarkably large, extending the full length of the caterpillar's body, and have a wet mass equal to approximately 1% of the total body mass in the fifth instar (Fitzgerald et al. 2014). The ducts of the glands are inserted into the apodemes of the adductor muscles of the mandibles, and their exudate is secreted as the caterpillars move about. Of particular relevancy to the study presented here is that the circular arena surrounding the base of the eggstick, to which the neonates confine their pre-excavation activity as described above, is marked with the mandibular pheromone that the neonates deposit along with their silk as they tentatively venture short distances from the eggstick. Furthermore, it is the pheromone rather than the silk that serves to contain the cohort to the base of the eggstick. This was demonstrated by spraying cladodes prior to the eclosion of the caterpillars with the mandibular gland pheromone (Fitzgerald et al. 2016). The result was that caterpillars failed to aggregate at the bases of their eggsticks, instead scattering independently over the surfaces of the cladodes upon eclosion, resulting in a significant loss of individuals, many of which fell from the plant. This suggested the possibility that a control strategy based upon this approach might be applied under field conditions. Thus, the pilot studies reported here were undertaken to determine the extent to which the elicitation of dispersal behavior of neonate caterpillars with the mandibular gland extract affects colony survivorship, and to assess the potential of the technique to serve as an alternative, eco-rational means of managing pest populations of the caterpillar.

## Materials and Methods

### EXPERIMENTAL PROCEDURES

Four experimental studies were conducted to determine colony survivorship when host plants were blanket sprayed with mandibular gland pheromone. The expectation was that the pheromone would elicit the independent dispersal of neonates and prevent them from aggregating in the numbers needed to successfully colonize the plant. All studies consisted of 3 groups of *Opuntia* spp. cladodes or whole plants that were either (1) sprayed with a hexane extract of the *C. cactorum* larvae, (2) sprayed with the hexane solvent alone or, (3) left untreated. The mandibular gland pheromone can be obtained by dissecting the mandibular glands and extracting them in hexane (Fitzgerald et al. 2015). However, the process is tedious and impractical when large quantities of the pheromone are required; the pheromone is more efficiently harvested by whole-body extraction of the caterpillars (Fitzgerald et al. 2016). Thus, to prepare the extract, previously frozen fifth instar caterpillars were macerated in hexane at the ratio

of 7.5 g of caterpillars per 100 mL of solvent. This caterpillar wet mass is the equivalent of 50 to 60 fifth instar caterpillars. The mixture was then filtered and centrifuged to remove particulate matter, and the supernatant drawn off. Cladodes were sprayed with this supernatant with Preval© sprayers (Preval, Coal City, Illinois, USA) at the rate of approximately 0.6 to 1.3 mL per cm<sup>2</sup> of cactus surface. These sprayers produced a fine mist, and allowed for complete coverage with a minimal loss of the solution due to over-spraying. To assure good overall coverage, 2 light sprays were applied, the second applied immediately after the first had dried. After plants were sprayed, eggsticks were attached to a cladode in a natural upright position with a small drop of hot glue (Surebonder Glue Gun and Glue Sticks, Wauconda, Illinois, USA). All eggsticks used in this study were obtained from a *C. cactorum* colony reared at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) Crop Protection and Management Research Unit, Tifton, Georgia, USA. Eggstick fates were followed on the plants and records kept of the number of viable eggs in each eggstick, date of larval eclosion, percentage of larvae eclosed, whether larvae were successful in entering cladodes, and percent survival of larvae from each eggstick determined by excavating the cladodes at variable intervals post-hatch, as noted below for each of the separate experiments. All laboratory or greenhouse experiments were conducted at the USDA-ARS Crop Protection and Management Research Unit facility, and the field experiments at the Plant Science Research and Education Unit, University of Florida (UF), Institute of Food and Agricultural Sciences (IFAS) facility near Citra, Florida, USA. To reduce the probability that a solvent residue left behind on the surface of the cactus would interfere with response of neonates to the gland extract, the extract was dissolved and applied in hexane, which fully evaporated within seconds after application. However, in 1 of the intended experiments, the solvent injured young cladodes on the plants. It was not possible to collect meaningful data from that experiment, and it was not considered in the results reported here.

### LABORATORY STUDIES WITH CLADODES

The first experiment, conducted in the laboratory, was initiated on 18 Sep 2014. Ten cladodes of *Opuntia ficus-indica* (L.) Miller (Cactaceae) that had been separated from field collected parent plants were randomly assigned to each of the 3 experimental categories. After the application of the extract and hexane sprays to cladodes, as described above, a single eggstick was attached to each of the cladodes. All cladodes were then housed individually at room temperature in sealed containers, and data collected as described above. Survivorship of larvae subjected to the 3 experimental treatments was determined by excavation of cladodes on 8 Oct 2014.

