1-1-1992

Comparison of food foraging behavior in the temperate apple maggot fly (Rhagoletis pomonella Walsh) and the tropical Mediterranean fruit fly (Ceratitis capitata Wiedemann).

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COMPARISON OF FOOD FORAGING BEHAVIOR IN THE TEMPERATE APPLE MAGGOT FLY (*RHAGOLETIS POMONELLA* WALSH) AND THE TROPICAL MEDITERRANEAN FRUIT FLY (*CERATITIS CAPITATA* WIEDEMANN)

A Dissertation Presented

by

JORGE P. HENDRICHS

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 1992

Department of Entomology

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ACKNOWLEDGEMENTS

I am dedicating my dissertation to my late father, Jorge Hendrichs, who introduced me to Entomology and who through his example has been my driving inspiration. This degree and recent developments in Europe would have made him so happy.

My immense gratitude goes to Marti, who in all times has been a great companion. Her endless love and understanding have made this all possible. I also thank Andrea and Jorge, who provided in their own way a great deal of patience and love and have been most wonderful and motivating.

Very special thanks to Ron Prokopy for his support and friendship. He not only initiated my in behavioral studies of fruit flies, but also in every sense has been a role model since our first cooperation in Guatemala in 1978. Dieter Enkerlin, Jorge Gutierrez Samperio, Carrol Calkins, Derrell Chambers, Don Lindquist and all my colleagues and friends at Moscamed have equally been of fundamental importance in my development. I could not have gotten to this point without their encouragement and friendship.

I owe special thanks to the members of my committee, Joseph Elkinton, John Stoffolano and John Buonaccorsi, as well as all other faculty members for valuable advice and support. I appreciate also the useful criticisms and stimulating discussions provided by Dick Drew, Brian Fletcher, Byron Katsoyannos, Carol Lauzon, and Dan Papaj.

Many friends have made our three year stay at Amherst a pleasant and fulfilling experience, primarily Jeff and Jung, Pieter, Linda and Rolando, who provided for the stimulating discussions on history and politics and to Pieter for those wonderful weekends in Vermont. Also our lab group: Sylvia and Patty, Sue Opp, Sue B. and Margareth, Martin, Dan, Tom and Duan. They all helped with advice, assistance, and their friendship. Special thanks to Linda and Ron for their year-round hospitality. Also many thanks to Dave, Frank, Don, and Randy, and to Joe, JoAnn, Paula, Kathy, Ruth, Amity, Maggie and Julia for being so supportive.

I have greatly benefitted from the outstanding assistance of Sylvia Cooley, a fruit fly expert in her own right. Also my thanks to Patty Powers, Maryam Mashayekhi, David Eagle, Jung Tang Wang, Josh Prokopy, Shifu Qu and Gabriela Gonzalez, who helped during different aspects of this work. I am also indebted to Trina Hoshmer and Stanley Lemeshow for advising on data analysis.

Special thanks are also extended to T. El-Abbasi, S. Bamumin, M. Chidiac, and S. Hassan at Plant Protection Research Institute and UNDP, Cairo, Egypt, and Byron Katsoyannos, University of Thessaloniki, for his hospitality on the Greek island of Chios.

I acknowledge important financial support by the Science and Education Administration of the USDA under grant No. 8700564 to Ron Prokopy, the University of Massachusetts, and the International Atomic Energy Agency.

ABSTRACT

COMPARISON OF FOOD FORAGING BEHAVIOR IN THE TEMPERATE APPLE MAGGOT FLY (RHAGOLETIS POMONELLA WALSH) AND THE TROPICAL MEDITERRANEAN FRUIT FLY (CERATITIS CAPITATA WIEDEMANN)

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The food foraging behavior of two frugivorous tephritid fruit flies, apple maggot fly (Rhagoletis pomonella Walsh) and the Mediterranean fruit fly (Ceratitis capitata Wiedemann) was compared by (1) assessing quantitatively fly feeding sites and activities over time and space in nature; (2) collecting substrates identified from feeding sites and assessing their contribution to fly maintenance and fecundity; (3) assessing fly intra-tree food-foraging behavior in field cages, as affected by food quality, and quantity. C. capitata feeding was studied in mixed orchards in Egypt and Greece. Females, dispersing and feeding more than males, foraged for food throughout most of the day requiring a substantial and varied diet that they often acquired away from the primary host. Feeding occurred at wounds and juice oozing from ripe fruits, as well on bird droppings. Male feeding on ripe fruit, occurred late in the day when they were least likely to find a mate. Fruit such

as grapes did not support fecundity, contributing only to longevity, whereas fig fruit sustained longevity and fecundity. Bird feces added to a fig diet significantly increased fly fecundity.

Apple maggot fly feeding was studied in an abandoned apple orchard in Massachusetts. Females, spend daily considerable time foraging for food on hosts and the surrounding vegetation, where they acquired food from foliage as well bird droppings. Fruit feeding played a minor role. Males remained mostly on fruiting host trees were they fed on leaf surfaces. Leaf surface bacteria did not support fly longevity or fecundity. Fly survival was sustained by leachates from host foliage, explaining the extensive "grazing" of flies there. Fly fecundity was sustained by bird droppings, supplemented by carbohydrates, as well as by aphid honeydew.

Intra-tree fly foraging time was positively related to total amount of food solute previously encountered though largely independent of food volume or concentration. Volume and concentration, however, affected significantly food "handling time" and "bubbling" behavior, the oral extrusion of liquid crop contents to concentrate ingested food by elimination of excess water by evaporation. Weight losses of flies during post-feeding bubbling were an order of magnitude higher than when not bubbling.

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CHAPTER 1

INTRODUCTION

1.1 Study Insects

The Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), originated in Subsaharan tropical Africa from where is has spread to the Mediterranean region during the last century, and to South America, Hawaii, Australia and Central America during this century (Huettel et al. 1980; Gasperi et al. 1991). It is one of the most destructive and costly agricultural pests in the world, attacking over 350 species of fruits and vegetables (Liquido et al. 1991). Weekly bait-pesticide applications are required during the fruiting period in areas where medfly is established to maintain fruit free of larval infestation. In spite of strict regulatory barriers and quarantine procedures, medfly has been introduced and eradicated from North America on a number of occasions (Klassen 1989; Carey 1991). Over the last decade a massive barrier of sterile flies has been maintained at the border between Guatemala and Southern Mexico to prevent the fly from becoming established in Mexico and North America (Schwarz et al. 1989).

The apple maggot fly, Rhagoletis pomonella (Walsh), is indigenous to the temperate North American climate where its principal native host is hawthorns, Crataegus spp. In the last two centuries, the host range of the apple maggot fly has broadened to include fruits of such introduced rosaceous

plants as apples, pears and stone fruits (Bush, 1966; Prokopy and Berlocher 1980). Apple maggot damage on this widening array of hosts has led to extensive pesticide applications by growers. The geographical range of apple maggot fly has also been expanding from northeastern North America to the midwest, south and, within the past decade, to the west, where it presents a very serious agricultural problem, threatening existing low-pesticide-use integrated pest management programs (AliNiazee and Brunner 1986; Dowell 1990).

1.2 Life Strategies of Frugivorous Tephritids

These two species of fruit flies selected as models for this study represent the two basic types of life systems or strategies used by frugivorous tephritids to exploit resources (Zwoelfer 1983). Flies utilizing the first strategy, represented by the Mediterranean fruit fly, are opportunistic polyphagous exploiters of pulpy fruits. They are multivoltine and have a high reproductive potential. Most species live in the tropical and subtropical regions of the world, where successive host resources are used (Bateman 1972). To allow bridging between host fruiting seasons, adults are relatively long lived and usually highly mobile. Both of these features, together with the high fecundity, increase the importance of regular intake of adult food (Zwoelfer 1983).

Fruit flies utilizing the other basic life strategy found in frugivorous tephritids, represented by the apple maggot fly, are specialized (i. e., stenophagous or monophagous) exploiters of pulpy fruits (Zwoelfer 1983). Members of this group are mostly univoltine, occurring in temperate climates where they spend most of their life in pupal diapause in the soil (Boller and Prokopy 1976). Precision in seasonal synchronization of adult emergence with availability of favourable substrates for larval development is more important than high reproductive potential, longevity and mobility (Zwoelfer 1983). However, these specialized fruit exploiters are also anautogenous flies, requiring regular intake of adult food for maintenance, sexual maturity and development of eggs. The apparent reason for the lack of nutritional reserves carried over from the larval stage is the need, as with most frugivorous insects, to leave the mature fruit as early as possible to escape predation by the dispersal agents of the fruit, such as frugivorous birds and mammals (Drew 1987).

A third group of tephritids not included in this study consists of specialized exploiters of relatively stable vegetative plant structures, galls or inflorescences. These non-frugivorous tephritids, much more numerous than frugivorous tephritids, are also mostly from the temperate regions of the New and Old World (Zwoelfer 1983). This group is characterized by a very close association with the host, which becomes a multipurpose resource, including provision

of flower nectar and pollen as adult food. Unlike frugivorous tephritids which compete with seed dispersers but generally do not destroy seeds in host fruit, non-frugivorous tephritids can reduce the reproductive potential of the host by causing damage to the host plant itself and have been deployed as biological control agents of weeds (Zwoelfer 1983, 1988).

1.3 Resource Foraging Behavior of Frugivorous Tephritids

Changes in the spatial and temporal distribution of potential resources elicit "decisions" in organisms that result in changes of behavior that may affect foraging efficiency and ultimately fitness. A fundamental question in behavioral ecology of how an organism adjusts its activities in response to the nature and distribution of resources is addressed by resource foraging theory (Hassel and Southwood 1978; Kamil and Sargent 1981; Pyke 1984). Dipteran movement within and between food patches that vary in distribution, quality and quantity has been addressed in laboratory studies by Bell (1990) and references therein. In addition, detailed mechanistic studies of Dipteran neurophysiological responses and feeding behaviors are described in Dethier's classic the "Hungry Fly" (1976). Understanding the basic principles underlying animal resource foraging behavior has not only theoretical value, but also holds practical significance. To manage agricultural pests effectively it is

vital to have a thorough understanding of their resource foraging behavior.

Present knowledge of the foraging behavior of fruit flies is generally still restricted to information gained at the population level by studying fly distribution and movement patterns in space and time. Some ground-breaking quantitative studies of tephritid foraging behavior of individual flies (reviewed by Prokopy and Roitberg 1989) have concentrated mainly on oviposition-site foraging behavior, and to a lesser degree on mate foraging behavior. Food foraging behavior, however, has never been examined in a systematic fashion in any tephritid. When one considers that control and eradication efforts are often restricted to large scale application of insecticide-food bait sprays that are imposed at a great cost against stiff environmental opposition, it is surprising that tephritid food foraging behavior in nature has so far received so little serious attention.

The objective of this dissertation is to provide a foundation of quantitative knowledge of food foraging behavior in frugivorous tephritids. The studies undertaken have potential strong practical impact on strategies and tactics for managing both medflies and apple maggot flies. In addition, they are part of a long term effort to gain a comprehensive understanding of resource foraging behavior of major fruit fly pests (Prokopy and Roitberg 1989). The behavioral-ecological investigations presented here proceed

from general questions addressed by systematic observational studies in nature to more specific questions addressed by experimental manipulation under controlled conditions in field cages or the laboratory.

The first research chapters, Chapters 2 and 3, concern two observational studies of medfly in nature. The objective was to assess quantitatively frugivorous tephritid food foraging activities over time and space, identifying sites and sources of adult fly feeding in nature. Both of these studies were carried out in the Mediterranean region, under high population density (Chapter 2), and under relatively low density (Chapter 3). A similar study of apple maggot fly feeding behavior was undertaken in New England (Hendrichs and Prokopy 1990 and unpublished data). These studies of feeding and other fly behaviors in a natural context formed the basis for the research objectives addressed in subsequent chapters.

One such objective was assessment of natural foods ingested by flies for their contribution to fly survival and egg development. This objective is addressed in Chapters 3 and 4. In Chapter 3, a laboratory study was carried out in Greece in which medflies were fed the principal natural foods identified during field observations. In Chapter 4, a similar series of laboratory as well as field cage tests was conducted on the apple maggot fly.

Chapter 5 concerns a field cage study in which feeding, food handling and post-feeding foraging activities of

individual apple maggot flies were recorded. The purpose was to establish food acceptance and ingestion thresholds and to contribute to the understanding of the dynamics of fly intra-tree foraging behaviors as affected by foods of varying quality and quantity as well as fly physiological state.

Finally, in Chapter 6, I studied oral droplet extrusion or "bubbling" behavior, which was observed in medfly, apple maggot fly and other frugivorous tephritids, and appears to be a phenomenon common to fluid feeding Diptera in general. Occurring regularly in the context of fly feeding, this behavior affected food processing time significantly and consequently food foraging efficiency. The objective of this last study was to determine the significance of this behavior in the biology of fruit flies and Diptera in general.

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CHAPTER 2

LOCATION AND DIEL PATTERN OF FEEDING AND OTHER ACTIVITIES OF MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE), ON FRUITING AND NONFRUITING HOSTS AND NONHOSTS

2.1 Introduction

The Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (subfamily Trypetinae), infests more than 200 species of fruits and vegetables (Christenson and Foote 1960). Over the last two decades various field programs of medfly suppression or eradication, utilizing the Sterile Insect Technique (SIT), have been conducted with varying degrees of success in Italy, Israel, Tunisia, Central America, Peru, Western Australia, and the United States (Burk and Calkins 1983). The largest ongoing medfly SIT program, in Southern Mexico and Guatemala, has prevented the spread of the medfly into Mexico and the rest of North America over the last ten years. However, this program has not yet eradicated medflies from Central America (Hendrichs et al. 1983, Schwarz et al. 1985, Ortiz et al. 1986). After analyzing medfly SIT programs, including the less-than-successful and much-publicized medfly SIT-eradication campaign in California (1980-1981), Burk and Calkins (1983) concluded that the SIT approach to medfly control is sound, with improvements depending not only on more efficient tactics, but also on a more accurate knowledge of the behavioral ecology of medflies under natural conditions. Surprisingly, such knowledge is still largely unavailable.

For example, little quantitative information exists on the location and diel pattern of adult medfly activities in nature. This is true for frugivorous tephritid fruit flies in general, except for studies in nature of Rhagoletis flies (Prokopy et al. 1972, Prokopy 1976, Smith and Prokopy 1981), Anastrepha flies (Burk 1983, Malavasi et al. 1983), and Dacus flies (Nishida and Bess 1957, Iwahashi and Majima 1986, Hendrichs and Reyes 1987).

Here, we present results of systematic observations on the spatial distribution and temporal activities of wild medfly populations in an undisturbed mixed orchard and surroundings.

2.2 Materials and Methods

Our study was carried out in an unsprayed and semi-isolated orchard (ca. 0.5 ha) on the edge of the Nile River valley approximately 10 km South of Luxor, Qena Governorate, in Southern Egypt. On the north, east and south the orchard was bordered by the desert. Only on the west was it connected by corn and sugarcane fields to similar non-commercial orchards. Predominant plantings in the orchard were guavas, oranges, mangoes and grapes.

Observations were conducted two days per week for a total of four weeks in September and October, 1984, corresponding to the second half of the guava and the beginning of the orange fruiting season, and to the fruiting of date palms and grapes. The population of medflies

studied over approximately one generation arose mostly from early fruiting guavas and late fruiting figs.

A representative tree, bush or vine of each type of fruiting and non-fruiting host and nonhost vegetation present in the orchard and surroundings was selected randomly for observation. Hosts included were: Baladi-orange Citrus sinensis (L.) Osbeck (fruiting), guava Psidium guajava L. (fruiting), apple Malus sylvestris L. (with some fruit), lime Citrus aurantiifolia (Christm.) Swingle (with some fruit), fig Ficus carica L. (with few fruit left), mango Mangifera indica L. (without fruit), and peach Prunus persica (L.) (without fruit). Possible nonhosts were grapes Vitis vinifera L. (fruiting) and date palm Phoenix dactilifera L. (fruiting). The nonhosts represented were the asclepidaceous Calotropis procera Ait. (fruiting), castor bean Ricinus communis L. (without fruit) and casuarina Casuarina sp. (without fruit). With the exception of some mandarine Citrus reticulata Blanco trees (with fruit), on which informal observations were carried out, there were no other trees or bushes in the area of the orchard. Most selected observation trees and bushes measured between ca. 3 and 4 m in height. Only grapes, growing on trellises, were smaller. Mango, date palm and Casuarina trees were taller.

Systematic observations were carried out by 2 observers, starting at sunrise (ca. 0615 h) and ending at dusk (ca. 1815 h). Every 2 hours, we recorded for 8 observer-minutes

per tree, the location and activities of flies observed on each of the selected trees and bushes. The order of the observation sites on the different trees was rotated systematically between observation periods and observation days, resulting in equal time for each tree and time of the day. The last census counts of each day were initiated 20 minutes earlier to allow for enough light to detect flies.

As no ladders were available, observations were restricted to between ca. 0.8 and 2.5 m above ground. Each of the observers surveyed respectively, 2 of the 4 observation areas on each selected observation tree. The 4 observation areas, of variable forms and dimensions depending on the configuration of branches, were delimited at each of the four cardinal points. Each contained ca. 1 m^3 of foliage (1.7 m high x 1 m wide x 0.6 m deep). The density of foliage (i. e., the number and size of leaves in each observation area) was highly variable between trees, within trees, and even within observation areas, as no pruning of branches or clearing of leaves was carried out. Nonetheless, an approximate relative ranking of densities was estimated based on leaf counts and leaf surface areas. Mangoes had the highest foliage density in the orchard, followed by citrus, apple, peach, fig and guava. The selected mango tree, the highest tree in the area (13 m), was climbed every 2 h for observation of fly presence at the top. In the case of the selected date palm, a bent stem

allowed inspection of the 6 m-high crown bearing mature dates.

Temperatures and relative humidities were measured with a hygrothermograph in the shade of a mango tree. Types of fly activity were defined as follows: feeding as a repetitive lowering of the proboscis to touch the surface on which the fly was situated, accompanied by an increased rate of turning. Ovipositing as the insertion of the ovipositor into a fruit (probing is included as "ovipositing" because census counts did not allow for the observation of actual ovipositor dragging at the end of a successful oviposition). The conspicuous presence in a male of a clear droplet in a pouch everted from the anal gland (Nation 1981), was defined as calling (puffing). The term resting was used for motionless flies, except for occasional cleaning. Interactions were all those behaviors, not included in other activity categories, involving intra- and intersexual, as well as interspecific encounters, in which at least one of the interacting flies orients and/or responds to another fly or predator. These included male-male territoriality, males or females approaching calling males, males pursuing females or males unsuccessfully attempting to mate a female or male, flies interacting with mating pairs, female-female encounters, and interspecific encounters. A lek was defined as an aggregation of at least three males calling simultaneously on adjacent leaves, with an estimated

distance of not more than 10-15 cm to the nearest calling neighboring male.

Throughout our study flies were regularly detected at dawn and dusk in the top of the canopy, including the tall mango tree. This prompted us to quantify the daily vertical movement of male medflies by placing Jackson-type white cardboard delta traps (baited with trimedlure attractive to medfly males) 1 and 12 m above ground on this mango tree. On non-observation days, and only for two 24-h periods (so as to avoid trapping too many flies), trap inserts were changed every 2 h and captured males counted. Data were analyzed statistically by ANOVA and regression analysis (SAS 1982, 57-82, 287-336) and means separated using Duncan's (1955) multiple range test. For pair-wise comparisons Chi-square analysis was used. No voucher specimens have been deposited.

2.3 Results

2.3.1 Diel Pattern of Fly Distribution Among Trees

The average number of medflies observed per hourly census is shown in Fig. 2.1. Overall, males represented 67% of all medflies sighted. Numbers of both sexes increased during the mid-morning hours, remained at a peak from 1000-1100 h for males ($F = 37.8$; $df = 6,252$; $P < 0.001$) and from 1000-1500 h for females ($F = 33.1$; $df = 6,252$; $P < 0.001$), and decreased in the later afternoon hours. Relatively few

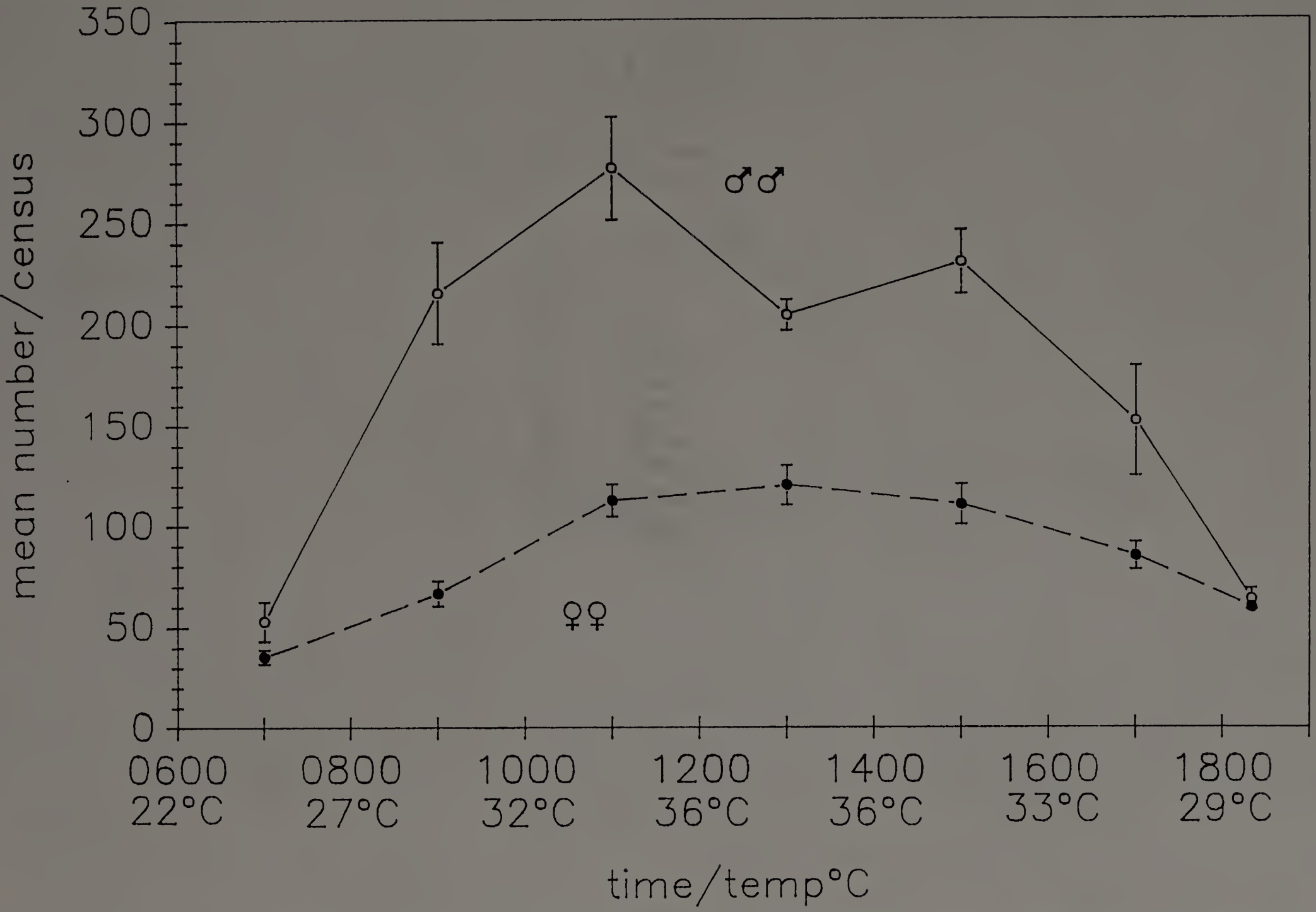

Fig. 2.1. Average number of male (n = 9,563) and female (n = 4,708) flies observed in census counts every 2 h on all host and nonhost vegetation in and around the mixed orchard, Qena, Egypt. Average temperatures in ^{o} C.

flies were sighted at dawn and dusk, probably because flies had moved to the tree tops and out of our view. Trimedlure trap counts of males at the top and bottom of the 13 m high mango tree (Fig. 2.2) confirm this pattern. The upper trap received most captures near dawn and dusk. Counts at the lower trap reflect a diel pattern of male movement similar to that recorded during census counts. The peak in numbers of males at ca. 1000 h in both observation and lower canopy trap catches corresponds with peaks in male courtship activities.

With the first daylight (ca. 0600 h), resting flies were detected near the top of mango and upper canopies of other trees, facing the rising sun. With increasing light-levels and rapidly increasing early morning temperatures, flies began walking and flying in areas of upper canopy foliage illuminated by the emerging sun. As temperatures continued to increase, flies moved progressively to more shaded positions lower in the canopy and away from the sun. By midday, the majority of flies was seen in the interior of the lower part of the canopy. Also at midday, flies tended to move from trees with open canopies to those with dense canopies. In later afternoon hours, as temperatures fell, flies moved to the western side of trees (unpublished data), which received the setting sun. From there they moved progressively to the upper part of the canopy.

