Attraction of face fly *Musca autumnalis* De Geer (Diptera：Muscidae) to ornamental plants *Euonymus europaeus* and *E*. *kiautschovicus*

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Abstract: Experiments in attraction of face fly *Musca autumnalis* De Geer females to ornamental plants *Euonymus europaeus* and *E*. *kiautschovicus* were conducted in the laboratory with Y-shaped glass olfactometer. The two plant species could attract face fly female adults，but *E*. *europaeus* was better in attraction of face flies than *E*. *kiautschovicus*. Female face flies responded more actively to odors of flowers of *Euonymus* plants than to odors of leaves，but leaves also played some roles in attraction when applied alone，and synergized the attraction of flowers when used together. Flowers of *E*. *europaeus* were extracted with several solvents，and the results showed that water or methanol extracts were better in attraction than hexane extracts. Frozen flowers showed similar attraction effects to fresh ones.

Key words: Face fly；*Musca autumnalis* De Geer；*Euonymus europaeus*；*E*. *kiautschovicus*；attraction；bioassays；volatiles；flower extracts

INTRODUCTION

Face fly，*Musca autumnalis* De Geer (Diptera：Muscidae)，native to Europe and western Asia (Cummings *et al*.，2005)，was introduced into North America in the early 1950's (Depner，1969) and has become a very important veterinary pest in the temperate regions of the northern hemisphere (Krafsur and Moon，1997). This pest feeds at the eyes and faces of cattle and horses and plays roles as vectors of various pathogens of cattle (*e*. *g*.，Geden and Stoffolano，1977)，for example，pinkeye (infectious bovine keratoconjunctivitis) (Cheng，1967) and *Thelazia* eyeworms (Ortanto *et al*.，2003). Heavy face fly populations can cause cattle to stop feeding and move into a shady location or cluster together to escape the flies，resulting in reduced grazing time and animal production (Schmidtmann *et al*.，1981；Arends *et al*.，1982；Schmidtmann and Valla，1982). Insecticide applications，such as pray-on，impregnated ear tags，mixture with syrup，*etc*，are primary remedial control tactics，but provide only limited or temporary control and may result in environmental pollution and toxic effects on animals (Krafsur and Moon，1997). Classical biological control (Chirico，1996；Sowig *et al*.，1997)，sterile insect technique (Pickens and Miller，1980)，area-wide control (Miller *et al*.，1984；Drummond *et al*.，1988) as well as other conventional control measures (*e*. *g*.，Miller，1989) were taken but not very effective. Thus，other complimentary control methods are necessarily available for safer and more effective management of face flies.

Face flies were found to be attracted to volatiles of two plant species *Euonymus europaeus* L. and *E*. *kiautschovicus* Loes. (Celastraceae)，which are commonly grown as ornamental plants. Bioassays were conducted in our present research in attempt to confirm the attraction of the face flies to the volatiles of plants，to compare which of the two plant species and which plant part were of potential for future use. Our goal is to develop an attractant or attracticide from plant bioactive chemicals or crude extracts for safe use in the control of face flies.

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1 MATERIALS AND METHODS

1.1 Plants

The experiments were conducted in July and August of 2003 when *Euonymus* flowers were blooming on the campus of Kansas State University, Manhattan, KS, USA. Flowers and leaves of *E. europaeus* L. and *E. kiautschovicus* Loes. were collected from plants within 30 min just before experiments started. Weighed flowers and leaves were used separately or in combination as odor sources. Because *Euonymus* flowers were only available for 3 – 4 weeks in July or August, *E. europaeus* flowers, which were proven to be the most attractive, were collected during blooming period and stored at –20℃ in a refrigerator for further use (see below).

1.2 Insects

Face fly *M. autumnalis* De Geer mated female adults (< 1 week old) were used for experiments. The face fly colony at Department of Entomology, Kansas State University, were kept under conditions of *ca.* 25℃, 75% relative humidity (RH) and photoperiod L16:D8, and fed with 10% sucrose solution. Mated female adults were isolated from the colony around 9:00 p.m., kept in a vial and supplied with water, and used for experiments around 9:00 a.m. the next day.