### GREENHOUSE STUDIES WITH WHOLE POTTED PLANTS

The second experimental study was initiated on 19 Sep 2014. The experiment employed the same experimental protocol as described above, but was conducted in a greenhouse using whole, potted *O. ficus-indica* plants housed individually in 1 m<sup>3</sup> screened cages. Survivorship of larvae subjected to the 3 experimental treatments was determined by excavation of cladodes on 8 Oct 2014.

### FIELD STUDY 1

A third study was initiated on 18 Sep 2014 using the same experimental protocol described above, except that the *O. ficus-indica* plants used in this study were whole, open grown plants that had been established in the small, experimental plantation at the UF-IFAS Plant Science Research and Education Unit facility in 2009. For this study, the

mandibular gland pheromone and unsprayed control treatments were replicated 20 times each, and the hexane-only treatment, 19 times. Of the total 32 plants used in the study, 12 were sprayed with the mandibular gland pheromone, 11 with hexane-only, and 9 served as unsprayed control plants. Eggsticks were attached to the current year's cladodes with hot glue. On those plants that had more than 1 eggstick attached, eggsticks were placed at a distance on separate cladodes to ensure that hatching larvae from different cohorts would not come into contact with each other. Ants attacked 17 of the 59 eggsticks before the caterpillars eclosed, and were replaced with new eggsticks on 20 Sep. Survivorship of larvae subjected to the 3 experimental treatments was determined by excavation of cladodes on 15 Oct 2014.

## FIELD STUDY 2

A fourth study was initiated on 8 Oct 2014 using the same experimental protocol described above for field study 1. The mandibular gland pheromone and hexane-only treatments were replicated 10 times, and the control 9 times. Eggsticks were attached with glue to the current year's cladodes of open grown plants. Survivorship of larvae subjected to the 3 experimental treatments was determined by excavation of cladodes on 27 Oct 2014.

## STATISTICAL PROCEDURES

Data were analyzed with SigmaPlot 12.5 (Systat Software, Inc., San Jose, California, USA) with appropriate tests as reported below. All statistical error terms are reported as standard errors.

## Results

### LABORATORY STUDY

The number of larvae that eclosed from eggsticks attached to cladodes that had been sprayed with the mandibular gland pheromone was  $41.3 \pm 1.1$ , those sprayed with hexane alone was  $42.7 \pm 1.8$ , and the unsprayed controls was  $43.3 \pm 1.4$ . Larvae began to eclose in all 3 experimental groups 7 days after the experiment was initiated, and eclosion was completed by 26 Sep. At that time, 2 of the 10 cohorts that eclosed on cladodes treated with pheromone, and all cohorts on cladodes sprayed with hexane and untreated controls had penetrated the cladode near the base of the eggstick, and caterpillars were feeding inside the plants. Caterpillars in the 8 cohorts on cladodes sprayed with mandibular gland pheromone that did not penetrate the cladode at the base of the eggstick dispersed independently over the surface of the cladode, though in 1 of these colonies a fragment of the cohort re-aggregated at a distance from the egg mass and successfully penetrated the cladode. Survivorship of cohorts on cladodes sprayed with mandibular gland pheromone was  $14.8 \pm 7.2\%$ , for those sprayed with hexane alone,  $85.0 \pm 3.3\%$ , and for unsprayed controls,  $84.0 \pm 4.4\%$  (Fig. 1). A 1-way ANOVA followed by Tukey's multiple comparison test showed that there was no significant difference between survivorship in the hexane only and control treatments ( $q = 0.19$ ), but that the pheromone treatment differed significantly from the hexane only treatment ( $q = 13.36$ ;  $P < 0.05$ ), and the non-sprayed control ( $q = 13.17$ ;  $P < 0.05$ ).

