This is to certify that the

thesis entitled

ONION FLY BEHAVIORAL RESPONSES TO BACTERIALLY
INFECTED ONION

presented by

Susan Marie Hausmann

has been accepted towards fulfillment
of the requirements for

M.S. degree in Entomology

James R. Miller
Major professor

Date 3-5-86

ONION FLY BEHAVIORAL RESPONSES TO BACTERIALLY INFECTED ONIONS

by

Susan Marie Hausmann

A THESIS
Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1986

ABSTRACT

ONION FLY BEHAVIORAL RESPONSES TO MICROBIALLY INFECTED ONION

by

Susan Marie Hausmann

When cultured on sterilized onion tissue, _Erwinia carotovora_ var. _carotovora_ (EC), a casual organism of soft rot, and _Klebsiella pneumoniae_ (KP), a predominate early colonizer of onions decomposing in the field, produced strong attractants and ovipositional stimulants of the onion fly _Delia antiqua_ (Diptera: Anthomyiidae). EC cultured on onion produced more potent attractants than axenic onion, KP-inoculated onion, EC-inoculated potato, or chemical synthetic attractants. EC cultured on potato (a nonhost of onion fly) produced attractants, but did not stimulate oviposition. EC cultured on onion is a reproducible source of attractants as well as ovipositional stimulants.

D. antiqua ovipositional response on mature onions with different levels of physical and microbial damage was well correlated with larval developmental performance on those treatments. The increased suitability of those damaged plants most preferred by ovipositing females may be due to the ability of first instar larvae to penetrate the bulb. Since damaged bulbs can be an excellent food source for fall generation onion flies, good sanitation practices in the fall may decrease the number of overwintering pupae.

This work is dedicated to my sisters, Kathy Moss and Carol Ann.

ACKNOWLEDGEMENTS

My deepest respect and appreciation goes to my major professor, Dr. James Miller, for his guidance, advice, and perhaps most of all for his trust in me to develop my own lines of scientific inquiry. I also thank the members of my guidance committee, Dr. Jack King, Dr. Karen Klomparens, and Dr. Ed Grafius, for their considerable time and effort devoted to editing manuscripts, offering unique insights, and sharing a bottle of champaigne with me after the final oral exam. The other members of the Miller lab, Marion, Joan, Paul, Jim, Martha, Dave, and Debie, have become my friends, as well as my colleagues. I will miss them very much. And thank you, Andrew, for everything.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GENERAL INTRODUCTION

The onion fly, Delia antiqua (Meigen), is an economically important insect pest of onion in the temperate Northern Hemisphere, including many onion-growing regions of the United States, Canada, Europe, and Asia (Ellis and Eckenrode, 1979). In the field, onion flies mate within one to two weeks after eclosion (Loosjes, 1976). Female flies oviposit on or near their host-plant, and the eggs hatch a few days later. Larvae enter the base of the plant and feed on subterranean plant tissues for two to four weeks before pupating in the soil. Most populations overwinter as diapausing pupae. Diapause is not obligatory, but is induced by lower temperatures and shorter photoperiods (Ramakers, 1973).

There are generally two to three generations a year, but exceptions occur at the limits of its geographical range. For example, only one generation occurs in northern Norway (Rygy, 1960). In southern Japan, both diapause and aestivation occur (Kato, 1958). In Ingham County, Michigan, there are three generations per year with temperatures and photoperiods conducive to diapause beginning in August and September (NOAA, 1984).

The primary host-plant of D. antiqua is onion, Allium cepa. Other plants of the same genus are also attacked, including shallot (A. ascalonicum), leek (A. ampeloprasum), Japanese bunching onion (A. fistulosum) and chive (A. schoenoprasum) (Ellis and Eckenrode, 1979). A characteristic common to alliums is the production of various volatile, sulfur-containing organic molecules (Bernhard, 1970), many of which are responsible for the familiar odors and tastes of this genus.

Because of some interesting and useful properties of Allium spp. extracts, scientists from fields as diverse as medicine and entomology recognized the importance of identifying the secondary metabolites. Allicin from garlic has antimicrobial (Cavallito and Bailey, 1944; Tansey and Appleton, 1975) and insecticidal (Amonkar and Banerji, 1971) properties. There is some evidence that consumption of onion and garlic produces hypoglycemic effects (Brahmachari and Augusti, 1962) and may reduce aortic atherosclerosis (Sainani et al., 1979). Many flavor components in Allium spp., especially onion and garlic, are also important in the food and flavor industry.

Analytical chemists and biochemists have revealed much about the molecular structures and biochemical pathways of onion flavor components. The flavor precursors in onion are S-alk(en)yl-L-cysteine sulfoxides, which are biosynthesized from L-cysteine through a series of complex reactions (Block, 1985). The most abundant precursor is trans-(+)-S-(propen-1-yl)-L-cysteine sulfoxide (Schwimmer and Friedman, 1972). Its biosynthesis in an intact onion (Figure 1) starts with L-cysteine (a) combining with 2-methylpropenoic acid (c), which is itself derived from valine (b). The product, S-(2-carboxypropyl)-L-cysteine (d), is decarboxylated to give propen-1-yl-L-cysteine (e), which is oxidised to the corresponding sulfoxide (f) (Vickery and Vickery, 1981). In mature onion, at least half of the trans-(+)-S-(propen-1-yl)-L- cysteine sulfoxide is bound as gamma-glutamyl peptide and is not accessible for flavor production (Krull et al., 1970).

When onion is cut or crushed, the integrity of the cells is destroyed. Alliinase enzymes (alliin allylsulphonate-lyases) are

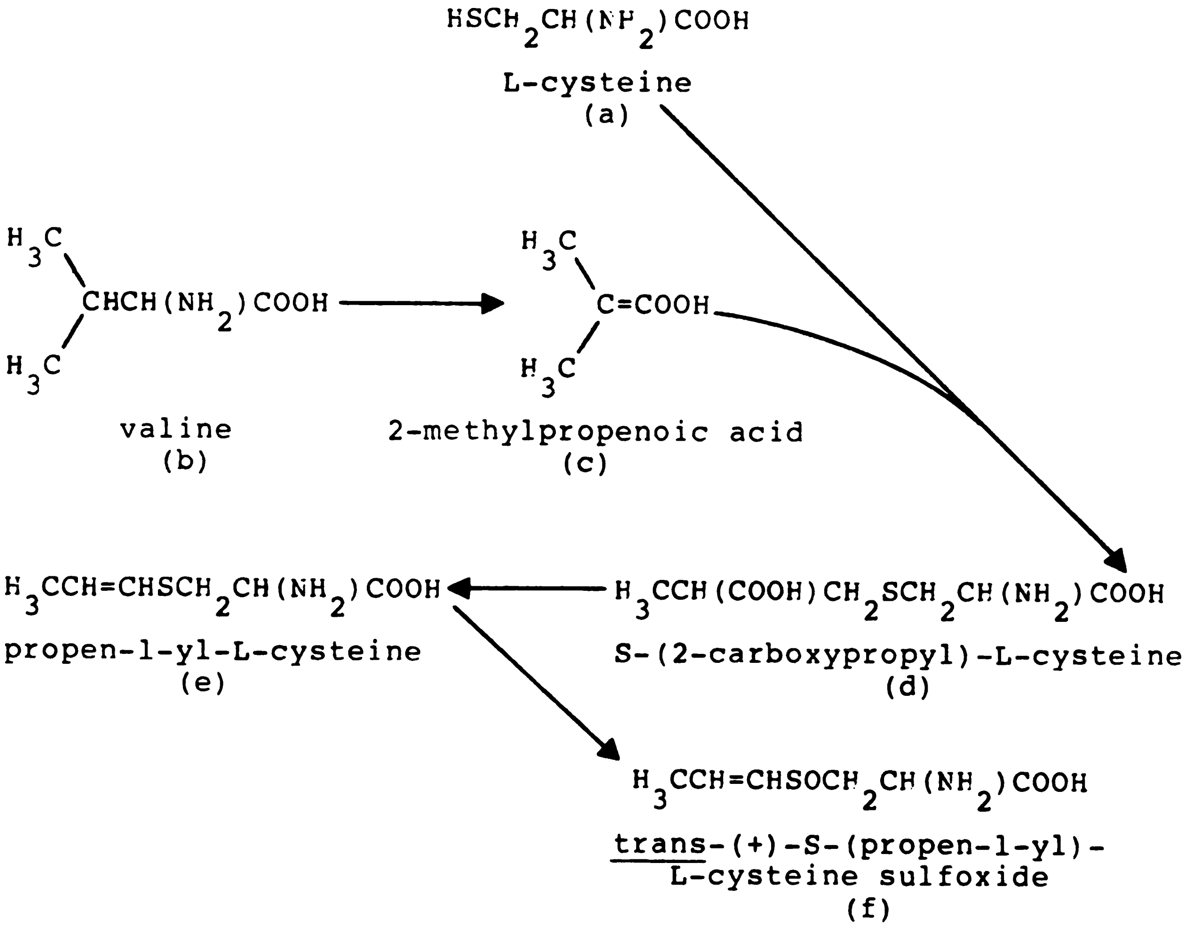

Figure 1. The biosynthesis of the onion flavor precursor trans-(+)-S-(propen-1-yl)-L-cysteine sulfoxide. L-cysteine (a) combines with 2-methylpropenoic acid (c), which is a derivative of valine (b). The product (d) is decarboxylated to give propen-1-yl-L-cysteine (e), which is oxidized, forming trans-(+)-S-(propen-1-yl)-L-cysteine sulfoxide (f) (adapted from Schwimmer and Friedman, 1972; Block, 1985).

released to catalyze the degradation of the S-alk(en)yl-L-cysteine sulfoxides. Sulfenic acids, RSOH, are formed with ammonia and pyruvate as by-products (Schwimmer and Friedman, 1972). Because sulfenic acids are highly reactive, they degrade and react spontaneously to form other sulfur-containing volatiles giving onion its characteristic odor and lacrimatory qualities (Block, 1985).

The formation of the lacrimator, thiopropanal sulfoxide provides a good illustration of the biochemical fate of flavor precursors in onion (Figure 2). When onion is cut or crushed, alliinases catalyze the hydrolysis of trans-(+)-S-propen-1-yl-L-cysteine sulfoxide (f) to propen-1-ylsulfenic acid (g), pyruvate, and ammonia (Block, 1985). By an internal transfer of hydrogens, this unstable sulfenic acid (g) forms the lacrimator (h), which can undergo hydrolysis to form propionaldehyde (i), sulfuric acid, and hydrogen sulfide (Block, 1985). Other degradation products from the sulfenic acid are also possible. For example, N-dipropyl disulfide is a predominate alkyl disulfide produced by onion.

The exact composition of onion flavor components depends on the specific methods and conditions of extraction (Block, 1985). For example, n-dipropyl disulfide and propionaldehyde are the major products obtained by steam distillation. A complex array of sulfur-containing flavor components and associated by-products of onion has been identified, including di- and tri-sulfides, thiosulphinates, and mercaptans (Neigisch and Stahl, 1956; Carson and Wong, 1961; Saghir et al., 1966; Bernhard, 1970; Boelens et al., 1971; Schwimmer and Friedman, 1972; Whitaker, 1976).