1.3 Test arena

A Y-shaped glass olfactometer, with the main arm (5 cm id) and two detachable branch arms (4.5 cm id), were placed on a table in a laboratory under 25 – 27℃, 65% RH and 650 lx illumination. Charcoal-filtered clean air, passing through a 250 mL flask containing distilled water, was introduced via a splitter tube into each of two 250 mL flask containing filter paper (control) or 15 g plant materials before entering one of the two upwind arms of the olfactometer. Wind speed inside the main tube was *ca.* 20 cm/s. A set of clean glass olfactometer was used for each replicate.

1.4 Bioassays with fresh plant materials

Three kinds of plant materials (flowers, leaves, flowers + leaves) were used as odor sources. Face flies kept in a jar were immobilized with compressed CO_2 or by placing them at –20℃ for *ca.* 1 min, and then introduced into downwind end (main arm, 5 cm id) of the olfactometer. Flies were given *ca.* 3 min for recovery. Around 16 flies were released each time. Their displacement, orientation and other behaviors were observed and recorded within 15 min. Complete randomized design was used for experiments. Each treatment were repeated for > 12 times.

1.5 Bioassays with frozen flowers

E. europeaus flowers showed most attractive effects among plant parts of two species, but were only available for a short period during summer, so flowers of this plant species were collected and stored at –20℃. In order to test whether frozen flowers were similar in attraction to fresh ones, olfactory bioassays were done using the olfactometer and experimental protocol mentioned above.

1.6 Bioassays with flower extracts

Three hundred frozen flowers (*ca.* 3 g) were kept in 50 mL of hexane, methanol and water, respectively, for 10 min. The extracts were concentrated to *ca.* 5 mL using a rotatory evaporator (except water extract). 50 μL of extract were applied onto 4 cm × 1 cm filter paper strip, allowed solvents to evaporate for 3 min. The filter paper was folded and inserted into a glass pipette, covered with aluminum foil.

All glassware used in the experiments was salinized with 5% dimethyl dichlorosilane in hexane or acetone, and then heated at 260℃ overnight.

1.7 Statistics

Differences between two odor sources were subject to t test at $P < 0.05$ level.

2 RESULTS AND DISCUSSION

Volatiles from flowers and/or leaves from either of the two plant species trapped female face flies (Fig. 1), but when volatiles from flowers of these two plant species were paired for comparison, *E. europaeus* performed much better in attraction (63.5% ± 12.70% face flies attracted) than *E. kiautschovicus* (35.8% ± 8.52% face flies attracted). For both plant species, flowers played primary parts in attraction. When used alone, flowers gave more attraction than control or leaves. Leaves alone just had little effect on the response of insects. However, leaves, if used with flowers, could increase, to some extent, the attractiveness.

Because volatiles from *E. europaeus* flowers was better in attraction than any other plant parts of either *E. europaeus* or *E. kiautschovicus*, further tests were focused only on *E. europaeus* flowers. Solvent extraction of flowers was done to confirm effects of attraction. The results indicated that, among the extracts with several solvents (water, methanol and hexane), methanol extract was the best in face fly attraction (Fig. 2), which indicated that the chemicals responsible for attraction were somewhat polar. Frozen flowers showed similar attractiveness to fresh ones, so frozen flowers could be used for collection of volatiles or bioassays when fresh flowers are not available.

Face fly is a veterinary pest, so studies, either basic or applied, have been focused on interactions of face fly-cattle or face fly-manures. Our results present the first report on interactions between face flies and plants.

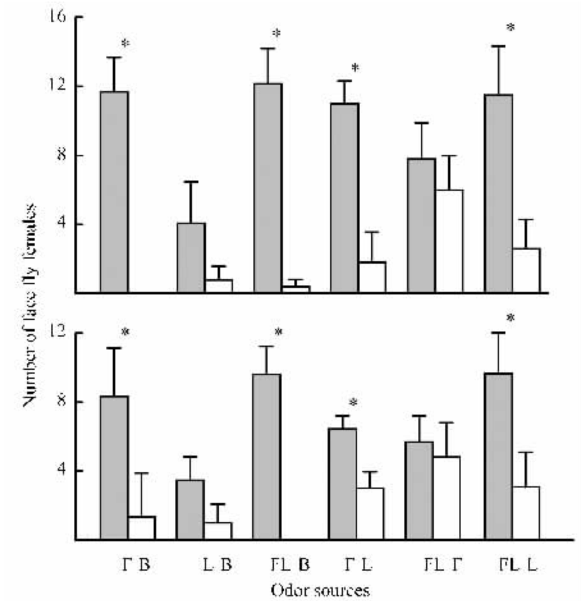

Fig. 1　Attraction of face fly females to *Euonymus europeaus* (top) and *E. kiautschovicus* (botton) in a Y-shaped olfactometer

Odor sources were paired to compare the numbers of face fly females trapped (mean ± *SE*). F = flowers; L = leaves; FL = flowers and leaves; B = blank (tissue paper). Significant difference (marked with an asterisk " * ") of paired odor sources was set at $P < 0.05$ level under *t* test.