Very few medflies were present on nonhost vegetation surrounding the orchard. The few that were sighted were on

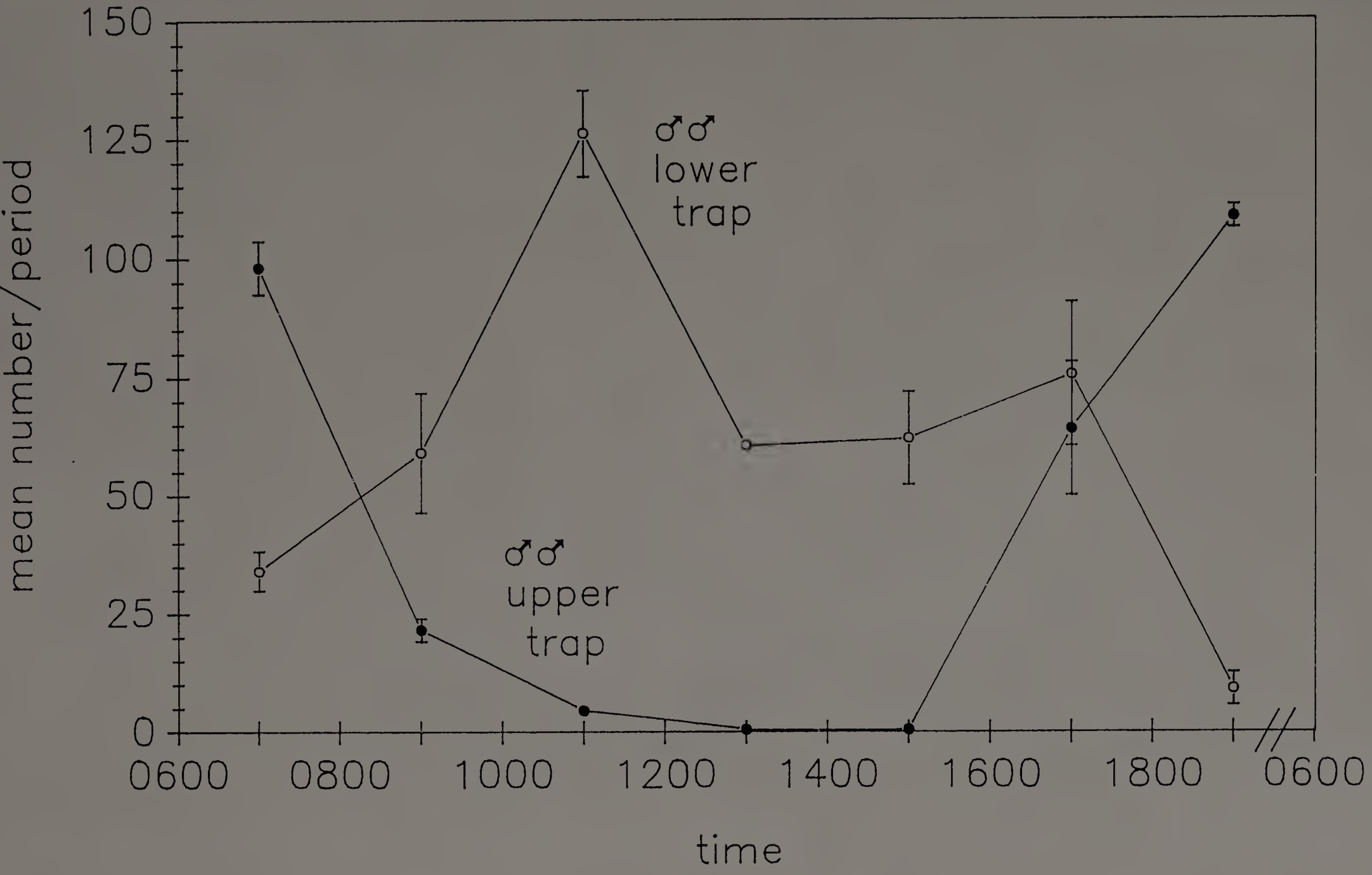

Fig. 2.2. Number of males captured every 2 h (over 24 h), by two Jackson traps baited with 2 ml trimedlure and placed at the bottom (1 m aboveground) and near the top (12 m above ground) of the same nonfruiting mango tree, Qena, Egypt.

grapes and date palms. On all host trees in the orchard, fruiting or non-fruiting, fly populations were consistently present, including the mandarine trees not under systematic observation (Table 2.1). Flies were significantly more abundant on fruiting host orange trees (males: $F = 91.8$; $df = 6,252$; $P < 0.001$; and females: $F = 41.7$; $df = 6,252$; $P < 0.001$). 38 % of all males and 24 % of all females were sighted there. During the principal male calling time (0800-1100 h), over half of all males were sighted on fruiting orange trees (Table 2.1). Among other fruiting or non-fruiting host trees in the orchard, there were no significant differences in male numbers. Only fig trees had significantly fewer males. In the case of females, guava followed orange in having a significantly higher female presence than all other trees in the orchard.

The diel pattern of fly presence on orange, guava and lemon peaked mainly in the morning, on fig it peaked around noon, and on apple, mango and peach, possibly because of receiving more afternoon sun, it peaked mainly in the afternoon (Table 2.1).

2.3.2 Diel Pattern of Types of Fly Activity

Flies were seen feeding throughout the day (Table 2.2). Relative to total numbers of each sex observed over the course of the study, significantly more female than male feeding events were observed ($P < 0.001$, chi-square). Feeding on fruit occurred mainly during mid-morning and late

Table 2.1. Percentages of C. capitata location at different times of day on host trees in a mixed orchard and surroundings[a].

	Fruiting hosts				Lightly fruiting hosts						Nonfruiting hosts			
Time, hours	Orange		Guava		Lemon		Apple		Fig		Mango		Peach	
0600-0700	3	5	8	6	8	9	3	5	3	3	9	13	3	4
0800-0900	23	14	17	10	28	20	12	7	7	8	10	9	13	7
1000-1100	29	21	25	23	12	18	19	17	18	16	21	16	21	17
1200-1300	15	20	21	22	6	9	19	17	36	34	19	19	19	24
1400-1500	15	15	17	15	13	11	25	26	32	30	23	14	26	28
1600-1700	12	15	7	14	24	18	15	16	3	7	9	13	14	14
1740-1820	3	10	5	10	9	15	7	12	1	2	9	16	4	6
Mean no. individuals[b]	523a	163p	173bc	141q	125c	69st	202b	86rs	51d	55t	127c	61t	162bc	94r

[a]All days, locations, and activities are combined. Flies were sighted only occasionally on grapes (, n = 14; , n = 12) and dates (, n = 7; n = 10). No flies were sighted on nonhosts Calotropis procera, Casuarina sp., and Ricinus communis.

[b]Means within rows followed by the same letter are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test). For both male and female data, there was also significance ($P < 0.01$; df = 36,252; ANOVA) for interactions of host versus time and host versus date of observation.

afternoon, whereas feeding on leaf surfaces occurred primarily during mid-day when flies were in lower parts of the canopy. However, it is probable that more feeding on leaf surfaces took place during early and late hours of the day than was observed, because flies on upper surfaces of leaves were out of our sight in upper parts of the canopy during those hours. Actually, analysis relating feeding events to fly presence indicates the highest percentages of feeding flies occurred during the early morning (females) and the late afternoon hours (both males and females).

Some oviposition activity was recorded in early morning hours (20%). It nearly ceased during the hot hours of the day, and then reached a peak in later afternoon hours (71%). Flies were observed resting throughout the day. However, resting peaked for both sexes during the high temperatures of midday (Table 2.2).

Courtship activities occurred throughout most of the day. Male calling was bimodal, with a main peak in the morning before the hottest part of the day, and a smaller one during the afternoon hours. Male sexual activities began at dawn in the uppermost foliage facing the sun in the east, shifted to more shaded positions in lower foliage during most of the day, and shifted again in late afternoon to higher foliage facing the setting sun in the west. The earliest calling males were observed shortly after 0600 h, and the last ones near dusk (Table 2.2). Only 4% of male calling was detected in the higher foliage (3% in the early

Table 2.2. Percentages of diel pattern of C. capitata activities[a].

	Feeding				Ovipositing	Resting	Calling	Mating		
Time, hours	On fruit		On leaves						x No.[b]	x No.[b]
0600-0700	14	9	5	6	11	6	6	3	0	85d	56d
0800-0900	14	19	21	12	9	10	11	26	13	345b	106c
1000-1100	13	23	4	12	4	20	18	26	29	443a	180a
1200-1300	6	9	31	21	2	23	23	11	21	327b	192a
1400-1500	4	9	24	31	5	19	21	21	13	369b	177a
1600-1700	30	17	5	12	41	12	12	12	17	234c	136b
1740-1820	19	14	10	6	28	10	9	1	7	101d	95c
n	140	156	58	69	337	4,214	3,416	4,165	267		

[a]All trees, locations, and days are combined. Interacting flies not included in the activity categories represented 719 and 463. Because of our inability to census the upper parts of tree canopies, activities during the early and late hours of the day are underrepresented.

[b]Means within columns followed by the same letter are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test).

morning, 1% before dusk). Significantly more male calling took place between 0800 and 1100 h (52%), ($F = 9.3$; $df = 6,36$; $P < 0.01$) with a smaller afternoon peak between 1400 and 1700 h (33%). The sightings of mated pairs followed a similar pattern, although they shifted 1 to 2 hours in time due to the ca. two-hour-long matings in medflies.

2.3.3 Distribution of Activities Within and Among Trees

The male/female sex ratio varied considerably among trees (Table 2.4). On orange, the ratio was 3.2, on guava 1.2, and on fig 0.9. These ratios reflect the fact that orange was the principal site of male calling, guava was one of the main oviposition sites, and fig was the principal site of feeding on foliage.

For both males and females, the main site of feeding was fruit (Table 2.3). Two thirds of all feeding recorded took place there, mainly on ripe fruits. These were, almost exclusively, ripe guavas oozing juice naturally, or ripe and some unripe oranges with wounds caused by feeding of birds or other agents (Table 2.4). Flies were regularly observed feeding in groups at these sites, often competing for access.

An additional and important feeding site was the upper surface of leaves, where approximately one third of all feeding events were recorded (Table 2.3). Surprisingly, nearly half of the feeding on leaves occurred on fig trees, where flies fed on shiny spots on leaf surfaces, which were

Table 2.3. Percentages of within-tree locations of C. capitata activities.[a]

Structure	Feeding		Ovipositing	Resting		Calling	Mating	x No.[b]	x No.[b]
Lower surface of leaves	0	0	0	97	94	98	97	2281a	963a
Upper surface of leaves	29	31	0	>0.5	2	0	1	21b	33b
Unripe fruit	5	11	66	1	2	>0.5	1	30b	98b
Ripe fruit	65	56	34	1	1	1	>0.5	53b	77b
Branches and stems	1	2	0	>0.5	1	>0.5	>0.5	6b	6b
n	198	225	337	4,214	3,416	4,165	267		

[a]All hours, days, and trees are combined. Interacting flies not included in the other activity categories represented 719 and 463.

[b]Means within columns followed by the same letter are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test).

Table 2.4. Percentages of among-tree locations of C. capitata activities.[a]

Tree	Sex ratio	Feeding				Ovipositing	Resting		Calling	Mating
		On fruit		On leaves						
Orange	3.2	34b	32b	3b	1b	45a	21a	16a	54a	49a
Guava	1.2	60a	64a	4b	12b	42a	17a	19a	8b	10b
Lemon	1.8	0c	0c	19ab	9b	5b	8b	11bc	10b	12b
Apple	2.3	5c	3c	14ab	10b	7b	18a	15ab	12b	10b
Fig	0.9	1c	1c	40a	48a	1b	8b	10c	0b	0b
Mango	2.1	0c	0c	3b	7b	0b	11b	11bc	8b	10b
Peach	1.7	0c	0c	17ab	13b	0b	17a	18a	8b	9b
Grape	1.1	0c	0c	0b	0b	0b	>0.5c	>0.5d	0b	0b
Date palm	0.9	0c	0c	0b	0b	0b	>0.5c	>0.5d	0b	0b
Other[b]	---	0c	0c	0b	0b	0b	0c	0d	0b	0b
n	--- 140	156	58	69	337	4,214	3,416	4,165	267	

[a]All hours, days, and locations are combined. Interacting flies not included in the other activity categories represented 719 and 463. Percentages of each activity total within the same column followed by the same letter are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test).

[b]Includes Casuarina sp., Calotropis procera, and Ricinus communis.

probably honeydew droplets of undetermined origin (Table 2.4). In relation to their numbers in the orchard, females fed here significantly more than males ($P < 0.001$, chi-square). As it rarely rains in this part of Egypt, leaves accumulate dust and other substances that form a thin crust on the foliage. Therefore, with the exception of 10.3% of feeding that occurred on fresh or dry bird droppings, it was not possible to determine the other substances on which flies were feeding.

Females oviposited mainly into greenish, unripe fruit (66%), with yellowish ripe fruit being less preferred (34%) (Table 2.3). Oviposition occurred predominantly in oranges and guavas (87%) (Table 2.4). No females were observed ovipositing grapes or dates despite the fact that these are hosts in other parts of the world and that they were much more common than apples, lemons, or figs, which had ovipositions.

Male and female resting occurred almost exclusively on the undersides of leaves. However, some males and females rested on branches and fruits (Table 2.3). Relative to their abundance in the orchard, significantly more females than males were resting ($P < 0.02$, chi-square). Although the numbers of resting flies differed significantly between trees in the orchard (males: $F = 27.3$; $df = 8,56$; $P < 0.001$; females: $F = 22.1$; $df = 8,56$; $P < 0.001$), no significant differences were found between trees in the proportion of sighted flies to be resting. It was only on the orange tree

that males rested significantly less ($P < 0.001$, chi-square) and females significantly more ($P < 0.05$, chi-square) than expected relative to their abundance there. Any sudden movements by other flies, arthropods, birds flying overhead, or human observers caused the fly to terminate its activity and to face the moving object. This occurred even if so much as a shadow moved over a leaf under which a fly rested.

Male pheromone-calling and mating pairs were seen nearly exclusively on the bottom surfaces of leaves (Table 2.3). Although there were many brief visits of males to fruit, increasing progressively during the afternoon, only about 1% of observations of males calling and of mating pairs were made on fruit (both ripe and unripe). No males called on the fig tree even though it apparently offering food on its foliage and it being located in the orchard between the fruiting orange and guava trees. Except for fig, calling males and mating pairs were seen on all other host trees in the orchard (Table 2.4). There was a significant linear regression ($F = 73.6$; $df = 1,54$; $P < 0.001$) between male presence and number of mating pairs. Sexual activities occurred significantly more on the orange trees, where about half of all recorded cases of calling ($F = 9.0$; $df = 6,48$; $P < 0.001$) and mating ($F = 13.1$; $df = 6,42$; $P < 0.001$) took place. The remainder of calling males and mating pairs was distributed rather evenly among all other host trees in the orchard, fruiting or non-fruiting.

Overall, 37% of calling was done by single males, whereas the other 63% occurred in leks in which males competed continuously while calling from single-leaf territories. Leks were concentrated at specific locations during the hours of principal calling activity, and these could be detected by us by smell alone. On orange, during mid-morning, for every single male calling nearly 5 other males were recorded calling in a lek. On mango, this ratio was 3:1; on lemon 2:1; on apple and guava 1:1. Only on peach was this ratio below 1:1.

Copulation was initiated mostly in leks; however, pairs already in copula were mostly detected apart from leks. Leks shifted position over time, partly due to predator disturbance and possibly changes in microhabitat conditions. Mating pairs generally moved away from male aggregations due to disturbance by males.

2.3.4 Weekly Pattern of Distribution and Activities

Over the four weeks of observation, the population of medflies increased to a peak in the third week (males: $F = 5.3$; $df = 7,252$; $P < 0.01$; females: $F = 9.2$; $df = 7,252$; $P < 0.01$), declining thereafter (Table 2.5). The male-female ratio, already favouring males at the beginning of this study, continued to increase through week 4, possibly indicating more female than male migration out of the orchard. The harvest of oranges, which occurred during the last week of observations, most likely accelerated this

Table 2.5. Weekly percentages of C. capitata flies and their activities.[a]

Week	Sex ratio	Feeding				Ovipositing	Resting		Calling	Mating	x No.[b]	x No.[b]
		On fruit		On leaves								
1	1.6	19	27	69	75	24	23	24	12	21	359c	226bc
2	2.0	26	19	12	10	31	24	23	25	39	497b	243b
3	2.0	39	34	13	9	28	32	33	33	27	602a	299a
4[c]	2.6	16	20	6	6	17	21	20	30	13	454bc	174c
n	---	140	156	58	69	337	4,214	3,416	4,165	267	----	----

[a]All trees, locations, and hours are combined. Interacting flies not included in the other activity categories represented 719 and 463.

[b]Means within the same column followed by the same letter are not significantly different ($P < 0.05$; Duncan's [1955] multiple range test).

[c]During the last week, the harvest of oranges for the local market was initiated.

process. After the second week, numbers of ovipositions observed decreased weekly (Table 2.5).

Feeding on the leaf surface substrates decreased drastically after the first week. Feeding on fruits, however, increased with the population. Besides nutrients, fruit juices apparently represent, under dry conditions, an important source of water for flies. During the third week, the orchard was not irrigated (as had been usual) and the importance of feeding on fruit juices relative to foliage feeding increased further. Matings peaked in the second week, and male calling peaked in week three. Resting increased in direct relation to the size of the weekly population.

2.3.5 Predation

Throughout this study, flies were continuously the target of predation attempts by different predators. In exposed locations, such as more open foliage (e.g. on figs), or on fruit, medflies were often attacked or ambushed. Overall flies seemed to be very successful in evading predation. For example, out of every twelve ambush attempts on the fruit be praying mantids, on average only one was successful. This rate increased to about one out of every four, however, when females had initiated boring with their ovipositor into fruit. Damselflies and dragonflies were the most conspicuous predators. These predators, as well as Mantids and Vespid wasps, followed a spatial and temporal

daily dispersion pattern in the orchard similar to fruit flies. Libellulid dragonflies, waiting on perches, seemed to specialize on flies flying into or out of the foliage. Mantids specialized in ambushing flies on the foliage in the mornings and next to fruits during the oviposition period. Zygopteran damselflies and Vespid wasps searched continuously fruit and the undersides of leaves for flies. Although their attacks on calling males inside the canopy did not result in any successful case of predation, they regularly disrupted male aggregation in leks.

2.4 Discussion

This study reports, for the first time, results of systematic observations of medfly feeding, mating and oviposition activities in time and space over a fly generation in nature.

Fly presence was largely restricted to fruiting larval host trees and surrounding non-fruiting host trees. Only on a few occasions were flies seen on nonhost vegetation, such as grapes and date palm. Sexual and oviposition activities were concentrated mostly on fruiting host-trees. Apparently to acquire food and shelter, flies moved regularly to non-fruiting host trees. The balance of sexual activities, which was observed on the latter trees, possibly represented overflow populations of males rejected by territoriality at saturated aggregation sites on the favored fruiting host (citrus). Results of a similar study on the Greek island of

Chios (Hendrichs et al., unpublished data), under lower populations densities, seem to confirm this explanation: all matings observed occurred on fruiting citrus.

2.4.1 Feeding

Both sexes of C. capitata fed mainly on ripe guava and bird-damaged orange fruits oozing fluid, followed in amount by feeding on leaf surfaces, mostly on honeydew and bird droppings, but apparently also on other unidentified sources. Feeding on fruit fluids, which most likely represent an important source of nutrients and water, has been observed previously in C. capitata (Sacantanis 1955; Katsoyannos 1983) and in subtropical and tropical Anastrepha spp. (Burk 1983) and Dacus spp. (Nishida 1980). Malavasi et al. (1983) reported for A. fraterculus (Wiedemann) that nutrients in fruit fluids, combined with probable additional nutrients supplied by microorganisms colonizing the fluids, apparently were sufficient for normal reproductive development. Christenson and Foote (1960), however, found in limited tests that D. dorsalis Hendel flies, which fed on rotting guavas and mangoes, did not lay eggs. However, D. dorsalis flies, which fed on bird dung, did mature sexually and lay eggs.

Our observations support these results. In spite of an apparently unlimited availability of fluid oozing from fruit on host guava and orange trees, medflies still moved to the foliage of non-fruiting hosts, probably in search of

additional nitrogenous food sources. Here, one out of every three feeding events recorded took place on honeydew, on the fig tree, on leaf surfaces, and on bird droppings, which where relatively common in the orchard.

Honeydew has generally been considered as the principal source of food of adult fruit flies (Hagen 1958, Moore 1960, Neilson and Wood 1966). Nevertheless, honeydew may generally not constitute a complete adult diet because various essential amino acids are often absent or present only in low concentration (Craig 1960).

Feeding on bird droppings has been observed previously in other fruit flies, tropical as well as temperate (Christenson and Foote 1960, Malavasi et al. 1983, Hendrichs and Prokopy, unpublished data). This nitrogenous resource is probably utilized in the form of bacteria colonizing the droppings. Adult fruit flies apparently use certain species of Enterobactereaceae as protein source (Drew and Lloyd 1990). Bird feces, splashed on the vegetation under bird perching sites, seem to be exploited opportunistically by adults of many other Diptera, Lepidoptera, and even Hemiptera species (Ray and Andrews 1980, Adler and Wheeler 1984, Young 1984). However, in tropical rainforests and/or during rainy seasons in subtropical regions, the availability of organic nitrogen is more restricted. In these cases, utilization of bird droppings may shift from an opportunistic basis to deliberate orientation. Fruiting vegetation, to which birds come to feed, represent a

predictable source of bird droppings. Feeding and oviposition sites on often inaccessible fruit, made available due to wounds caused by birds, are additional benefits for fruit flies resulting from bird presence. In India, Grewal and Kapoor (1986) correlated bird attack on fruit with initial buildup of fruit fly populations. Flies may learn to respond to combinations of odors from bacterial breakdown products of droppings and odors from fruiting trees.

Tephritid fly feeding on leaf surfaces bearing no obvious sign of food has been reported (Christenson and Foote 1960, Bateman 1972). Gow (1954) showed that microbial breakdown products of nitrogenous food sources are attractive to fruit flies. More recently, Drew et al. (1983) and Drew and Lloyd (1990) have elucidated the role of leaf and fruit surface bacteria in the diet of Dacus flies. These bacteria, which presumably grow on plant leachates suitable in nutrients (Tukey 1971, Last and Warren 1972), represent apparently a major source of organic nitrogen in tropical tephritids.

It is very likely that feeding on leaf surface resources has gone largely unreported in previous fruit fly studies because of the inaccessibility to human observers of upper crown areas of large trees. Flies, after spending the night in the tops of tall trees, might feed during early morning hours on bird droppings under bird-perching sites and other food sources on leaf surfaces. Despite our limited access

to the fly population during the early morning hours, our results seem to confirm observations of Boyce (1934) and Christenson and Foote (1960) that early morning hours are devoted primarily to food foraging. In our study, feeding females were most common during these hours: <50% of all females were feeding at this time. During the rest of the day flies appeared to feed opportunistically upon encountering food rather than to actively search for it.

2.4.2 Sexual Behavior

This study shows that temperature is the predominant regulator of the diel pattern of mating behavior. Under the hot weather conditions prevalent during our study in Egypt, the diel pattern of sexual behavior was bimodal, with a period of reduced sexual activity during midday. In another locale (highlands of Guatemala during winter [Prokopy and Hendrichs 1979]), temperatures high enough for sexual activity occurred only near the middle of the day.

The diel pattern of sexual behavior is unlike that of other lek-forming tropical tephritids so far studied. These are early-morning maters such as A. fraterculus (Malavasi et al. 1983), late-afternoon maters such as Anastrepha suspensa (Loew) (Burk 1983), or dusk maters such as A. ludens (Loew) (Aluja et al. 1983), Dacus cucurbitae Coquillet (Iwahashi and Majima 1986) and Dacus tryoni (Frogatt) (Tychsen 1977). Only in Anastrepha obliqua (Macquart) (Aluja et al. 1983) and the papaya fruit fly, Toxotrypana curvicauda Gerstaecker

(Landolt and Hendrichs 1983) is the diel pattern of sexual activity similar to that of C. capitata, spanning most of the warmer hours of the morning and afternoon.

In the field, we saw numerous leks, confirming previous reports on lekking behavior under field cage (Prokopy and Hendrichs 1979; Zapien et al. 1983) and laboratory conditions (Arita and Kaneshiro 1985), and in a field study of released sterile flies (Van der Valk 1987). Characteristics of lek formation sites, where an initial male releases pheromone and is subsequently joined and challenged by other males, remain largely unknown but seem to require: sufficient foliage density and close branch structure that furnishes protection from predation pressure; the presence of fruiting host tree odor (the attractiveness of citrus may be due to alpha-copaene from ripening citrus fruit) (Teranishi et al. 1987); and illumination, temperature, and possibly other microhabitat properties (Arita and Kaneshiro 1985, 1989, Van der Vals 1987, Sivinski 1989).

Several hundred matings were observed in leks at sites with these characteristics. However, it is not yet clear which of several plausible hypotheses might account for why matings are much less common on host fruit and why females approach displaying males in aggregations. High predation levels on flies on the fruit (our observations and Van der Valk 1987) could be proposed as a complementary model to the "female-preference", "hotshot" and mainly the "hotspot"

models of lek mating systems (Beehler and Foster 1988), a combination of which is currently the most adequate explanations for tephritid lekking behavior (Hendrichs 1986).

The "hotspot" model (Bradbury 1985), proposes that male accumulations originated in microhabitats favorable to females and consequently where they are more likely to be found. The fact that a clumped distribution of lek-forming fruit flies has been confirmed in the field by Van der Valk (1987) and Sivinski (1989), not only for males but also for receptive and non-receptive females, lends some support to the "hot spot" model. The movement to protected "roosting refugia", resulting from intense predation pressure on the fruit and exposed foliage, may have influenced the origin of tropical tephritids' mating aggregations. Predation on more exposed lekking or swarming males is common in Diptera, with some predators specializing on male prey (Peckham and Hook 1980).

The quality of shelter offered by the host plant or tree and the intensity of predation pressure apparently determine in many instances where sexual activities of a species occur. In host plants that are annuals or have open canopies such as the papaya trees (Landolt and Hendrichs 1983), and fig trees studied here, predation pressure may have caused flies to move to more protected encounter sites.

Within the tephritids, rendezvous sites for mating vary considerably. They include oviposition sites, larval host

tree foliage, and non host foliage. In temperate non-lek-forming tephritids, probably because of a less intense predation pressure, males do monopolize oviposition sites. In comparison, in lek forming fruit flies, sexual activities take place on apparently resourceless encounter sites on the foliage, at varying distances from the fruit (Prokopy 1980). In C. capitata, as in D. tryoni (Tychsen 1977), matings are initiated mainly on canopies of fruiting host trees and on nearby protective vegetation. In A. fraterculus (Malavasi et al. 1983) and T. curvicauda (Landolt and Hendrichs 1983), most sexual activity occurs on surrounding nonhosts, and in D. cucurbitae (Iwahashi and Majima 1986) and D. frontalis (Becker) (Steffens 1983), which infest annual plants, all matings apparently take place on more distant protective nonhost trees.

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CHAPTER 3

MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN NATURE: SEX DIFFERENCES IN MOVEMENT BETWEEN FEEDING AND MATING SITES AND TRADEOFFS BETWEEN FOOD CONSUMPTION, MATING SUCCESS AND PREDATOR EVASION

3.1 Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), (hereafter referred to as medfly), is one of the primary parasites of fruits and vegetables. Because of its wide host range of over 200 species, including many commercially important crops (Christenson and Foote 1960), it is a pest especially feared by major fruit exporting countries such as the USA, Chile, Mexico, Australia and New Zealand that have subtropical or tropical climates and are still largely or completely free of this pest (Baker et al. 1990; Bateman 1979; Wilson 1983). Drastic eradication measures, often based on large scale aerial insecticide-bait sprays, are immediately undertaken in medfly-free countries in response to the detection of medfly introductions (Baker 1984; Klassen 1989; Hendrichs et al. 1983; Schwarz et al. 1989). Insecticide-bait sprays are also the conventional approach to medfly control in countries in which this agricultural pest has become established (Roessler 1989).

When one considers that medfly control and eradication efforts are often restricted to large scale aerial insecticide bait sprays that are imposed at great cost

against stiff environmental opposition (Dreistadt 1983; Scribner 1983), it is surprising that medfly food foraging behavior in nature and medfly natural history in general have so far received little serious attention (Burk and Calkins 1983). Such a knowledge would promote a more directed application of baits and generally facilitate the design of environmentally sounder control strategies. With the objective of expanding the understanding of medfly behavior under field conditions, particularly of dispersal between natural feeding and other activity sites, the Mediterranean region was selected to carry out studies of medfly behavior in relatively undisturbed natural or agricultural situations. The first reported study was conducted in southern Egypt (Hendrichs and Hendrichs 1990). The study we report here was carried out on the Greek island of Chios, at much lower population densities and in a more agricultural setting. An additional objective of this study was to assess natural foods (those fed upon by flies) for their contribution to fly longevity and fecundity. With the exception of a few limited studies (Baker 1944; Christenson and Foote 1960; Neilson and Wood 1966; Hendrichs et al. 1990), the contribution of natural foods to tephritid fly fecundity has not been assessed.