The role of non-pathogenic microorganisms in the mediation of onion

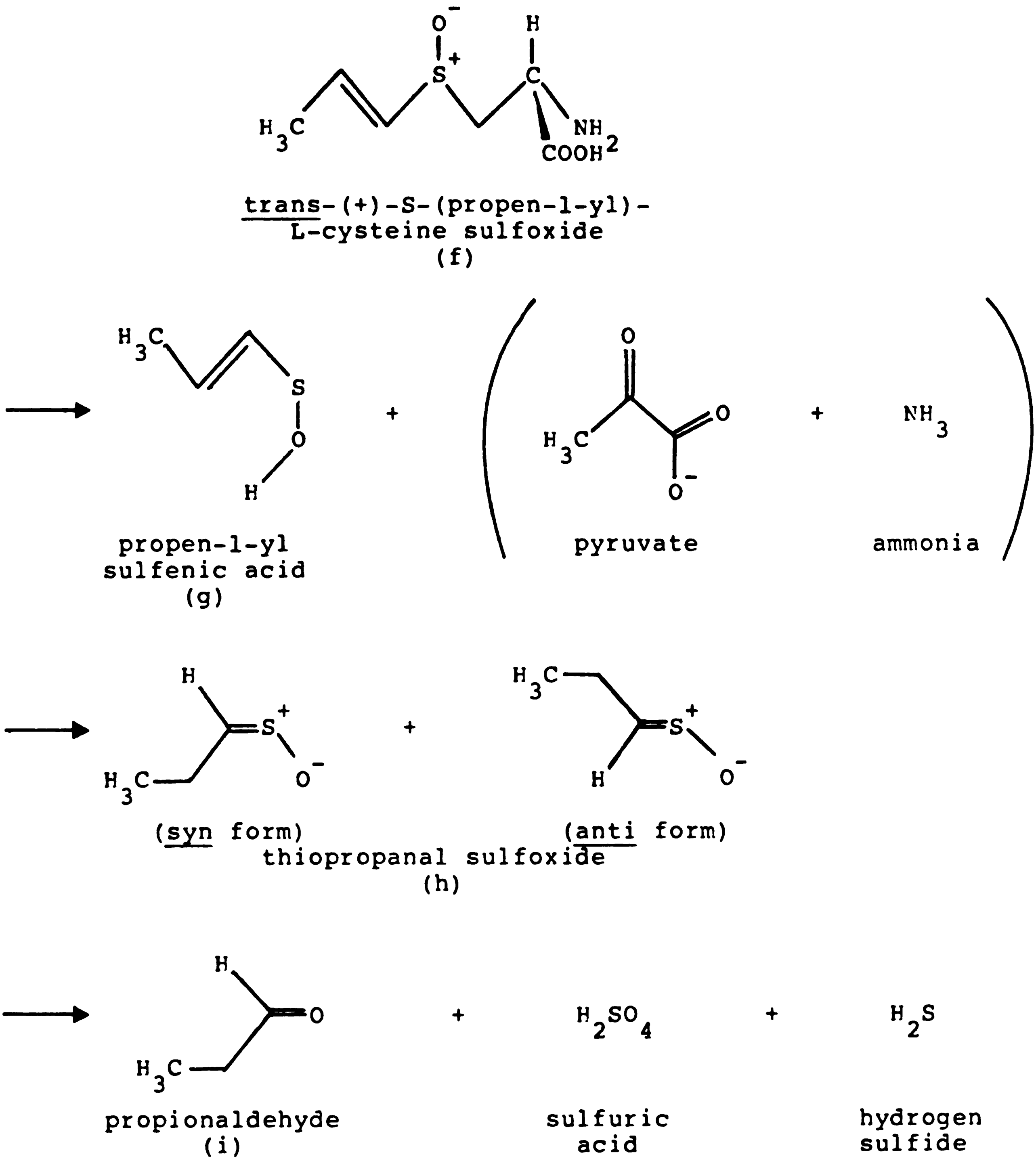

Figure 2. The degradation of trans-(+)-S-(propen-1-yl)-L-cysteine sulfoxide when onion is cut or crushed. The hydrolysis of the sulfoxide (f) is catalyzed by alliinases to give propen-1-ylsulfenic acid (g), and the by-products, pyruvate and ammonia. A transfer of internal hydrogens in the sulfenic acid (g) forms the lacrimator, thiopropanal sulfoxide (h). After hydrolysis, propionaldehyde (i), sulfuric acid, and hydrogen sulfide are formed (adapted from Schwimmer and Friedman, 1972; Block, 1985).

metabolite production has received some attention. Intact onions do not release large amounts of sulfenic acids or their derivatives (Schwimmer and Friedman, 1972). Yet, some sulfoxide precursors may be exuded by the roots into the soil where common soil bacteria can convert them to sulfides and related compounds (King and Coley-Smith, 1969). Following the lead of Ellis et al. (1979), Hough et al. (1981) found that female onion flies lay significantly more eggs on seedlings exposed to non-pathogenic microorgansms than on axenic seedlings. Increased release of sulfur-containing compounds from non-sterile, healthy seedlings probably stimulated oviposition (Hough et al., 1981).

The earliest conclusive studies linking onion fly behavioral responses to specific onion chemicals reported that n-dipropyl disulfide stimulates oviposition of the onion fly (Matsumoto and Thorsteinson, 1968) and functions as an attractant in the field (Matsumoto, 1970). At least 23 sulfur containing compounds, including mercaptans, monosulfides, disulfides, and one trisulfide, have since been shown to stimulate oviposition in the laboratory (Matsumoto and Thorsteinson, 1968; Vernon et al., 1978; Pierce et al., 1978; Ishikawa et al., 1978). The attractancy of some of the compounds has been demonstrated in field trials as well (Matsumoto, 1970; Loosjes, 1976; Vernon et al., 1981; Vernon and Borden, 1983).

Several of these sulfur-containing onion fly attractants/ovipositional stimulants have not been reported as constituents of onion, e.g., dipropyl sulfide and propenylpropyl sulfide catch significant numbers of onion flies when placed in field traps (Vernon et al., 1981). Dipropyl sulfide, methylpropyl sulfide, and dipropenyl sulfide stimulate oviposition in the laboratory (Vernon et al., 1978; Ishikawa et al., 1978); yet, none of these compounds have been found in onions.

Many host- and nonhost-produced ovipositional stimulants of onion fly contain a thiopropyl moiety that may be responsible for olfactory receptor stimulation resulting in an ovipositional response (Vernon et al., 1978; Ishikawa et al, 1978). Active compounds generally have a single sulfur bonded to a saturated hydrocarbon chain 3-5 carbons long (Vernon et al., 1978).

Blending onion chemical synthetics to more closely resemble those released by onion has led to some increase in attractancy (Vernon et al., 1981; Dindonis and Miller, 1981a). Yet, to elicit normal insect responses, release rates of chemical baits as well as chemical composition are important. For example, super- or subnormal concentrations of the pheromone components emitted by female oriental fruit moth Grapholitha modesta significantly decreases field trap catches (Baker et al., 1981). If too little (E)-8-dodecenyl acetate is released relative to (Z)-8-dodecenyl acetate, the male moth loses contact with the odor plume. If too much is released, the male moth is arrested within the plume downwind of the source.

Controlled release rates have been achieved by putting volatile chemicals in polyethylene enclosures (Kuhr et al., 1972). Optimizing release rates of dipropyl disulfide in polyethylene enclosures significantly increased onion fly attractancy in the field (Dindonis and Miller, 1981b). Nevertheless, no synthetic chemical tested singly, in a blend, or with an optimized release rate is consistently more attractive than freshly cut or intact onion plants (Dindonis and Miller, 1980; Dindonis and Miller, 1981a; Ishikawa et al., 1981; Vernon et al., 1981).

Decomposing onions at some stages of microbial and physical damage produce more potent onion fly attractants and oviposition stimulants than healthy or fresh cut onions (Tomioka, 1977; Dindonis and Miller, 1981a; Ishikawa et al., 1981; Miller et al., 1984). Vernon et al. (1981) showed attractancy of decomposing onion in the field increased with longer incubation periods. This changing attractancy was correlated with qualitative and quantitative changes in the head space profile as analyzed with gas-liquid chroatography. Miller et al. (1984) baited traps with chopped onion exposed to microorganisms in the field and also found that attractancy varied as decomposition progressed, yet the attractancy of any decomposing onion proved difficult to predict. In field trials conducted in May and June, attractancy of onion tissue increased with incubation time (Figure 3), which ranged from one to five days. In field trials conducted in late August, trap catches increased with baits aged for one to three days, but then dramatically declined with baits aged for four to five days (Miller et al., 1984) (Figure 3). The authors hypothesized that warmer temperatures increased the rate of decomposition during the August trials. Yet, even during the same week, similiarly treated baits of decomposing onion placed in different fields varied in attractancy (J. R. Miller, personal communication).

The present research focused on the influence of bacterially infected onion on onion fly behavior. The first objective was to isolate and identify one or more bacteria associated with decomposition that produced onion fly attractants and ovipositional stimulants when cultured on onion (Chapter 1). The purpose was to clarify the role of bacteria in the production of attractants/ovipositional stimulants, as well as to define a reproducible source of these compounds that may be used in future work aimed at identifying the potent chemical components.

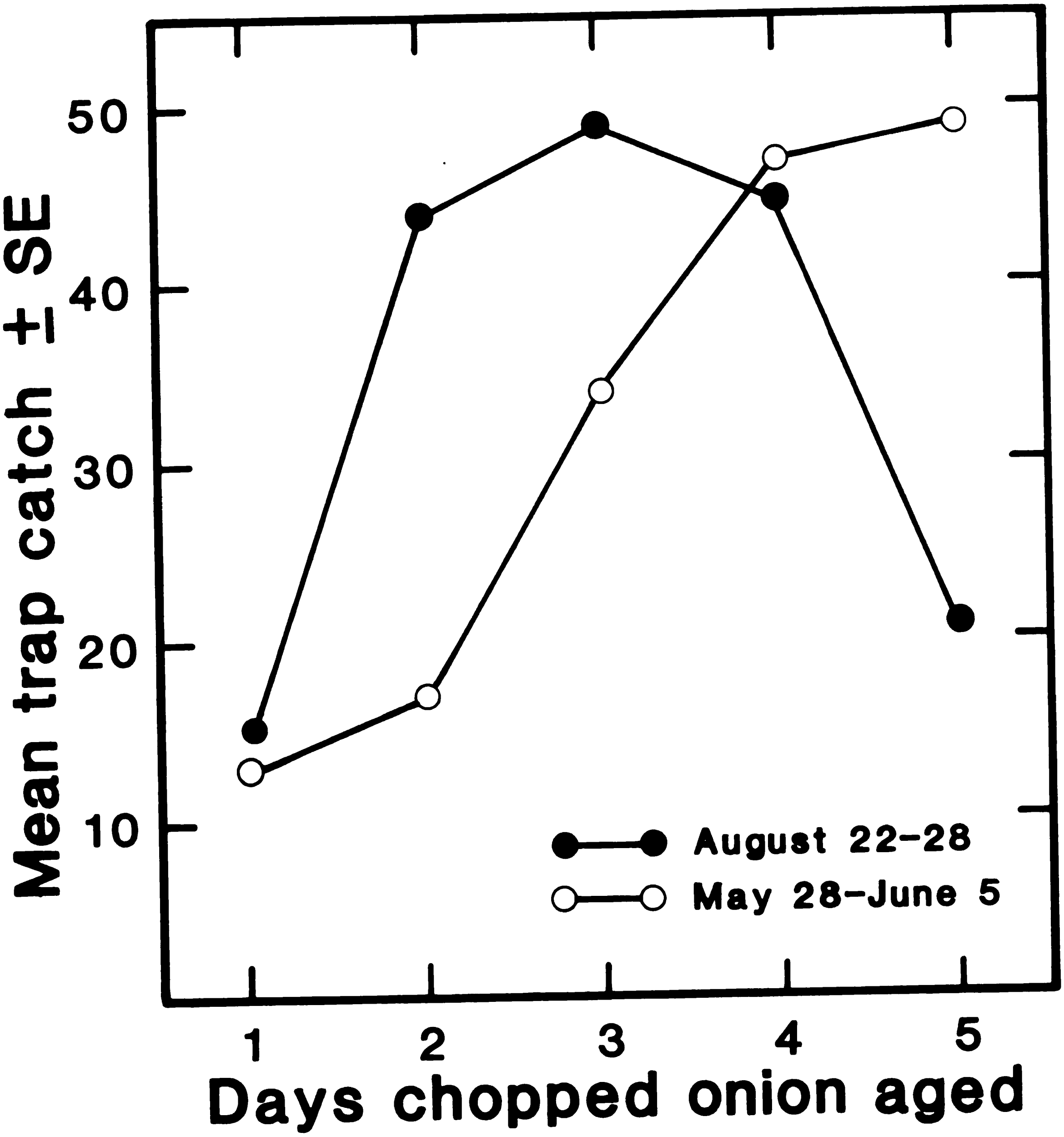

Figure 3. Onion fly catch as affected by onion bait aged in the field after chopping (adapted from Miller et al., 1984).

The second objective was to document how _D. antiqua_ ovipositional preference and subsequent larval performance was influenced by different levels of physical and microbial damage to maturing onions (Chapter 2). In the fall, female onion flies must oviposit on maturing bulbs. Their offspring feed on these older plants and overwinter as diapausing pupae. After emergence and mating the following spring, females oviposit on onion seedlings. Onion maggot damage in the spring accounts for most of the crop losses during the growing season. Any pest management scheme aimed at reducing the number of overwintering _D. antiqua_ pupae, must begin with an understanding of ovipositional preference and larval performance on the maturing bulbs in the fall. An important goal of this study was to determine whether ovipositional preference may have adaptive value for fall generation onion flies.

CHAPTER 1

Production of onion fly attractants and ovipositional stimulants by bacterial isolates cultured on onion

CHAPTER 1

Introduction

Decomposing onion produces more potent onion fly attractants and ovipositional stimulants than does healthy or freshly cut onion (Tomioka, 1977; Dindonis and Miller, 1981a; Ishikawa et al., 1981; Miller et al., 1984), but only at certain successional stages of microbial and physical damage (Ishikawa et al., 1981; Vernon et al., 1981; Miller et al., 1984). Using gas-liquid chromatography (GLC), Vernon et al. (1981) demonstrated that changes in onion fly attractancy were associated with qualitative and quantitative changes in the headspace chemical profile of onion as decomposition progressed.