### GREENHOUSE STUDY

Caterpillars eclosed from eggsticks between 26 to 29 Sep. The number of larvae that eclosed from eggsticks attached to cladodes sprayed with mandibular gland pheromone was  $44.2 \pm 1.5$ , those sprayed with hexane alone,  $43.7 \pm 1.3$ , and unsprayed controls,  $49.9 \pm 1.4$ . Three

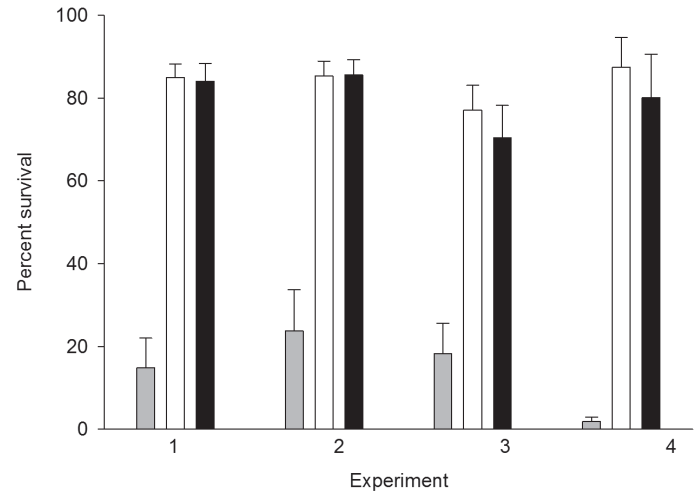


Fig. 1. Percent survival of caterpillars in cohorts of *Cactoblastis cactorum* on plants sprayed with caterpillar extract (gray bar), solvent-only (white bar), or unsprayed (black bar) for 4 separate experiments. Experiment 1 = laboratory study; experiment 2 = greenhouse study; experiment 3 = field study 1; experiment 4 = field study 2.

of the 10 cohorts that eclosed on plants treated with the mandibular gland pheromone, all of the cohorts on the cladodes sprayed with hexane-only, and all unsprayed controls penetrated the cladode near the base of the eggstick, and caterpillars entered plants. Caterpillars in the 7 cohorts that did not penetrate the cladode at the base of the eggstick dispersed widely over the surface of the cladode. Survivorship of caterpillars in cladodes sprayed with mandibular gland pheromone was  $23.7 \pm 10.0\%$ , for those sprayed with hexane alone,  $85.3 \pm 3.6\%$ , and for unsprayed controls,  $85.6 \pm 3.7\%$  (Fig. 1). A Kruskal-Wallis 1-way ANOVA on ranks followed by Tukey's multiple comparison test showed that there was no significant difference between survivorship in hexane only and control treatments ( $q = 0.14$ ), but that the pheromone treatment differed significantly from the hexane only treatment ( $q = 5.1$ ;  $P < 0.05$ ), and non-sprayed control ( $q = 4.98$ ;  $P < 0.05$ ).

### FIELD STUDY 1

Hatching of original and replaced eggsticks occurred between 24 to 28 Sep. The number of larvae that eclosed from eggsticks attached to cladodes sprayed with mandibular gland pheromone was  $37.7 \pm 1.5$ , those sprayed with hexane alone,  $35.6 \pm 1.8$ , and unsprayed controls,  $36.8 \pm 2.2$ . Survivorship of caterpillars in cladodes sprayed with mandibular gland pheromone was  $18.3 \pm 7.3\%$ , for those sprayed with hexane alone,  $77.1 \pm 6.0\%$ , and for unsprayed controls,  $70.4 \pm 7.8\%$  (Fig. 1). A Kruskal-Wallis 1-way ANOVA on ranks followed by Dunn's multiple comparison test showed that there was no significant difference between survivorship in the hexane only and control treatments ( $q = 0.55$ ), but that the pheromone treatment differed significantly from the hexane only treatment ( $q = 4.40$ ;  $P < 0.05$ ), and non-sprayed control ( $q = 3.89$ ;  $P < 0.05$ ) (Fig. 1). For 10 of the replicates on plants sprayed with the mandibular gland pheromone, caterpillars scattered upon eclosion, did not enter the plant, and perished. For the remaining replicates, fragments of colonies entered the plant either near the base of the eggstick (7 colonies) or a site distant from the eggstick (3 colonies). Survivorship for these colonies was  $36.6 \pm 12.4\%$ .

### FIELD STUDY 2

Eggsticks attached to experimental cladodes hatched between 10 to 12 Oct. The number of larvae that eclosed from eggsticks attached

to cladodes sprayed with mandibular gland pheromone was  $38.4 \pm 1.1$ , those sprayed with hexane alone,  $35.8 \pm 1.9$ , and unsprayed controls,  $33.3 \pm 0.9$ . Survivorship of caterpillars in cladodes sprayed with mandibular gland pheromone was  $1.84 \pm 1.84\%$ , for those sprayed with hexane alone,  $87.4 \pm 7.2\%$ , and for unsprayed controls,  $80.1 \pm 10.5\%$  (Fig. 1). A Kruskal-Wallis 1-way ANOVA followed by Dunn's multiple comparison test showed no significant difference between survivorship in hexane only and control treatments ( $q = 0.64$ ), but that the pheromone treatment differed significantly from the hexane only treatment ( $q = 3.9$ ;  $P < 0.05$ ), and non-sprayed control ( $q = 3.15$ ;  $P < 0.05$ ) (Fig. 1).