3.2 Materials and Methods

3.2.1 Field Observations of Fly Activities

The first part of our study was carried out on the farm (ca. 4 ha) of Byron Katsoyannos, on the south-eastern plain of Talaros on the island of Chios (Greece), in the Aegean Sea, 10 km from the coast of Turkey. The farm produces mainly citrus and vegetables, and has been used for previous medfly studies (Katsoyannos 1983; 1987a,b; Papaj et al. 1989). In and around the cultivated area are fig, grapes, pear, mulberry, pomegranate and olive trees. On all sides, the orchard is bordered by similar mixed orchards and vegetable fields.

Observations were conducted daily over a week in late September and early October, corresponding to the fruiting of the late-ripening fig varieties and grapes and the beginning of the orange fruit ripening season. At this time, most orange fruit were ca. 7 cm in diameter and still green. The population of medflies studied originated mostly from earlier figs and pears. Population levels were considerably lower than those of the population studied in southern Egypt (Hendrichs and Hendrichs 1990).

Representative trees or bushes of the dominant host and nonhost trees in the grove and surroundings were selected for observation. Hosts included Valencia-type orange, Citrus sinensis Ob. (fruiting), and fig, Ficus carica L. (fruiting). Nonhosts included pomegranate, Punica granatum

(fruiting), and mulberry, Morus sp. (without fruit). Informal observations were also made on grapes, Vitis vinifera L. (fruiting). Other vegetation such as vegetables (mainly tomatoes and cucurbitaceous crops), pear trees (no fruit), olive trees (with fruit), and various other trees such as Pistacia terebinthus L. were not included in the observations, although McPhail traps with food baits placed in the upper canopies of some of these trees regularly detected the presence of flies there (including olive trees). The selected orange and fig trees were ca. 3 - 4 m high. Grapes, growing on trellises, and the pomegranate tree were smaller. The mulberry tree was ca. 10 m tall. No pruning of branches or clearing of leaves was carried out. The orange canopy was smaller than the fig canopy (respectively, ca. 2 and 4 m diameter). However, leaves in the orange canopy were much more numerous, denser and darker.

Systematic observations were carried out, starting at sunrise (ca. 0615 h) and ending at dusk (ca. 1815 h). Every hour, two observers carefully examined foliage and fruit and recorded for 15 min (30 observer-min per tree type) the location and activities of flies observed on the selected fig, orange, mulberry and pomegranate trees. Equal observation time was assigned to the upper half and lower half of each tree canopy. Ladders were used to survey the fig and orange tree tops. The mulberry tree was climbed every hour. The order of observation periods on the

different trees was rotated systematically between hours and observation days, resulting in equal observation time for each tree type and time of day. Temperatures were measured every hour in the shade of an orange tree.

Types of fly activity were defined as follows: **feeding**= arrestment (or high rate of turning) with repetitive lowering of the proboscis to touch the surface on which the fly was situated; **ovipositing**= insertion of the ovipositor into a fruit (boring or probing is included as "ovipositing" because observations did not allow for the verification of actual ovipositor dragging at the end of a successful oviposition); **calling**= conspicuous presence on a male of a clear droplet of pheromone everted from the anal gland (Nation 1981). A **lek** was defined as an aggregation of at least three males calling simultaneously on adjacent leaves, with an estimated distance of not more than 10-15 cm between neighboring males. For all statistical analysis we used Statistix 3.1 (Analytical Software, St. Paul, Minn.). Data were analyzed by ANOVA. Means were compared by Tukey's HSD test.

3.2.2 Laboratory Fly Fecundity on Natural Food Sources

Substrates collected in the field (on which medflies were observed feeding) were assessed in the laboratory in Thessaloniki for their contribution to fly longevity, fecundity and fertility. These substrates included ripe figs, bird feces (both collected in Chios at the time of

observations) and grapes (collected in Thessaloniki). Bird feces were collected mainly from citrus, fig and grape. Fruits and bird feces were kept refrigerated until they were placed into laboratory cages. Pupae, obtained from figs infested in the field (Chios), were transported to Thessaloniki and kept in the laboratory at 25^{o}C, ca. 60 % R. H., and a 14:10 L:D cycle (500-1000 lux). Upon emergence, 5 males and 10 females were transferred into each Plexiglass laboratory cage (15x15x15 cm) with water (3 males and 5 females in the second test), a food treatment (either natural or laboratory food), and 6 black ceresin wax oviposition domes (Katsoyannos et al. 1986). In each test there were four replicates (cages) for each treatment. In the first test flies had access ad libitum to water and one of the following treatments: (a) 2 open ripe figs; (b) 2 open ripe figs and ca. 1 g of dry bird feces; and (c) enzymatic yeast hydrolysate-sucrose mixture 1:4 (hereafter referred to as laboratory food). In the second test we compared the following treatments: (a) no food, only water; (b) 1 g of sucrose; (c) 4 open ripe grapes; (d) ca. 1 g of dry bird feces; and (e) laboratory food. Dry bird droppings were placed on wet filter paper in a petri dish. Eggs were collected every other day at which time female and male mortality was recorded, remaining feces were humidified with a few water drops and the position of cages was rotated. Fruit and laboratory food was replaced every 6 days. Collected eggs were placed on humid black filter paper. Egg

fertility was recorded after larval hatch. Female fecundity and female and male longevity were evaluated up to 9-10 weeks after fly emergence, although some flies lived longer. Data were transformed (square root + 0.5) and analyzed by ANOVA. Means were compared by Tukey's HSD test.

3.3. Results

3.3.1 Field Observations of Fly Activities

The overall average number of medfly males and females sighted during observation periods throughout the day is presented in Table 3.1. For both sexes there were significant differences between hours in the numbers of flies observed ($F=11.95$; $df=5,90$; $P<0.01$). Overall more females than males were sighted ($F=10.95$; $df=1,94$; $P<0.01$). Although there were no differences in numbers of each sex observed during morning hours (0600-1200 h), in the afternoon (1200-1800 h) the number of females observed was consistently larger. This greater number of females observed, corresponding to a shift of females onto the fruit, may partly reflect (to the observer) increased female apparency on fruit compared with apparency on foliage and branches.

Average daily temperatures (Table 3.1) were relatively constant, with cool early mornings. As a result and as found in Guatemala highlands (Prokopy and Hendrichs 1979), but unlike southern Egypt (Hendrichs and Hendrichs 1990), medfly

Table 3.1. Average temperatures and average numbers of male and female medflies sighted per 2 h period over 7 observation days on all trees (i.e. orange, fig, mulberry and pomegranate).

Observation Period (hours)	Temperature (°C)		Average Number Flies/Census*		Total Mating Pairs
	Mean	Range	Males	Females	
0600-0800	18.8	18-20	1.1b	1.8c	0
0800-1000	21.8	21-24	5.8ab	4.9bc	2
1000-1200	23.9	23-26	7.6a	7.6ab	4
1200-1400	25.8	24-27	7.5a	11.4a	4
1400-1600	26.0	25-28	5.4ab	10.6a	1
1600-1800	24.2	21-27	4.1ab	12.3a	0

*Numbers in same column followed by the same letter are not significantly different ($P<0.05$ Tukey HST).

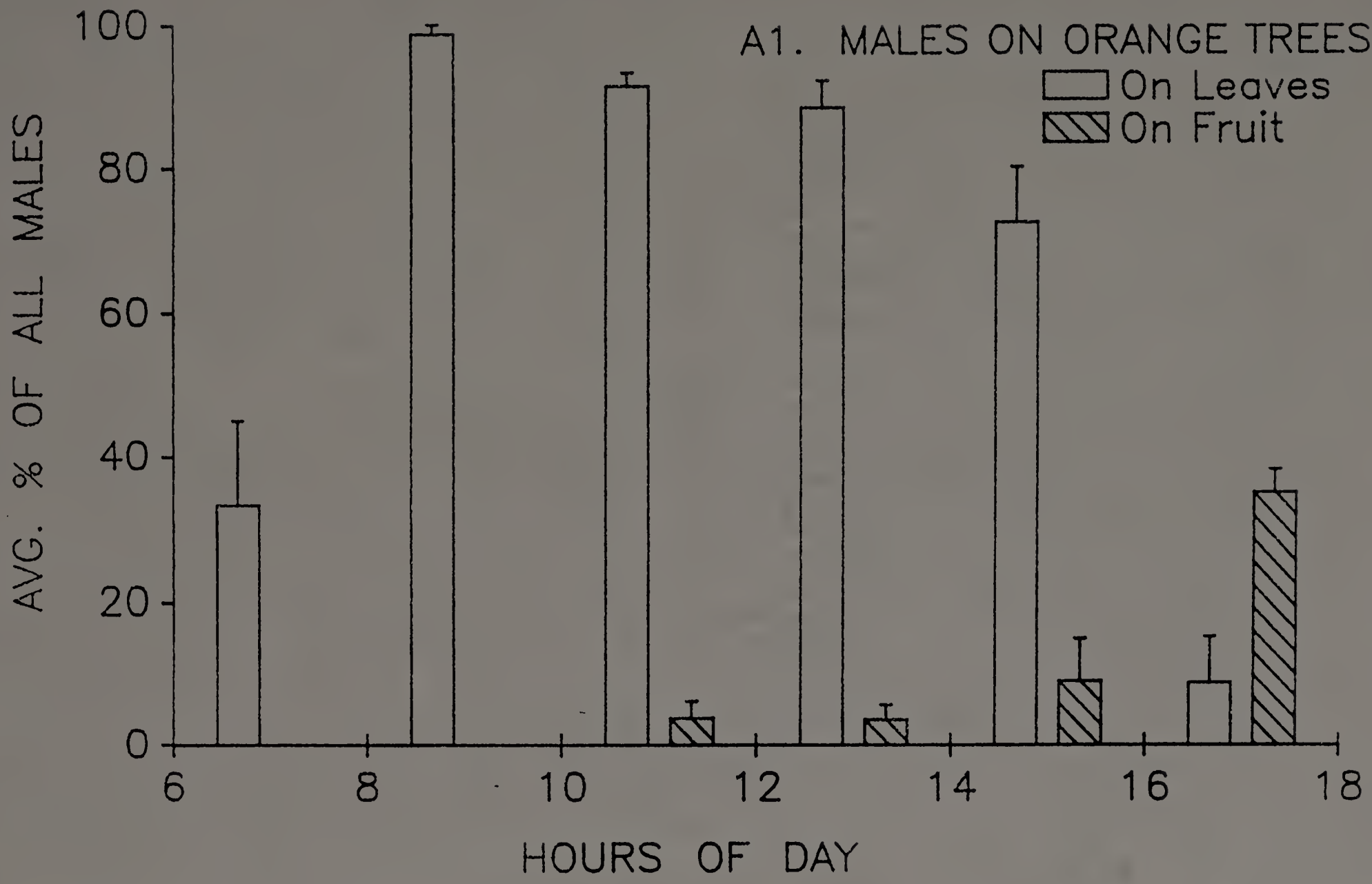

Fig. 3.1.A1. Percentage distribution (± S. E.) by tree type and site of C. capitata in flies an orange grove and surroundings, Chios, Greece. A1. Males on leaves and fruit of orange trees, and A2. males on leaves and fruit of fig and other trees. B1. Females on leaves and fruit of orange trees, and B2. females on leaves and fruit of fig and other trees. (Continued next page)

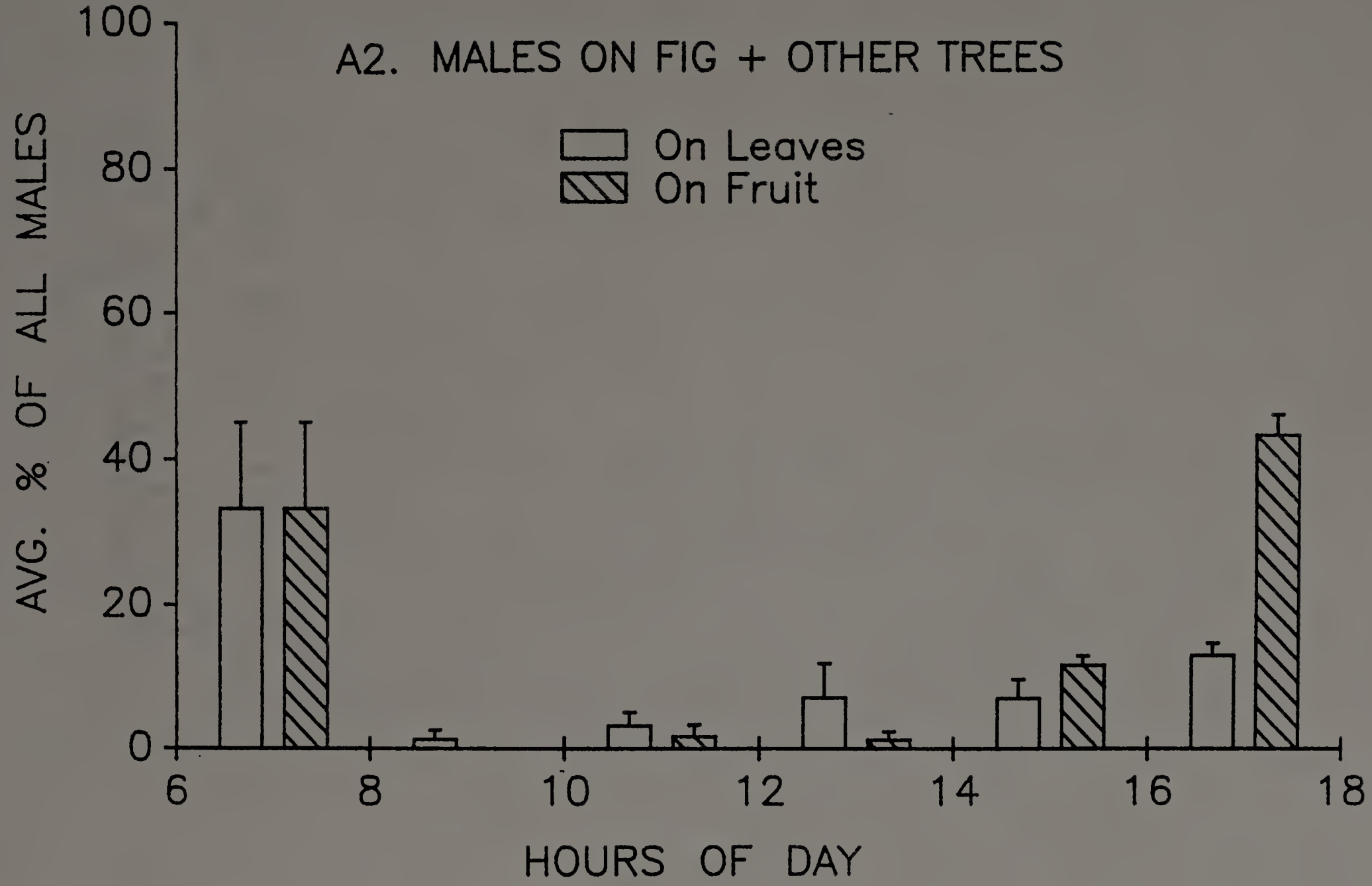

Fig. 3.1.A2. (Continued next page)

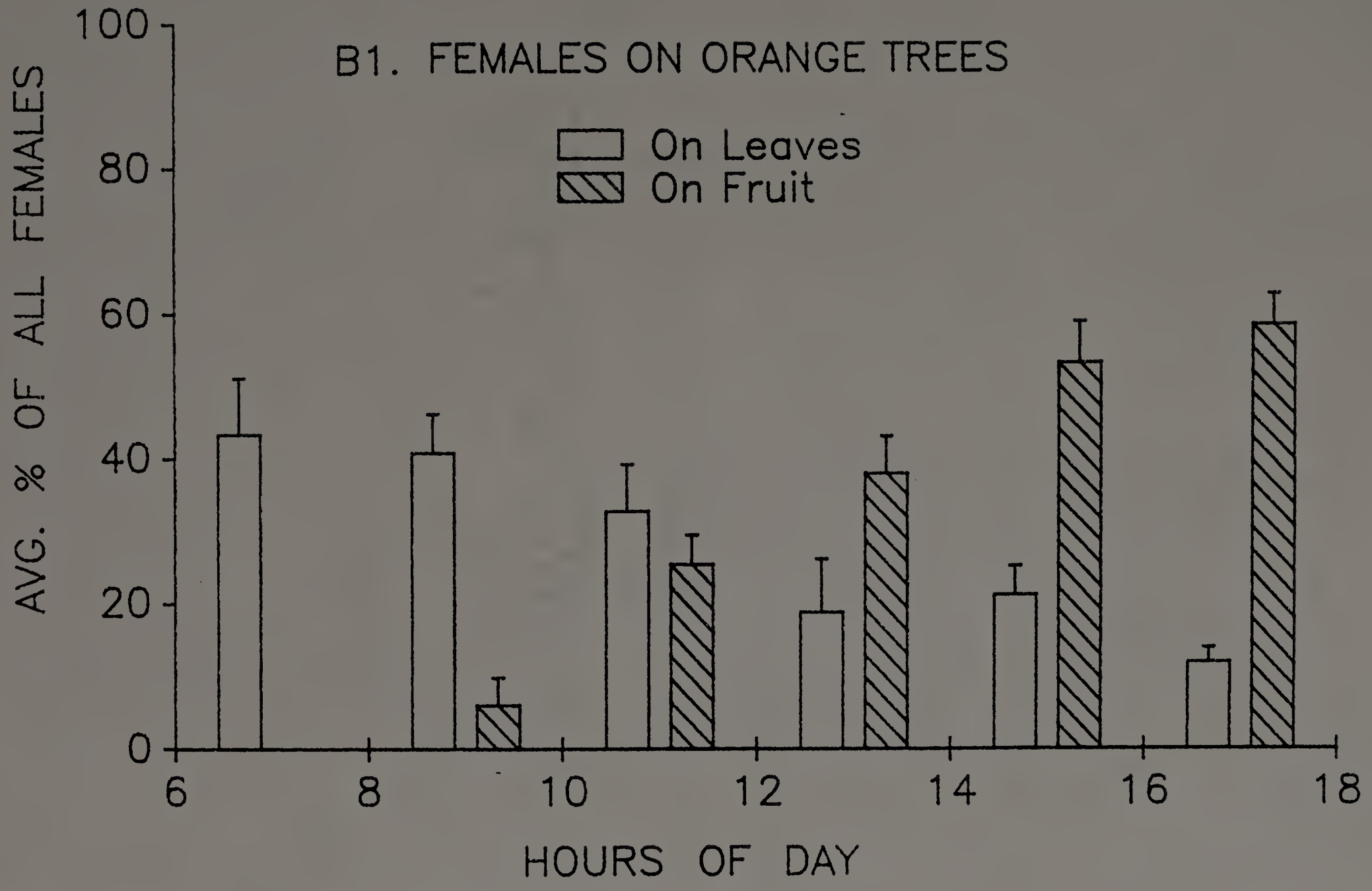

Fig. 3.1.B1. (Continued next page)

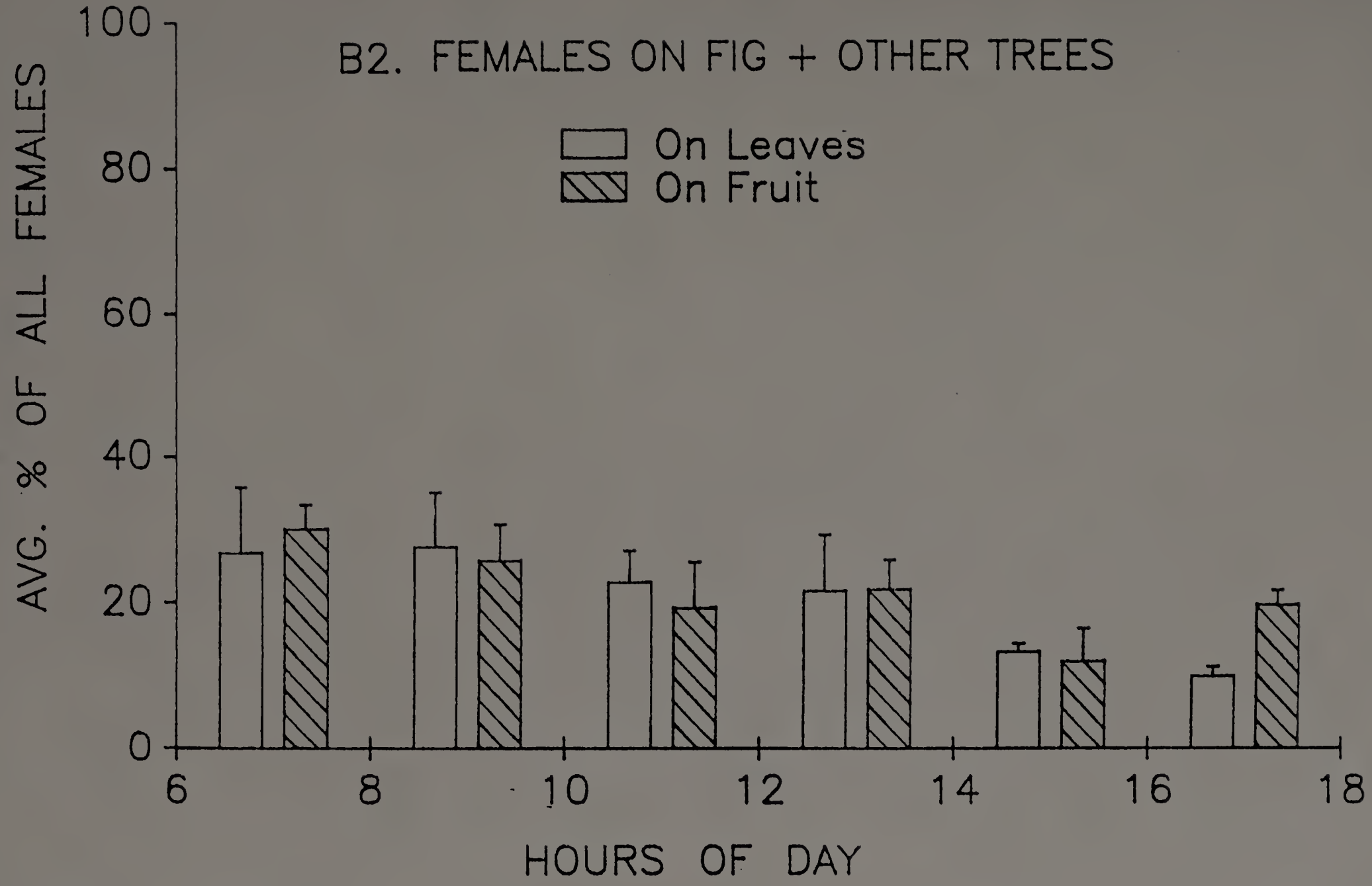
B2. FEMALES ON FIG + OTHER TREES
On Leaves
On Fruit
% OF ALL FEMALES
AVG.
100
80
60
40
20
0
6
8
10
12
14
16
18
HOURS OF DAY

Fig. 3.1B2.

sexual activities and time of pair formation peaked during the warmest hours of the day (Table 3.1).

The diel pattern of male and female location is presented in Fig. 3.1. There was a significant interaction between trees (orange vs. fig and others), site (leaves vs. fruit) and observation periods both for males ($F=6.53$; $df=5,69$; $P<0.01$) and females ($F=10.88$; $df=5,69$; $P<0.01$) (Fig. 3.1). With the exception of early morning (when more than half of observed males were found on fig leaves and fruit), males spent most of the morning and afternoon on orange foliage ($F=36.03$; $df=23,69$; $P<0.01$). In the late afternoon (1600-1800 hours) a majority of males shifted to fruit, both oranges and figs. Rarely were males found on the mulberry or pomegranate trees. Females, on the other hand, shifted gradually throughout the day from fig and other trees to the orange tree, and from orange foliage to orange fruit, so that by late afternoon a majority of females was on orange fruit ($F=7.13$; $df=23,69$; $P<0.01$).

Overall, females fed significantly more than males ($F=18.24$; $df=1,5$; $P<0.01$). Fig fruit was the main site of medfly feeding. Olive flies, Dacus oleae were also found feeding there (see also Katsoyannos 1983). When mature, many figs open naturally. Others are opened by birds, thereby allowing easy access to flies foraging for food. All medfly male feeding events recorded occurred on ripe fig fruit ($n=31$), including figs already on the ground. However, of female feeding events recorded ($n=70$), not all were on figs:

13% of female feeding observed was on bird feces and other undetermined substances on foliage and corresponded mostly to females foraging during midmorning to early afternoon on the foliage of host and non-host trees under observation. The informal observations made on other vegetation, not included in our systematic study, revealed further female feeding on bird feces. In addition, informal observations on grapes indicated regular feeding by both females and males on fruits where skins were broken by bird pecking, wasp feeding or cracks due to turgidity.

The diel pattern of fly activities was linked to the daily shift of fly location from foliage to fruit and between host trees. Male feeding (Fig. 3.2) took place mainly in late afternoon ($F= 16.06$; $df= 5,18$; $P<0.01$) and corresponded largely to male presence on fig fruit. Although female feeding (Fig. 3.2) occurred throughout the day, corresponding to equal female presence on fig fruit and the foliage of fig and other trees, it likewise peaked during late afternoon ($F= 12.83$; $df=5,18$; $P<0.01$). Female feeding on foliage and fruit during the morning, when sexual activities take place, possibly corresponded to non-receptive females. These were either immature (foraging for food during these hours as well as during the rest of the day), or already mated mature females (foraging for food during the morning hours and shifting to oviposition sites during the afternoon).