Many factors influence volatile metabolite production in decomposing onion. A complex interaction of environmental factors such as temperature and relative humidity, the characteristics of the host-plant, and the presence of competing microorganisms and their metabolites may influence which organisms and volatile metabolites predominate.

A bacterial species on a nutritive medium produces a headspace profile that is often unique. Indeed, using GLC for the qualitative and quantitative determination of volatile compounds has become an accepted technique in the identification of anaerobic bacteria (Zechman and Labows, 1984). By analyzing the headspace profile of pure cultures, anaerobic bacteria have been identified to the species level (Larsson et al., 1980). The anaerobic bacteria infecting urine or other body fluids have been detected and identified both by direct headspace analysis of the clinical sample and by subculturing the bacteria onto an appropriate medium before analysis (Taylor, 1980; Mardh et al., 1981). Some aerobic bacteria are also known to produce distinctive volatile metabolites (Bowman et al., 1983; Pittard et al., 1982; Freeman et al., 1976; Lee et al., 1979).

The variable attractancy of decomposing onion has hindered work aimed at identifying potent onion fly attractants and ovipositional stimulants. A reproducible source of these compounds was obtained by culturing bacterial isolates, which are associated with onion decomposition, on sterilized onion tissue. The experiments described in this chapter clarify the role of such bacteria in the production of onion fly attractants.

Materials and Methods

Insects

Parental stock originated from commercial onion fields in Eaton Rapids, MI. Insects used were 5-7 generations removed from the field. Adults were fed water and a dry artificial diet (Ticheler, 1971) and housed as described by Schneider et al. (1983), except sprigs of onion seedling foliage was replaced by green surrogate stems (Harris and Miller, 1983) in ovipositional dishes. Larvae were reared in sand-filled boxes provisioned with an excess of cross-sectioned onion bulbs.

All behavioral bioassays were conducted at 35 ± 5% RH, 23 ± 1° C, and in a 16:8 L:D light regime (alternating cool 55 W and warm 85 W white flourescent bulbs 10 cm above the cages), corresponding to light during the hours 0700-2300. Three days before assaying, flies from the laboratory culture were placed in cylindrical, metal-screened cages (42 cm diameter x 46 cm height). Between assays, flies were given food, water, and access to ovipositional dishes of freshly cut onion covered with moist sand. In the center of each dish was placed a surrogate onion stem (4mm diameter x 9 cm length above substrate) constructed from a hollow, sealed, glass cylinder, enclosing a strip of green paper. Before each assay, the water dish was placed in the center of the cage, but food and ovipositional dishes were removed.

Bacterial isolates

Erwinia carotovora var. *carotovora* (EC) is a casual organism of soft rot on a wide range of vegetables. Onion maggots moving through infested soil may spread the disease (Agrios, 1978). EC was isolated from damaged Spartan Banner hybrid stored onions potted in moist muck soil taken from a commercial onion field in East Lansing, MI. Damage was inflicted by 20 *D. antiqua* second instars taken from the laboratory culture and added to each potted bulb. After five days of larval feeding, 10 g of the decomposing plant tissue was homogenized in 25 ml sterile buffer (0.2 M KH_2PO_4 and 0.2 M K_2HPO_4, pH 7.0) with a sterilized mortar and pestle. Dilutions of the homogenate were made in buffer and plated on CVP, a selective medium for the isolation of soft-rot bacteria (Cuppels and Kelman, 1974). This medium, which contains crystal violet and sodium polypectate, was modified by the addition of manganous sulphate, an anti-foam agent (O'Neill and Logan, 1975). Plates were incubated overnight at $25 \pm 2^{\circ}$ C.

Colonies of *Erwinia* spp. formed deep, cuplike depressions in the selective medium. They could be distinguished from pectolytic *Pseudomonas* spp., which form shallow, wider depressions (Cuppels and Kelman, 1974). A bacterial isolate of *Erwinia* sp. was obtained and designated as strain EC6. Using current taxonomic keys and texts, the isolate was identified (Kelman and Dickey, 1980; Lelliott, 1974; Dye, 1969).

Klebsiella pneumoniae was cultured from an isolate stored at -65° C in 4% DMSO:nutrient broth (v:v). Nutrient broth consists of 3 g beef extract and 5 g peptone (Difco) per liter of distilled water. The original strain, JM1, was isolated and identified as a predominant microorganism from decomposing onions that were five times as attractive in the field than fresh cut onions (Miller et al., 1984).

For use in experiments, cultures of both species were grown on nutrient agar (nutrient broth with 15 g/l of Bacto-agar) at 25 ± 2° C. Stock cultures were maintained on nutrient agar in the refrigerator at 4-6° C.

Preparation of axenic onion and potato

Axenic plant tissue was prepared from Russet Burbank potatoes and an undetermined cultivar of yellow onions from commercial storage. The outer, papery scales and upper and lower 2 cm of bulb were removed; pototoes were peeled. Plant tissue was soaked in 75% ethanol for 20 minutes and flame sterilized 1-2 minutes. The flamed outer 0.5 cm of potato and the flamed outer 2 onion scales were removed with sterilized instruments and discarded.

Two sizes of potato and onion tissue were cut. Squares of tissue measuring 3.5 x 3.5 x 0.5 cm and 2 x 2 x 0.5 cm were placed individually in polystyrene petri dishes (diameters of 5.5 cm and 3.5 cm, respectively). Onion squares were cut only from the 2-3 outer scales. The scale thickness (ca. 0.5 cm) varied slightly. Potato squares were cut from transverse tuber sections 0.5 cm thick. Enough sterilized, distilled water was added to cover the dish bottom. Tissues were incubated at 25 ± 2^{o} C. The concentration of sulfur- and nitrogen-containing compounds in onion are known to differ in the outer and inner scales (Freeman, 1975), so only tissue taken from the same onion scale (or the same potato) was used within the same cage for bioassays.

Experiment 1 - Attractancy of axenic onion

Approximately 100-125 flies of both sexes and of mixed ages were placed in each cylindrical cage. Between hrs 1100 and 2200, treatments were assayed simultaneously by putting different test materials into traps equally spaced along the periphery of cylindrical cages. After the bioassay, flies in each trap were sexed and counted.

Traps are described in Weston and Miller (1985). They were constructed from 400-ml glass beakers with three equally spaced holes (1 cm diam) around the circumference of the base. Glass tubing (0.8 cm ID x 4 cm length) was inserted through the holes until ca. 1 cm projected outside the beaker. Before addition of test materials, traps were autoclaved. To minimize direct contact with captured flies, test materials were placed in an autoclaved wire-screen basket suspended from the top of the trap. A beaker lid made from a sterile, polystyrene petri dish and the glass tubing were sealed onto the trap with Parafilm^{TM}.

Axenic onion was an important control in all the behavioral bioassays, therefore, the influence of time axenic onion tissue was held before use in the bioassays had to be established. In each of four cages, traps were baited with an axenic onion squares (3.5 x 3.5 x 0.5 cm) cut immediately before assaying, cut axenic onion held for 1, 2, and 3 days before assaying, and a petri dish filled with sterilized, distilled water (water control). At the time of cutting, two parallel grooves ca. 1 cm apart were cut into each square. Onion tissues were assayed in the original water-filled polystyrene petri dishes with the covers removed.

Experiment 2 - Attractancy of bacteria cultured on onion over time

To determine the minimal length of time necessary to incubate bacteria with onion to obtain significant differences in attractancy, traps were baited with axenic onion inoculated with one of the bacterial isolates and incubated 1, 2, or 3 days before assaying. These treatment were compared to freshly cut axenic onion, and a water control. To inoculate onion with bacteria, two parallel grooves ca. 1 cm apart were cut into each square. A loopful of 24 hr inoculum was placed in each groove. Tissues were incubated at 25 ± 2^{o} C. Experiments using EC and using KP were replicated seven times as described in Experiment 1.

Experiment 3 - Relative attractancy of EC- and KP-inoculated onion

To determine the relative attractancy of the two isolates cultured on onion, traps were baited with onion inoculated with EC, KP, or EC plus KP and incubated for three days at 25 ± 2^{o} C before assaying and with freshly cut axenic onion. Onion was inoculated as described in Experiment 2 with EC or KP inoculum placed in both grooves of a square or with EC inoculum in one groove and KP inoculum in the other groove. Eight replications were conducted as described in Experiment 1.

Experiment 4 - Attractancy of EC-inoculated host- and nonhost-plant

The volatile onion fly attractants produced by EC-inoculated onion may be due to EC, regardless of the nutritive medium, or to the interaction of EC with onion, the primary host plant of the onion fly. To determine the importance of the host plant in the production of attractants, EC was cultured on axenic onion and axenic potato, a nonhost plant of onion fly, as described in Experiment 2. The bioassay was similiar to that described in Experiment 1 with traps baited with EC-inoculated onion, EC-inoculated potato, axenic onion, axenic potato, and a water control. All tissues were incubated three days before assaying, except axenic onion, which was freshly cut. Experiments were replicated seven times.

Experiment 5 - Attractancy of EC-inoculated onion vs. synthetics

In a bioassay similiar to that described in Experiment 1, the attractancy of onion inoculated with EC and incubated 3 days before assaying was compared to a water control and synthetic chemical attractants.

One chemical attractant was a solution of 100 ul 2-phenylethanol (2-PhEt) and 25 ul pentanoic acid was mixed in 50 ml distilled water. These chemicals were identified by Ishikawa et al. (1984) as attractants extracted from decomposing onions. This formulation was reported to give an optimal release rate for onion fly attractancy in the field (Ishikawa et al., 1984). A ten-ml glass beaker with 10 ml of this aqueous solution was placed in traps immediately before testing.

A second chemical attractant was n-dipropyl disulfide (Pr_2S_2), a predominate secondary metabolite obtained by steam distillation of fresh onions (Block, 1985). For an optimal release rate (ca. 100 ug/hr), 100 ul of Pr_2S_2 (purity 99%) was placed into size 3 BEEM^{TM} (Ted Pella Co., Box 510, Tustin, CA, 92680) polyethylene enclosures (Dindonis and Miller, 1981b). To attain a stable release rate, filled capsules were held for six hours prior to assaying. One capsule per trap was used. The experiment was replicated four times.

Experiment 6 - Oviposition on EC-inoculated host- and nonhost-plant

Six to eight ovipositing female onion flies were placed in each cage. In the laboratory, peak oviposition occurs 9-11 hrs (1600-1800) into scotophase (Miller, personal communication). Treatments were assayed between hrs 1500 and 2100; the numbers of eggs were counted by water flotation.

Ovipositional dishes (35 ml) were filled with sterilized sand moistened with sterilized, distilled water. A surrogate onion stem was placed in the center of each dish. By spacing ovipositional dishes equally along the cage periphery, different test materials were assayed simultaneously.

Two separate experimental set-ups were used. In the first experiment, the small squares of plant tissue (2.0 x 2.0 x 0.5 cm) along with associated water was placed ca. 0.5 cm beneath the sand surface immediately before assaying. No plant material was placed in the cup designated as a water control.

To determine whether test material volatiles alone are sufficient to stimulate oviposition, a second experiment was conducted. Petri dishes containing the larger squares of tissue (3.5 x 3.5 x 0.5 cm) and water were placed in sterilized glass quart canning jars. A GLC septum was inserted into each of two holes drilled into the jar lid. Teflon^{TM} tubing (1 mm ID) was inserted into each septum. One tubing passed through the side of the screened walls of a cage and was threaded through a septum inserted into a hole in the side of an ovipositional cup. The tubing opened ca. 5 cm from the surrogate stem and ca. 1.25 cm below the sand surface. The second tube led from the jar lid to a small plastic manifold and then to the filter of a compressed air tank. Air flowed at a rate of 10mm/min through each tube leading from the tank to a jar. Air carrying volatiles from the jar was then pumped into an ovipositional cup.

In both experiments, ovipositional dishes or jars with tubes leading to ovipositional dishes were baited with onion or potato inoculated with EC and incubated for 3 days before assaying, axenic potato held for 3 days before assaying, freshly cut axenic onion, and a water control. Preparation of treatments followed the procedure outlined in Experiments 1 and 2. Both experiments were replicated four times.

Results

Identification of Erwinia carotovora var. carotovora

Bacterial cells of EC6 were motile, straight, short rods, occurring singly and sometimes in pairs or short chains. This isolate was gram-negative, facultatively anaerobic, grew at 36^{0} C on nutrient agar, did not produce reducing substances from sucrose, and utilized Simmon's citrate. Based on these results and characteristic growth on CVP medium, EC6 was identified as a strain of Erwinia carotovora var. carotovora.

