Response of *Lucilia sericata* (Diptera: Calliphoridae) to Screwworm Oviposition Attractant

M. F. CHAUDHURY,1,2 J. J. ZHU,3 AND S. R. SKODA4

ABSTRACT The sheep blowfly, *Lucilia sericata* Meigen (Diptera: Calliphoridae), causes sheep myiasis in various parts of the world. Female flies are attracted to sheep following various olfactory cues emanating from the sheep’s body, and oviposit on suitable substrates on sheep ultimately causing myiasis. Earlier workers attempted to reduce fly population in the field, with some success, using traps baited with various attractants. This research was conducted to determine if *L. sericata* would respond to a recently developed synthetic attractant that has attracted gravid screwworms, *Cochliomyia hominivorax* Coquerel, and stimulated them to oviposit. Results of the laboratory bioassays demonstrated that gravid females *L. sericata* were attracted to substrates treated with the synthetic screwworm attractant composed of five compounds—dimethyl disulfide, dimethyl trisulfide, phenol, p-cresol, and indole. Tests with various combinations of these compounds suggest that the sulfur compounds and indole are the most important compounds to elicit attraction and stimulate oviposition, while phenol and p-cresol may have minor roles. Semiochemical baits based on these compounds may be useful in the field to trap gravid *L. sericata*.

KEY WORDS dimethyl disulfide, dimethyl trisulfide, insect trap, sheep blowfly, semiochemical

The sheep blowfly, *Lucilia sericata* Meigen (Diptera: Calliphoridae) is a species of economic importance, causing sheep myiasis in temperate north-western parts of Europe (Wall et al. 1992a) and parts of the Southern Hemisphere (Tenquist and Wright 1976). Closely related *Lucilia cuprina* Wiedemann is prevalent in Australia, where this species is the main cause of cutaneous myiasis of sheep (Anderson et al. 1988). Females of these blowflies lay eggs in the fleece of sheep (Barton Browne et al. 1979, Wall 1993) and the larvae feed on sheep skin tissue and secretions (Evans 1936), thereby causing extensive sheep lesions, which in turn reduces sheep body weight and fertility (Heath et al. 1987). Current control of sheep blowflies relies primarily on application of insecticides; however, this method is undesirable because of the accumulation of chemical residues in fleece and the risk of blowfly resistance to pesticides (Levot 1995). Reducing fly numbers using blowfly traps with attractants was shown to be a promising control strategy; however, because of lack of effective synthetic attractants, improvements are needed in trap-attractant design (Urech et al. 2004).

Extensive research has been conducted to identify volatile components emitted by natural sources that attract sheep blowflies. The responses of *L. sericata* and *L. cuprina* to various semiochemicals, odor baits, and host animals are similar (Ashworth and Wall 1994); it appears that they respond first to sulfur-rich volatile compounds, for upwind orientation and landing on the oviposition substrate and later respond to ammonia-rich compounds as well as moisture and other cues for actual oviposition. Normally, more females are attracted than males and more gravid than nongravid females are attracted.

Cues used by *L. sericata* for oviposition are predominantly olfactory (Hobson 1936a). Indole-treated sheep and carrion-baited traps preferentially attract gravid females (Hobson 1937, Wall et al. 1992b, Wall 1993). Hobson (1938) suggested that a combination of two factors were responsible for attraction to sheep and subsequent oviposition—one factor supplied by the living sheep causing long-distance attraction, and a factor produced by bacterial putrefaction of wool keratin inducing actual oviposition, which could be mimicked by the compounds such as indole, 3-methyl indole, or ammonium carbonate. Further research showed that a blend of ammonium carbonate, indole, and hydrogen sulfide was attractive bait for *L. sericata* females (Cragg and Ramage 1945). Later, Cragg and Thurston (1950) reported that a blend of dimethyl disulfide or ethanethiol with hydrogen sulfide resulted in strong attractants for female *L. sericata*. Cragg (1950) showed that the pad treated with organic sulfur compounds and placed on sheep were more attractive than those treated with inorganic sulfur compounds or untreated pads. The mode of action of these semiochemicals is not well understood, although it is likely that both semiochemicals and host odorants are involved.
treated with ammonium carbonate. There was no oviposition on pads treated with sulfur compounds; however, pads treated with both sulfur compounds and ammonium carbonate stimulated oviposition. Ashworth and Wall (1994) suggested that the differences in responses of L. sericata in these studies were probably due to the fact that chemicals were used alone in some studies, in traps, or combined with wool on a sheep in others.