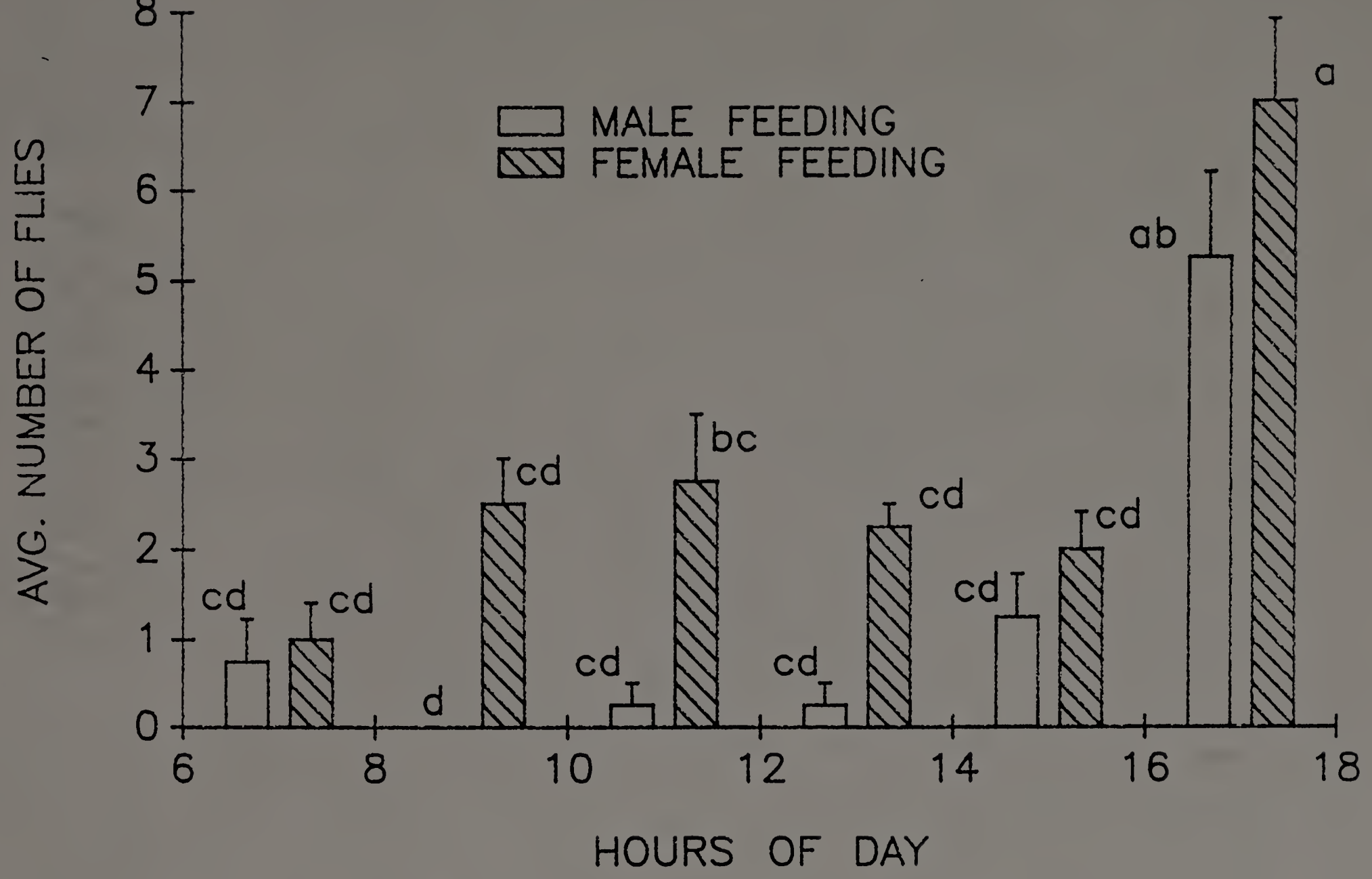

Fig. 3.2. Average numbers (± S. E.) of C. capitata males and females feeding in an orange grove and surroundings, Chios, Greece. Comparison of means by Tukey HSD test (Q= 4.96; d.f.= 12,33; P<0.05).

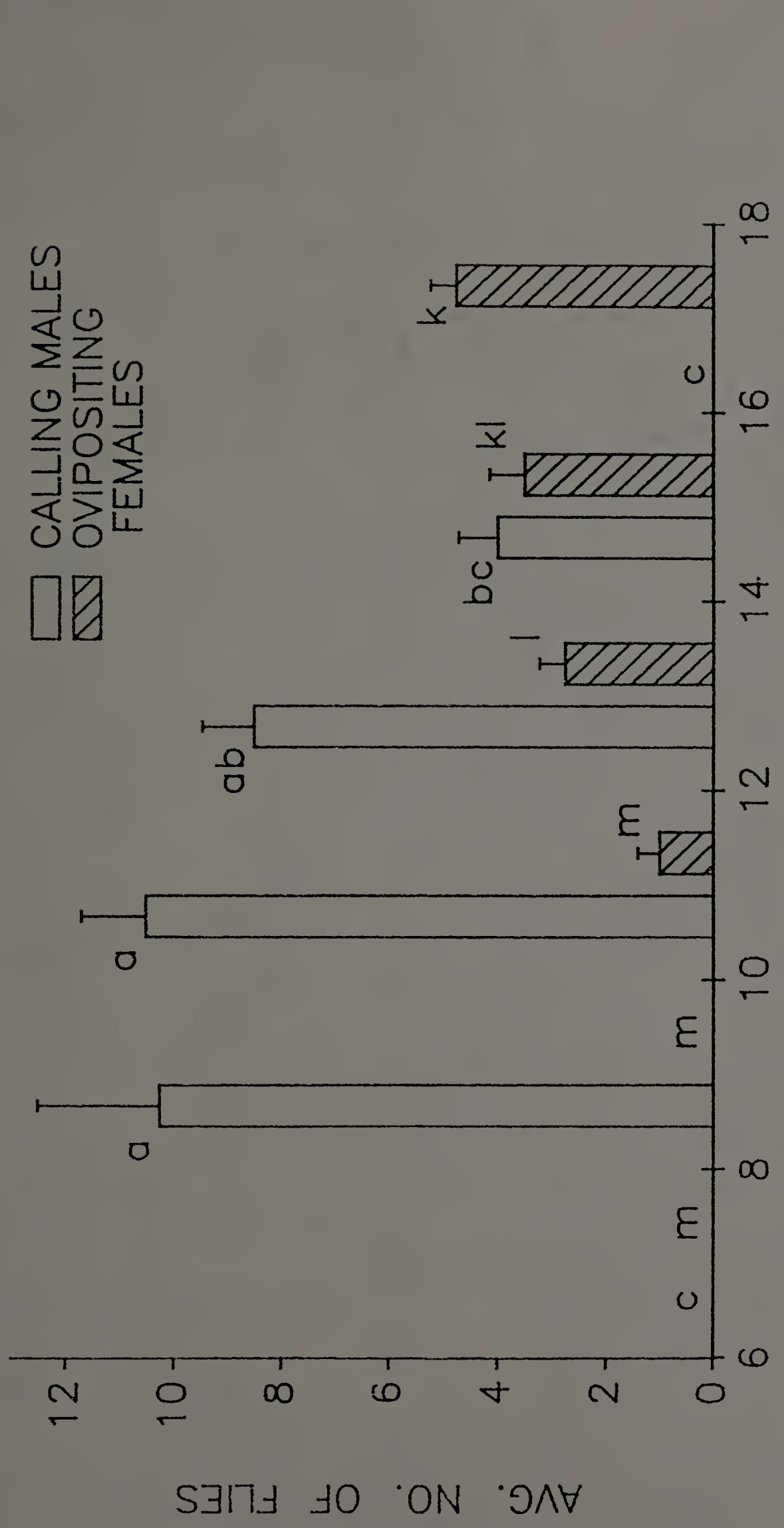

Fig. 3.3. Average numbers (± S. E.) of C. capitata males pheromone calling and females ovipositing in an orange grove and surroundings, Chios. Comparison of means by Tukey HSD test (respectively for males and females Q= 4.59; d.f.= 6,15; P<0.05).

Male calling (Fig. 3.3), was greatest from mid-morning to mid-afternoon (0800-1400 hours) ($F= 18.13$; $d.f.=5,18$; $P<0.01$). Male calling, lek formation, male-male interactions, visits by receptive females to calling males, and formation of mating pairs occurred exclusively on the orange trees and corresponded to male and female presence there (Fig. 3.1). All these activities relating to mating behavior took place mainly in illuminated but protected areas of the foliage of the orange trees, and mostly in the upper and central parts of the canopy.

Female oviposition (Fig. 3.3) occurred almost entirely in the afternoon (1200-1800 h) ($F= 22.32$; $df= 5,18$; $P<0.01$) and corresponded to female presence on greenish oranges and a few ripening figs. In the late afternoon (1600-1800 hours), about as many males moved from orange tree foliage to fig fruit as to orange fruit (Fig 3.1), although there was apparently no food on the orange fruit (i.e. no flies observed feeding on orange fruit). This situation allowed us to distinguish males present on fruit for the apparent purpose of feeding from those that switched from the main sexual strategy of calling and lekking on foliage to a secondary mating strategy of searching for females on fruit and attempting to mate with them (Prokopy and Hendrichs 1979). Most male visits to orange fruit were much shorter than female visits to orange fruit, a fact that probably caused us to underestimate the number of males pursuing this strategy during late afternoon.

European yellowjacket wasps, Vespula germanica (Fabricius), were very conspicuous predators of adult flies on fruit and foliage, and occasionally of fly larvae in wounded figs. During mid-morning to mid-afternoon hours, these wasps were regularly observed approaching and penetrating into dense orange foliage, resulting in the dispersion of aggregated pheromone-calling males. The density of the foliage did not allow us to see the outcome of these predation attempts. During the afternoon hours, when flies shifted to fruit, wasps were also seen to forage on fruit. In a parallel study on Chios (Papaj et al. 1989), we regularly observed wasps in the afternoons attacking flies on fruit. Regularly, wasps were successful in capturing females with the ovipositor inserted into fruit.

3.3.2 Assessment of Fly Fecundity on Natural Food Sources

Results of laboratory assessment of fly fecundity on natural food sources are presented in Table 3.2. In test I, both at 5 and 10 weeks, there was no significant difference in longevity among flies that fed upon a diet of yeast and sucrose and those that fed upon a diet of figs or figs plus bird feces, although longevity of the former was somewhat shorter. Neither was there any difference in longevity between females and males. On the other hand, flies that fed on the standard laboratory food of yeast and sucrose laid significantly more eggs ($F=29.37$; $df= 2,42$; $P<0.01$) than those that fed on the other two treatments. However, both

Table 3.2. Average total egg hatch (% fertility), and average male and female longevity (% survival), and fecundity (E/F/D= eggs/female/day) of wild medflies after 5 and 9-10 weeks of feeding (starting with emergence) on water and natural food sources or the laboratory diet of enzymatic yeast hydrolysate and sucrose (1:4).

	After 5 weeks*			After 9-10 weeks*			
Food Source	% Survival		E/F/D**	% Survival		E/F/D**	% Total
	Males	Females		Males	Females		Fertility
TEST I							
Yeast and sucrose	95.0a	92.5a	2.52a	60.0a	40.0a	3.76a	85.0a
Ripe figs and bird feces	90.0a	97.5a	1.21b	60.0a	45.0a	1.24b	81.4a
Ripe figs	90.0a	97.5a	0.73c	65.0a	47.5a	0.97b	88.0a
TEST II							
Yeast and sucrose	85.0a	85.0a	2.41a	65.0a	40.0b	4.41a	87.0a
Grapes	95.0a	90.0a	0.18b	85.0a	65.0ab	0.17b	73.9b
Sucrose	95.0a	100.0a	0.11b	65.0a	90.0a	0.08b	71.0b
Bird feces***	0.0b	0.0b	0.00b	0.0b	0.0c	0.00b	----
Water	0.0b	0.0b	0.00b	0.0b	0.0c	0.00b	----

*Numbers in same column followed by the same letter are not significantly different ($P<0.05$; Tukey HSD-test).

**Averaged over total lifetime of flies, including the pre-reproductive period

***Does not include bird feces replicate that contained fruit pieces and sustained longevity and some fecundity

the fig diet and the fig plus bird feces diet also sustained a continuous, although lower, fly fecundity. Over the 10 week test period, there was no significant difference in number of eggs laid by females that fed on ripe figs versus ripe figs plus bird feces. During the first 5 weeks, however, the diet with the bird feces gave rise to more eggs laid than that of figs alone ($P<0.05$; Tukey HSD test). There was no difference in fertility levels between the three treatments ($F=1.52$; $df=2,49$; $P<0.05$).

In test II (Table 3.2) fly longevity differed among treatments both at 5 weeks ($F= 191.3$; $df=4,12$; $P<0.01$) and at 10 weeks ($F=12.47$; $df=4,12$; $P<0.01$). The diet of bird feces alone and that of water alone did not sustain fly longevity. However, one of the cages with bird feces only, unlike all other replicates, did sustain fly longevity and some egg-laying. Inspection of the droppings in that cage showed that they contained undigested parts of fig and possibly other fruits. The diets of grape alone and sucrose alone did sustain female and male longevity as much as the laboratory diet of yeast and sucrose. At the end of the 10 week test, flies fed sucrose alone suffered a lower mortality than those fed yeast and sucrose. Flies fed yeast and sucrose were the only ones that sustained a continuous level of egg production ($F=26.97$; $df=2,48$; $P<0.01$). Among all the other treatments, including the treatment of water only, there were no significant differences in fly fecundity, although the grape alone diet and sucrose alone

diet yielded a few eggs, generally of a lower fertility level.

3.4 Discussion

3.4.1 Dispersal, Feeding and Fecundity

With few exceptions, frugivorous tephritid fruit flies are anautogenous, requiring constant intake of carbohydrates for maintenance. In the case of females, additional meals of protein and other nutrients such as minerals, vitamins and sterols are necessary for egg maturation and daily oviposition (Teran 1977; Webster and Stoffolano 1978; Tsitsipis 1989). Because they need a more diverse and substantial diet, females are expected to disperse more than males to the extent that at least one of the required food sources is off the primary host tree. Both the premise and the expectation are consistent with our results. Females fed more than males, foraged for considerable periods off the primary host, orange, and realized higher fecundity when feeding on a more diverse diet. Males, on the other hand, did not forage much away from the primary host, fed mostly during a late afternoon period when they were least likely to miss a potential mate, and did not have increased longevity on a more diverse diet. Such sex differences in food foraging behavior, more emphasized in vertebrate literature (Baker 1978; Greenwood 1980; Raymond et al. 1990), have apparently been less studied in insects

(Southwood 1962; Stinner et al. 1983; Fletcher 1989; Bell 1990), although they may be as common a phenomenon as in vertebrates (Nishida 1980; Drew 1987; Haslett 1989; Hendrichs and Prokopy 1990).

As in another study carried out under different field conditions and much higher medfly population densities (Hendrichs and Hendrichs 1990), fruits and their juices, together with bird droppings and other undetermined leaf surface substances, were the main sites and substrates of medfly feeding in nature. Although feeding on fruit fluids had been reported previously for medfly (Sacantanis 1955; Katsoyannos 1983; 1987a) and in subtropical/tropical Anastrepha and Batrocera (Dacus) spp. (Baker 1944; Nishida 1980; Burk 1983; Malavasi et al. 1983), our fecundity studies have shown for the first time that some host fruits, such as figs, can sustain substantial egg production in medfly females. Christenson and Foote (1960) found that Batrocera (Dacus) dorsalis flies which had only fed on overripe guavas or mangoes did not lay eggs. The protein content of grapes, guavas, mangoes and oranges is about 1 % (National Academy Sciences 1961), i. e. approximately one third to one quarter the amount present in figs. However, the results of Christenson and Foote (1960) seem to have been obtained in a rather limited test with no replicates.

Our fecundity studies also showed that bird droppings, a common feeding site for medfly (Hendrichs and Hendrichs 1990) and other fruit flies (Malavasi et al. 1983; Hendrichs

and Prokopy 1990; Hendrichs et al. 1990), significantly increase fecundity on a fruit diet. It remains to be determined whether bird droppings represent an additional source of scarce nitrogen or whether they provide complementary nutrients required by females. Also to be explored in fruit flies is the occurrence of bacteria that can degrade uric acid from bird droppings. In some tropical cockroaches that likewise are nitrogen scavengers feeding on bird droppings, the presence of uricolytic bacteria has been implicated in breakdown of nitrogenous waste products (Schal and Bell 1982; Cockran 1985). According to Terra (1990) the cyclorraphous dipteran adult digestive tract often possesses adaptations to handle a diet consisting mainly of bacteria that develop in liquids associated with materials in various degrees of decay. It is therefore likely, although it remains to be determined, that the source of at least some of the amino acids both in fruit juices and in bird feces is bacterial (Drew et al. 1983; Drew and Lloyd 1990).

Protein as a source of insect food may be scarce in the tropics (Price 1984; Cockran 1985). In an undisturbed tropical environment, widely dispersed single fruiting host trees may represent not only the larval food of frugivorous tropical fruit flies, as well as the encounter site of the sexes and adult shelter, but also the main male feeding site and one of the main female feeding sites. As many tropical trees fruit intermittently over a number of months, offering fruit in various stages of development at any one time, such

trees may represent a source of food for successive generations of multivoltine species. Tropical fruit flies can utilize the nectar of host flowers (Hendrichs and Reyes 1987), feed on different stages of maturing host fruit (facilitated by vertebrate or insect damage), and even consume rotting fruit on the ground (Baker 1944). Additionally, they may be attracted to and feed on feces of birds also attracted to fruiting trees. Consequently, it is likely that tropical fruit flies respond not only to host and male-produced pheromone volatiles but also to the odor of fruit in various stages of decay (Robacker 1990), products of bacterial breakdown of amino acids (Bateman and Morton 1981; Mazor et al. 1987; Drew and Lloyd 1987; Hedstroem 1988) and to the synergistic interactions of all these products (Bartelt et al. 1986; Galun et al. 1985; Schaner et al. 1987; Sharp and Chambers 1983).

Our field observations have shown that flies adjust their food foraging activities in response to dynamic changes in the spatial, temporal, and seasonal distribution of food resources. For example, on Chios, flies were often seen feeding on grapes, although grape juices did not support fecundity and appeared to be only sources of water and carbohydrates. In the study by Hendrichs and Hendrichs (1990), grapes, although available, were rarely a medfly feeding site. There, the main feeding site available to flies was juice oozing from ripe guavas. By feeding on this juice, medflies apparently also satisfied simultaneously

their water requirements. In Chios, ripe figs, although a richer fly food in terms of amino acids and carbohydrates, appeared not to satisfy fly requirements for water. The juice of grapes, apparently, represented for flies a complementary source of water and other nutrients. Such adjustment in fly food-foraging activities probably also bears upon the variable effectiveness of such management tools as food-baited monitoring traps and insecticidal-bait sprays. Cunningham et al. (1978) have shown that under dry orchard conditions, typical of those in our study, "wet" traps (such as McPhail traps) perform better than where flies have continuous access to water.

Under natural conditions, fly food foraging may be highly dynamic, varying not only with the combination of hosts available, but also over time with local host phenologies of fruit and non-fruit trees. Under monoculture conditions (commercial plantations or orchards), fly food foraging may be less complex and therefore more predictable. Even so, food and water availability greatly affect on practices of monitoring and controlling flies. In relatively food-scarce commercial orchards, for example, one can expect that immigrating flies might inhabit largely the perimeter rows of trees because of their need to move back and forth regularly to the surrounding vegetation to obtain food. Such an obligatory fly movement would increase many-fold the effectiveness of food-baited interception traps placed

around orchards and of insecticide bait sprays applied specifically to orchard perimeters.

3.4.2 Food Consumption and Mating System

Our field observations have confirmed the daily shift between two mating systems described as a dual mating strategy by Prokopy and Hendrichs (1979). In later afternoon, males shift from the main lek mating site on foliage to a secondary resource based mating site on host fruit to intercept ovipositing females there. Although this secondary strategy is less effective because females on fruit are generally less receptive, males shift to the fruit because they have a better probability of encountering and mating with a female on fruit at this time than in a lek, because this is where the majority of females are located. Finally, males feed toward the end of the day possibly because it is the time when they were least likely to find a mate. Even at this time, however, both males and females could be found sometimes in close proximity feeding on cracks and wounds on ripe figs (see also Katsoyannos 1987). Burk (1983) suggested that encounters with these feeding females on ripe fruit, unlike encounters with unreceptive ovipositing females on fruit, are potentially more rewarding to males because of the number of less unresponsive virgin females involved.

With knowledge that the formation of a majority of mating pairs is confined to certain areas of the foliage of

the primary host orange, we might expect tradeoffs to be expected between food consumption and mating success. These tradeoffs might differ between the sexes particularly when the mating site is spatially restricted relative to food resources. (We found no evidence to support the suggestion by Galun et al (1985) that lek sites may be selected to coincide with feeding sites. Females are mainly food-limited, unless sperm depleted and therefore receptive, and need not restrict food foraging to the mating site. By contrast, to the extent that sperm quality does not depend critically on nutrition (Webster and Stoffolano 1978), male fitness depends strongly on the overall number of matings achieved. One might likewise expect selection against males dispersing to non-host foliage in order to mate with widely dispersed and mostly unreceptive females. Rather, selection would favor males who wait on the primary host to which females have to return. In fact, males form leks on foliage in anticipation of the arrival of receptive females (Prokopy and Hendrichs 1979). In theory, male calling and lek formation could even have evolved as adaptations for further "concentrating" the highly dispersed female sex in certain areas of the host foliage. However, this is unlikely because similar sex differences in dispersal in relation to food resources exist in the non-lek-forming apple maggot fly, Rhagoletis pomonella (Hendrichs and Prokopy 1990). More likely is that medfly females, subject to high predation on fruit, might have driven the evolution of male calling and

the lek mating system by having selected for ready location of males on the host foliage, as well as for an arena in which males are sorted by intrasexual selection and in which female choice is facilitated.

3.4.3. Mating System and Predator Evasion

Since Tychsen (1977) and Prokopy and Hendrichs (1979) described a lek mating system respectively in the Queensland fruit fly, Batrocera (Dacus) tryoni Frogatt, and medfly, such mating systems have been found in many other species of tropical and subtropical frugivorous fruit flies (Aluja et al. 1983; Malavasi et al. 1983; Iwahashi and Majima 1986; Shelly and Kaneshiro 1991). In temperate fruit flies, by contrast, males monopolize fruit and there intercept and mate with females that arrive to oviposit (Boyce 1934; Prokopy et al. 1972). Bradbury and Gibson (1983), Beehler and Foster (1988), Reynolds and Gross (1990) and others have put forward models directed mainly at the evolution of vertebrate lek mating systems. Prokopy (1980) and Burk (1981) put forward a model to explain the dichotomy in mating systems of fruit flies, a model which has held for a decade. They proposed that the resource-based mating system of fruit flies in temperate climates is the result of fly monophagy and univoltinism. Under these conditions, a male strategy of monopolizing fruit to wait for females maximizes male mating success. In tropical flies, which are generally multivoltine and polyphagous, such a male strategy is

presumably less effective than a lek mating system because female presence is less predictable in space and time due to the greater variability and lower predictability of host fruit resources.

Based on the protected characteristics of favoured lek sites, together with a remnant of the resource based mating system on the fruit and the narrow time frame for oviposition, possibly an adaptation to saturate predators of flies on the fruit, Hendrichs and Hendrichs (1990) proposed that differential predation is responsible for the dichotomy of mating systems in tephritid fruit flies and therefore possibly a driving force of the lek mating system in frugivorous tropical species. This relationship between mating system and intense predation on the fruit in the polyphagous medfly is supported by our results and those of Papaj et al. (1989). Apparently as a consequence of the high mortality suffered by ovipositing females on the fruit, females minimize predation risk by utilizing existing oviposition punctures (Papaj et al. 1989), and by mating selectively in leks on the host foliage. By contrast, in the temperate non-lek-forming apple maggot fly, Rhagoletis pomonella, predation on fruit is presumably not intense enough to favor the exploitation of existing oviposition punctures (not only in small hawthorn fruit populations but also in apple populations where larval competition is low) (Averill and Prokopy 1989). Further evidence in support of the linkage between the existence of a lek mating system and

intense predation pressure on fruit can be found in the largely monophagous, though tropical, papaya fruit fly, Toxotrypana curvicauda Gerstaecker. The extremely open foliage of papaya trees offers no protection to papaya fruit flies from predation. Male leks are formed away from the host tree (Landolt and Hendrichs 1983), female oviposition takes place during a narrow time frame and papaya fruit flies are Batesian mimics of vespid wasps, varying geographically with the local wasp predators (Landolt et al. 1990).

In Chios, pheromone-calling medfly males were found exclusively on orange foliage. Unlike a study in Egypt under much higher population densities (Hendrichs and Hendrichs 1990), the number of calling males in our study apparently did not saturate appropriate calling sites on orange trees. Although other factors may also account for the differences found between the Egyptian site and the site of this study, calling males could not be found away from citrus foliage, and leks were discrete, with no overflow onto exposed sites, neighboring leks, and other non-host vegetation. As reported for medfly by Arita and Kaneshiro (1989) and for the oriental fruit fly, Batrocera (Dacus) dorsalis, by Shelly and Kaneshiro (1991), calling males were hidden in protected sites in the center of the tree foliage. Perhaps the greater the population level the larger the portion of males displaced (through increased agonistic male-male interactions) from prime sites for male calling and

aggregation. Such males may be constrained to display from less protected sites in host or nearby non-host foliage, where they possibly have less access to attracted females and are most likely subject to higher predation (Burk 1982).

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CHAPTER 4

CONTRIBUTION OF NATURAL FOOD SOURCES TO THE LONGEVITY AND FECUNDITY OF *RHAGOLETIS POMONELLA* FLIES (DIPTERA: TEPHRITIDAE)

4.1 Introduction

The evolution of Diptera in the Triassic is considered to have depended in part on availability of honeydews of Homoptera, abundant since the Permian, that provided nutrients to Diptera before nectar from flowering plants appeared much later in the Cretaceous (Downes and Dahlem 1987). Various differences between Diptera and other orders of Neoptera, such "dancing behavior" (Dethier 1957), and the presence of sugar receptors on the tarsi are accordingly explained in relation to this original nutritional dependence on Homoptera honeydews (Downes and Dahlem 1987).

Based on early observations by Lintner (1885), Silvestri (1914), Back and Pemberton (1917), Boyce (1934), and Batra (1954) and demonstration through various feeding tests (Middlekauf 1941; Hagen 1958; Matsumoto and Nishida 1961; Neilson and Wood 1966), it has become widely accepted that homopteran honeydews, which provide nutrients required for maintenance and egg development, remain the principal natural food source of many species of frugivorous adult tephritids. Observations by several workers, however, have revealed occasions on which tephritid flies have been seen feeding on other food sources in nature (Christenson and Foote 1960; Bateman 1972; Boller and Prokopy 1976; Nishida

1980). In fact, reports of an apparent paucity of insect honeydew on host trees and plants in the vicinity of host orchards supporting large Rhagoletis fly populations (Dean and Chapman 1973; Webster et al. 1979) suggest that Rhagoletis flies may obtain or even require nutrients from other or multiple sorts of natural food.

Most of adult tephritids are anautogenous, requiring frequent access to an extrinsic supply of carbohydrate and water throughout life for survival and maintenance, and in addition regular intake of nutrients such as amino acids, vitamins, minerals and sterols for normal egg production, though apparently not for spermatogenesis (Neilson and McAllen 1965; Tsiropoulos 1977a; Webster and Stoffolano 1978; Tsitsipis 1989). In the apple maggot fly, Rhagoletis pomonella (Walsh), alfa-glucosidase is the only glucosidase in the fore- and midgut (Ross et al. 1977). Consequently, only sugars in the form of disaccharides that contain an alfa-glycosidic linkage can be hydrolysed. In addition, tephritid flies lack proteases and can not break down peptides and proteins. Therefore, they need food containing free amino acids. For most tephritids, nutrient requirements can be satisfied in the laboratory by an artificial diet of sucrose and enzymatic yeast hydrolysate (Fluke and Allen 1931; Hagen and Finney 1950; Hagen 1952; Neilson and McAllan 1965). Wild flies starve if they are fed only aqueous yeast extract as a protein source and develop few or no eggs if they are fed only sucrose.