Experiment 1 - Attractancy of axenic onion

Freshly cut, sterilized onion tissue was a significantly more attractive bait than the water control ($P \leq 0.05$) (Figure 4). Sterilized onion tissues incubated 1 day or more before being used as bait lost activity ($P \leq 0.05$). Indeed, the numbers of flies caught with traps baited with sterilized onion tissues incubated 1, 2, and 3 days

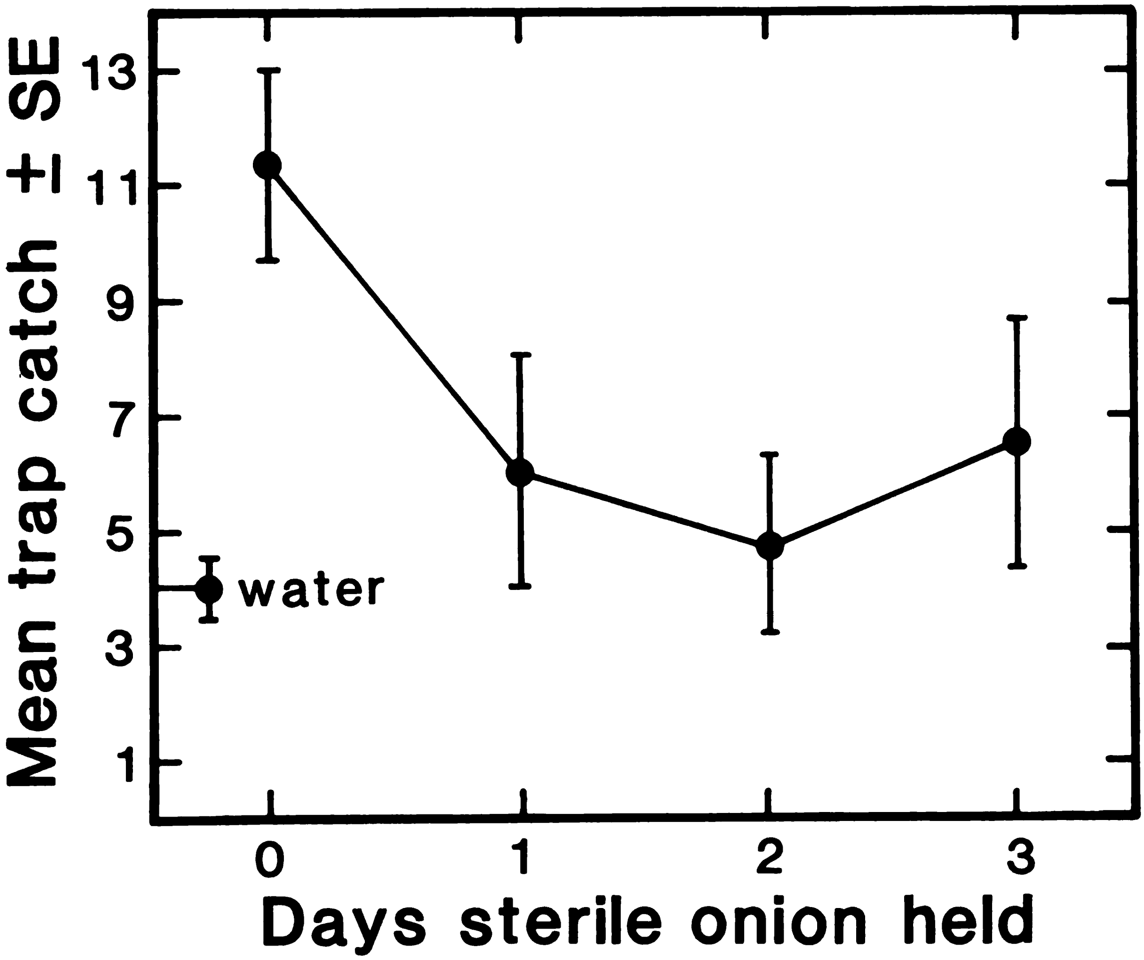

Figure 4. Onion fly attractancy as influenced by length of time sterilized onion tissue was held before assaying. Orthogonal contrasts: freshly cut onion vs. onion incubated 1, 2, and 3 days ($P \leq 0.05$); freshly cut onion vs. water control ($P \leq 0.05$); and water control vs. onion incubated 1, 2, and 3 days ($P > 0.20$).

before assaying or with the water control were not significantly different ($P > 0.20$). In all remaining bioassays, sterilized onion tissue controls were cut immediately before assaying.

Experiment 2 - Attractancy of bacteria cultured on onion over time

Attractancy of EC-inoculated onion increased with incubation times from one to three days (Figure 5), however, the differences in mean trap catch were significant only for EC-inoculated onion incubated 3 days vs. 1 day ($P \leq 0.05$). Onion inoculated and incubated 2 or 3 days caught significantly more flies than either non-inoculated onion or the water control ($P \leq 0.05$). Few flies were caught in traps baited with non-inoculated onion, EC-inoculated onion incubated only 1 day, and the water control. The mean differences between these three treatments were not significant ($P > 0.20$).

A similiar pattern of increasing attractancy with incubation times from one to three days was seen for KP-inoculated onion tissue (Figure 6). Traps baited with KP-inoculated onion incubated 3 days caught significantly more flies than those baited with 1-day incubated tissue ($P \leq 0.10$). KP-inoculated onion that was incubated 2 days was intermediate in attractancy between tissue incubated 1 and 3 days, but these differences were not significant ($P > 0.20$). Regardless of incubation time, KP-inoculated onion was more attractive than non-inoculated onion ($P \leq 0.10$) and the water control ($P \leq 0.05$). Freshly cut, sterile onion also caught significantly more flies than the water control ($P \leq 0.05$).

A three day incubation period was sufficient to obtain significant differences in attractancy between bacterially-inoculated onion vs. sterilized onion. In the remaining bioassays, EC- or KP-inoculated

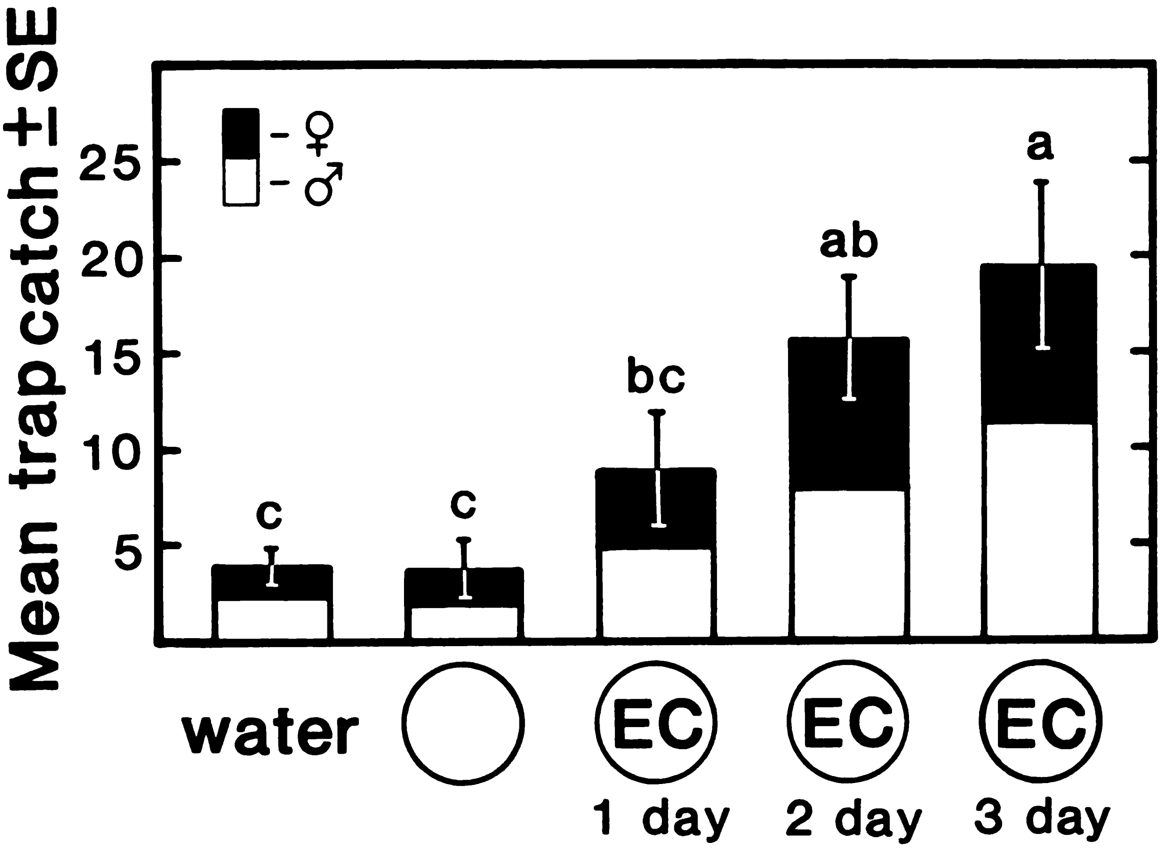

Figure 5. Influence of incubation time on onion fly attractancy of EC-inoculated onion. Sterilized onion tissue (○) inoculated with *Erwinia carotovora* var. *carotovora* (EC) and incubated 1, 2, or 3 days before the bioassay. Treatments marked by different letters are significantly different ($P \leq 0.05$). ANOVA, Tukey's HSD all pair-wise comparisons.

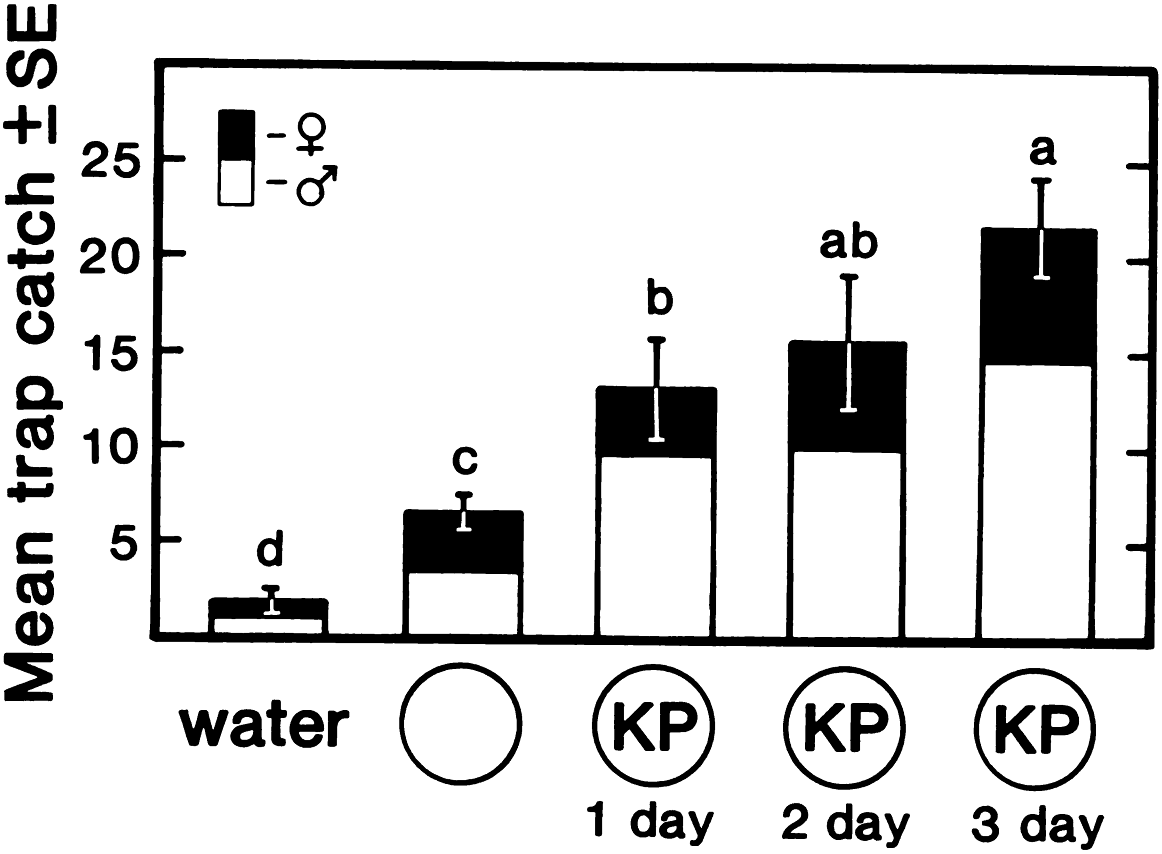

Figure 6. Influence of incubation time on onion fly attractancy of KP-inoculated onion. Sterilized onion tissue (○) inoculated with *K. pneumoniae* (KP) and incubated 1, 2, or 3 days before the bioassay. Treatments marked by different letters are significantly different ($P \leq 0.10$). ANOVA, Tukey's all pair-wise comparisons.

tissue was incubated three days before use in experiments.