Working to develop a female-specific attractant for the screwworm Cochliomyia hominivorax Coquerel, we have recently tested a five-compound blend against the screwworms C. hominivorax and Cochliomyia macellaria (F.) in the laboratory with promising results and potential to be useful in the field to trap gravid screwworm flies (Chaudhury et al. 2014). This blend, known as the screwworm oviposition attractant, consisted of dimethyl disulfide, dimethyl trisulfide, phenol, p-cresol, and indole. At least two of these compounds (dimethyl disulfide and indole) have been previously used for trapping L. sericata, as well as three of the compounds (dimethyl disulfide, phenol, and indole) for trapping L. cuprina (Urech et al. 2004). In the present study, we conducted laboratory bioassays to assess the response of L. sericata to the five-compound screwworm oviposition attractant as well as other combinations of these five compounds, demonstrating importance of these compounds in eliciting oviposition response in L. sericata.

Materials and Methods

Insects and Chemicals. Adult L. sericata used for the experiments were obtained from a colony maintained at the U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) laboratory in Lincoln, NE, which originated from pupae supplied by Dr. Jeff Tomberlin, Texas A & M University, College Station, TX. Upon emergence, adult flies were offered a mixture of milk powder and sugar (50:50) in a Petri dish, and water in a plastic bottle fitted with a dental wick. Adults were held at a temperature of 26 ± 1°C and 55 ± 5% relative humidity. Synthetic chemicals and ethanol were purchased from Sigma-Aldrich (St. Louis, MO). Synthetic blends of dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), phenol, p-cresol, and indole were prepared according to the descriptions by Chaudhury et al. (2014). One microliter of ethanol stock solution contained 335 µg of DMDS, 200 µg of DMTS, 57 µg of phenol, 1 µg of p-cresol, and 12 µg of indole. Various blends that were used in this study are detailed in Table 1. Essentially, the blends were prepared to determine the relative importance of each of the five compounds. Blend 1 included all five compounds; blend 2 to 6, excluded one of the compounds in sequence, thus including four of the remaining compounds; blend 7 was prepared to test the two sulfur compounds and indole together.

Landing Response. Experiments were conducted under aforementioned temperature and humidity conditions, as described by Chaudhury et al. (2014) with some modifications. Wire mesh cages (0.5 by 0.5 m) with solid metal base were used for these experiments. In total, 100 flies of mixed age and sex, previously marked with a dot on the thorax with different colors of artists’ acrylic paint to identify the following four differed age and sex groups: 1) 25 10-old gravid females; 2) 25 4-old pre-gravid females; 3) 25 10-old males; and 4) 25 4-old males, which were introduced in the cage through the front opening that was secured with a stretched cotton/polyester sleeve. Flies were allowed to settle in the cage for 15 min. Meanwhile, two clear plastic containers (11 cm top diameter, 9 cm bottom diameter, 8 cm in height) were prepared by lining the bottom of each with a 9-cm filter paper on which 20 g of fresh chicken liver was placed. In each container, one 2 by 2-cm clear plastic piece was placed on the top of the liver on which a piece of dental wick 1 cm in length was positioned. The dental wick was treated with either 0.1 ml of a 10-fold diluted stock solution of the blend or 0.1 ml of ethanol. The chemical-treated (test) and ethanol-treated (control) containers were then introduced into the cage and placed on the base of the cage diagonally ~10 cm apart. The top of the cage was covered with a white plastic bag to provide a diffused and homogeneous light condition inside the cage. Flies were allowed to land in the containers for 30 min, after which they were captured in the respective containers by placing the lid on top of the containers. The captured flies were immobilized by cooling in a freezer and the number trapped was recorded according to age and sex for each container. Each experiment was replicated five times on different days using different cohorts of flies and by alternating positions of the treated and control containers on the base of the cage.

Oviposition Tests. Oviposition assays were conducted using wire mesh cages (30 by 30 by 30 cm³) with solid metal base, according to the procedures described by Chaudhury et al. (2014) with some modifications. For assay various chemical blends, nine cages per replicate were prepared to test seven blends an ethanol control and a liver control. Twenty-five 10-old gravid females were transferred to each cage. A disposable plastic Petri dish (10 cm in diameter) was used to prepare the oviposition substrate test sample for each cage. Each of the petri dishes was lined with filter paper on which 20 g of fresh chicken liver was placed. A piece of clear plastic (2 by 2 cm) was placed on top of the liver and a 1-cm-long piece of dental wick was placed on the plastic piece. All blends were diluted 10-fold, and the dental wick was treated with either.