Analyses of tephritid larval food (host fruit) (Burroughs 1970; Mattson 1980) and adult food (homopteran honeydews) (Auclair 1963; Miyazaki et al. 1968; Boush et al. 1969; Hagen and Tassan 1972; van Vianen 1989) have revealed that both of these food types are low in nitrogen and lack one or more essential amino acids. Therefore, these and other researchers have speculated that tephritids form obligate symbiotic relationships with certain species of microorganisms that provide missing essential amino acids.

In an attempt to identify the types of food consumed by tephritid adults in nature, Chang et al. (1977) chemically analyzed sugar profiles of crops dissected from Bactrocera (Dacus) flies and sought to correlate crop sugar profiles with chemically analyzed sugar profiles of sap exuding from fruit on trees harboring flies. Despite a rather intense effort, no clear correlation could be established, suggesting that this sort of indirect approach may not be useful. In another study using an indirect approach, Nishida (1980) presented information on differences in crop color contents among individual Bactrocera (Dacus) flies collected from nature. He postulated that differences would reflect variation in local availability of certain food types and the kind of food ingested. Again, however, such an indirect sort of approach falls short of what is needed for accurate determination of fly feeding sites and the sorts of food consumed by adults in nature.

Until recently, systematic quantitative field studies examining through direct observation the types of different food sources encountered and consumed by adults in nature have been unavailable for any species of tephritid. Such studies provide the foundation for assessing in a biologically meaningful way the contribution of different natural foods to fly longevity and fecundity. We undertook quantitative field studies of this temporal sort to determine spatial variation in sites and sources of food of R. pomonella flies (Hendrichs and Prokopy 1990) and Mediterranean fruit flies, Ceratitis capitata (Wiedemann) (Hendrichs and Hendrichs 1990). We found that insect honeydew was largely absent in observed orchards and surroundings during times of peak fly population. Substantial numbers of flies were observed feeding on various other substances.

The main objective of this study was to collect substances identified as natural feeding sites of R. pomonella flies and assess their contribution to fly longevity and fecundity in laboratory cage tests as well as in tests on potted host trees in large field cages. Findings for C. capitata have been reported elsewhere (Hendrichs et al. 1991).

4.2 Materials and Methods

Only wild R. pomonella flies were used in our experiments, as nutrient carry-over from the larval stage appears to occur in some laboratory cultured tephritid flies reared for generations on a rich larval diet (Bustamante et al., unpublished data). They originated from larvae from apples collected the previous year from unsprayed trees in Amherst, Massachusetts and surroundings. Larvae pupated in moist vermiculite, and were stored at least six months at 3°C before being placed at 25°C, 80% RH. Emerging adults were maintained at 24±2°C, 60±5% RH, and 15-h photophase with dry sucrose and water in holding cages (20x20x20 cm). From here they were transferred within 24-48 h to different treatment conditions in laboratory cage tests or within 48-96 h in field cage tests.

Natural food was collected three times per week from the same abandoned apple orchard and surroundings in Amherst wherein systematic fly feeding observations had been carried out (Hendrichs and Prokopy 1990). Types of food collected corresponded to those upon which flies had been observed feeding: bird droppings from foliar surfaces (deposited by blue jays, Cyanocitta cristata, and other unidentified species of feral birds); insect frass from the surface of apple fruit (deposited by codling moth larvae feeding on the fruit flesh); and mixtures of wind blown pollen obtained by collecting pollen masses concentrated on vegetation by rain. Disposable plastic gloves were used for handling food.

Natural foods were presented to flies both without and with sucrose as a carbohydrate source to determine the separate effect of each on fly longevity and fecundity.

4.2.1 Laboratory Cage Tests

These tests were conducted under the same environmental conditions (described above) at which emerging flies were held. Unless stated otherwise, a replicate consisted of 6 females and 6 males placed in a 10x10x10-cm screen-plexiglass cage that had been carefully cleaned and washed with bleach and hot-water. There were 6 replicates per treatment. Food and water placed in each cage were renewed three times per week. Controls consisted of standard laboratory food (a 1:4 mixture of enzymatic yeast hydrolysate and sucrose on dry filter paper strips) or sucrose alone on dry filter paper strips. Only sucrose of high purity was used (Grade II Crystalline). Where apple foliage was tested, a single unwashed twig with 8-12 leaves (depending on leaf size) was used per cage. The base was inserted into a water pic containing Hoagland's solution (Hoagland and Arnon 1950). For some treatments only foliage that appeared (upon careful scrutiny) to be free of honeydew, insect frass or bird droppings was used. For other treatments, the foliage was partially covered with honeydew from Aphis pomi De Geer. Masses of wind blown pollen (ca. 0.5-1.0 g) were presented together with the apple leaves on which they were found concentrated by rain. Bird droppings

or insect frass (ca. 5-10 g) were placed on moistened filter paper on petri dish lids. Detailed methodology for collecting and presenting preparations of apple leaf bacteria is described in Lauzon et al. (1992).

A week after emergence flies were provided aseptically with egg-laying sites. Artificial fruit were in the form of two ceresin wax domes that provided appropriate size, shape, color, and texture cues for oviposition (Prokopy 1967; Prokopy and Boller 1971). They were placed on glass microscope slides on the cage floor. Natural fruit were uninfested hawthorns, Crateagus mollis (Torr. et Gr.) Scheele. The previous summer they were protected from infestation by cloth bags, picked when orange-red and stored in vented plastic bags at 3°C for up to a year. They were soaked in warm water for 5 min and then gently but thoroughly washed before placement on the cage floor (2/cage). In some tests, wax-covered hawthorn fruit were used. Immediately after soaking in warm water, these were dipped briefly into transparent (undyed) hot ceresin wax. All ovipositional substrates were renewed three times per week, when number of eggs laid and number of living females per cage was counted. Percent egg hatch was not determined for each experiment because egg fertilization is largely a result of mating status and not adult diet (Neilson 1975; Opp and Prokopy 1986). Females were allowed to oviposit for up to one month after introduction of the first oviposition substrates.

4.2.2 Field Cage Tests

Although laboratory cage tests allowed for control of environmental factors as well as use of artificial oviposition devices devoid of possible nutrients, tests were extended to field cages with potted host trees to determine whether the quantity as well as the quality of nutrients on foliage was a limiting nutritional factor. We placed one apple tree and one hawthorn tree (both non-fruiting) into each 3 m tall x 3 m diam screen mesh field cage. The canopy of each tree was ca. 1 m^3 in size, and was lightly pruned so that flies had approximately the same foliage surface available in all cages (1290 ± 333 hawthorn leaves, 952 ± 206 apple leaves). All trees received a recommended standard dose (1 tablespoon per gallon of water) of soluble inorganic 20-20-20 NPK fertilizer in spring. Developing fruit were removed manually. A band of Tangletrap (The Tanglefoot Col., Grand Rapids, Michigan) was applied to the base of trunks to exclude ants feeding upon and displacing flies from the food. For each test a new set of apple and hawthorn trees was utilized. At the start of each test, trees were thoroughly hosed with water. Each field cage top was covered by a tarpaulin to prevent rain washing away food resources, although this may have interfered with atmospheric particles settling on the tree foliage. We released individually 20 immature females and 5 males in each field cage. Limited

supply of cages and potted trees did not permit use of more than one cage per treatment.

All treatment foods, except honeydew and bird droppings, were placed on cotton wicks in 10 ml glass vials. Five vials were hung on each tree (10 per treatment). Vials contained either a 0.1 M aqueous solution of sucrose, a 1 % aqueous solution of enzymatic yeast hydrolyzate, a solution of leaf surface bacteria prepared as for the laboratory cage tests, or water. Bird droppings were presented on filter paper in 5 Petri dishes hung from each tree. In the honeydew treatment, each tree received 2 twigs bearing 8-12 aphid-honeydew-covered leaves placed in water pics. When flies neared maturity (7 days of age), 5 thoroughly washed unwaxed or waxed hawthorn fruit, hung with thin wire by the pedicel, were evenly distributed on the branches of each potted tree (10 per cage). In a preliminary test, we found that wax domes were not accepted by females for oviposition and therefore could not be used in field cages. We also found that fly fecundity could not be determined with precision on the basis of the number of females originally introduced because, in spite of having cages with closed floors, walls and ceilings, occasionally spiders or other predators managed to enter a cage and kill some flies. As a result, each day cages were searched thoroughly for predators. Dead flies were collected before ants removed them. In addition, 3 times per week all flies were captured to determine the number of living flies present in each field cage, after

which flies were re-released. Three times per week the soil holding all trees was watered, the foliage was lightly misted to compensate for the presence of the rain-shielding tarpaulin, food and fruit were renewed and the fruit were dissected to count the number of eggs laid, and samples of cotton wicks and leaves were sampled to analyze for bacteria populations present using standard techniques for isolation and identification of bacteria (Lauzon et al. 1992).

Fecundity was assessed as number of eggs/female/day (E/F/D) by dividing the number of eggs laid in each replicate (cage) by the period since the last egg collection (2 or 3 days) and the number of living females in that cage at the end of the period. E/F/D data were then transformed by squareroot (x+0.5) for two-way analyses of variance (treatments by oviposition periods). Preoviposition periods were determined by averaging fly age at first egg-laying across the 6 replicates of a treatment. Fly longevity is presented as the average percentage of flies alive after 30 days across the 6 replicates of a treatment. All means were compared using Tukey's HSD-test. Statistical analyses were carried using Statistix 3.1 (Analytical Software, St. Paul, Minn. 55113).

4.3 Results

4.3.1 Effect of Fly Excrement Nutrients

Experiment 1. Prior to the laboratory tests, we needed to establish whether R. pomonella flies could obtain nutrients from their own excrement after emergence or after a protein meal and therefore would have to be transferred regularly to clean cages. Two treatments were compared: twice transfer of flies into clean cages (days 1 and 3) after 24 h access once per week for 5 weeks to enzymatic yeast hydrolysate (rest of each week access only to sucrose and water) versus non-transfer of flies on the same diet regimen.

The resulting fly fecundity was not significantly different between the two treatments ($p = 0.88$) (Table 4.1), indicating that regular transfer of flies to clean cages after protein meals was unnecessary for succeeding laboratory tests. Also, there was no significant difference between treatments in longevity to 30 days (males, $p = 0.26$; females, $p = 0.92$), nor was there a difference in preoviposition period ($p = 0.93$). The principal effect of having access to yeast hydrolysate only one day per week, in comparison to normal laboratory practice in which flies feed ad libitum on yeast hydrolysate, was a lower overall fecundity (1.4 vs. 4-6 eggs/female /day) as well as an extension of the normal preoviposition period (14 vs. 7-11 days).

Table 4.1. Average fly survival and average number of eggs laid into artificial fruit (wax domes) by apple maggot flies confined to laboratory cages without host foliage when flies were transferred (days 1 and 3) or not transferred into clean cages after 24 h access once per week for 5 weeks to enzymatic yeast hydrolysate (rest of each week access only to sucrose and water).*

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days**	Mean Number Eggs/Fem /Day
Transfer	72.2a	80.5a	13.7a (6)	1.47a
No transfer	83.3a	81.4a	13.8a (6)	1.44a

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

**In parentheses, number of replicates in which eggs were laid.

4.3.2 Effect of Host Fruit Nutrients

In various previous tephritid feeding studies, host fruit has been used as an oviposition substrate without confirming first whether it contributes nutrients to flies. The objective of the next four laboratory cage tests was to assess the nutritive value of natural host hawthorn fruit to R. pomonella fly longevity and fecundity.

Experiment 2. First, we compared natural and artificial fruit with and without sucrose to the standard laboratory food. Data in Table 4.2 indicate significant differences in fecundity among treatments. Flies in the control treatment of yeast, sucrose and hawthorn fruit had by far the highest fecundity, significantly greater than in any other treatment. Flies with sucrose and hawthorn fruit laid significantly more eggs than flies with sucrose and artificial fruit (wax domes). In fact, flies given only sucrose and artificial fruit were essentially unable to produce eggs. In both treatments in which flies had no access to sucrose (hawthorn fruit alone or artificial fruit alone), they laid no eggs. Male and female longevity in these two treatments was significantly lower than in all treatments with sucrose. No flies without sucrose survived to sexual maturity. We conclude that flies obtain insufficient carbohydrate from hawthorn fruit for survival, but do obtain sufficient other nutrients (possibly amino acids) for at least some egg development.

Table 4.2. Average fly survival and average number of eggs laid into hawthorn fruit or artificial fruit (wax domes) by apple maggot flies confined to laboratory cages.*

Treatment	Mean % Survival (at 30 days)** Males	Females	Mean Preoviposition Period in days***	Mean Number Eggs/Fem/Day
Yeast & sucrose & hawthorn fruit	77.8a	86.1a	9.7 (6)	6.49a
Sucrose & hawthorn fruit	83.6a	86.1a	14.8 (6)	0.33b
Sucrose & artificial fruit	77.7a	80.6a	16.5 (2)	0.02c
No sucrose & hawthorn fruit	0.0b	0.0b**	---- (0)	0.00c
No sucrose & artificial fruit	0.0b	0.0b**	---- (0)	0.00c

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

** The average age of the last flies to die was 4.1 days (no sucrose-hawthorn) and 3.9 days (no sucrose-artificial fruit).

*** In parentheses, number of replicates in which eggs were laid.

Experiment 3. We next assessed whether, to obtain nutrients from hawthorn fruit, flies require access to the flesh of the fruit (provided by cutting open fruit and exposing the flesh) or need access only to the fruit surface. In addition, by evaluating surface-sterilized fruit (1 min dip in a 10 % clorox (sodium hypochloride solution) followed by rinsing in sterilized water), we tested whether microorganisms growing on the fruit surface might be furnishing nutrients.

Results presented in Table 4.3 show a significant difference in fly fecundity only between the control treatment with yeast hydrolysate and the other three treatments without yeast hydrolysate. There was no difference in fecundity between flies that had free access to surface-sterilized versus non-sterilized intact hawthorn fruit. Also, there was no difference in fecundity between these two treatments and surface sterilized fruit that was opened to allow flies free access to the flesh. In terms of preovipositional period, likewise there was no difference among these last three treatments. Inasmuch as all treatments included sucrose, no significant differences were found in fly longevity. Although bacterial absence was not verified in the surface-sterilized fruit, this experiment confirmed findings of the previous experiment in that flies obtain some nutrients supporting egg development from hawthorn fruit. The absence of fecundity differences between flies exposed to sterilized versus non-sterilized hawthorns

Table 4.3. Average fly survival and average number of eggs laid into intact or opened, sterilized or non-sterilized hawthorn fruit by apple maggot flies confined to laboratory cages with sucrose.*

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days****		Mean Number Eggs/Fem/Day
Yeast & sucrose & intact sterilized hawthorn fruit**	78.8a	70.8a	8.2a	(6)	6.45a
Sucrose & intact sterilized hawthorn fruit	63.2a	58.3a	11.3ab	(6)	0.58b
Sucrose & intact non-sterilized hawthorn fruit	82.1a	79.2a	10.8ab	(6)	0.54b
Sucrose & opened sterilized hawthorn fruit***	61.9a	45.8a	11.8b	(6)	0.53b

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

** Fruit surface was sterilized by placing fruit in 10% sodium hypochloride solution for one minute. Only fruit with no openings or wounds were selected.

*** Surface-sterilized fruit were cut in half to allow flies access to the fruit interior.

**** In parentheses, number of replicates in which eggs were laid.

suggests that microorganisms or their products do not appear to provide nutrients obtained by flies from the surface of washed hawthorn fruit. Furthermore, the results also suggest that fruit need not be damaged or opened to permit fly access to nutrients. There were no cases, both in the field observations of Hendrichs and Prokopy (1990) and informal observations in the laboratory, in which flies fed on oviposition punctures. Apparently, nutrients leach out through the fruit surface.

Experiment 4. In this experiment, our objective was to determine whether the duration of time and number of hawthorn fruit to which flies had access influenced the supply of fruit nutrients and therefore fly fecundity. Consequently, this experiment evaluated not only the supply of hawthorn fruit leachates but also allowed for varying periods of growth of microorganisms on sterilized (sodium hypochloride-treated) fruit surfaces. All treatments were provided with sucrose but no yeast.

Results (Table 4.4) show that flies in contact with the same individual fruits for 7 days realized significantly lower fecundity than flies with access to hawthorn fruit twice per day (same overall quantity of fruit). The standard renewal of fruit (3 times/week, overall same quantity of fruit) gave rise to an intermediate level of fecundity, not significantly different from either of the previous treatments. Fecundity in the artificial fruit treatment was significantly lower than in any hawthorn fruit treatment.

Table 4.4. Average fly survival and average number of eggs laid by apple maggot flies into sterilized hawthorn fruit to which flies were exposed for three different lengths of time (however same overall quantity of fruit was used for each treatment). Flies were confined to laboratory cages with sucrose.*

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days**	Mean Number Eggs/Fem/Day
Hawthorn Fruit Renewed 2-times/Day	86.1a	72.4a	13.5 (6)	0.26a
Hawthorn Fruit Renewed 3-times/Week	83.3a	75.0a	12.2 (6)	0.22ab
Hawthorn Fruit Renewed Once/Week	80.6a	83.3a	14.2 (6)	0.15b
Artificial Fruit Renewed 3-times/Week	80.6a	83.3a	16.0 (4)	0.05c

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).
** In parentheses number of replicates in which eggs were laid.

These findings suggest that any potential buildup of microorganisms transmitted by flies to fruit surfaces does not appear to play a nutritional role under the test conditions used. The possibility exists that with age fruit became less acceptable to flies for oviposition. However, the effect is probably minor when considering that the final appearance of hawthorn fruits in the 0.5, 2-3 and 7 day treatments was similar and that females had no choice between the fruit of the respective treatments. Inasmuch as all treatments were provided with sucrose, fly longevity to 30 days was not different among treatments.

Experiment 5. Next we tested whether covering hawthorn fruit with a thin wax layer (ca. 0.5 mm) interfered with fly access to nutrients from hawthorn fruit leachate that flies appear to utilize for egg development. All treatments were provided with sucrose. Results (Table 4.5) indicate no significant difference in fecundity among flies confined with wax-covered hawthorn fruit or artificial fruit. Only on non-wax-covered hawthorn fruit did flies exhibit significantly higher fecundity, although still a rather limited amount. These results confirmed that in the absence of other food, nutrients from intact hawthorn fruit can be ingested by flies and utilized to contribute to egg development. In terms of longevity at 30 days, there were no differences among treatments, as again all flies were provided with sucrose.

Table 4.5. Average fly survival and average number of eggs laid by apple maggot flies into sterilized hawthorn fruit, wax-covered hawthorn fruit, and artificial fruit (wax domes). Flies were confined to laboratory cages with sucrose.*

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days**	Mean Number Eggs/Fem/Day
Sterilized Hawthorn Fruit	80.6a	83.3a	12.2 (6)	0.29a
Wax-Covered Hawthorn Fruit	88.8a	83.3a	13.6 (5)	0.09b
Artificial Fruit	86.1a	91.7a	17.0 (3)	0.02b

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).
**In parentheses number of replicates in which eggs were laid.

4.3.3 Assessment of Field-Collected Substances for Contribution to Fecundity

Experiment 6. In the sixth experiment, we tested field-collected substances placed in laboratory cages with sucrose (and artificial fruit as egglaying sites) for their contribution to fly fecundity. The substances chosen were the most common sites of apple maggot fly feeding identified during our field observations in an abandoned apple orchard and surroundings (Hendrichs and Prokopy 1990). Results (Table 4.6) indicate that, except for the yeast plus sucrose treatment, only treatments that included bird droppings plus sucrose (with or without apple leaves) yielded any appreciable egglaying. Fecundity was significantly greater in the former than the latter 2 treatments, but was not significantly greater among the latter 2 treatments and the remaining treatments (sucrose plus apple leaves, sucrose plus codling moth frass or sucrose alone) . No significant differences were found among treatments in fly longevity to 30 days of age.

Experiment 7. Next we proceeded to test various other field-collected substances for their potential contribution to fly fecundity. The substances tested were not sites where apple maggot flies where regularly observed feeding in the study of Hendrichs and Prokopy (1990). Results (Table 4.7) indicate that fecundity was significantly greater in the yeast-sucrose control than in the treatment with sucrose plus aphid honeydew on apple foliage, which in turn yielded

Table 4.6. Average fly survival and average number of eggs laid into artificial fruit (wax domes) by apple maggot flies confined to laboratory cages with different natural foods and sucrose.*

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days**	Mean Number Eggs/Fem/Day***
Yeast hydrolysate & sucrose	89.5a	86.7a	9.3 (6)	5.43a
Bird droppings Apple leaves & sucrose	71.7a	86.7a	17.3 (6)	0.45b
Bird droppings & sucrose	85.0a	89.2a	19.0 (5)	0.39b
Apple leaves & sucrose	90.0a	85.0a	21.0 (2)	0.06b
Codling moth frass & sucrose	78.3a	79.0a	18.6 (2)	0.04b
Sucrose	86.7a	90.0a	20.5 (2)	0.03b

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

** In parentheses, number of replicates in which eggs were laid.

*** Egg hatch averaged 79.4% for the yeast control, 69.2% for the combined treatment, 92.3% for bird droppings, 58.7% for the apple leaves, 54.5% for frass and 46.8% for sucrose.

Table 4.7. Average fly survival and average numbers of eggs laid into artificial fruit (wax domes) by apple maggot flies confined to laboratory cages with different field-collected substances and sucrose.

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days**	Mean Number Eggs/Fem/Day
Yeast hydrolysate & sucrose	75.0a	83.3a	7.5 (6)	2.41a
Honeydew & sucrose	83.3a	91.6a	15.5 (6)	0.94b
Apple foliage bacterial solution & sucrose	75.0a	66.7a	16.0 (3)	0.05c
Pollen mixture & sucrose	91.6a	75.0a	17.0 (2)	0.03c
Sucrose	91.6a	75.0a	14.0 (3)	0.06c

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).
** In parentheses, number of replicates in which eggs were laid.

significantly greater fecundity than treatments of sucrose plus a mixture of pollen, sucrose plus a solution of various bacteria isolated from apple leaf surfaces or sucrose alone. No differences in fly longevity were found among treatments.

Experiment 8. In this experiment, we presented flies with pure culture preparations of Klebsiella oxytoca and Enterobacter cloacae bacteria. Each of these species was offered in two forms, either in dry, lyophilised form from cultures grown in tripticase soy broth (TSB), or as live cells collected from trypticase soy agar. We also included an additional control treatment with TSB, as well as one of uric acid crystals, the main component of bird feces. All cages were provided with sucrose, and artificial fruit as oviposition devices.

Results (Table 4.8) show significantly greater fecundity from flies on the yeast-sucrose control treatment than on any other treatment. TSB was the only other treatment in which flies exhibited significantly greater fecundity than flies with sucrose alone. Live E. cloacae and lyophilised E. cloacae or K. oxytoca cells in TSB yielded levels of fly fecundity not significantly different from TSB alone. In all treatments except the yeast-sucrose control, preoviposition periods were exceptionally long. No significant differences in fly longevity to 30 days were found among treatments. We conclude that under the laboratory cage conditions of our tests, flies did not seem to obtain sufficient nutrients from either species of bacterium to produce many eggs. Nor

Table 4.8. Average fly survival and average numbers of eggs laid into artificial fruit (wax domes) by apple maggot flies confined to laboratory cages with uric acid or different bacterial preparations (TSB= trypticase soy broth). All cages were provided with sucrose.*

Treatment	Mean % Survival (at 30 days) Males	Females	Mean Preoviposition Period in days**	Mean Number Eggs/Fem/Day
Yeast hydrolysate& sucrose	96.1a	92.6a	10.4 (6)	3.45a
TSB & sucrose	88.3a	83.3a	22.2 (5)	0.38b
Lyophilised K. oxytoca in TSB & sucrose	91.7a	91.7a	23.0 (2)	0.12bc
Lyophilised E. cloacae in TSB & sucrose	95.0a	100.0a	22.5 (2)	0.10bc
Live cells of K. oxytoca & sucrose	95.0a	85.0a	28.0 (2)	0.01c
Live cells of E. cloacae & sucrose	81.7a	75.1a	27.0 (3)	0.24bc
Uric acid crystals& sucrose	88.9a	74.9a	26.7 (3)	0.02c
Sucrose	94.4a	83.3a	23.8 (4)	0.02c

* Six replicates per treatment. Due to limited availability of bacteria as food, only 2 females and 1 male were used for each replicate in bacterial treatments. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

** In parenthesis number of replicates in which eggs were laid.

were flies apparently able to utilize uric acid crystals as nutrients supporting egg development.

4.3.4 Assessment of Field-Collected Substances for Contribution to Longevity

In the following 2 laboratory experiments with artificial fruit as egglaying sites, we evaluated several previously-tested substances as well as some additional field-collected substances (all presented to flies 3 times a week without sucrose) for contribution to apple maggot fly longevity.

Experiment 9. In this experiment we evaluated the 2 substances most commonly fed upon by apple maggot flies in the study of Hendrichs and Prokopy (1990) (apple foliage and bird feces) as well as codling moth frass and apple fruit. Results (Table 4.9) indicate that few or no flies survived to sexual maturity (10 days) and none laid eggs when presented, in the absence of sucrose, with either apple leaves, bird droppings, codling moth frass, wounded apple fruit (wounds caused by birds or insects), or a treatment combining all these substances. The average age of the last flies to die was greatest (9-11 days) when wounded apple fruit was present alone or combined with other field-collected substances. In contrast, significantly more flies of each sex (80% or more) survived to 30 days of age in the control treatments of sucrose plus yeast or sucrose alone.

Table 4.9. Average fly survival and average number of eggs laid into artificial fruit (wax domes) by apple maggot flies confined to laboratory cages with different field-collected substances but without sucrose.*

Treatment	Mean % Survival (at 30 days)** Males	Females	Mean Preoviposition Period in days***	Mean Number Eggs/Fem/Day****
Yeast hydrolysate & sucrose	90.0a	86.7a	8.9 (6)	4.48a
Sucrose	86.7a	83.3a	19.0 (2)	0.04b
Apple leaves & no sucrose	0.0b	0.0b	---- (0)	0.00c
Bird feces & no sucrose	0.0b	0.0b	---- (0)	0.00c
Codling moth frass & no sucrose	0.0b	0.0b	---- (0)	0.00c
Wounded unwashed apple fruit & no sucrose	0.0b	0.0b	---- (0)	0.00c
Composite of above***** & no sucrose	0.0b	0.0b	---- (0)	0.00c

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

** The average age of the last flies to die was 3.1 days for apple leaves, 3.4 days for bird droppings, 6.2 days for codling moth frass, 8.9 days for wounded apple fruit, and 11.2 days for the composite.