Experiment 3 - Relative attractancy of EC- and KP-inoculated onion

Traps baited with onion inoculated with EC caught significantly more flies than those baited with KP-inoculated onion ($P \leq 0.01$) (Figure 7). Onion tissue inoculated with both bacteria did not catch significantly more flies than onion inoculated with either bacterial isolate alone ($P > 0.20$). Regardless of the bacterial isolate used, all inoculated onion baits caught significantly more flies than traps baited with non-inoculated onion ($P \leq 0.01$). The remaining experiments focused on the bacterial isolate EC.

Experiment 4 - Attractancy of EC-inoculated host- and nonhost-plant

The effects of the two factors in this experiment, i.e., plant tissue and the presence or absence of EC, interacted significantly ($P \leq 0.01$) (Figure 8). The highest mean trap catch was attained with EC-inoculated onion. Traps baited with EC-inoculated potato caught significantly more flies than those baited with non-inoculated potato ($P \leq 0.01$), but significantly fewer flies than those baited with EC-inoculated onion ($P \leq 0.01$). No significant differences in mean fly catch were seen in traps baited with sterilized onion or potato ($P > 0.20$).

Experiment 5 - Attractancy of EC-inoculated onion vs. synthetics

Traps baited with EC-inoculated onion caught significantly more flies than any other bait used ($P \leq 0.01$) (Figure 9). The mean fly catches obtained with traps baited with either chemical attractant or with non-inoculated onion were all low and did not differ significantly from each other ($P \geq 0.20$). All baits tested caught significantly

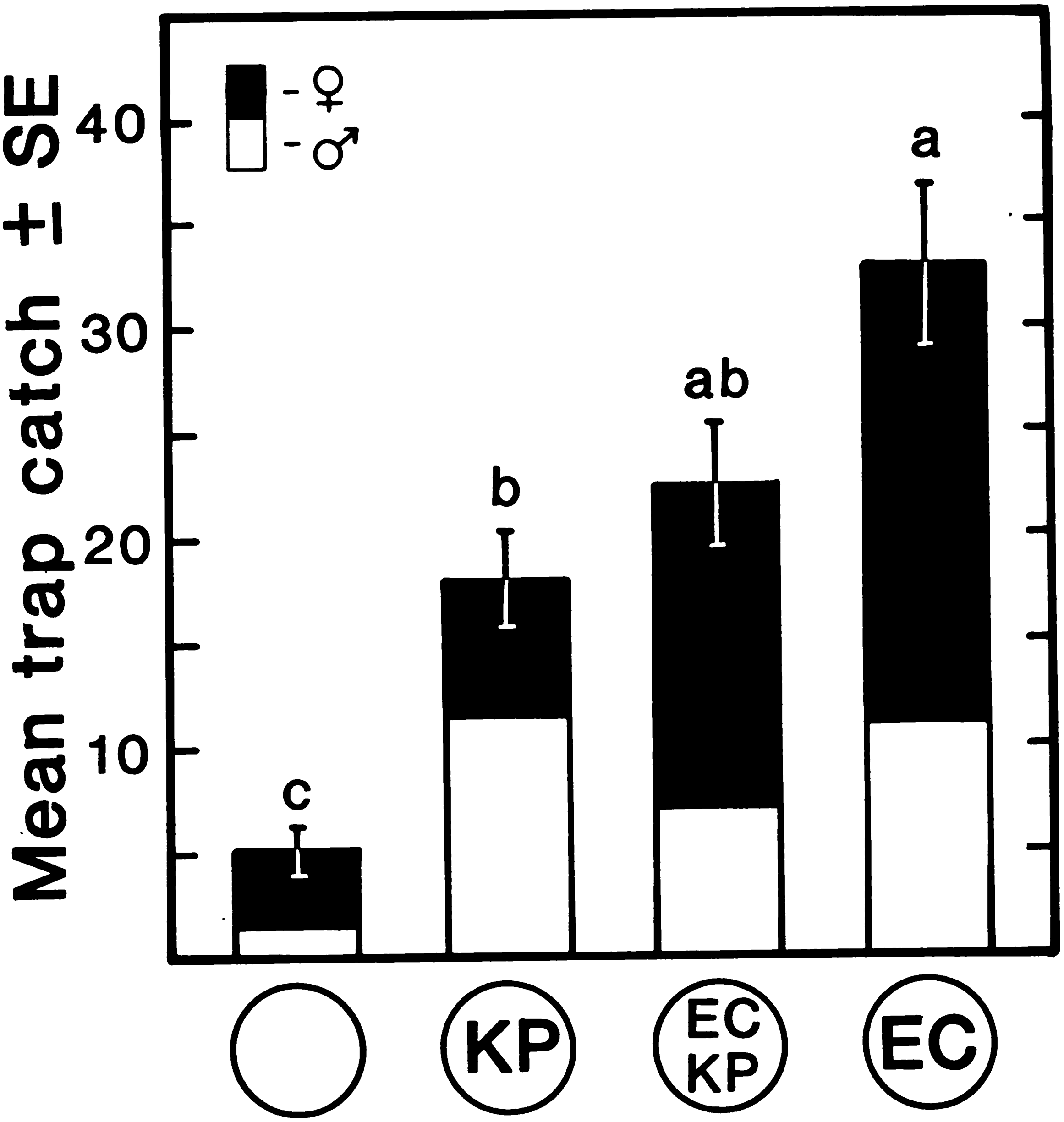

Figure 7. Relative onion fly attractancy of EC and/or KP inoculated on sterilized onion tissue (○) and incubated 3 days before the bioassay. Treatments marked by different letters are significantly different ($P \leq 0.01$). ANOVA, Tukey's HSD all pair-wise comparisons.

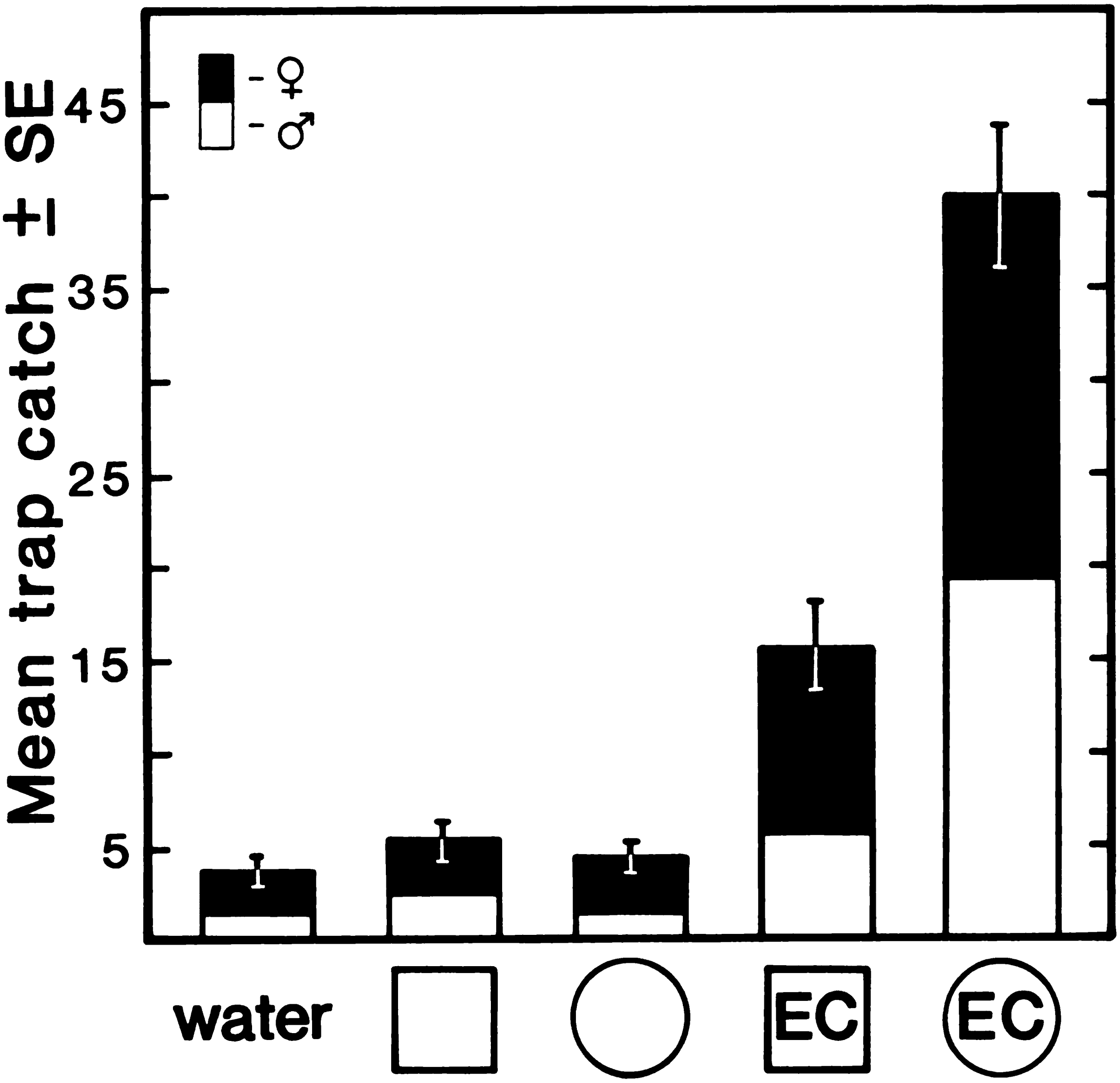

Figure 8. Production of onion fly attractants by the interaction of EC on onion fly host- and nonhost-plant tissue. Sterilized onion tissue (○) and sterilized potato tissue (□) inoculated with EC and incubated 3 days before the bioassay. Two-way ANOVA of data transformed to $(X + 0.5)^{1/2}$. Significant interaction between plant tissue and presence or absence of EC ($P < 0.01$). Orthogonal contrasts: potato vs. EC-inoculated potato ($P \leq 0.01$); onion vs. EC-inoculated onion ($P \leq 0.01$); EC-inoculated potato vs. EC-inoculated onion ($P \leq 0.01$); potato vs. onion ($P > 0.20$).

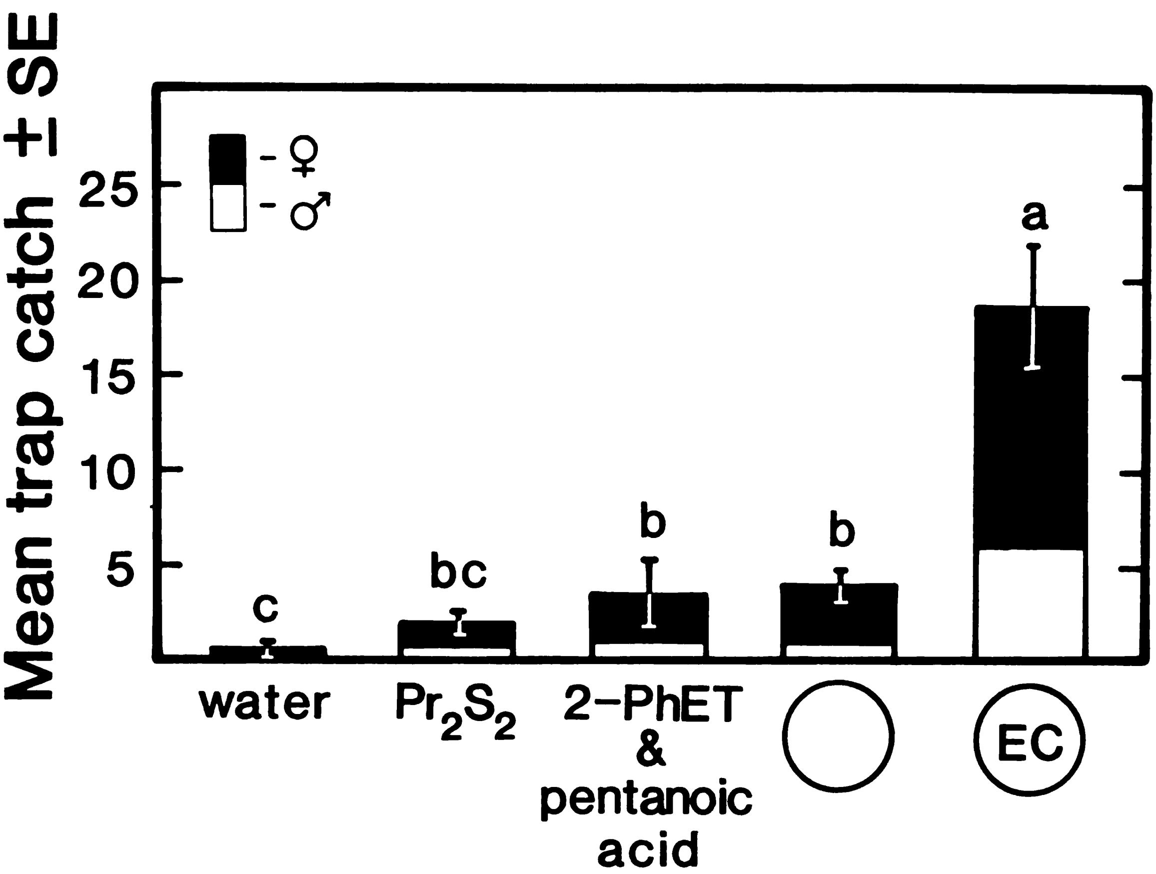

Figure 9. Comparison of chemical synthetics and EC-inoculated onion as onion fly attractants. Baits include dipropyl disulfide (Pr_2S_2) released from polyethylene enclosures, an aqueous solution of 2-phenylethanol (2-PhEt) and n-pentanoic acid, and sterilized onion (○) inoculated with EC and incubated 3 days before the bioassay. Treatments marked by different letters are significantly different ($P \leq 0.05$). ANOVA, Tukey's HSD all pair-wise comparison.