Materials and Methods

Table 1. Formulation of synthetic chemical blends used for testing response of L. sericata

<table>
<thead>
<tr>
<th>Compounds (µg/ml)</th>
<th>Blends</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dimethyl disulfide (DMDS) (335)</td>
<td>+</td>
</tr>
<tr>
<td>Dimethyl trisulfide (DMTS) (200)</td>
<td>+</td>
</tr>
<tr>
<td>Phenol (57)</td>
<td>-</td>
</tr>
<tr>
<td>P-Cresol (1)</td>
<td>+</td>
</tr>
<tr>
<td>Indole (12)</td>
<td>-</td>
</tr>
</tbody>
</table>

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0.1 ml of the blend, 0.1 ml of ethanol (ethanol control), or left untreated (liver control). The Petri dishes were introduced into respective cages and placed on the center of the base. The cages were covered with black plastic bags to reduce the ambient light inside the cages. Flies were given the opportunity to oviposit for 3 h, after which petri dishes were removed and the egg batches in each dish were collected and weighed. Flies were dissected to determine if they had oviposited. Each oviposition test was replicated five times with different cohorts of flies on different days.

Statistics. Landung response was calculated by subtracting the number of flies responding to ethanol controls from the flies responding to each respective blend of synthetic chemicals (if the result was <0 then the response was indicated to be 0). Landung response was analyzed as a three-factor analysis of variance (ANOVA) in a randomized complete block (days = blocks); all treatment interactions were investigated and significant differences (P < 0.01) were further analyzed with appropriate contrasts between means of interest. Further investigation of differences in least squares means of landing response for the sex x age and sex x age x blend combinations were done using Tukey’s test (adjusted for multiple comparisons; SAS Institute 2008, Cary, NC). ANOVA was used to determine treatment effects on oviposition for all nine treatments (seven chemical blends and two controls); a significant F-value (P < 0.01) was further examined using Tukey’s honest significant difference to separate the means. All statistics were performed with SAS statistical software (2008).

Results

Results of the landing response of females and males of L. sericata to the treated and control substrates are presented in Table 2. The overall ANOVA for treatment effects on landing response was significant (F = 23.36; df = 31, 108; P < 0.01); all individual treatments and their interactions were significant with the exception of blocks (replications over days). The comparison (contrast) of 10-d-old gravid females, 4-d-old pre-gravid females, 10-d-old males, and 4-d-old males was significant (F = 149.77; df = 1, 108; P < 0.01) and showed that more gravid females landed on the substrates than did young females and both old and young males. Gravid females landed on the substrates with blends 1, 2, 3, 4, and 7 in significantly higher amounts (mg) of eggs laid by L. sericata during 3-h period on substrates treated with each of the seven synthetic chemical blends (BL1 = 335 μg of dimethyl disulfide, 200 μg of dimethyl trisulfide, 57 μg of phenol, 1 μg of p-cresol, and 12 μg of indole; BL2 = 335 μg of dimethyl disulfide, 200 μg of dimethyl trisulfide, 57 μg of phenol, 1 μg of p-cresol; BL3 = 335 μg of dimethyl disulfide, 200 μg of dimethyl trisulfide, 57 μg of phenol, and 12 μg of indole; BL4 = 335 μg of dimethyl disulfide, 200 μg of dimethyl trisulfide, 1 μg of p-cresol, and 12 μg of indole; BL5 = 335 μg of dimethyl disulfide, 57 μg of phenol, 1 μg of p-cresol, and 12 μg of indole; BL6 = 335 μg of dimethyl disulfide, 57 μg of phenol, 1 μg of p-cresol, and 12 μg of indole; BL7 = 335 μg of dimethyl disulfide, 200 μg of dimethyl trisulfide, and 12 μg of indole), ethanol (ETOH), and untreated liver. Plot values not accompanied by the same letter (above plot lines) are significantly different from each other by Tukey’s test after significant F-value.