*** In parenthesis number of replicates in which eggs were laid.

**** Egg hatch averaged 79.9% for the yeast hydrolysate and sucrose treatment and 42.9% for the sucrose treatment.

***** Composite of apple leaves, apple fruit, bird feces and frass.

Experiment 10. In our final laboratory experiment, we evaluated other field-collected substances visited (and in some cases fed upon) by apple maggot flies in the study by Hendrichs and Prokopy (1990): foliage of buckthorn (Rhamnus cathartica L.); juice of bird-wounded buckthorn fruit on buckthorn foliage; buckthorn fruit juice plus blue-colored bird feces on buckthorn foliage deposited by birds that apparently had fed on ripe buckthorn berries; foliage of plum (Prunus nigra Ait.); and foliage of maple (Acer saccarum Marh.).

Results (Table 4.10) indicate that no flies survived to sexual maturity (all died between days 2 and 5) when confined with foliage of buckthorn, maple or plum. Significantly more survived to 30 days on the combination of buckthorn foliage, buckthorn fruit juice, and bluish bird feces (69-83%) or buckthorn fruit juice and buckthorn foliage (40-43%). No significant differences in fecundity were found among treatments, although eggs were produced in four of the six replicates of the buckthorn juice, bird feces, buckthorn foliage treatment and in two of six replicates of the buckthorn juice, buckthorn foliage treatment. In both these treatments, however, preoviposition periods were very long.

4.3.5 Effect of Host Foliage Nutrients

As the surface of apple foliage was a major apple maggot fly feeding site in the study of Hendrichs and

Table 4.10. Average fly survival and average number of eggs laid into artificial fruit (wax domes) by apple maggot flies confined to laboratory cages with different field-collected substances but without sucrose.*

Treatment	Mean % Survival (at 30 days)** Males	Females	Mean Preoviposition Period in days***	Mean Number Eggs/Fem/Day****
Buckthorn fruit juice, Buckthorn foliage, Blue bird feces & no sucrose	69.2a	82.8a	27.5 (4)	0.12a
Buckthorn fruit juice, Buckthorn foliage & no sucrose	40.0ab	43.3b	25.5 (2)	0.06a
Buckthorn foliage & no sucrose	0.0b	0.0c	---- (0)	0.00a
Plum foliage & no sucrose	0.0b	0.0c	---- (0)	0.00a
Maple foliage & no sucrose	0.0b	0.0c	---- (0)	0.00a

* Six replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

** The average age of the last flies to die was 3.5 days for plum leaves, 3.7 days for maple leaves, and 4.7 days for buckthorn leaves.

*** In parenthesis number of replicates in which eggs were laid.

**** Egg hatch averaged 39.1% for the buckthorn juice, buckthorn foliage treatment and 66.7% for the buckthorn juice, buckthorn foliage, bird droppings treatment.

Prokopy (1990), we hypothesized that the low longevity of flies confined with apple foliage alone may have been due to insufficient quantity rather than quality of nutrients available on the limited amount of foliage provided in the small laboratory cages. To evaluate this hypothesis and to corroborate laboratory cage findings relating to fly fecundity, we conducted 3 tests in large field cages in the presence of larger amounts of host foliage.

Experiments 11-A and 11-B. Of the first two field cage tests (each with three field cages), one was carried out using natural hawthorn fruit and a second one using wax-covered hawthorn fruit. In each, we compared the same 3 treatments: yeast plus sucrose, sucrose, and no yeast or sucrose.

Results using natural hawthorn fruit (Table 4.11A), indicate significantly greater fecundity in the yeast plus sucrose control than in the two other treatments, between which there was no significant difference. In all treatments, a majority of each sex survived to the end of the 20-day test period. Results using waxed hawthorns (Table 4.11B) reveal that similar numbers of eggs were laid in the yeast plus sucrose control as in Experiment 11-A, even though females appeared to have difficulty, at least under cooler temperatures, ovipositing into the wax-covered fruit. Once again, there was significantly lower fecundity in the other two treatments, between which there was no significant difference. Again, in all treatments, a majority of each sex

Table 4.11. Average fly survival and average number of eggs laid by apple maggot flies confined in field cages with apple and hawthorn trees having 10 natural hawthorn fruit (11-A) or 10 wax-covered hawthorn fruit (11-B).

Treatment	Mean % Survival (at 20 days) Males	Females	Mean Preoviposition Period in days	Mean Number Eggs/Fem/Day*
A. Natural Hawthorn Fruit				
Yeast & sucrose	60.0	85.0	9	1.99a
Sucrose	80.0	65.0	15	0.42b
No yeast or sucrose	60.0	55.0	11	0.36b
B. Wax-covered Hawthorn Fruit				
Yeast & sucrose	60.0	65.0	10	1.86a
Sucrose	80.0	65.0	14	0.07b
No yeast or sucrose	60.0	45.0	13	0.05b

* Field cage tests were not replicated. However, each fruit was considered as a replicate for statistical analysis. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).

survived to day 20. Average fecundity in the sucrose treatments and no yeast or sucrose treatments was about 6 times greater in the presence of natural hawthorn fruit (Table 4.11A) than waxed hawthorn fruit (Table 4.11B).

Together, these 2 field cage tests confirmed our laboratory cage findings that apple maggot flies do obtain some amount of nutrients important for egg development from natural hawthorn fruit, but apparently not from host foliage. In addition, the results support our hypothesis that large amounts of washed host foliage appear to provide flies with enough carbohydrate for longevity.

Experiment 12. The objective of our final experiment was to corroborate, in the presence of host foliage, results of assessment of fly response to natural food substances obtained under laboratory conditions. Owing to the limited number of field cages available, not all substances or combinations could be evaluated. Even so, we used 7 field cages (treatments) and replicated each treatment twice. Unwaxed hawthorn fruit (10/cage) were provided as oviposition sites. In the first field cage, the flies' diet (potentially present on the foliage of the 2 trees and the hawthorn fruit) was supplemented with sucrose and yeast, in the second with aphid honeydew and sucrose, in the third with bird droppings and sucrose, in the fourth with sucrose plus a preparation of bacteria isolated from apple foliage (same as in laboratory experiment), and in the fifth with sucrose alone. The sixth and seventh cages received no

sucrose. In the sixth, the same potted trees remained in the cages through the entire test. In the seventh, the potted trees were replaced with new trees every 4 days.

The results (Table 4.12) confirm the principal findings of laboratory cage experiments 2, 6, 7 and field cage experiment 11. As in the previous field cage experiment, the fecundity of flies in the control treatment of yeast plus sucrose was lower than in the laboratory cage tests, possibly because the food was presented in a dilute rather than dry form. Even so, it was significantly greater than with aphid honeydew plus sucrose or bird droppings plus sucrose, which in turn yielded greater fecundity than the remaining 4 treatments (which were not different from one another). Probably, in the 4 treatments without yeast, honeydew or bird droppings, flies obtained nutrients required to sustain the observed low level of egglaying largely from the hawthorn fruits which were provided as egglaying sites. There was no difference among treatments in percent flies surviving to 20 days.

4.4 Discussion

4.4.1 Survival

Besides being able to obtain carbohydrate (in nature) from insect honeydew and other sources such as buckthorn fruit juice, R. pomonella flies confined on field caged hawthorn and apple trees were found to obtain sufficient

Table 4.12. Average fly survival and average number of eggs laid into hawthorn fruit by apple maggot flies confined to field-caged apple and hawthorn trees.*

Treatment	Mean % Survival (at 20 days) Males	Females	Mean Preoviposition Period in days	Mean Number Eggs/Fem/Day
Yeast & sucrose	50.0a	87.5a	9.5a	1.79a
Honeydew & sucrose	60.0a	62.5a	12.5ab	1.14b
Bird droppings & sucrose	40.0a	65.0a	11.0ab	0.81b
Leaf-bacteria** & sucrose	70.0a	72.5a	15.0b	0.43c
Sucrose	30.0a	67.5a	14.5ab	0.42c
No sucrose & no tree change	50.0a	52.5a	13.5ab	0.44c
No sucrose & tree change***	40.0a	62.5a	13.0ab	0.28c

* Two replicates per treatment. Within columns, numbers followed by the same letter are not significantly different at the 5 % level (Tukey's HSD test).
** Bacteria of the genera Bacillus, Enterobacter and Micrococcus.
*** Tree renewal every 4 days.

carbohydrate from leaf surfaces alone to satisfy some basic energy requirements for survival and maintenance (Tables 4.11 and 4.12). In contrast, in a previous field study in which lower limbs of apple trees were covered with saran screening, Neilson and Wood (1966) found that R. pomonella flies were not able to survive on apple leaf surfaces alone, even though both carbohydrate and amino acids were established as being present on apple foliage and fruit surfaces. As illustrated by the contrasting results obtained between our laboratory and field tests with host foliage alone (Tables 4.9, 4.11 and 4.12), one possible cause of this discrepancy may lie in the smaller amount of host foliage included by Neilson and Wood (1966) in their small field cages. Boyce (1934), on the other hand, obtained results similar to ours. He showed that some walnut husk flies, Rhagoletis completa Cresson, survived up to 70 days in field cages containing only small walnut trees devoid of honeydew.

Apple maggot fly feeding on substances on leaf surfaces invisible to the human observer has been described in numerous reports (e.g. Middlekauff 1941; Prokopy et al. 1972; Webster et al. 1979). Similar observations of "grazing" on leaf surfaces have been made on other fruit flies (Bateman 1972). In our systematic field observations of R. pomonella food foraging behavior, we confirmed and quantified this behavior (Hendrichs and Prokopy 1990). We found that in the absence of honeydew, food foraging flies

actively move from apple leaf to apple leaf inspecting upper leaf surfaces, a behavior in which they spend considerable time and energy. Upon encountering substances invisible to the human observer, flies engage in area concentrated search (Bell 1990), extending the proboscis and applying the labellum directly to these surfaces in apparently indiscriminate fashion. Our findings suggest that flies are indeed ingesting nutrients, apparently mainly carbohydrates, that are present on host leaf surfaces. Flies do not seem to obtain enough nutrients from apple leaf surfaces to contribute to fecundity, however (Tables 4.9, 4.11 and 4.12). Our combined findings may explain why, in Neilson's (1971) study using a radio-active label incorporated into artificially-placed food and in field observations of Hendrichs and Prokopy (1990), males departed from fruiting host trees less often than females, which foraged for food extensively on non-host vegetation in the surroundings. Because males appear to move less frequently and are of smaller size than females, their energy requirements may be less and, unlike females, they require little proteinaceous food (Webster and Stoffolano 1978). Consequently, even though maturing males respond to ammonia and bird feces odour (Hendrichs et al. 1990; Prokopy et al. 1992a) and bacteria of fecal origin are found in their alimentary canals (Lauzon et al. 1992), mature males seem able to fulfill most of their nutritional requirements on host trees.

What is the nature and origin of nutrients that apple maggot flies consume while feeding on host leaf surfaces and that, unlike honeydew, are not visible to the human observer? Under natural conditions, on occasion the nutrients might be in part minute residues of insect honeydew remaining after weathering. In our field cage tests, however, the potted trees were thoroughly rinsed with water before initiation of tests. Moreover, potential nitrogen contribution through rainfall was excluded by an overhead tarpaulin. In any event, nitrogen in rainfall is minute in quantity (< 3 ppm) and is largely unavailable to flies because it is in an inorganic form (Mattson 1980). A more likely source of nutrients on host foliage might be pollen grains. Fluke and Allen (1931), however, failed to maintain apple maggot flies on squash pollen and water. Also Tsiropoulos (1977b), found that various pollens (either dry or suspended in distilled water) did not support D. oleae fly longevity.

A further alternative, which may explain our findings best (Hendrichs et al. 1992), involves nutrient leaching from leaf surfaces that resulted from daily misting of the foliage of potted trees. Leaching is a process of widespread occurrence in nature, supported by a wealth of evidence based on use of radioisotope techniques showing that inorganic and organic materials of plant origin pass through outer plant tissues into water from light rain, dew, mist or fog in contact with plant surfaces (reviewed by Tukey 1971,

and Godfrey 1976). In addition to consumption of foliage leachates, flies may feed on nutrients from guttation liquids, which are forced out through leaf hydathodes found near leaf margins on upper leaf surfaces (Frossard 1981). Carbohydrates account for the majority of materials reaching the phylloplane from leaching or guttation. For apple trees, losses of carbohydrates through leaching and guttation have been estimated to be as great as 800 kg per hectare per year (Tukey 1971). During both leaching and guttation, most nutrients are lost from upper leaf surfaces, where exudates are most pronounced over veins and at leaf margins (Collins 1976). In field observations of food foraging flies (Hendrichs and Prokopy 1990), R. pomonella were observed to search and feed on upper leaf surfaces and to rest on lower leaf surfaces. Older leaves lose considerably more leachate and contain much more carbohydrate than younger leaves (Collins 1976). This may be a further explanation of the discrepancy between the results of Neilson and Wood (1966) (who employed trees with expanding foliage in June and July) and results from our field cage tests (carried out in August and September on trees withe older foliage).

4.4.2 Fecundity

Our results indicate that bird droppings, when complemented with a source of carbohydrate (sucrose), can sustain R. pomonella egg production to a degree comparable to that of aphid honeydew (Tables 4.6 and 4.12). This

finding illuminates the value of the most common pattern of apple maggot fly food foraging behavior recorded in field observations of Hendrichs and Prokopy (1990). Flies foraged mostly upon a diet of bird droppings complemented by frequent grazing on leaf surfaces devoid of apparent honeydew. The significant contribution of bird droppings to tephritid fecundity has been shown for Ceratitis capitata (Wiedemann) (Hendrichs et al. 1991), which similarly is frequently found feeding on bird feces in nature (Hendrichs and Hendrichs 1990). On both bird droppings and aphid honeydew, the level of fecundity realized by R. pomonella was, however, significantly below that resulting from feeding on yeast hydrolysate. Interestingly, when the main component of bird feces, uric acid, was presented together with sucrose, the fecundity of R. pomonella flies was not increased over a diet containing only sucrose (Table 4.8). Dean (1938) reported that R. pomonella fecundity was slightly greater than on the sucrose control for the following non-proteinaceous nitrogen sources: urea, ethylamine, ammonium hydroxide and tartrate. Although it is possible that we presented uric acid in too high a concentration to elicit feeding and egg development, it is more likely that other components of bird droppings, such as microorganisms (yeasts and enteric bacteria) and other partially digested and undigested nutrients were responsible for the egg development obtained. In addition, uricolytic bacteria have been shown to be involved in the breakdown of

nitrogenous products in bird feces. Such bacteria have been implicated as food for tropical cockroaches that likewise are nitrogen scavengers, feeding on bird droppings (Schal and Bell 1982).

Our results further indicate that nutrients supportive of at least some (though limited) egg development are obtained by R. pomonella flies from the surface of hawthorn fruit, as shown by comparison of data using natural fruit versus artificial oviposition domes (Tables 4.2 and 4.5). Although this finding may be of little practical relevance to nature (where R. pomonella infrequently were observed to feed on fruit surfaces - Hendrichs and Prokopy 1990), it is nevertheless of consequence when comparing the contribution of other natural substances to fly fecundity. The fact that fly access to nutrients on hawthorn fruit was interrupted by covering fruit with wax (Table 4.5), while it was not interrupted by surface sterilization (Table 4.3), points again to the involvement of leachate (on fruit surface following washing) as a contributing factor. Soft fruit is especially susceptible to leaching, particularly just prior to harvest (Tukey 1971; Godfrey 1976). Although carbohydrate available to flies from leachate on the surface of the few fruit supplied was apparently not enough to sustain fly longevity, other nutrients in the leachate allowed the development of a limited number of eggs in the presence of sucrose.

A significant body of literature has accumulated on the grazing of canopy micro-epiphytes by arthropods (Carroll 1981). Oakeshott et al. (1989) showed that partitioning of resources in Drosophila species is strongly associated with the distribution of different components of the microbial flora. Drew et al. (1983) and Drew and Lloyd (1987) have provided evidence indicating that Bactrocera (Dacus) fruit flies are able to obtain all nutrients essential for egg development from select members of the family Enterobacteriaceae (Klebsiella oxytoca and Enterobacter cloacae primarily) that they isolated from alimentary tracts and oesophageal bulbs of flies and from bacterial colonies growing on fruit surfaces. Drew and Lloyd (1989) report that adults inadvertently deposit K. oxytoca and E. cloacae bacteria from their alimentary canals on fruit and foliage. The bacteria then form colonies, using plant surface nutrients, spreading over foliage and fruit surfaces and furnishing flies with protein of bacterial origin. Drew and Lloyd (1989) conclude, however, that K. oxytoca and E. cloacae are not true symbionts of alimentary canals of Bactrocera flies but rather are ingested during feeding and are then used directly as sources of nutrients following autolysis in the fly gut.

In R. pomonella, extensive bacterial isolations from digestive tracts and oesophageal bulbs of field-collected flies have been made since the 1930's (reviewed by Howard and Bush 1989). Recent studies (Dean and Chapman 1973;

Rossiter et al. 1983; Howard et al. 1985) agree that apple maggot flies are most frequently associated with the same Enterobacteriaceae, K. oxytoca and E. cloacae, as the Bactrocera flies studied by Drew and Lloyd (1989) in Australia. Although this may appear surprising, these enteric bacteria are widely distributed in nature, where they are acquired by adult flies from vegetation, either directly from leaf surfaces or indirectly from bird droppings on leaf surfaces. Dean and Chapman (1973) showed that the only proteinaceous material in crops of R. pomonella flies was in the form of bacterial cells of K. oxytoca and that numbers of these cells decreased progressively from the crop to the rectum. Ratner and Stoffolano (1982) studied the development of the oesophageal bulb in R. pomonella and suggested the possibility that this organ may contain a feeder culture of bacteria for slow release to the crop. Although Howard and Bush (1989) argue against the premise of symbiosis between R. pomonella larvae and bacteria, they do not dismiss the possibility that bacteria may represent an important food of adult apple maggot flies.

Results from our study indicate that preparations of Klebsiella, Enterobacter, Bacillus and Micrococcus bacteria (all isolated from host foliage visited by R. pomonella) provided together with sucrose under both field and laboratory conditions (Tables 4.7 and 4.12) had no detectable effect on R. pomonella fecundity. Possibly this

was a consequence of the form in which the bacteria were presented to flies (an aqueous mineral solution). However, pure lyophilised or live cell preparations of the two most common bacteria had no effect (K. oxytoca) or only a minor effect (E. cloacae) on fly fecundity (Table 4.8). Finally, build-up of fly-type bacteria on vegetation following initial bacterial deposition by flies, reported by Drew and Lloyd (1987, 1989) to furnish abundant nitrogen nutrients to Bactrocera species, did not appear to occur with R. pomonella. Neither access to the same hawthorn fruit (Table 4.4) nor to the same host foliage (Table 4.11 and 4.12) for extended periods (7-20 days), potentially allowing for bacterial build-up, enhanced significantly fly fecundity compared with short exposure periods.

Like bacteria, other natural substances we offered with sucrose (codling moth frass and pollen grains) did not contribute significantly to R. pomonella fecundity (Tables 4.6 and 7). Neither of these substrate types should be dismissed as potential sources of nutrients for R. pomonella on the basis of our limited tests, however. Thus, only one type of insect frass was evaluated. Furthermore, nutrient leaching from pollen grains may have occurred before presentation to flies. Even so, our tests represent the only evaluation to date of either of these substrate types as potential nutrients for R. pomonella. A previous attempt to assess the contribution of pollen (from squash) to the fecundity of R. pomonella was not successful because pollen

was presented to flies without sucrose (Fluke and Allen 1931). For Dacus oleae, however, Tsiropoulos (1977b) demonstrated that various pollens from wind-pollinated plants, supplemented with sucrose, did indeed yield considerable egg production. Assessing the contribution of floral nectars to R. pomonella fecundity is worth further consideration, as various frugivorous tephritids have been observed feeding on flowers (Bateman 1972). The presence of free amino acids in the nectar of many flowers, including some Rosaceae on which we occasionally saw R. pomonella flies, has been confirmed (Baker and Baker 1973; Baker et al. 1978). Because of the extreme rarity with which R. pomonella were observed on roses or other flowers by Hendrichs and Prokopy (1990), we did not evaluate Rosaceous pollen or nectar here.

4.5 Conclusions

Gaps identified in our work underscore aspects of R. pomonella nutritional ecology that remain to be studied. Furthermore, future studies of fruit fly nutritional ecology should include provision of combinations of all identified natural food substances to allow for diet balancing through self-selection of diets (Waldbauer and Friedman 1991; Simpson and Simpson 1990). We can state with confidence from our investigations here, however, that R. pomonella flies, and probably a majority of frugivorous tephritid flies, are not dependent on homopteran honeydew to satisfy their

nutritional requirements. In fact of the 246 species of honeydew feeding insects listed by Zoebelein (1956a, 1956b), best represented by Diptera and Hymenoptera, most are facultative feeders and only a few species, such as certain ants, are obligate honedew-feeders. Our findings indicate that, in the absence of insect honeydew, apple maggot flies can satisfy their needs for carbohydrate from host foliage alone and from other natural sources such as juice from ripe buckthorn berries. Nutrients suitable for egg development, however, are obtained by flies mostly from non-host sources. Ingestion of bird droppings sustains fecundity at a level comparable to that provided by aphid honeydew, but significantly below that provided by a laboratory diet of yeast hydrolysate and sucrose.

The fact that females may depend on locating nitrogenous substances away from host trees to achieve significant egg development has practical implications for fly control. Measures such as maintaining commercial orchards comparatively free of important natural food sources (through sanitation, adjusting of pruning regimes to remove aphid-infested water sprouts and using Scare-Eye balloons to discourage birds), placing food-baited interception traps around orchards, or confining bait sprays specifically to orchards perimeters, may successfully contribute to a more environmentally oriented management program for fruit flies. In addition, the odor of bird feces has been shown to be much more attractive than the odor of protein hydrolysate

bait spray droplets in both R. pomonella (Prokopy et al. 1992a) and C. capitata flies (Prokopy et al. 1992b). As a result, efforts are under way to develop improved fruit fly attractants based on the identification of volatiles from bird droppings.

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CHAPTER 5

EFFECTS OF DIFFERENT FOODS, CONCENTRATIONS AND VOLUMES ON FOOD FORAGING BEHAVIOR IN *RHAGOLETIS POMONELLA* FRUIT FLIES (DIPTERA: TEPHRITIDAE)

5.1 Introduction

Foraging behavior through which an organism acquires essential resources such as food, mates, egg laying sites and refugia is shaped by natural selection, just as is the physiology and morphology of an organism. Foraging "decisions" in organisms result in adjustments of behavior that may affect foraging efficiency and ultimately fitness. The foraging behavior of an organism can be expected to reflect tradeoffs between efficient search and assessment mechanisms to satisfy different types of resource requirements on the one hand, and handling costs and risk reducing mechanisms during foraging on the other hand (Prokopy and Roitberg 1989; Bell 1990). Thus, a fundamental question in behavioral ecology is how an organism adjusts its activities in response to the quality, quantity, and spatial and temporal distribution of potential resources (Hassel and Southwood 1978; Kamil and Sargent 1981; Pyke 1984; Stephens and Krebs 1986; Mangel 1990).

With the exception of quantitative studies of the foraging behavior of individual tephritid fruit flies carried out largely in Rhagoletis species (reviewed by Prokopy and Roitberg 1989), present knowledge of the foraging behavior of tephritid flies generally remains

restricted to qualitative information gained at the population level by studying distributions of various species in space and time. Moreover, even in Rhagoletis species, the focus of investigation has been largely on oviposition-site foraging behavior, with minor attention to mate foraging behavior. The food foraging behavior of tephritid flies has been essentially neglected. This is surprising in that knowledge of the food foraging behavior of frugivorous tephritids is central to more judicious and environmentally-sound application of widely used Malathion bait sprays for population suppression (Roessler 1989) and to more effective deployment of food-baited traps for detecting and monitoring flies.

Recently, we initiated an investigation of food foraging behavior of the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), and the apple maggot fly, Rhagoletis pomonella (Walsh). This has involved systematic observations in nature to determine feeding sites and natural food substrates, as well as field-cage studies to assess the contribution of identified field-collected natural food substrates to fly survival and fecundity. Our field observations indicated that flies of both species regularly leave host trees to forage for food on non-host vegetation, where the main natural food sources were found to be bird droppings, fruit juices and insect honeydews (Hendrichs and Hendrichs 1990; Hendrichs and Prokopy 1990; Hendrichs et al. 1991a). Field-cage studies have confirmed that throughout their lives,

frugivorous tephritids require a nearly constant supply of carbohydrate and water for survival, and a periodic supply of amino acids, minerals, vitamins and sterols for normal egg production (Tsisipis 1989; Hendrichs et al. 1990a), although not for spermatogenesis (Webster and Stoffolano 1978). In both species, none of the observed natural food substrates contributed to fecundity at a level equalling that of the standard laboratory diet of sucrose-enzymatic yeast hydrolysate (Hendrichs et al 1990b; Hendrichs et al. 1991a).

In addition, as suggested previously by others (Bateman 1972; Prokopy et al. 1972; Webster et al. 1979), our field observations confirmed that R. pomonella flies spend considerable time foraging for and feeding on food sources distributed diffusely on dry or wet foliage surfaces of host and non-host trees (Hendrichs and Prokopy 1990). As with R. fausta (Osten Sacken) (Prokopy 1976), searching by R. pomonella flies for small amounts of food on foliage occurs in a stereotyped fashion that involves meandering across the top surface of a leaf and hopping to the next leaf further up. Having detected a food substrate with its tarsal receptors, a R. pomonella fly, like other flies (Dethier 1957, 1976; Bell 1985), switches from a unidirectional walk to a convoluted searching pattern of walking and turning. Whenever a fly detects more food while conducting local area-restricted searches on a leaf, another bout of concentrated area search occurs. Our field-cage studies

confirmed that R. pomonella does indeed gain some nutrients from leaf and fruit surfaces, apparently in the form of plant surface leachates (Hendrichs et al. 1991b) and possibly also yeast and bacteria growing on these (Drew and Lloyd 1987). This "grazing" type of feeding may be typical under conditions of scarcity of concentrated food sources which are detectable visually or by odor (Downes and Dahlem 1987).

With these findings as background, we sought to quantitatively assess the dynamics of food-foraging behavior of tephritid flies. Movement of dipteran adults within and between patches that vary in distribution, quality and quantity of food has previously been addressed extensively in laboratory studies by Bell and co-workers (reviewed by Bell 1990). Furthermore, Dethier's classic "The Hungry Fly" (1976) provides detailed mechanistic analysis of dipteran neurophysiological responses to food and feeding behavior. Here, we report on the effect of initial food quantity, concentration and total volume on feeding and subsequent food foraging behavior of R. pomonella flies on foliage of apple tree branchlets. In addition, we deal with specific situations or contexts in which regurgitation behavior, observed in various tephritids, occurs in R. pomonella (Hendrichs 1986; Aluja 1989; Hendrichs et al. 1991c), and the relationship of regurgitation behavior to foraging behavior. Based on this information we eventually hope to quantify the dynamics of food foraging behavior of tephritid

flies of different age, sex, feeding and reproductive status, as they forage for competing natural and artificial foods such as bait spray droplets.