## Discussion

Although sex pheromones have been employed widely to survey and manage populations of phytophagous insects, trail pheromones have been studied little in either of these contexts, and the conventional wisdom has been that, in comparison to sex pheromones, they hold little promise as agents for pest management. Targeting the stage with the greatest reproductive value, the pre-ovipositional female, and preventing her insemination offers the most direct and efficient means of curbing the spread of an insect. Furthermore, compared with their use of sex pheromones as adults, the larvae of relatively few major plant pests are known to employ trail pheromones. As a consequence, the study reported here is 1 of only a few to explore the possibility of using trail pheromones as control agents.

The pre-eminent insect trail followers are ants, and considerable research has been conducted to identify and synthesize their trail pheromones (Holldobler & Wilson 1990). Several investigations also have been undertaken to determine the potential of trail pheromones for managing populations of various pest species. Studies of 2 economically important invasive species, the Argentine ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) (Suckling et al. 2008, 2010a, 2011; Tanaka et al. 2009; Nishisue et al. 2010) and the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Suckling 2010b, 2012), showed that exposure to synthetic trail pheromone interfered with the ability of the ants to follow their authentic trails, and negatively impacted the insects' foraging behavior. However, there was no indication in these studies that such disruption caused direct mortality, or resulted in a reduction of population size even when the ants were exposed to the synthetic pheromone for the duration of 2 consecutive field seasons (Nishisue et al. 2010). In another study, Sunamura et al. (2011) showed that combining insecticide baits with trail pheromone dispensers resulted in better control of ants than did baits alone. Choe et al. (2014) obtained similar results by mixing trail pheromone with an insecticide. Studies by Westermann et al. (2014) suggested that long term disruption of foraging behavior of the Argentine ant with a synthetic trail pheromone might reduce their competitive ability, leading to collapse of populations in favor of less harmful, indigenous species. However, the difficulty of achieving significant control of ants by any of these techniques is attributable to the fact that colonies of ants are not fully dependent on their communal trail system for survival, but also can search independently and orient directly to food odors (Suckling et al. 2010a).

In contrast to studies with ants, the results reported here for *C. cactorum*, and a previous study of tent caterpillars (Fitzgerald 2008), indicate that trail marking caterpillars are more vulnerable to colony collapse when their trail systems are disrupted. For the 4 experiments reported here, the average survivorship of *C. cactorum* larvae from colonies on cladodes sprayed with mandibular gland pheromone was approximately 15% compared with 80% on unsprayed control plants.

Similarly, losses of colonies of the tent caterpillars, *Malacosoma disstria* Hübner and *Malacosoma americanum* (F.) (Lepidoptera: Lasiocampidae), on trees sprayed with a 1 ppm formulation of the trail pheromone mimic  $5\beta$ -cholestan-3-one, resulted in the loss of 83% of the former colonies and 87% of the latter colonies (Fitzgerald 2008). As with *C. cactorum*, new eclosed caterpillars of both tent caterpillar species dispersed independently instead of aggregating en masse on their egg mass, their normal behavior. Although specific causes of *C. cactorum* larval loss in our study were not determined, tent caterpillar larvae typically perished by losing purchase on the branches of the host tree and falling to the ground (Fitzgerald 2008). A previously reported laboratory study suggested that falls from the plant also may constitute a major mortality factor for *C. cactorum* (Fitzgerald et al. 2016). When moving over the surface of the host plant en masse, the collective spinning efforts of a cohort of social caterpillars allows them to lay down a broad mat of silk that adheres tightly to the plant and engages their crochets, affording them secure purchase on the often smooth and slippery surfaces of the plant. In contrast, a single strand of silk produced by an isolated individual affords the caterpillar more tenuous purchase (Fitzgerald 1993, 1995).