Table 2. Landing response of combined groups of L. sericata females and males of two different ages (mean ± SE)

<table>
<thead>
<tr>
<th>Blends</th>
<th>Test</th>
<th>Control</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2 ± 0.4</td>
<td>2.2 ± 0.7</td>
<td>5.6 ± 0.7</td>
<td>3.6 ± 0.5B</td>
</tr>
<tr>
<td>2</td>
<td>1.0 ± 0.4</td>
<td>1.5 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>3.8 ± 0.7B</td>
</tr>
<tr>
<td>3</td>
<td>2.0 ± 0.4</td>
<td>1.6 ± 0.5</td>
<td>5.6 ± 1</td>
<td>4.0 ± 0.4B</td>
</tr>
<tr>
<td>4</td>
<td>3 ± 0.9</td>
<td>2.6 ± 0.2</td>
<td>6.2 ± 0.7</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>2.8 ± 0.6</td>
<td>2.2 ± 0.6</td>
<td>6.2 ± 0.4</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>2.6 ± 0.5</td>
<td>0.8 ± 0.5</td>
<td>3.5 ± 1</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>1.8 ± 0.4</td>
<td>2.6 ± 0.7</td>
<td>4.6 ± 0.5</td>
<td>2.8 ± 0.9</td>
</tr>
</tbody>
</table>

- Mean for treatments (age by sex) not followed by the same letter (lower case) are significantly different based on the contrast comparing 10-d-old females to the other three age by sex combinations (F = 149.77; df = 1, 108; P < 0.01) and interpretation of Tukey’s test.

- Means in the column for 10-d-old females not followed by the same letter (upper case) are significantly different based on the following contrasts: 10-d-old females by blends 1 versus 3 (F = 5.05; df = 1, 108; P = 0.0267); 10-d-old females by blends 1 versus 5 (F = 10.83; df = 1, 108; P = 0.0001); 10-d-old females by blends 1 versus 7 (F = 5.05; df = 1, 108; P = 0.0267); 10-d-old females by blends 3 versus 5 (F = 17.2; df = 1, 108; P < 0.0001); 10-d-old females by blends 3 and 4 versus 5 and 6 (F = 26.34; df = 1, 108; P < 0.0001) – and interpretation of Tukey’s test using least squares means (adjusted for multiple comparisons; SAS 2008).
numbers (see Table 2 for results of contrasts comparisons) than on those with blends 5 and 6. Interpretation of Tukey's test (adjusted for multiple comparisons) indicated no significant differences between landing response of the young females or the two male age groups (Table 2).

The overall ANOVA of the oviposition tests with seven experimental blends and ethanol and fresh liver as controls were significant ($F = 26.31; df = 12, 32; P < 0.01$) with no significant effect for replication but significant treatment effects ($F = 39.14; df = 8, 32; P < 0.01$; Fig. 1). Blends 1 and 7 resulted in the highest oviposition (221.4 ± 9.4 and 213.6 ± 10.4 mg, mean ± SE, respectively) followed by blend 4 (198.6 ± 10.7), 3 (194 ± 10.7), 6 (155.2 ± 5.4), 5 (142.5 ± 5.7), and 2 (109.4 ± 8.7). Both ethyl alcohol and liver controls resulted in the least amount of oviposition (77 ± 8.6 and 82 ± 7.2, respectively).

Tukey's mean separation indicated no significant differences between means of eggs deposited with blends 1, 7, 4, and 3; blends 3 and 6 were not different from each other; blends 5 and 6 did not differ; blends 5 and 2 did not differ; blend 2 did not differ from the ethanol or liver controls, which had the lowest numbers of eggs deposited (Fig. 1).

Discussion

The attraction of various blowfly species, including screwworms and sheep blow flies, particularly that of female flies to their hosts involves a series of sequential behavioral manifestations. This begins with initial activation of olfactory sensilla, then orientation to and location of specific hosts acting through olfactory and visual senses, then landing on the potential oviposition substrates, and ultimately resulting in oviposition possibly acting through some tactile cues to select suitable oviposition site (Ashworth and Wall 1994). Although considerable amount of work has been done on blowfly responses to hosts and baited traps, conflicting reports are numerous (Ashworth and Wall 1994). In the present study, gravid females of L. sericata responded positively to the synthetic blend of the compounds developed for screwworms indicating that the same blend may serve as an oviposition attractant for L. sericata females; this blend was shown earlier to be attractive to both species of screwworms—C. hominivorax and C. macellaria—in laboratory bioassays (Chaudhury et al. 2014). In preliminary tests, we could not induce oviposition simply offering treated dental wicks, indicating that a dental wick was not a suitable substrate for oviposition; consequently, we added fresh chicken liver as the substrate, which apparently provided additional factor(s), potentially tactile, such as moisture, to influence the oviposition behavior. In our study, a few eggs were deposited on the untreated liver control. Earlier studies reported that moisture was an important stimulus for oviposition by L. sericata (Davies and Hobson 1935). Additionally, these and other studies on the response of L. sericata show that a mixture of ammonium carbonate, indole, and hydrogen sulfide placed on sheep was attractive to L. sericata females (Cragg and Ramage 1945), indicating a possible additional influence of a "sheep factor." Later on, Cragg and Thurston (1950) reported promising results from dimethyl disulfide and ethaneethol. More recently, Eisemann (1995) demonstrated in a laboratory bioassay that L. cuprina was attracted to mixtures of 2-mercaptoethanol, indole, and hydrogen sulfide.