Table 1. Onion fly ovipositional responses to host- and nonhost-plant tissue inoculated with EC. Treatments presented in ovipositional dishes (Experiment A) and as volatiles pumped into ovipositional dishes (Experiment B).

	Mean[1] eggs laid	
Treatment	Experiment A	Experiment B
EC-inoculated onion	$73.5^{a} \pm 23.87$	$47.5^{a} \pm 10.54$
non-inoculated onion	$12.5^{b} \pm 2.96$	$1.0^{b} \pm 0.56$
EC-inoculated potato	$1.0^{c} \pm 1.00$	$1.0^{b} \pm 0.41$
non-inoculated potato	0.0^{c}	0.0^{b}
water control	0.0^{c}	0.0^{b}

[1] Means ± SE followed by the same letter within a column are not significantly different, one-way ANOVA of data transformed to $(X + 0.5)^{1/2}$ followed by Tukey's all-pair wise comparisons ($P \leq 0.05$) (Gill, 1978).

more flies than the water control ($P \leq 0.05$), except Pr_2S_2 ($P > 0.20$).

Experiment 6 - Oviposition on EC-inoculated host- and nonhost-plant

In the first ovipositional choice experiment, test materials were placed directly in the ovipositional dishes (Table 1, Experiment A). Dishes containing EC-inoculated onion received significantly more eggs than any other treatment ($P \leq 0.01$). Freshly cut, sterilized onion received significantly fewer eggs than EC-inoculated onion ($P \leq 0.01$), but significantly more eggs than all other treatments ($P \leq 0.05$). No other significant differences were found ($P > 0.20$).

In the second ovipositional choice test, volatiles of these same treatments were pumped into the ovipositional dishes (Table 1, Experiment B). Dishes with volatiles from EC-inoculated onion received significantly more eggs than any other treatment group ($P \leq 0.01$). No other differences were found among the remaining treatments ($P > 0.20$), all of which received very few eggs.

Discussion

It was not unexpected that freshly cut, sterilized onion tissue was more attractive to onion flies than tissue incubated 1 day or more before bioassaying. The unstable sulfenic acids released after cutting onion tissue degrade to form various sulfur-containing volatiles that, in part, give onion its characteristic odor and taste (Figure 2) (Block, 1985). Many of these volatiles, such as dipropyl disulfide, are known to be attractive to and stimulate oviposition of onion fly (Matsumoto and Thorsteinson, 1968; Vernon et al., 1978; Ishikawa et al., 1978). Since the release of volatiles from damaged onion cells is exhausted rather quickly, the attractancy of freshly cut sterilized onion would be expected to decrease with time. Freshly cut sterilized onion was chosen as a more appropriate control for the remaining assays testing the influence of bacteria cultured on sterilized onion tissue.

Trap catches increased as the length of time EC- or KP-inoculated onion was incubated before assaying. This increased activity may have been due to changes in the volatiles produced over time. Quantitative changes in volatiles may occur as the bacterial population increases, perhaps producing larger amounts of pectolytic and cellulolytic enzymes (Echandi et al., 1957). Qualitative changes are possible if the initial bacterial and plant metabolites react chemically or are re-metabolized by the bacteria, resulting in time-dependent changes in headspace metabolites. In addition, as bacteria exhausted the supply of available metabolites, one would expect changes in the composition of volatiles produced.

The experiments demonstrating the effect of incubation time on attractancy of bacteria cultured on onion illustrate the importance of standardizing environmental factors. If conditions were not held constant, the rate of successional changes in headspace metabolites may have been altered. Factors such as temperature, size of plant tissue, and length of incubation time all were controlled to produce a fairly consistent headspace profiles from inoculated plant tissue.

Both KP and EC produced attractants when inoculated on onion. EC was a better candidate under the given experimental conditions because of its significantly higher attractancy than KP or KP plus EC when cultured on onion. If the two bacteria on the same onion tissue had produced more potent attractants than either one alone, it would have been evidence that at least some of the attractant volatiles produced were unique to one bacterium and capable of increasing the attractancy of the other.

Human olfaction detects little differences between sterilized and EC-inoculated potato tissue. Indeed, many vegetables infected with E. carotovora var. carotovora alone do not release the unpleasant odors we commonly associate with decomposing plants. Saprophytic bacteria, which colonize plant tissue after the pathogen, generally are responsible for the foul odor. Two notable exceptions are crucifers and alliums, both of which release unpleasant sulforous odors when inoculated with the soft rot pathogen (Agrios, 1978).

Peterson (1924) demonstrated that the addition of ethanol, 2-propenol, and isopropanol to honey increased the attractancy of these sweetened baits. EC-inoculated potato tissue may release some simple fermentation products, such as short-chained alcohols that are not particularly odoriferous but may still may function as mild onion fly attractants. Since EC-inoculated potato was not nearly as attractive as EC-inoculated onion (Figure 8), the potency of EC-inoculated onion was due to the interaction of the bacterium with the primary host-plant of D. antiqua.

The aqueous solution of 2-PhEt and pentanoic acid was far less attractive than EC-inoculated onion tissue (Figure 9), indicating that one or more potent volatile attractants associated with decomposing onions remain to be identified. Indeed, the low fly catches obtained with 2-PhEt/pentanoic acid as well as with Pr_2S_2 demonstrate that currently known synthetic attractants do not closely simulate fresh or maximally attractive decomposing onion.

In behavioral bioassays designed to test the efficacy of attractants or deterrents, the use of appropriate controls is sometimes neglected or infeasible. The relative effectiveness of a candidate chemical synthetic must be compared not only with a negative control, e.g., an unbaited or water-filled trap in attractancy assays, but also with a positive control, e.g. the optimal source of the attractant.

Although aqueous solutions of 2-PhEt/pentanoic acid tend to catch more flies than unbaited traps (Ishikawa et al., 1984; Ishikawa et al., 1986), few data have been available on its efficacy relative to decomposing onions of proven attractancy. The strength of 2-PhEt/pentanoic acid as an onion fly attractant in laboratory and field trials was reported by Ishikawa et al. (1984) to be similiar to that of onion pulp exposed to undefined microorganisms and aged for 8 days at 25^{0} C. Since it is not known whether such onion pulp was a reliable source of onion fly attractants, traps with freshly cut onion should have been included in these experiments as a standard to which the attractancy of the decomposing pulp (the positive control) could be compared.

EC-inoculated onion, whether presented as tissue or as volatiles, stimulated the most oviposition in choice tests. Since female onion flies will oviposit in response to freshly cut onion volatiles alone (Matsumoto and Thorsteinson, 1968; Vernon et al., 1978; Ishikawa et al., 1978), some oviposition on this treatment in Experiment B was expected. Yet, because of the low response to any treatment except EC-inoculated onion, the relative ovipositional preference among the remaining treatments was not extant.

Even though attracted, female onion flies did not accept EC-inoculated potato as an ovipositional site. Oviposition is a specific behavioral response generally restricted to stimuli from the host-plant. On the other hand, attractancy responses include both host-finding and food-finding. Thus, onion flies may be attracted to and feed on decomposing non-host plants (Miller et al., 1984), but may not accept them for oviposition.

CHAPTER 2

Onion fly ovipositional preference and larval performance as influenced by physical and microbial damage to mature onion bulbs

CHAPTER 2

Introduction

Previously damaged and decomposing onions in the field have been reported to be more susceptible to colonization by onion fly, *Delia antiqua* (Meigen), than undamaged bulbs (Armstrong, 1924; Workman, 1958; Crete and Tartier, 1972). Since newly hatched onion maggots are not able to migrate very far from their initial feeding site (Kendall, 1932), especially under dry environmental conditions, larval survival depends strongly on the ability to utilize the plant on which oviposition occurs. No study clearly demonstrates whether the observed susceptibility of damaged, maturing, field onions is due to increased oviposition, higher larval survival, or both.

Fall generation onion maggots feed on maturing bulbs then overwinter as diapausing pupae. Upon emergence and mating the following spring, the females oviposit on the new crop of onion seedlings. Onion maggot damage in the spring accounts for most of the crop losses during the growing season. Any pest management scheme aimed at reducing fly populations in the fall, thus the subsequent number of overwintering pupae, must begin with an understanding of onion fly ovipositional behavior and the suitability of commericial onions available in the fall for larval survival and development. The objective of this study was to determine the influence of different levels of physical and microbial damage to maturing bulbs on ovipositional preference and subsequent larval performance.

Materials and Methods

Insects

Parental stock originated from commercial onion fields in Eaton Rapids, MI. Insects used were 5-7 generations removed from the field. Adults were fed water and a dry artificial diet (Ticheler 1971) and housed as described by Schneider et al. (1983), except foliage was replaced by green surrogate stems (Harris and Miller, 1983) in ovipositional dishes. Eggs used in host plant suitability studies were removed from laboratory culture ovipositional dishes by water flotation. Larvae used to inflict damage to onions were taken directly from sand-filled, rearing boxes provisioned with an excess of cross-sectioned onion bulbs.

Host plant

Mature (5.5 - 6.5 cm diameter) Spartan Banner Hybrid onion bulbs were transplanted from the field on September 29 - 30, 1984, into plastic pots (15 cm diameter x 25 cm height) containing field-collected muck soil. The senescing foliage was lodging, but the roots were intact. Plants were kept at 22^{o} C, 25-35% RH, and in a 16:8 L:D light regime (cool 55 W and warm 85 W flourescent bulbs ca. 30 cm from plants).

To obtain different levels of plant damage, groups of 20 late second instar D. antiqua larvae were added to some of the pots at five day intervals. Individuals pupated ca. 5-8 days later. Fifteen days after transplanting, four qualitative treatment groups had been obtained: (1) undamaged onions with no added larvae, (2) onions damaged by 5 days of active feeding with decomposition localized at the base of the bulb, (3) onions damaged by 10 days of active feeding with bacterial decomposition invading inner tissues, but bulbs firm to touch, and (4) onions damaged by 15 days of active feeding with advanced decomposition throughout the inner tissues and the majority of the outer tissues, and bulbs soft to touch.

Ovipositional choice test

Behavioral bioassays were conducted in a randomized complete block design. Two cages of flies were used during two different ovipositional periods. Approximately 300 adult flies, one to two weeks old, were placed in rectangular cages (3 x 1 x 1 m) with food and water. At ca. 20^{o} C, maximal rates of oviposition begin ten to twelve days after pupal eclosion.

At the start of the first experimental ovipositional period, five plants from each treatment group were randomly placed equidistant from each other in each cage. These were removed after 24 hours and replaced with another set of plants for the second ovipositional period of 48 hours. Eggs were collected by water flotation. The total numbers of eggs on all five plants from each treatment group and in each cage were counted.

Host plant suitability

Using a white wheat flour and water mixture, groups of 20 eggs were pasted gently on squares of black paper (1.5 x 1.5 cm) and subsequently placed on top of the soil 1 cm from each plant. Eggs that did not hatch after four days (less than 5%) were removed and replaced by newly hatched larvae for a total of 20 larvae per plant. Ten replicates of plants with 5-, 10-, and 15-day damage and twenty replicates of undamaged plants were included. Soil was kept moist and was sifted every five days to remove and discard the pupae that developed from second instars added to cause the initial damage. Within each plant treatment group, larvae added as newly hatched first instars pupated ca. 10 after the late second instars.

When possible, a single individual was weighed as both a fresh pupa and an emerged adult or as a fresh and a freeze-dried pupa. When larvae added as first instars were collected as pupae, they were placed individually in 5 dram glass vials containing moist sand. Pupae were kept in glass vials for five days before weighing to standardize pupal moisture content. Unfortunately, many began to show evidence of bacterial contamination before weighing. Perhaps the sand-filled vials were too moist and temperatures too warm for healthy development of the pupae to the adult stage. In these cases, only freeze-dried pupal weights were taken. Pupae not eclosing after three weeks were freeze-dried and weighed to avoid difficulties with possible bacterial contamination. Some individuals emerged as adults before being removed from the soil, so no fresh pupal weight was available. Adults were weighed within twelve hours of emergence.