The success of this pilot study of *C. cactorum* caterpillars can be attributed, most notably, to the stability and persistence of the caterpillars' pheromone, and to the identification and targeting of the most vulnerable stage of the insect's life cycle. Because the mandibular gland pheromone of the cactus caterpillar was applied up to 10 days in advance of caterpillar eclosion, its persistence in active form was essential to its ability to draw caterpillars away from the eggstick and their siblings. Furthermore, studies of cactus caterpillars and tent caterpillars targeted the earliest active stage in the life cycle of the insects, interfering with the ability of neonates to form stable post-eclosion aggregates, essential to the colonies' survival. *Cactoblastis cactorum* and the tent caterpillar *M. americanum* present a particularly narrow window of vulnerability. In the case of the tent caterpillar, survivorship rates on treated and control trees did not differ significantly when trees were sprayed with the pheromone mimic after the first instar caterpillars had constructed a tent, even when the pheromone concentration was increased 100-fold (Fitzgerald 2008). While the reason for this is not fully known, the robust and spatially concentrated trail marking of the caterpillars when aggregated appears to allow them to distinguish their authentic trails from surfaces marked with the synthetic pheromone. Similarly, exposure of cactus caterpillar neonates to the mandibular gland pheromone is limited to the brief interval between the time the caterpillars eclose and the time of penetration, because they thereafter confine almost all their activity to the interior of the host. Although the later instars of *C. cactorum* may mark and follow trails over the surface of cactus when moving between cladodes (Dodd 1940; Pettey 1947), and have been shown to follow pathways marked with mandibular gland extract (Fitzgerald et al. 2015), the extent to which blanket-spraying plants with pheromone might affect the ability of maturing caterpillars to stay together is unknown. Furthermore, even if the pheromone were to interfere with the ability of the cohort to maintain a tight aggregate, such disruption might have little impact on colony survival. Dodd (1940) and Pettey (1947) reported that dispersing colonies of older caterpillars commonly fragment into smaller units that successfully penetrate and tunnel cladodes.

Were this technique to be adapted to manage pest populations of *C. cactorum*, it would be necessary to formulate the active ingredient with a non-phytotoxic carrier. A largely water-based formulation used to successfully apply the tent caterpillar pheromone in the disruption studies discussed above (Fitzgerald 2008; T. D. F. unpublished), indicate that a similar formulation can be used for the cactus caterpillar. Moreover, while a sprayable formulation might be prepared from

whole body larval extract, as in the present study, a preferable approach would require the identification of the bioactive components of the mandibular glands and their synthesis in quantities sufficient for abatement programs. Chemical analysis of the mandibular gland indicates that the fluid consists of a large number of chemically distinct compounds (Fitzgerald et al. 2015). Particularly prominent are a series of 2-acyl-1, 3-cyclohexanediones. Laboratory bioassays show that one of these, 4-hydroxy-2-oleoyl-1, 3-cyclohexanedione, is as effective on a per unit volume basis in eliciting trail following from neonates as whole mandibular gland extract (Fitzgerald et al. 2015). Moreover, the compound is non-volatile and chemically stable, important characteristics of a chemical that needs to be persistent under field conditions. Although a molecule lacking the hydroxyl group in position 4 can be prepared directly from commercially available starting materials, the addition of the hydroxyl group requires 3 additional synthetic steps, significantly increasing the task of its preparation, and potentially making it too costly for use in a control program. While less effective than 4-hydroxy-2-oleoyl-1,3-cyclohexanedione, studies show that sub-components of the compound, as well as other 2-acyl-1, 3-cyclohexanediones, also elicit trail following responses in neonate *C. cactorum*, raising the prospect of designing and synthesizing a cost-effective trail following molecule (Fitzgerald et al. 2015). Moreover, bioassays of fractionated mandibular gland extract indicate that other yet unidentified chemical components of the mandibular gland, chemically distinct from the 2-acyl-1, 3-cyclohexanediones, also elicit trail following, though it is not known the extent to which any of these compounds are competitive with whole mandibular gland extract. The capability of these unidentified mandibular gland components to disrupt the collective excavation behavior of neonates has not been investigated.

Particularly problematic for the effective application of this technique for disrupting the establishment of pest populations of caterpillars is the need for effective timing of application. The tent caterpillars represent the ideal situation. These insects have 1 generation per yr, the eggs hatch in close synchrony over large geographic areas, and the date of hatching is highly predictable (Fitzgerald 1995). In contrast, the cactus caterpillar has multiple generations and egg hatching is largely asynchronous. Dodd (1940) and Pettey (1947) recorded winter and summer generations in Australia and South Africa, each with flight periods lasting about 1.5 mo for the winter generation and just over 1 mo for the summer generation. In the southeastern US, there are 3 non-overlapping generations per yr, each flight period lasting approximately 2 mo (Hight & Carpenter 2009). Thus, management of the cactus caterpillar by disrupting the establishment of neonates would require long-term presence of the disruptor pheromone. This would largely limit the applicability of the technique to heavily managed, commercial plantations and, depending on the effective longevity of the pheromone, require repeated application of the chemical. It remains to be determined if the potential ecological benefit of high target specificity and the added market value of an “organically” managed crop would warrant the use of this procedure over conventional approaches involving pesticides.

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