In the present study, we demonstrated that the landing response of gravid females was significantly higher with blends comprising dimethyl disulfide, dimethyl trisulfide, and indole than those where one of the sulfur compounds was missing. Additionally, we observed that the substrate treated with a blend with no indole (blend 2) resulted in significantly less oviposition (Fig. 1), although the same blend elicited highest attraction in the gravid females in the landing response tests (Table 2). It is possible that the sulfide compounds act as attractant, whereas indole is effective in arresting the females on the substrate for longer period, thus allowing oviposition. In behavioral sequence, the first reaction from the flies is the perception of an attractant through olfactory mechanisms and then orientation and flight towards the source; our interpretation of the work here indicates that this is achieved mainly due to sulfur-rich compounds. Once this is achieved, flies then land on the substrate where they are arrested, eventually to oviposit, through possible chemosensory mechanisms caused by compound(s) such as indole and other ammonium-rich compounds, as suggested by earlier workers (reviewed by Ashworth and Wall 1994). Our tests show clear differences in attraction and landing of 10-d-old gravid females on chemical blend-treated and ethanol-treated substrates (Table 2). Ideally, one would not expect any flies landing on control dishes having no chemical blends. However, a few flies that were trapped in control dishes were probably attracted to moisture arising from the liver or trapped as a result of random flight, not oriented to a particular attractant. In all other groups of flies there were no significant differences between the landing responses to test and control dishes (Table 2). These results indicate no attraction to any of the blends exhibited by males and young females, suggesting that the olfactory mechanisms in these flies for perceiving these attractant chemicals are not developed or not as functional as compared with gravid females. Low electroantennogram responses of males and young females of C. macellaria to these compounds suggest such mechanisms (J. J. Z, unpublished data). Cragg and Thurston (1950) reported that a mixture of ethaneethol and hydrogen sulfide was more attractive than the ammonium carbonate, indole, and hydrogen sulfide mixture used previously (Cragg and Ramage 1945). Cragg (1950) showed that the pads treated with organic sulfur compounds and placed on sheep were more attractive than those treated with ammonium carbonate. There was no oviposition with sulfur compound alone. However, oviposition occurred on pads treated with sulfur compounds in conjunction with carbonate (Cragg 1950). These authors also indicated the importance of putrefactive sulfur-based chemicals in attraction concluding that the attraction to sheep wool was largely due to the breakdown of cystine in the wool keratin by bacteria, thereby releasing volatile sulfur compounds (Cragg and Ramage 1945). Consequently, it was suggested that the elements of behavioral response of L. sericata are generated by two distinct sets of semiochemicals—an attraction and
landing response regulated by sulfur-rich volatiles and an oviposition response to ammonia rich compounds (Ashworth and Wall 1994). Volatiles emitted from bacteria-inoculated and incubated bovine blood have been shown to be attractive to gravid C. hominivorax (Chaudhury et al. 2010). Compounds identified from these volatiles include dimethyl disulfide, dimethyl trisulfide, indole, among others (J. J. Z, unpublished data). Volatiles emitted from rotten chicken liver, which contained both dimethyl disulfide and dimethyl trisulfide were found to attract C. nucellaris (Zhu et al. 2013). Early in research with L. sericata, Hobson (1936b) showed that indole, 3-methyl indole, and ammonium carbonate were important compounds that induced oviposition in this species. It is possible that the compound such as indole, which appears to act as an arrestant, may also contribute to searching and landing behavior mediated by olfactory mechanisms; however, further research will be needed to ascertain these possibilities.

Our results indicate that this five-compound synthetic blend (blend 1) developed as an oviposition attractant for C. hominivorax may also serve as an oviposition attractant for L. sericata. Blend 7 was equally effective for C. nucellaris (M. F. C, unpublished data) and could also be used as an attractant for L. sericata. These blends may prove superior to some existing baits for trapping C. hominivorax and C. macellaria (blend 1) developed as an oviposition attractant for L. sericata, Hobson (1936b) showed that indole, 3-methyl indole, and ammonium carbonate were important compounds that induced oviposition in this species. It is possible that the compound such as indole, which appears to act as an arrestant, may also contribute to searching and landing behavior mediated by olfactory mechanisms; however, further research will be needed to ascertain these possibilities.

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References Cited


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