5.2 Materials and Methods

We used apple maggot flies obtained as puparia the previous year from apples collected in nature in Amherst, Massachusetts. From eclosion until 5 - 7 days afterward (the testing age), flies were held in plexiglass-screen cages and fed *ad libitum* on dry sucrose and spring water. They were deprived of protein during this period. In experiments in which enzymatic yeast hydrolysate was offered as food, flies had free access to sucrose up to testing. In experiments in which sucrose was presented, flies were deprived of sucrose 12 - 18 h before testing.

Tests took place in Amherst, MA, in summer 1988. Mosts tests were carried out in field cages (or in a well-illuminated laboratory room on rainy days) and were conducted simultaneously by three or four observers. Replicates of different treatments were equalized among observers. We employed fresh-picked apple branchlets, each with 10 leaves. The stem end was placed in a vial containing Hoagland's solution (Hoagland and Arnon 1950) mounted on a pole about 1 m long. Immediately before use, each branchlet was washed thoroughly with a 1% commercial detergent solution, rinsed under tap water for about 3 min, and handled only with plastic disposable gloves. This was

necessary to ensure that flies had access to no food other than the droplets of food deposited on the release leaf during tests. Observations indicated that touching rinsed leaves with bare fingers apparently resulted in deposition of substances which flies detected and fed upon.

A fresh leaf on which the fly was released was used for each replicate. It was pinned to the branchlet just before fly release. Its position was always between the second and third lowermost leaves of the branchlet. At this time a droplet of known volume and concentration liquid food solution was placed with a micropipete on the release leaf. In the case of dry food treatments, release leaves were prepared at least 12 h before to allow droplets to dry by natural evaporation of the water. Release leaves with drying droplets were left attached to branchlets up to testing time, when they were removed and pinned to test branchlets. The very small dry yeast particles for Experiment 6 were obtained by progressive dilution of the lowest droplet concentration used in Experiment 5, and allowing once more droplets to dry.

For testing, a single fly was placed on the release leaf near the liquid droplet or dry particle. The fly was transferred from a holding cage using a piece of cardboard (5 x 10 mm) mounted on the tip of a probe. The cardboard had been dipped previously into a 0.1% solution of the food substrate to be tested in the experiment. As a result, during the transfer local search behavior was stimulated

(Dethier 1957; Bell 1985), bringing the fly into contact with the liquid droplet or dry particle on the release leaf. For the sake of uniformity only females were used, although preliminary tests indicated that males behaved similarly. Recording of data commenced at the moment a fly arrived at the food droplet or particle on the release leaf and lasted until the fly left the branchlet or for a maximum of 30 min. Six experiments were performed: four with enzymatic yeast hydrolysate (protein) as the food substrate on the release leaf and two with sucrose (carbohydrate). In each case, either the total droplet volume, the amount of food solute in the droplet, or the concentration of food in the droplet of a treatment was held constant (Table 5.1). Sucrose solutions were expressed as percent concentration rather than molar concentration to facilitate comparing yeast with sucrose solutions, and dry particles with liquid droplets. For each experiment (with the exception of Experiment 6 in which only 15 flies were tested per treatment), treatments were replicated 30 times (i.e., 30 flies were tested individually and only once). Temperatures at which replicates were conducted ranged from 15 - 35^{o} C.

Parameters recorded for each experiment were: length of time feeding, time resting, time foraging, total time on branchlet, and number of leaves visited. Also, it was noted whether flies ingested the entire amount of food droplet or dry particle presented, and whether flies engaged in oral droplet extrusion behavior (bubbling). The following

Table 5.1. Constant or variable state of the three main independent parameters in 6 experiments conducted to assess the effect of initial food quantity and quality on food acceptance and subsequent food foraging behavior, quiescence and regurgitation of R. pomonella flies.

EXPT.	FOOD SOURCE	n	DROPLET OR DRY PARTICLE			TEMPERATURE °C	
			VOLUME	SOLUTE	CONCENTR.	MEAN	RANGE
1.	YEAST	180	**CONSTANT**	VARIABLE	VARIABLE	31.3	26-35
2.	SUCROSE	180	**CONSTANT**	VARIABLE	VARIABLE	22.6	17-27
3.	YEAST	210	VARIABLE	**CONSTANT**	VARIABLE	27.9	21-34
4.	SUCROSE	180	VARIABLE	**CONSTANT**	VARIABLE	22.9	15-27
5.	YEAST	180	VARIABLE	VARIABLE	**CONSTANT**	26.4	22-33
6.	YEAST	75	VARIABLE	VARIABLE	**CONSTANT**	24.0	21-29

definitions were used for parameters measured: ingestion of total food particle or droplet = consumption of more than 90 % of food offered on the release leaf; feeding time = mean time a fly fed on a particle or droplet (i.e., sum of periods within a maximum of 30 min in which the fly proboscis was extended and touching the droplet or particle); resting time = mean time during which a fly, although not motionless, did not move more than the equivalent of about one body length; foraging time = mean time after the initial meal on the release leaf during which a fly walked or hopped more than one body length; total patch residence time = mean time a fly spent on a branchlet from moment of contact with the food droplet on the release leaf to the moment it departed from the branchlet or 30 min had elapsed; number of leaves visited = mean number visits to another leaf on the branchlet, including the release leaf; bubbling = engagement, during resting periods, in oral droplet extrusion behavior; handling time = sum of feeding and resting time.

Data for each experiment were analyzed separately by a one-way analysis of variance for each dependent variable measured. Means were compared by Tukey's HSD-test. For comparing acceptance thresholds of yeast versus sucrose food, two-way analyses of variance were carried out combining data from the same treatments across Experiments 1 and 2, and across Experiments 3 and 4. To measure association between temperature and duration of each

activity recorded, linear regression analysis was carried out on the combined data for all 6 experiments. The same analysis was used to compare the overall relationship between handling or foraging time and total patch residence time.

To determine the context in which fly bubbling behavior occurred, we assessed the relationship of proportion of flies bubbling to each of the other variables recorded. Because bubbling is a binary (dichotomous) dependent variable, logistic regression analysis of the combined data across all 6 experiments (n= 1005) was performed by maximum likelihood estimation (Hosmer and Lemeshow 1989). First, we performed univariate logistic regression analysis of the relationship of incidence of bubbling to temperature. Additionally, we performed a multivariate logistic regression analysis of incidence of bubbling as a function of all independent variables measured in this study (temperature, food state and type, droplet volume, solute weight, percent concentration of food solute in a droplet), as well as of certain dependent variables (time feeding and whether a droplet or particle was eaten entirely or not). For the polytomous independent variable of food state, design or dummy variables were created (Hosmer and Lemeshow 1989) for discrete outcomes: liquid food, water, dry food and no food (these latter two, used as reference group, were combined when they were found to be very similar). To avoid excessive complexity, only interactions involving

temperature (entered as products of the main effects) were included. Significance of regression coefficients was tested by the Wald statistic and likelihood ratio tests (deviance analysis). Non-significant parameters were eliminated from the model. Model fit was assessed both by the accuracy of the cross-classification of predicted values above and below the 0.5 cut off point as well as by the Hosmer-Lemeshow goodness-of-fit test (based on Pearson Chi-square statistic) by grouping estimated probabilities according to deciles of risk (Hosmer and Lemeshow 1989). All analyses were carried out using the software package Statistix 3.1 (Analytical Software, PO Box 130204, St. Paul, Minnesota 55113).

5.3 Results

5.3.1 Feeding, Resting and Foraging

First, we evaluated feeding and post-feeding responses of flies to a constant droplet size (0.5 ul) of decreasing nutritious value (decreasing amount of solute and of concentration) of either yeast hydrolysate (Experiment 1) or sucrose (Experiment 2). Preliminary tests showed that 0.5 ul of a food solution is a volume that hungry, average-sized R. pomonella flies can easily ingest in one meal. By increasing progressively the degree of dilution of food in droplets presented, ingestion thresholds were reached for food quality at which flies consumed food droplets only partially or not at all. At equivalent dilutions, ingestion was

significantly greater for yeast than for sucrose droplets (F= 71.5; p= 0.0001) (Table 5.2). The percentage of flies ingesting an entire droplet decreased significantly with decreasing droplet quality, both for yeast hydrolysate (F= 148.6; p= 0.0001) and for sucrose (F=24.9; p= 0.0001), to the point where there was no significant difference in ingestion between the lowest food concentrations offered in each experiment and the two controls (no droplet and water droplet). Similarly, decreasing droplet quality was directly related to the other parameters measured: decreasing time feeding on a droplet (i. e., the more concentrated the food in a droplet, the longer it took a fly to ingest the droplet)(for yeast: F= 38.3; p=0.0001; for sucrose: F= 41.7; p= 0.0001); decreasing fly resting time (for yeast: F= 17.3; p= 0.0001; for sucrose: F= 5.4; p= 0.001), decreasing fly foraging time on the apple branchlet (for yeast: F= 26.6 ; p= 0.0001; for sucrose: F= 9.48; p= 0.001), decreasing total time on the branchlet (for yeast: F= 33.9; p= 0.0001; for sucrose: F= 9.3; p= 0.001), and decreasing number of leaves visited (for yeast: F= 29.5; p= 0.0001; for sucrose: F= 7.3; p= 0.001). For all of these parameters, there were no significant differences between the low-quality droplet treatments and the no-food controls.

In both of these experiments, following ingestion of a droplet and sometimes after having moved first to the more protected leaf-underside, a proportion of flies engaged during quiescent periods in regurgitation or bubbling

Table 5.2. Effect of quality of food (yeast in Expt. 1 or sucrose in Expt. 2) presented in droplet size of constant volume (but decreasing concentration and weight of solute) on food ingestion and feeding time, and subsequent resting, food foraging, and total patch residence time, number of leaves visited and regurgitation behavior (bubbling) of R. pomonella flies.

DROPLET VOLUME (ul)	SOLUTE CONC. (%)	SOLUTE WEIGHT (ug)	FLIES INGESTING ENTIRE DROPLET (%)	TIME (min) FEEDING	RESTING	FORAGING	TOTAL	LEAVES VISITED (number)	FLIES BUBBLING (%)
EXPT. 1: YEAST[a]									
0.5	30.00	125.00	100 a	0.87 a	11.2 a	7.9 a	19.1 a	19.6 a	67 a
0.5	3.00	12.50	97 a	0.41 b	11.8 a	5.9 b	18.1 a	14.4 b	80 a
0.5	0.30	1.25	57 b	0.17bc	6.0 b	3.9 c	10.1 b	6.9 c	47ab
0.5	0.03	0.12	3 c	0.01 c	2.3 b	2.7 c	5.0 c	5.9 c	3 b
0.5	0.00	0.00	0 c	0.00 c	2.5 b	2.3 c	4.8 c	4.9 c	3 b
0.0	0.00	0.00	0 c	0.00 c	2.0 b	2.4 c	4.4 c	5.6 c	0 b
EXPT. 2: SUCROSE[a]									
0.5	30.00	130.00	77 a	0.32 a	5.2 a	4.5 a	10.0 a	8.9 a	10 a
0.5	15.00	65.00	80 a	0.23 b	4.8 a	3.7ab	8.8ab	7.0ab	13ab
0.5	7.50	32.50	67 a	0.18bc	2.6 b	3.6ab	6.4bc	5.3bc	7ab
0.5	3.00	13.00	30 b	0.11cd	1.9 b	2.3bc	4.3bc	4.6bc	3ab
0.5	0.30	1.30	10bc	0.04de	1.9 b	1.2 c	3.1 c	3.3 c	0 b
0.0	0.00	0.00	0 c	0.00 e	1.2 b	1.3 c	2.5 c	3.2 c	0 b

[a] Numbers in the same column within each experiment followed by the same letter are not significantly different at the 0.05 level (comparison of means by Tukey's HSD-test). Thirty flies were tested for each treatment.

behavior. This behavior consisted of the oral extrusion of a large droplet of liquid crop content that was held externally by a pumping proboscis. Occasionally, bubbling was accompanied either by defecation or by oral deposition onto the leaf substrate of a series of small droplets of crop contents that were subsequently reabsorbed after varying periods of time. We hypothesized that this behavior occurred mainly in the context of feeding on diluted food, possibly to eliminate excess water by evaporation to concentrate crop contents. Thus, the following experiments, in addition to determining response and ingestion thresholds according to varying droplet volume (Experiments 3 and 4) and varying amount of solute (Experiments 5 and 6) were designed to test the hypothesis that larger and more diluted droplet volumes would increase fly bubbling behavior.

In Experiment 3 (yeast) and Experiment 4 (sucrose), droplet volume and dilution increased while the amount of food solute in droplets was held constant (Table 5.3). Again, overall fewer sucrose than yeast droplets were totally ingested by flies ($F= 84.5$; $p=0.0001$). In both experiments, however, flies were able to ingest a droplet volume of only about 1.0 ul in a single feeding bout or meal. Even so, the percentage of flies eating the entire droplet decreased significantly only at the largest volumes (for yeast: $F= 53.0$; $p=0.0001$; for sucrose: $F= 29.6$; $p=0.0001$). This was due to the fact that a majority of flies, after becoming fully engorged following the initial

Table 5.3. Effect of form of presentation of a constant quantity (solute weight) of food (yeast in Expt. 3 or sucrose in Expt. 4) presented in droplet size of increasing volume and decreasing concentration on food ingestion and feeding time, and subsequent resting, food foraging, and total patch residence time, number of leaves visited and regurgitation behavior (bubbling) of R. pomonella flies.

DROPLET VOLUME (ul)	SOLUTE CONC. (%)	SOLUTE WEIGHT (ug)	FLIES INGESTING ENTIRE DROPLET (%)	TIME (min) FEEDING	RESTING	FORAGING	TOTAL	LEAVES VISITED (number)	FLIES BUBBLING (%)
EXPT. 3: YEAST[a]									
0.15	100.00	125.0	97 a	2.70 a	2.8 c	4.7 a	10.3 c	13.3 a	3 a
0.50	30.00	125.0	100 a	0.64bc	4.8 c	5.2 a	10.6 c	11.8 a	20 a
1.00	15.00	125.0	97 a	0.88bc	13.9 b	5.1 a	20.0 b	9.7ab	83 b
2.00	7.50	125.0	93 a	1.51 b	20.6 a	4.3 a	26.4 a	6.0bc	100 b
4.00	3.75	125.0	83ab	2.68 a	19.7 a	4.6 a	27.0 a	4.4bc	97 b
8.00	1.87	125.0	67 b	2.86 a	19.5 a	3.6ab	26.0 a	3.2 c	87 b
0.00	0.00	0.0	0 c	0.00 c	1.4 c	1.8 b	3.2 d	4.2bc	0 a
EXPT. 4: SUCROSE[a]									
0.15	100.00	130.0	87 a	1.75 a	3.9bc	4.7 a	10.4 b	8.3 a	0 c
0.50	30.00	130.0	80 a	0.31cd	6.4bc	4.7 a	11.4ab	9.1 a	13bc
1.00	15.00	130.0	83 a	0.57bc	9.5ab	4.6 a	14.7ab	8.2 a	50ab
2.00	7.50	130.0	63 a	0.90 b	12.1 a	4.3 a	17.3 a	6.4ab	57 a
4.00	3.75	130.0	17 b	0.45 c	6.7bc	2.9ab	10.1 b	3.5 b	27bc
0.00	0.00	0.0	0 b	0.00 d	1.8 c	1.4 b	3.2 c	3.1 b	0 c

[a] Numbers in the same column within each experiment followed by the same letter are not significantly different at the 0.05 level (comparison of means by Tukey's HSD-test). Thirty flies tested for each treatment.

meal, engaged for extended periods in regurgitation behavior. Subsequently, they usually returned to the food droplet, where they fed again, each time ingesting a smaller volume, and reinitiating bubbling behavior immediately thereafter. As a result, a majority of flies was able to ingest an entire droplet within the 30 min of observation time. The percent of flies engaged in bubbling behavior increased therefore in direct relation to increasing droplet volume (for yeast: $F= 74.6$; $p= 0.0001$; for sucrose: $F= 9.5$; $p= 0.001$), as did the related resting time (for yeast: $F= 47.9$; $p= 0.0001$; for sucrose: $F= 7.0$; $p= 0.001$). Feeding time increased significantly with droplet volume and for the dry food treatments (for yeast: $F= 25.4$; $p=0.0001$; for sucrose: $F= 39.6$; $p= 0.0001$). Foraging time was similar for all droplet volumes, except for the shorter foraging time in the no-food treatment (for yeast: $F= 4.4$; $p= 0.001$; for sucrose: $F= 5.8$; $p=0.001$). As a result, total time on an apple branchlet was directly related to increasing droplet volume (for yeast: $F= 51.5$; $p=0.0001$; for sucrose: $F= 8.96$; $p= 0.001$), largely determined by time spent bubbling during resting periods.

In Experiment 5 (yeast) and Experiment 6 (yeast) (Table 5.4), flies were presented with droplets of a constant concentration (dry food) but decreasing solute weight and total volume. We asked three major questions: (1) what is the upper threshold of food quantity that inhibits further appetite and food foraging behavior in R. pomonella?,

Table 5.4. Effect of quantity of food (yeast) presented in droplet size of decreasing volume and amount of solute (but constant concentration) on food ingestion and feeding time, and subsequent resting, food foraging, and total patch residence time, number of leaves visited and regurgitation behavior (bubbling) of R. pomonella flies.

DROPLET VOLUME	SOLUTE CONC. (%)	SOLUTE WEIGHT (ug)	FLIES INGESTING ENTIRE DROPLET (%)	TIME (min) FEEDING	RESTING	FORAGING	TOTAL	LEAVES VISITED (number)	FLIES BUBBLING (%)
EXPT. 5: YEAST[a]									
1.200ul	100	1000.0	17 a	21.7 a	3.0 a	1.6 c	26.3 a	2.0 c	0 a
0.600ul	100	500.0	57 b	11.9 b	2.4ab	3.0bc	17.3 b	5.2bc	0 a
0.300ul	100	250.0	83 c	7.6 c	2.7ab	4.4ab	14.7 b	10.1ab	0 a
0.150ul	100	125.0	83 c	3.9 d	1.8bc	4.3ab	10.0 c	9.7ab	0 a
0.075ul	100	62.5	100 c	2.2de	1.9bc	5.2 a	9.3 c	11.9 a	0 a
0.000ul	0	0.0	0 a	0.0 e	0.8 c	2.0bc	2.8 d	4.9bc	0 a
EXPT. 6: YEAST[a]									
0.7500nl	100	0.625	87 a	0.089a	1.4 a	2.4 a	3.9 a	4.7 a	0 a
0.0750nl	100	0.062	67 a	0.049b	0.9 a	2.2 a	3.2 a	4.5 a	0 a
0.0038nl	100	0.031	27 b	0.011c	1.1 a	2.1 a	3.2 a	4.4 a	0 a
0.0007nl	100	0.006	7 b	0.001c	0.7 a	1.8 a	2.5 a	4.3 a	0 a
0.0000nl	0	0.000	0 b	0.000c	1.0 a	2.1 a	3.1 a	4.4 a	0 a

[a] Numbers in the same column within each experiment followed by the same letter are not significantly different at the 0.05 level (comparison of means by Tukey's HSD-test). Thirty flies tested for each treatment in Expt. 5, 15 flies per treatment in Expt. 6.

(2) what is the smallest quantity of food that a hungry fly canno longer detect when foraging over leaf surfaces?, and (3) what is the effect of food ingested in dry form on post-feeding quiescence and regurgitation behaviors?

In relation to food quantity or solute weight that a protein-deprived fly could ingest during the 30 min observation time (Experiment 5), our findings indicate that even though average feeding time ($F= 85.3$; $p=0.0001$) and average total time on a branchlet ($F= 54.8$; $p= 0.0001$) increased significantly with increasing solute weight, significantly fewer flies were able to ingest in entirety the larger amounts of food available ($F= 42.6$; $p= 0.0001$). Presented with a dry yeast particle weighing from 62.5 ug to 250 ug, a majority of flies (83-100%) was able to consume it entirely. For a larger amount (500 ug) only 57% consumed it completely. Finally, when the amount was 1000 ug, few flies (17 %) ate the entire amount, although feeding time increased significantly to an average of 21.7 min. Feeding on dry food apparently elicited considerably more cleaning of mouthparts and tarsi than did feeding on liquid food. Resting periods between feeding bouts, mostly dedicated to this cleaning activity, increased with feeding time ($F= 4.4$; $p= 0.001$). No bubbling behavior was observed during resting time while ingesting dry food. Foraging time was significantly longer ($F= 8.0$; $p= 0.001$) and number of leaves visited was significantly greater ($F= 6.6$; $p= 0.001$) following feeding on particle sizes which most flies were

able to ingest entirely (62.5 - 250 ug) compared with the no-food control or largest particle size tested (1000 ug), which most flies were not able to consume totally.

In Experiment 6 we found that protein-deprived flies would generally ingest dry yeast particles on a leaf surface down to a size of 0.625 - 0.0625 ug, provided that they walked directly onto the particle, that is, that their tarsi would come into contact with the particle. Significantly fewer flies ingested smaller particles ($F= 16.9$; $p= 0.0001$), even though the tarsi appeared to make contact with the particle. For all other parameters measured, no significant differences between treatments and the no-food control were found. Only feeding time decreased significantly with decreasing particle size ($F= 37.6$; $p= 0.0001$).

For Experiments 1 - 6, total time on a branchlet (fly patch residence time) was composed of two types of activities: time handling and processing food (feeding, cleaning and bubbling), and time foraging before leaving the patch. Linear regression analysis of the combined data over all six experiments for these two types of activity in relation to total patch residence time indicate handling time was more closely associated with total patch residence time ($r^2 = 0.91$, $F = 9990$, $p < 0.001$) than was foraging time ($r^2 = 0.24$, $F = 318$, $p < 0.001$). Linear regression analyses of the combined data also indicate some association between duration of one or another activity and environmental temperature: for total patch residence time, $r^2 = 0.02$, $F =$

20.5, $p < 0.001$; for resting time, $r^2 = 0.014$, $F = 14.6$, $p < 0.001$; for foraging time, $r^2 = 0.011$, $F = 11.6$, $p = 0.001$. Only for feeding time was there no significant relationship to temperature: $r^2 = 0.002$, $F = 1.6$, $p = 0.21$.

5.3.2. Bubbling Behavior

The fitted equation from a univariate logistic regression analysis describing the relation between bubbling behavior and environmental temperature was $y = -4.51 + 0.124 * \text{Temp}$. The temperature coefficient ($b=0.124$) was significant at a >0.001 level (Wald statistic = 6.36, deviance = 1024). Overall, this univariate model based on temperature alone fits the data poorly ($p= 0.19$). When temperature is plotted against bubbling behavior (Fig. 5.1), one can observe that although the threshold for initiating bubbling behavior decreases with increasing temperature, the probability of bubbling always remains below 50%, even under temperatures as high as 35°C. An increase in temperature by itself therefore appears unlikely to trigger bubbling behavior in R. pomonella.

Table 5.5 summarizes the results of the multivariate logistic regression analysis of the occurrence of bubbling behavior in relation to droplet state, droplet volume, consumption of the entire droplet or particle, temperature, time feeding and weight of solute as independent variables. As the two-way interactions with temperature were non-significant (difference in likelihood ratio for a model with

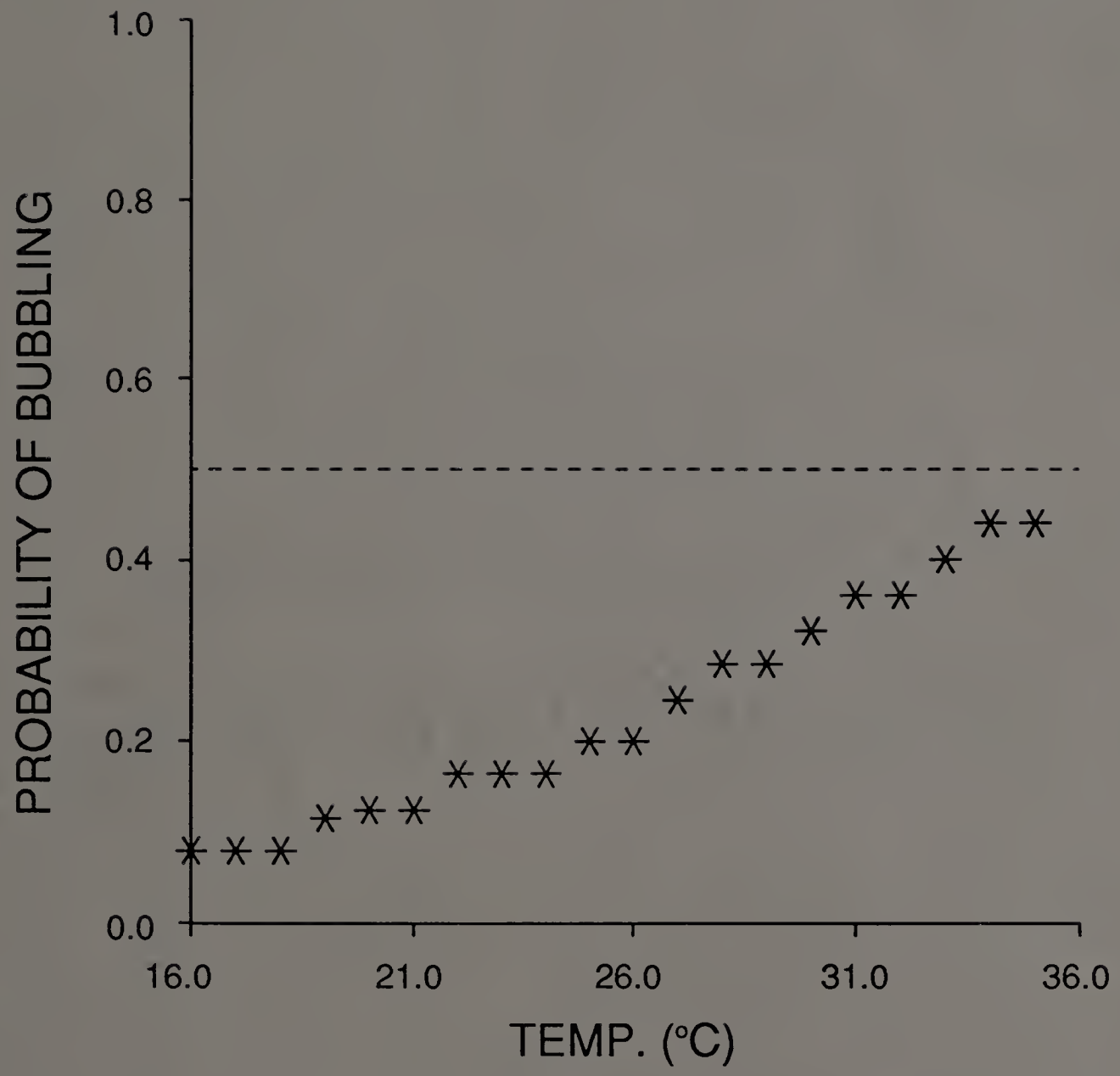

Fig. 5.1. Probability of occurrence of bubbling behavior in R. pomonella as a function of temperature. The logistic transform of bubbling was obtained by univariate logistic regression analysis of bubbling in relation to temperature.