Results

Few eggs were laid on undamaged plants (Table 2). Females laid significantly more eggs on plants with 5 or 10 days of active feeding and microbial damage. Host plant acceptability decreased significantly with increasing damage and decomposition beyond 10 days of active feeding. Females showed no significant ovipositional preference for 15-day damaged vs. healthy plants.

A plant was considered to be successfully colonized if at least one first instar pupated. Only 15% of the healthy plants were successfully colonized (Table 3). Nearly all of the 5- and 10-day

Table 2. Onion fly oviposition on undamaged and damaged onions

Treatment	Mean number of eggs per group of five plants
Undamaged	76.5
5-day damaged	584.5[*]
10-day damaged	384.8[*]
15-day damaged	227.8[ns]

[*] Means were significantly different from oviposition on undamaged onions ($P < 0.05$), randomized complete block ANOVA of data transformed to $(X + 0.5)^{1/2}$ followed by Dunnett's test (Gill 1978).

[ns] Mean was not significantly different from oviposition on undamaged onion ($P > 0.20$).

Table 3. Colonization rates of undamaged and damaged onions.

Treatment	% Plants colonized
Undamaged	15
5-day damaged	100
10-day damaged	90
15-day damaged	20

Table 4. Survival of first instar *D. antiqua* larvae to pupal stage on undamaged and damaged onions.

	Mean[1] larval survival per onion (max = 20)	
Treatment	Colonized onions	All onions
Undamaged	16.0 ± 1.6[a]	2.4 ± 1.3[b]
5-day damaged	17.2 ± 0.8[a]	17.2 ± 0.8[a]
10-day damaged	6.3 ± 2.0[b]	5.2 ± 1.8[b]
15-day damaged	1.0	0.2

[1] Means ± SE within a column followed by the same letter were not significantly different, one-way ANOVA with Scheffe's all pair-wise comparison for unbalanced data ($P \leq 0.05$) (Gill, 1978).

Table 5. Weights of pupae and adults from *D. antiqua* larvae reared on undamaged and damaged onions.

	Mean[1] weight, mg		
	Pupae		
Treatment	Fresh wt. (n)[2]	Dry wt.	Emerged adults
Undamaged	16.4 $\pm$ 0.4^{a} (44)	5.5 $\pm$ 0.2^{a} (44)	14.3 $\pm$ 0.9^{a} (3)
5-day damaged	15.8 $\pm$ 0.4^{a} (66)	5.4 $\pm$ 0.1^{a} (98)	12.2 $\pm$ 0.2^{ab} (74)
10-day damaged	13.6 $\pm$ 0.7^{b} (33)	4.5 $\pm$ 0.2^{b} (39)	11.2 $\pm$ 0.7^{b} (13)
15-day damaged	-	-	-

[1] Means ± SE within a column followed by the same letter were not significantly different, one-way ANOVA with Scheffe's all pair-wise comparison for unbalanced data ($P \leq 0.05$) (Gill, 1978).

[2] n is the number of individuals weighed. The total numbers of individuals recovered from undamaged, 5-, 10-, and 15-day damaged onions were 47, 172, 52, and 2, respectively. Data for two weight categories were available for some individuals.

damaged onions were successfully colonized, but the colonization rate dropped from 90% to 20% between the 10-day and 15-day damaged plants, respectively (Table 3).

On the few healthy plants that were colonized, the number of larvae surviving and developing to the pupal stage was not significantly different from that on the 5-day damaged onions (Table 4). Yet, because of very low colonization rates, overall larval survival on healthy onions was significantly lower than that on 5-day damaged plants (Table 4). In spite of the high colonization rate on the 10-day damaged plants (90%), the number of larvae surviving to the pupal stage was significantly lower than the number surviving on 5-day damaged onions. Overall larval survival on both 10-day damaged and healthy plants was quite low and not significantly different (Table 4). In the 15-day damage treatment group, only a single larva on each of two plants survived to the pupal stage (Table 4).

Pupal and adult weights did not differ significantly between larvae reared on undamaged and 5-day damaged onions, but weights decreased significantly with more severe decomposition (Table 5). Since only two pupae (fresh weights of 6.4 and 7.2 mg) were recovered from 15-day damaged onions, their weights were not included in the analysis.

Discussion

The high colonization and overall larval survival rates in the 5-day damaged onions indicate that these onions were more suitable host plants for D. antiqua development than healthy mature bulbs. Apparently, if first instars penetrate and begin sustained feeding on a healthy bulb, survival and weight gains will be similiar to those attained on the more suitable onions that have some damage and microbial infection. Since the soil was kept fairly moist throughout the experiment, larval death due to dessication was unlikely. Increased suitablity of 5-day damaged bulbs preferred by ovipositing females may be due, in part, to increased ease of penetration by first instars. Bulbs with more severe damage may be less suitable for larval survival and development because of a decrease in food quality associated with decomposition and in food quantity.

Microorganisms associated with decomposition also may contribute to larval nutrition. Although larvae reared on pieces of axenic onion tissue survive and develop nearly as well as those reared on onion contaminated with naturally occurring microorganisms, inclusion of microbes in the diet tends to accelerate developmental rates (Schneider et al., 1983). Microorganisms may contain important nutrients or may increase the availability of nutrients in onion (Eymann and Friend, 1985). Possible differences in the nutritive value of undamaged and the more suitable 5-day damaged plants probably do not fully account for the inability of first instars to successfully utilize a healthy, mature bulb as food. Indeed, the few healthy plants that were colonized resulted in larval survival rates and weight gains similiar to those on 5-day damaged plants.

Field traps baited with decomposing onions at certain stages of microbial succession are attractive to adult onion flies (Ishikawa et al., 1981; Vernon et al., 1981; Miller et al., 1984). Since the traps prevent visual or physical contact with the bait before capture, the flies must be responding to volatile compounds released from the bait. Female onion flies ovipositing on decomposing onion bulbs may also be responding to volatile attractants. In addition, bulbs at various stages of physical and microbial damage may release metabolites that influence ovipositional responses after females alight on or near the plant. These metabolites may be nutritive or have other attractant or deterrent properties that influence behavior.

Finch and Eckenrode (1985) showed that a high percentage of overwintering pupae develop on damaged bulbs left in the field after fall harvest. The results presented here support their conclusion that damaged bulbs can serve as an excellent food source for fall generation onion flies. In addition, this study demonstrates that the highest densities of onion maggots are found on recently damaged fall onion bulbs, both because of D. antiqua ovipositional preference and higher offspring fitness. In these experiments, there appears to be a good correlation between female ovipositional preference and subsequent larval performance.

GENERAL DISCUSSION

Volatile metabolites produced by Erwinia carotovora var. carotovora (EC) and Klebsiella pneumoniae (KP) cultured on sterilized onion tissue were more attractive to adult onion flies than volatiles of freshly cut, axenic onion. During a three day incubation period, the attractancy of onion inoculated with either bacterial isolate increased. Qualitative or quantitative changes in the composition of volatiles during this time were not determined. EC cultured on onion produced more potent attractants than KP on onion. Furthermore, the attractancy of volatiles produced by onion inoculated with both isolates was not significantly different than those produced by onion inoculated by either isolate alone.

Onion flies were attracted to headspace metabolites produced by EC cultured on potato, a nonhost plant of onion fly. Yet, these metabolites were significantly less potent than those produced by EC cultured on onion, the primary host of D. antiqua; therefore, the interaction of EC and onion, and not EC itself, was responsible for its strong activity toward onion fly.

In laboratory bioassays, the efficacy of EC-inoculated onion as an onion fly attractant was significantly better than an aqueous solution of 2-phenylethanol and pentanoic acid, attractants reportedly extracted from decomposing onion and formulated for an optimal release rate (Ishikawa et al., 1984). One or more volatile components responsible for the potent attractancy of some bacterially infected onion remain to be identified.

Whether presented as plant tissue or as volatiles, EC-inoculated onion stimulated significantly more onion fly oviposition than axenic onion. Onion flies were attracted to EC cultured on potato, but females did not accept it for oviposition, a specific behavioral response generally restricted to stimuli from the host plant. Attractancy comprises many behaviors, including host-finding by ovipositing females food-finding responses. Therefore, female onion flies may be attracted to and feed on bacterially infected, nonhost-plants without accepting them for oviposition.

Female onion flies preferentially oviposited on those damaged, fall onions most suitable for larval survival and development. Ovipositional preference for decomposing bulbs significantly decreased as the physical and microbial damage increased; the numbers of eggs laid on bulbs with the most severe damage and on healthy bulbs were not significantly different.

While colonizing 90-100% of the damaged bulbs (5-day and 10-day damaged) preferred by ovipositing females, newly hatched larvae successfully colonized only 15% of the healthy plants. Pupal and adult weights were not significantly different for larvae reared on undamaged or 5-day damaged bulbs, but did significantly decrease with further plant damage. Overall larval survival was lowest for undamaged and severely (15-day) damaged bulbs, which were also the plant groups stimulating the least oviposition.

These results support the hypothesis that damaged bulbs are an excellent food source for fall generation onion flies. Healthy onions may, in part, be resistant to onion maggot attack because of the inability of first instars to penetrate undamaged bulbs. The high densities of onion maggots found on recently damaged, fall onions was due to both D. antiqua ovipositional preference and higher offspring fitness.

A logical step to the work reported in this thesis would be to identify the biologically active, volatile metabolites from EC-inoculated onion. Direct headspace analysis is possible and avoids some of the complications introduced by extraction with solvents. It is feasible to withdraw an aliquot of headspace volatiles with a syringe and directly inject them into a gas-liquid chromatograph, but the concentration of component chemicals may be too low for detection. An automated headspace concentrator and high-resolution columns allow one to obtain reproducible, detailed profiles of the headspace volatiles.

After identification, these chemicals may be purchased or synthesized in pure form for further behavioral and neurophysiological studies. Basic research needs to be directed toward the mechanisms by which these attractants and ovipositional stimulants function. Their reception by the peripheral nervous system and subsequent integration in the central nervous system is an important area of research.

Attractants are currently used commercially as baits to monitor or detect pest populations and for use in attracticides. For example, traps baited with controlled-release attractants are used to detect accidental importation of agricultural pests. To make informed pest management decisions, growers and field scouts also may use baited traps to monitor pest populations throughout a growing season. Using traps baited with attractants to significantly decrease pest populations, i.e., mass trapping, has met with little success in crops and orchards, although this approach may be viable in confined areas such as greenhouses or warehouses. Attracticides, which are attractants laced with an insecticide, currently are used commercially. Since attracticides often may be applied with conventional pesticide equipment, they may be easily integrated into present pest management practices. Although combining an attractant with an insecticide may lead to more efficient use of smaller quantities of toxins, not all attractants have high specificity. For example, yeast hydrolysate based attracticides may attract and kill natural enemies of the pest along with the target insect.

Applied uses for ovipositional stimulants have not been explored in depth, but the study of ovipositional stimulants in the laboratory and field has some advantages making them useful tools in the research and development of behavior-modifying chemicals. Oviposition is a specific, quantifiable, behavioral response. In many bioassays designed to measure attractancy responses, it is not always possible to determine whether insects are responding to food-, host-, or mate-finding stimuli. An understanding of which insect behaviors are influenced by a particular attractant is an important step in developing applications. Of course, if only adult female insects at certain stages of development respond, the use of ovipositional stimulants in the field may be limited.

If new uses for behavior-modifying chemicals are to be developed for agriculture, the ecology and economics of the crop system must be considered. The influence of environmental factors, such as climate, availability of food, presence of natural enemies, and the age structure of the crop and insect pest must be studied in conjunction with optimized, behavioral, laboratory studies. Furthermore, if the goal is to develop a new product or technique, it must inexpensive enough to warrent its use. More basic and applied research is needed to realize the potential of behavior-modifying chemicals in management of agricultural insect pests.

LITERATURE CITED

LITERATURE CITED

Amonkar, S. U. and A. Banerji. 1971. Isolation and characterization of larvacidal principle of garlic. Science 174: 1342-1344.

Agrios, G. N. 1978. Bacterial soft rots. In Plant Pathology. Academic Press, New York. pp. 477-483.

Armstrong, T. 1924. Onion maggot studies in the district of Montreal, Quebec 1924. A. Rep. Ent. Soc. Ont. 54: 42-45.

Baker, T. C., W. Mayer, and W. L. Roelofs, 1981. Sex pheromone dosage and blend specificity of response by oriental fruit moth males. Ent. exp. appl. 30: 269-279.

Bernhard, R. A. 1970. Chemotaxonomy: Distribution studies of sulfur compounds in Allium. Phytochem. 9: 2019-2027.

Block. E. 1985. The chemistry of garlic and onion. Scientific American 252: 114-119.

Boelens, M., P. DeValois, H. Wobben, and A. van der Gen. 1971. Volatile flavor components from onion. J. Agric. Food Chem. 19: 984-1000.

Bowman, E. V., L. R. Freeman, D. W. Later, and M. L. Lee. 1983. Comparison of volatiles produced by selected pseudomonads on chicken skin. J. Food Sci. 48: 1358-1359.