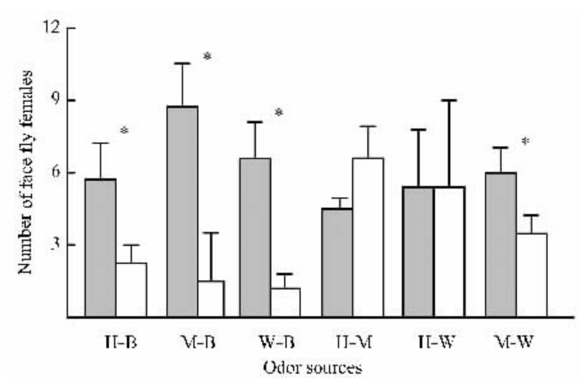

Fig. 2　Responses of face fly females to fresh flower extracts of *Euonymus europeaus* in an olfactometer

Paired odor sources were compared in the numbers of face fly females trapped (mean ± *SE*). Extractions of fresh flower were done with methanol (M), water (W) or hexane (H). B = solvent only. Significant difference (marked with an asterisk " * ") of paired odor sources was set at $P < 0.05$ level under *t* test.

Actually all non-blood-sucking flies need to eat sugars daily, for example, honeydew, extrafloral nectarines or pollens, which indicate that plants, as well as cattle and manure, can influence behaviors and development of veterinary insects. Findings in interactions of plant-veterinary insects could not only widen the areas of veterinary pest ecology but also shed a light on development and application of bio-insecticides of plant origin in management of these pests.

Like other dipterans, face flies are equipped with very sensitive antennae. Face fly antenna funicular sensilla were observed with electron microscope, and male and female funiculi are similar in size, type and distribution of sensilla (Bay and Pitts, 1976). Roles of manure or cattle volatiles in mediating host location for face flies were investigated (Birkett *et al*., 2004). Face fly males stay and feed on plants (Lysyk, 2003), and our present results showed that female face flies find the locations of *Euonymus* spp. mediated by the plant volatiles. Therefore, orientation of face fly females to *Euonymus* spp. volatiles is to locate mates and food sources. Volatiles of flowers play primary roles in attraction of face fly females, indicating that orientation of face fly females to the plants is to feed nectar or pollen as well.

Bio-insecticides of plant origin can be developed in two ways, either by use of pure chemical blends, or by direct applications of plant crude extracts. Screening of bioactive compounds from *E. europaeus* by using gas chromatography-electro-antennographic detection (GC-EAD) will help develop lures used in traps. Once the bioactive compounds of *E. europaeus* flowers are identified, blends of chemicals could potentially be used in management of face fly populations. However, crude extracts are sometimes much better in biological properties than a pure single chemical or blend of chemicals. Further work is needed in development of face fly control tactics from volatiles of *E. europaeus* flowers.

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秋家蝇对两种卫矛植物的趋性

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摘要：卫矛是常见的绿化植物，而秋家蝇 *Musca autumnalis* De Geer 是畜牧业的重要昆虫。在室内利用 Y 型嗅觉仪对欧洲卫矛 *Euonymus europaeus* 和胶东卫矛 *E*. *kiautschovicus* 气味对秋家蝇 *Musca autumnalis* De Geer 的引诱效果进行了研究。结果表明：两种植物都对秋家蝇的雌性成虫有引诱作用，而欧洲卫矛引诱效果更好。进一步对叶和花的气味进行的观察表明，卫矛吸引秋家蝇主要依靠花的气味，但叶的气味也有一定的吸引作用；叶的气味对花的引诱效果有增效作用。利用几种试剂(水、乙醇和正己烷)对欧洲卫矛花的气味物质进行了抽提，测试结果表明水或乙醇抽提物比正己烷抽提物引诱效果更好；冷冻花与新鲜花的引诱效果没有显著的差异。

关键词：秋家蝇；欧洲卫矛；胶东卫矛；引诱作用；生物测定；气味物质；花抽提物

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