Brachmachari, H.D. and K. T. Augusti. 1961. Hypoglycaemic agent from onion. J. Pharm. Pharmocol. 13: 128.

Carson, J. F. and F. F. Wong. 1961. The volatile flavor components of onions. J. Agric. Food Chem. 9: 140-143.

Cavallito, C. J. and J. H. Bailey. 1944. Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action. J. Amer. Chem. Soc. 66: 1950-1954.

Crete, R. and L. Tartier. 1972. Chemical control of onion smut in organic soils of southwestern Quebec in 1971. Can. Dept. Agric. Pestic. Res. Rep. pp. 280-281.

Cuppels, D. and A. Kelman. 1974. Evaluation of selective media for isolation of soft-rot bacteria from soil and plant tissue. Phytopath. 64: 468-475.

Dindonis, L. L. and J. R. Miller. 1980. Host-finding responses of onion and seedcorn flies to healthy and decomposing onions and several synthetic constituents of onion. Environ. Entomol. 9: 467-472.

Dindonis, L. L. and J. R. Miller. 1981a. Onion fly and little house fly host finding selectively mediated by decomposing onion and microbial volatiles. J. Chem. Ecol. 7: 419-426.

Dindonis, L. L. and J. R. Miller. 1981b. Onion fly trap catch as affected by release rates of n-dipropyl disulfide from polyethylene enclosures. J. Chem. Ecol. 7: 411-418.

Dye, D. W. 1969. A taxonomic study of the genus Erwinia. II. The "carotovora" group. N. Z. Jl Sci. 12: 81-97.

Echandi, E., S. D. Van Gundy, and J. C. Walker. 1957. Pectolytic enzymes secreted by soft-rot bacteria. Phytopath. 47: 549-552.

Ellis, P. R. and C. J. Eckenrode. 1979. Factors influencing resistance in Allium sp. to onion maggot. Bull. Entomol. Soc. Am. 25: 151-153.

Eymann, M. and W. G. Friend. 1985. Development of onion maggots (Diptera: Anthomyiidae) on bacteria-free onion agar supplemented with vitamins and amino acids. Ann. Entomol. Soc. Am. 78: 182-185.

Finch, S. and C. J. Eckenrode. 1985. Influence of unharvested, cull-pile, and volunteer onions on onion maggot (Diptera:Anthomyiidae). J. Econ. Entomol. 78: 542-546.

Freeman, G. G. 1975. Distribution of flavour components in onion (Allium cepa L.), leek (Allium porrum) and garlic (Allium sativum). J. Sci. Fd Agric. 26: 471-481.

Freeman, L. R., G. J. Silverman, P. Angelini, C. Merritt, Jr., and W. B. Essler. 1976. Volatiles produced by microorganisms isolated from refrigerated chicken at spoilage. Appl. Environ. Microbiol. 32: 222-231.

Gill, J. L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences, Vol. 1. Iowa State Univ. Press, Ames, IA.

Hough, J. A., G. E. Harman, and C. J. Eckenrode. 1981. Microbial stimulation of onion maggot oviposition. Environ. Entomol. 10: 206-210.

Harris, M. O. and J. R. Miller. 1983. Color stimuli and oviposition behavior of the onion fly, Delia antiqua (Meigen) (Diptera: Anthomyiidae). Ann. Entomol. Soc. 76: 766-771.

Ishikawa, Y., T. Ikeshoji, and Y. Matsumoto. 1978. A propylthio moiety essential to the oviposition attractant and stimulant of the onion fly, Hylemya antiqua Meigen. Appl. Ent. Zool. 13: 115-122.

Ishikawa, Y., T. Ikeshoji, and Y. Matsumoto. 1981. Field trapping of the onion and seed-corn flies with baits of fresh and aged onion pulp. Appl. Ent. Zool. 16: 490-493.

Ishikawa, Y., Y. Matsumoto, M. Tsutsumi, and Y. Mitsui. 1984. Mixture of 2-phenylethanol and n-valeric acid, a new attractant for the onion and seed-corn flies, *Hylemya antiqua* and *H. platura* (Diptera:Anthomyiidae). Appl. Ent. Zool. 19: 448-455.

Ishikawa, Y., Y. Matsumoto, M. Tsutsumi, Y. Mitsui, K. Yamashita, M. Yoshida, and E. Shirai. 1986. Controlled release formulation of the attractant for the onion and seed-corn flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae). Submitted to Appl. Ent. Zool.

Kato, S. 1958. The turnip maggot and other species of *Hylemya* (Diptera: Muscidae) of economic importance in Japan. Proc. int. Congr. Ent. 10: 445-447.

Kelman, A. and R. S. Dickey. 1980. Soft rot or 'carotovora' group. *In* Laboratory Guide for Identification of Plant Pathogenic Bacteria (N. W. Schaad, ed.). Amer. Phytopath. Soc. St. Paul, MN.

Kendall, E. W. 1932. Notes on the onion maggot (*Hylemya antiqua* Meig.). A. Rep. Ent. Soc. Ont. 62: 82-84.

King, J. E. and J. R. Coley-Smith. 1969. Production of volatile alkyl sulphides by microbial degradation of synthetic alliin and alliin-like compounds, in relation to germination of sclerotia of *Sclerotium cepivorum* Berk. Ann. appl. Biol. 64: 303-314.

Kuhr, R. J., A. Comeau, W. L. Roelofs. 1972. Measuring release rates of phermone analogues and synergists from polyethylene caps. Environ. Entomol. 1: 625-627.

Krull, L. H., D. E. Gibbs, and M. Friedman. 1970. 2-Vinylquinoline, a reagent to determine protein sulfhydryl groups spectrphotometrically. Analyt. Biochem. 40: 80.

Larsson, L., E. Holst, C. G. Gemmell, and P.-A. Mardh. 1980. Characterization of *Clostridium difficle* and its differentiation from *Clostridium sporogenes* by automatic head-space gas chromatography. Scan. J. Infect. Dis. Suppl. 22: 37-40.

Lee, M. L., D. L. Smith, and L. R. Freeman. 1979. High resolution gas chromatographic profiles of volatile organic compounds produced by microorganisms at refrigerated temperaturesnviron. Microbiol. 37: 85-90.

Lelliott, R. A. 1974. Description of the species of the genus *Erwinia*. *In* Bergey's Manual of Determinative Bacteriology, 8th ed. (R. F. Buchanan and N. E. Gibbons, eds.). The Williams and Wilkins Co., Baltimore, MD.

Loosjes, M. 1976. Ecology and genetic control of the onion fly, *Delia antiqua* (Meigen). Agric. Res. Rep. (Versl. landbouwk. Onderz.) 857. Pudoc, Wageningen. 179 pp.

Mardh, P. -A., L. Larsson, and G. Odham. 1981. Head-space gas chromatography as a tool in the identification of anaerobic bacteria and diagnosis of anaerobic infections. Scand. J. Infect. Dis. Suppl. 26: 14-18.

Matsumoto, Y. and A. J. Thorsteinson. 1968. Effect of organic sulfur compounds on oviposition in onion maggot, Hylemya antiqua Meigen (Diptera: Anthomyiidae). Appl. Ent. Zool. 3: 5-12.

Matsumoto, Y. 1970. Volatile organic sulfur compounds as insect attractants with special reference to host selection. In Control of Insect Behavior by Natural Products (D. L. Wood, R. M. Silverstein, and M. Nakajima, eds.). Academic Press, New York, pp. 133-160.

Miller, J. R., M. O. Harris, and J. A. Breznak. 1984. Search for potent attractants of onion flies. J. Chem. Ecol. 10: 1477-1488.

Niegisch, W. D. and W. H. Stahl. 1956. The onion: gaseous emanation products. Fd. Res. 2: 657-665.

NOAA (National Oceanic and Atmospheric Administration). 1984. Local climatological data. Annual summary with comparative data, Lansing, MI. Temperature normals, means, and extremes based on the 1951-1980 record period.

O'Neill, R. and C. Logan. 1975. A comparison of various selective isolation media for their efficiency in the diagnosis and enumeration of soft rot coliform bacteria. J. appl. Bact. 39: 139-146.

Peterson, A. 1924. Some chemicals attractive to adults of the onion maggot and the seed corn maggot. J. econ. Ent. 17: 87-94.

Pierce, H. D., Jr., R. S. Vernon, J. H. Borden, and A. C. Oehlschlager. 1978. Host selection by Hylemya antiqua (Meigen), identification of three new attractants and oviposition stimulants. J. Chem. Ecol. 4: 65-72.

Pittard, B. T., L. R. Freeman, D. W. Later, and M. L. Lee. 1982. I dentification of volatile organic compounds produced by flourescent pseudomonads on chicken breast muscle. Appl. Environ. Microbiol. 43: 1504-1506.

Ramakers, P. M. J. 1973. Diapauze-induktie bij de uievlieg, Hylemya antiqua (Meigen), onder invloed van fotoperiode en temperatuur. Doctoraalverslag Afd. Entomologie, Landbouwhogeschool Wageningen, 37 pp.

Rygy, T. 1960. The onion fly (Hylemya antiqua Meig.). Investigations on its biology and control in Norway. (Norwgian, Eng. summary) Medd. St. Plantev. 18: 1-56.

Saghir, A. R., L. K. Mann, M. Ournbey, R. Y. Berg. 1966. Composition of volatiles in relation to taxonomy of American Alliums. Am. J. Bot. 53: 477-484.

Sainani, G. S., D. B. Desai, M. N. Natu, K. M. Katrodia, V. P. Valame, and P. G. Sainani. 1979. Onion, garlic, and experimental atherosclerosis. Jpn. Heart J. 20: 351-358.

Schneider, W. D., J. R. Miller, J. A. Breznak, and J. F. Fobes. 1983. Onion maggot, Delia antiqua, survival and development on onions in the presence and absence of microorganisms. Ent. exp. & appl. 33: 50-56.

Schwimmer, S., M. Friedman. 1972. Genesis of volatile sulphur-containing food flavours. Flavour Ind. 3: 137-145.

Tansey, M. R. and J. A. Appleton. 1975. Inhibition of fungal growth by garlic extract. Mycologia. 67: 407-413.

Taylor, A. J. 1980. The application of gas chromatographic headspace analysis to medical microbiology. In Applied Headspace Gas Chromatography (B. Kolb, ed.). Heyden & Son, Inc. Philadelphia, PN. pp. 140-154.

Ticheler, J. 1971. Rearing of the onion fly, Hylemya antiqua (Meigen), with view to release sterilized insects. In Sterility Principle for Insect Control or Eradication. (Proc. Symp., Athens, 1970). IAEA, Vienna pp. 341-346.

Tomioka, T. 1977. Plant Protection. (Shokubutsu Boeki) 31: 206-209.

Vernon, R. S., H. D. Pierce, Jr., J. H. Borden, and A. C. Oehlschlager. 1978. Host selection by Hylemya antiqua: identification of oviposition stimulants based on proposed active thioalkane moieties. Environ. Entomol. 7: 728-731.

Vernon, R. S., G. J. R. Judd, J. H. Borden, H. D. Pierce, Jr., and A. C. Oehlschlager. 1981. Attraction of Hylemya antiqua (Meigen) (Diptera: Anthomyiidae) in the field to host-produced oviposition stimulants and their nonhost analogues. Can. J. Zool. 59: 872-881.

Vernon, R. S. and J. H. Borden. 1983. Dispersion of marked-released Hylemya antiqua (Meigen) (Diptera: Anthomyiidae) in an onion field. Environ. Entomol. 12: 646-649.

Vickery, M. L. and B. Vickery. 1981. Sulphur compounds derived from cysteine. In Secondary Plant Metabolism. University Park Press, Baltimore, MD.

Weston, P. A. and J. R. Miller. 1985. Influence of cage design on precision of tube-trap bioassay for attractants of the onion fly, Delia antiqua. J. Chem. Ecol. 11: 435-440.

Whitaker, J. R. 1976. Development of flavor, odor, and pungency in onion and garlic. Adv. Food Res. 22: 73-133.

Workman, R. B. 1958. The biology of the onion maggot (*Hylemya antiqua* Meig.) under field and greenhouse conditions. *Oregon State* College, Ph.D. Thesis, 82 pp.

Zechman, J. M. and J. N. Labows, Jr. 1984. Volatiles of *Pseudomonas aeruginosa* and related species by automated *headspace concentration*-gas chromatography. Can. J. Microbiol. 31: 232-237.

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1986-2

Title of thesis or dissertation (or other research projects):

Onion fly behavioral responses to microbially infected onion

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)

Susan Marie Hausmann

Date 3-2-86

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 2 of 2 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Delia antiqua Meigen	MI: Grant Newaygo Co. P.Weston 1986		28		10	10	10		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Susan Marie Hausmann

Date 3-2-86

Voucher No. 1986-2

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date