Table 5.5. Multivariate logistic regression analysis of bubbling behavior as a function of droplet or particle volume, dry or liquid food, ingestion of entire food droplet, feeding time, temperature and solute weight: logistic regression coefficients (b), their standard errors (SE), Wald Statistic (b/SE) and Odds Ratios ($OR=e^b$).

VARIABLES	b	(SE)	Wald	p	OR
STATE OF FOOD					
Dry/No Food					1.00
Water	3.200	1.650	1.94	0.0526	24.53
Liquid Food	4.781	1.257	3.80	0.0002	119.25
VOLUME	5.270	1.033	5.10	0.0000	
$VOLUME^2$	-1.391	0.337	-4.13	0.0000	
$VOLUME^3$	0.104	0.028	3.74	0.0002	
0.1ul					1.67
0.5ul					9.98
1.0ul					53.68
2.0ul					332.95
TIME FEEDING	2.054	0.492	4.18	0.0000	
$TIME\ FEEDING^2$	-0.280	0.082	-3.42	0.0007	
$TIME\ FEEDING^3$	0.007	0.002	3.15	0.0017	
0.05min					1.11
0.5 min					2.61
5.0 min					65.59
10.0 min					0.93
INGESTING ALL					
No					1.00
Yes	2.111	0.431	4.90	0.0000	8.25
TEMPERATURE	0.231	0.034	6.76	0.0000	
1^oC					1.26
5^oC					3.18
10^oC					10.09
15^oC					32.04
SOLUTE WEIGHT	-0.008	0.003	-2.65	0.0081	
1ug					0.99
10ug					0.92
100ug					0.45
1000ug					0.00
CONSTANT	-16.347	1.502	-10.89	0.0000	

Deviance = 398.0 df = 993 p = 1.000

main effects together with interactions versus a model with main effects only = 6.1, df = 4, p = 0.19), the final model included main effects only. Type of food (yeast or sucrose), concentration of food, and experiment type were also nonsignificant variables. The significant effect of liquid food (Wald = 3.80; p = 0.0002) and the marginally significant effect of water (Wald = 1.94; p = 0.0526) are reflective of the much greater probability of these two variables causing bubbling (respective odds ratios: $e^{4.781}$ = 119.25 and $e^{3.200}$ = 24.53) than the reference variable of dry/no food (odds ratio: 1.00). Both droplet volume and feeding time had significant non-linear effects. Compared with a 0.1 ul droplet, a droplet volume of 0.5 ul increased the probability of bubbling about five-fold. A further droplet volume increase from 0.5 ul to 1.0 ul resulted in yet again about a five-fold increase in the likelihood of bubbling behavior. For a feeding time of 0.5 min, the odds ratio that bubbling would occur was about 25 times less than for a feeding time of 5.0 min, but about twice as great as for a feeding time of 10.0 min. The effect of consuming an entire droplet was also significant, with the odds that bubbling might occur being about 8 times greater following full droplet consumption. When considered together with all the other variables, the effect of temperature on the expression of bubbling was even higher than when temperature was considered alone (Wald = 6.76, p = >0.0001). For example, the odds ratio that bubbling would occur is about

30 times greater for an increase in temperature of 15 ^{o}C (from 20 ^{o}C to 35 ^{o}C). Weight of food solute also influenced the probability of bubbling significantly (Wald = -2.65, p = 0.0081). Here, however, the effect was inverse: the greater the amount of solute, the lower the probability of bubbling. For example, flies that fed on a droplet containing 100 ug of solute were about half as likely to engage in bubbling as flies that fed on a droplet of the same volume but with only 10 ug of solute.

Estimation of the probability of bubbling, computed as $e^{logit}/(1+e^{logit})$, is presented in Table 5.6 for liquid food droplets of different combinations of volume, amount of solute, temperature and whether droplets were eaten entirely or not. Again one can observe the importance of droplet volume and temperature in determining the occurrence of bubbling behavior. Also, whether a fly does or does not eat an entire droplet (most likely a reflection of fly hunger and therefore linked to the need to eliminate excess water to allow further feeding), appears important in triggering bubbling behavior. The effect of solute amount on the probability of bubbling is smaller, as potential increases in bubbling caused by more diluted droplets are partially offset by the shorter feeding times that correspond to more diluted solutions.

The goodness-of-fit tests for the fitted logistic regression model in Table 5.5 are presented in Tables 5.7

Table 5.6. Probability of bubbling[a] computed for different combinations of droplet volume, amounts of solute, temperature and whether or not R. pomonella flies consumed the entire droplet.

		Approximate[b] Probability of Bubbling (%)									
		Entire droplet not consumed					Entire droplet consumed				
Droplet Volume[c] (ul)	Weight of Solute (ug)	15°C	20°C	25°C	30°C	35°C	15°C	20°C	25°C	30°C	35°C
0.5	100	0.3	0.8	2.6	7.7	21.0	3.0	9.0	23.9	49.8	75.9
	10	0.4	1.4	4.3	12.4	30.9	4.3	12.7	31.5	59.4	82.3
1.0	100	2.1	6.2	17.5	40.2	68.0	21.6	46.7	73.6	89.8	96.6
	10	3.1	9.1	24.1	50.2	76.2	26.8	53.7	78.7	92.1	97.4
2.0	100	18.3	41.6	69.3	87.8	95.8	79.0	92.3	97.4	99.2	99.7
	10	22.4	47.9	74.5	90.3	96.7	79.6	92.5	97.5	99.2	99.7

[a] Probability computed as $e^{logit}/(1+e^{logit})$
[b] Feeding times used were based on extrapolation of results obtained in observations, taking into account droplet volumes and concentrations.
[c] In all cases liquid food = 1; water = 0; dry/no food = 0

Table 5.7. Goodness-of-fit test for bubbling, using the fitted logistic regression model presented in Table 5.6. Observed (Obs) and expected (Exp) frequencies within each decile of risk for each outcome: bubbling and no bubbling, and Hosmer-Lemeshow statistic (c).

DECILE OF RISK	PROBABILITY OF BUBBLING	CASES (n)	BUBBLING Obs.	BUBBLING Exp.	NO BUBBLING Obs.	NO BUBBLING Exp.
1	.0000 - .0000	100	0	0.000	100	100.000
2	.0000 - .0001	100	0	0.003	100	99.997
3	.0001 - .0010	100	0	0.025	100	99.975
4	.0010 - .0078	100	0	0.393	100	99.607
5	.0078 - .0233	100	1	1.409	99	98.591
6	.0235 - .0885	100	8	5.289	92	94.711
7	.0885 - .1917	100	12	13.396	88	86.604
8	.1958 - .5276	100	33	35.241	67	64.759
9	.5281 - .9024	100	75	72.958	25	27.042
10	.9041 - .9986	105	102	102.300	3	2.700
	TOTAL	1005	231	231.015	774	773.985
	c = 2.644		df = 8		p = 0.9547	

and 5.8. The Hosmer-Lemeshow goodness-of-fit test (Table 5.7) confirms the value of the developed model, indicatingno significant difference between observed outcomes for bubbling and model-based expected outcomes for bubbling ($c = 2.644$, $df_{10-2} = 8$, $p = 0.947$). In addition, cross-classification of outcomes above and below the 0.5 cut off point, presented in Table 5.8, yielded a 92.1 % correct classification, i.e. only 79 of the 1005 cases predicted an outcome opposite to the one actually observed. Of these 79 incorrectly predicted cases, 86 % corresponded to the borderline volumes of 0.5 ul (61 %) and 1.0 ul (25 %).

Incorporation of resting time improved significantly the fit of the model (Table 5.5) for predicting bubbling behavior (difference in likelihood ratio for model with resting time included versus model without inclusion of resting time = 200.1, df = 1, $p < 0.0001$). Nevertheless, resting time was not included in the multivariate logistic regression analysis because quiescence was considered as a dependent variable of bubbling.

5.4 Discussion

5.4.1 Feeding, Resting and Foraging

A thorough understanding of resource foraging behavior requires integration of mechanistic approaches to behavior analysis which accentuate proximal causation with evolutionary-ecological approaches that accentuate adaptive

Table 5.8. Cross-classification table for bubbling to test the fitted logistic regression model presented in Table 5.5. Cut off point: 0.5.

		OBSERVED		
		Bubbling	No Bubbling	Total
PREDICTED	Bubbling	184	32	216
	No Bubbling	47	742	789
	Total	231	774	1005

Predictive value for not bubbling:	742/ 789	94.0%
Predictive value for bubbling:	184/ 216	85.2%
Sensitivity:	184/ 231	79.7%
Specificity:	742/ 774	95.9%
Total correct classification:	926/1005	92.1%

significance. Answering specific questions associated with foraging behavior analysis has proven challenging, mostly due to the lack of sufficient background information on the physiology, behavior and ecology of the insect being investigated (Prokopy and Roitberg 1989). The results obtained here represent a contribution to methodology and foundational elements of information relevant to tephritid fly food foraging behavior, perhaps the least known aspect of tephritid behavior.

In insects, feeding thresholds fluctuate considerably depending on the general physiological state and nutrition of the individual. With sucrose, for example, Dethier (1976) and co-workers demonstrated that electrophysiological thresholds of glucose-sensitive neural sensilla in Phormia regina (Meigen) fly tarsi can vary over several orders of magnitude of sugar concentration. The behavioral acceptance levels of food type, quality and quantity obtained in our study correspond to those of hungry immature R. pomonella flies, reflecting the state of food deprivation to which flies were subjected. The amount of food ingested by each sex of R. pomonella is greatest prior to reaching reproductive maturity. It then declines gradually throughout life (Webster et al. 1979). Differences observed here between protein and sucrose acceptance also may simply reflect different requirements for these resources at the age flies were tested in addition to different degrees of deprivation from each of these nutrients. Webster et al.

(1979) using R. pomonella, showed that the sort of cyclically recurring protein consumption associated with oviposition reported for female blowflies, Calliphora erythrocephala (Meigen), and for P. regina (Strangways-Dixon 1961; Dethier 1961; Belzer 1970) did not occur in the apple maggot fly. Unlike these other flies that cyclically lay large batches of eggs, the apple maggot fly matures oocytes asynchronously, depositing eggs on a daily basis (Webster and Stoffolano 1978). One could hypothesize that R. pomonella females therefore require regular small protein meals throughout their life to support a continuous level of egg production. Results from studies in nature (Hendrichs and Prokopy 1990), from fecundity studies in field cages (Hendrichs et al. 1991b), and from this study (Expt. 6) tend to confirm this hypothesis in the sense that hungry flies, while foraging from leaf to leaf in the absence of discrete food sources readily detectable by a human, do indeed detect and ingest minute food particles on plant surfaces. Quantification of daily apple maggot fly nutrient intake by Webster et al. (1979) has shown, however, that when non-deprived flies are allowed to feed ad libitum on protein and sucrose, intake is very substantial (mean of about 100-300 ug/female/day of protein and about 3 - 4 times as much sucrose).

We used two food presentation schemes to explore the response of R. pomonella flies to food quantity and quality. In the first, we varied either food concentration (Table

5.2) or volume (Table 5.4), keeping respectively volume or concentration constant and thereby varying the gross nutrient reward available at each concentration. In the second (Table 5.3), we varied both volume and concentration so that the gross nutrient reward was equal for all food droplets or particles, thereby decoupling high concentration from large nutrient reward. As expected from studies by Dethier (1957) on P. regina and Fromm (1988) on Musca domestica, the relationship between food ingestion and time invested in subsequent foraging was significant, i. e., the larger the quantity and quality of food consumed by R. pomonella flies, the longer the foraging time following feeding before leaving the branchlet. Both for sucrose and yeast, fly assessment of availability of food on a branchlet was apparently based on the total amount of food solute present in the initially consumed droplet or particle, largely independent of food state (liquid or dry), food volume or food concentration (Table 5.2 and Table 5.4). Consequently, again both for sucrose and yeast, foraging times were about the same for particles or droplets of varying concentration and volume, but constant amount of food solute (Table 5.3).

As expected, foraging or giving up time (searching time following food consumption until departing from a branchlet or 30 min expired) was directly related to food quality and quantity ingested. Total patch residence time (total time on a branchlet), however, was closely linked to food handling

and processing time (feeding time and cleaning and bubbling during resting time). In view of the relatively large average daily nutrient intake (Webster et al. 1978), fly decisions affecting food handling and processing time therefore have a strong effect on overall food foraging behavior, largely determining subsequent time available to a fly for further food foraging and other resource foraging.

Feeding on dry food cost R. pomonella more time than feeding on liquid droplets of food. It required not only liquification by salivary secretion before uptake, but also considerable cleaning of mouthparts during resting periods between feeding bouts. Also, feeding on dry food possibly may be potentially more costly in terms of vulnerability to predation, as flies remain next to dry food on the upper surface of foliage for long periods. As expected from a fluid feeder, food uptake time in R. pomonella was faster the more diluted the food solution. As has been elegantly demonstrated in other fluid-feeders, mainly nectivorous butterflies (Heyneman 1983; Pivnick and McNeil 1985; May 1985) but also nectivorous birds (Mitchell and Paton 1990), feeding duration increases significantly with increased food concentration in a droplet. Rate of nutrient intake, the currency assumed to be maximized, at least over the long term, in various foraging models (Stephens and Krebs 1986; Cartar and Dill 1990), is generally maximized at concentrations of about 30-50% (Pivnick and McNeil 1985). This range of nutrient concentration is preferred by

foragers: (a) that are presumed to be under strong selection pressure for high foraging efficiency, such as honeybees or bumblebees (Waller 1972; Bertsch 1984), (b) for which foraging costs are high, such as sphinx moths or hummingbirds that hover while feeding, (c) for which foraging entails vulnerability to various dangers (Pivnick and McNeil 1985), or (d) for which water is limited, such as honeybees whose preferences shift under dry conditions to flowers with lower nectar concentration (Southwick and Pimentel 1981). However, when distances between resource patches are large and transport costs become increasingly important, then greater handling time associated with ingestion of more highly concentrated food (or food more difficult to handle in general) becomes less important (Heinrich 1991; Lima 1985).

5.4.2. Bubbling Behavior

Even though, in general, handling costs related to R. pomonella feeding time decreased with dilution, below a certain threshold of food dilution (and total volume ingested), overall handling-processing costs actually increased. Engorged flies entered extended quiescent post-feeding periods, during which they "processed" the ingested liquid food by engaging in oral extrusion of liquid crop contents (bubbling). After returning to the diluted food solution, they reinitiated feeding, followed by additional bubbling and feeding bouts. On occasion, flies also

regurgitated droplets onto leaf surfaces and reingested the remaining dry solids once the droplets had dried. We found that these behaviors occurred not only in females, but also in males (unpublished data). Although some flies avoided predation risk during bubbling by moving to the underside of a leaf before initiating bubbling, most flies, including those depositing droplets onto the substrate, remained next to the source of initial food on the upper leaf surface.

Based on observations of bubbling under field and laboratory conditions by the Caribbean fruit fly, Anastrepha suspensa, (Hendrichs 1986), various other Anastrepha species (Aluja et al. 1989), and the Mediterranean fruit fly, Ceratitis capitata, (Hendrichs, unpublished data), we speculated that bubbling might be a mechanism to eliminate excess water to concentrate liquid crop contents. Our results here, both for yeast and sucrose droplets, supported this hypothesis. During longer resting times corresponding to larger drop volumes consumed, hungry R. pomonella flies apparently evaporated through bubbling behavior sufficient excess water to enable them to continue progressive ingestion of small meals (totalling up to 8 ul of diluted food) interspersed with bubbling periods. The significance of the positive relationship that was found between foraging time and bubbling time is indicative of the possibility that hungrier flies, presumably those that foraged more extensively, engaged in more bubbling behavior in order to be able to ingest more liquid food.

Gelperin (1972) and Dethier (1976) determined that crop emptying is regulated only by osmotic blood pressure. As a result, they found that dilute solutions can be ingested by blowflies in greater quantity (though not at a single meal) than concentrated solutions because dilute solutions empty from the crop more rapidly, allowing flies to feed again. Although in R. pomonella flies rapid crop emptying may be partially responsible for rapid lowering of feeding thresholds after ingestion of very dilute solutions, it cannot account for the prompt elimination of a large part of liquid in the crop. Defecation accompanies bubbling behavior only occasionally. In a follow-up study using a precision balance (Hendrichs et al. 1991c), we have demonstrated that through bubbling behavior, R. pomonella flies eliminate by evaporation most of the weight of excess water just ingested with a liquid meal. In nature, tephritid flies often ingest food in a liquid state. Such food may include juice oozing from fruit, which is possibly the most common food available to tropical tephritid flies in nature (Hendrichs 1986; Hendrichs et al. 1991a), floral nectar and various types of nutrients (including plant leachates) suspended in droplets of dew or guttation.

The multivariate logistic regression model we developed here predicting the occurrence of bubbling behavior in R. pomonella fits the data well but remains to be tested independently. It describes the context in which bubbling behavior occurs: hungry flies ingesting a sufficient volume

of liquid food of low-to moderate concentration of solute are highly likely to extrude liquid droplets orally. Over 3/4 of the cases predicted incorrectly by the logistic regression model corresponded to droplet volumes of 0.5 - 1.0 ul. These were apparently border-line volumes, where other non-recorded factors, such as fly size, recent water ingestion, variation in relative humidity, etc., may have played a determinant role influencing whether or not bubbling behavior occurred.

Bubbling behavior was observed under a broad range of temperatures. Even though thresholds for engaging in bubbling decreased with increasing temperature (possibly reflecting a secondary evaporative cooling function), bubbling was not triggered by warmer temperatures alone, but only in the context of feeding on diluted nutrient solutions. In our follow-up study using a precision balance (Hendrichs et al. 1991c), we were able to elicit bubbling at temperatures as low as 17 ^{o}C. We confirmed thereby that bubbling is probably not primarily a mechanism for evaporative cooling, but rather a mechanism to concentrate ingested dilute food to allow hungry flies to feed further on dilute food sources and thus also to reduce probable costs associated with movement in an engorged state.

Our results provide evidence against the concentration hypothesis of the control of drinking in flies (Barton Browne 1964; Barton Browne and Dudzinski 1968) and in support of the volumetric hypothesis (Dethier 1976).

Apparently, in R. pomonella, abdominal stretch receptors activate as a result of increasing (albeit very diluted) volume in the crop. Resulting inhibition of feeding is reversible, depending on state of liquid volume in the crop, as revealed by reinitiation of feeding immediately after a bout of excess water discharge.

Not a single R. pomonella fly involved in bubbling was seen moving. The close relationship between bubbling behavior and resting time is indicative of the fact that quiescence in bubbling flies appears to be an integral part of bubbling behavior. Whereas activity in flies generally increases with food deprivation, feeding in general inhibits fly appetitive behavior (Evans and Barton Browne 1960; Strangways-Dixon 1961; Dethier 1976). Green (1964a; 1964b) showed that blood constituents are involved in the mechanism whereby feeding affects locomotion. Two food-deprived P. regina flies were placed in parabiosis. After one was fed, the flies were separated. Both exhibited inhibition of activity. The rate at which activity was resumed increased as the concentration of nutrients decreased. Quiescence in bubbling R. pomonella flies, on the other hand, decreases with increasing concentration of crop content (and volume), and therefore appears to be under a different control, possibly volumetric.

Our findings have shown that although post-ingestion food foraging time in R. pomonella was directly related to quality and quantity of food consumed, overall total patch

residence time was more closely linked to food handling-processing time. Decisions affecting food handling and processing costs therefore seem to have at least as much effect on overall foraging behavior as food foraging itself. R. pomonella flies should be expected to prefer food solutions that not only minimize handling costs, but also maximize the rate of nutrient intake.

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CHAPTER 6

BUBBLING BEHAVIOR IN DIPTERA: EVAPORATION OF EXCESS WATER TO FACILITATE FURTHER UPTAKE OF DILUTED FOODS IN GORGED FLIES

6.1 Introduction

In the preceding study of food foraging behaviour in Rhagoletis pomonella (Walsh) flies, we showed that quality and quantity of food ingested influenced post-feeding behaviour and subsequent foraging activity. Hungry flies that became gorged by ingesting food in a diluted form engaged subsequently in regurgitation behaviour, after which they re-initiated feeding. Such behaviour, which occurred during quiescent post-feeding periods, reduced time available to flies for subsequent food foraging and other activities.

Regurgitation behaviour in tephritid flies consists of: oral extrusion of liquid crop contents to the surface of the mouthparts, where droplets exposed to air envelope the extended proboscis; rhythmic extrusion and retraction ('pumping') of the proboscis, which can be observed moving inside the extruded liquid; and eventual swallowing of the liquid (Hendrichs, 1986). Droplet size can grow with each proboscis pump until it reaches nearly that of the head (at least 1 ul) (Hewitt, 1912; Headrick and Goeden, 1990). Oral droplet extrusion behaviour has been referred to as 'bubbling' (Hendrichs, 1986; Aluja et al., 1989; Thomas, 1991), a term we will use here. It should not be confused with 'bubble blowing', a behaviour apparently of

significance in the courtship of certain otitid flies, in which a membrane (often bright orange) expands from the proboscis (Foote, 1967; Allen and Foote, 1975). On some occasions, bubbling in tephritids is accompanied by deposition of regurgitate onto the substrate in curving lines of individual droplets which are later partially or totally re-ingested (Hendrichs, 1986; Drew and Lloyd, 1987).

Oral droplet extrusion behaviour or bubbling has been observed in various frugivorous and non-frugivorous tephritids. In the walnut husk fly, Rhagoletis suavis (Loew), Brooks (1921) described such behaviour in flies feeding upon sap that exuded from oviposition punctures in the surface of walnuts husks. Fluke and Allen (1931) reported that in R. pomonella flies "after feeding, a droplet of liquid would often appear on the proboscis; this droplet would then disappear, only to reappear a few moments later. This was repeated several times." We have observed bubbling in nature and in the laboratory in the Mediterranean fruit fly, Ceratitis capitata (Wied.) (Hendrichs, unpublished data), and in Anastrepha suspensa Loew (Hendrichs, 1986). In addition, Drew and Lloyd (1987) reported oral droplet deposition behaviour in the Queensland fruit fly Bactrocera (Dacus) tryoni (Froggatt), and Aluja et al. (1989) observed it in Anastrepha ludens (Loew), A. obliqua (Macquart), and A. serpentina (Wied.). Headrick and Goeden (1990) described the extrusion of golden-coloured

droplets and proboscis pumping in resting adults of Paracantha gentilis Hering, a thistle-infesting tephritid.

Bubbling behaviour is apparently not restricted to tephritids. Thomas (1991) describes the exposure to air of fluid droplets extruded from the tip of the proboscis of screwworm flies, Cochliomyia homonivorax (Coquerel). The blow fly, Phormia regina Meigen, engages in bubbling behaviour after feeding on a liver meal (J. G. Stoffolano, personal communication). Some tachinid flies have been observed to extrude droplets orally after feeding (R. Lopez, personal communication). Gerling (1982) reported a similar behaviour in nectar feeding carpenter bees. Hewitt (1912), in a book on the house fly Musca domestica L., clearly distinguished between "fly specks" resulting from defecation, and "vomit spots" resulting from oral deposition of regurgitate. In addition, Hewitt included a drawing in which a house fly, with a large liquid droplet hanging from the proboscis, is apparently engaged in droplet extrusion behaviour. He stated (pp. 30): "The fly does not always deposit the regurgitated fluid. In many cases it will regurgitate a drop of fluid and repeatedly and alternately reabsorb the drop. One fly was seen alternately and regularly to regurgitate and absorb a drop of fluid eight times, each regurgitation and absorption occupying one and a half minutes". He suggested that extrusion of crop contents was concerned primarily with the digestion of food by mixing it with saliva.

Although oral extrusion or regurgitation behaviour has been observed in several species of insects, to our knowledge no attempt has been made to understand its proximate or ultimate functions. Attention has been devoted only to deposition of regurgitate on substrates in species of medical importance, owing to potential involvement in disease transmission (Lamborn, 1937; Sieyro, 1942; Gross and Preuss, 1951; Dipeolu, 1982; Glass and Gerhardt, 1984; Booth, 1987; Coleman and Gerhardt, 1988; Kloft and Hesse, 1988). Even here, however, it is surprising that only the medical implications of regurgitation behaviour have been studied, not the biological significance to the regurgitating insect.

Having established in a preceding food-foraging study the context in which regurgitation behaviour is exhibited in R. pomonella (Hendrichs et al., 1992), we evaluated here the hypothesis that this behaviour is primarily a mechanism by which fully gorged flies that have ingested fluid food evaporate excess water from crop contents, thus releasing volume in the crop to permit continued feeding on liquid food sources.

6.2 Materials and Methods

Wild R. pomonella flies used for the tests originated from infested hawthorn fruit, collected the previous year from unsprayed trees in Amherst, Massachusetts. Upon emergence, both sexes were held together in Plexiglass

screened cages and provided with dry sucrose and bottled spring water, which were removed shortly before testing. Half of the flies tested were 5-8 days old, half 10-14 days old.

We used a Cahn/Ventron 27 Automatic Electrobalance in which fly weights were given every other second to the nearest 0.001 mg. A fibre optic light was used to illuminate the balance. Temperature and relative humidity were recorded for each trial inside the closed scale, that is with sliding glass windows shut. Laboratory temperatures and relative humidities under which bubbling tests were carried out ranged from 17 - 26oC (median 21.5oC) and 40 - 72 % RH (median 50%). A magnifying glass was used to facilitate observation of a fly during testing.

For convenience we tested females, although we have shown previously (Hendrichs et al., 1992) that oral droplet extrusion behaviour occurs as often in males as females. Fly wings were partially clipped for easier handling. Immediately after clipping wings, individual females were transferred, with a small piece of cardboard mounted on the tip of a probe, to the platform of the electrobalance. Pre-feeding weights were recorded for c. 5 min, or until the fly hopped off the platform. During this time, none of the flies extruded droplets orally, although some pumped the proboscis. A fly was then transferred carefully to a petri dish, where several droplets of food (3 % enzymatic yeast hydrolysate, 97% spring water) had been pipetted onto the

floor of the dish to facilitate female encounter with the droplets. Feeding time was recorded. Flies which fed less than 10 s or not at all were discarded. Immediately after cessation of one feeding bout (at which time bubbling was often initiated), flies were transferred back to the electrobalance platform. Fly weights were recorded continuously until flies ceased bubbling. At this time quiescence usually also ended and flies often left the electrobalance. For 25 flies, complete records were obtained of weight loss before and after feeding. In 20 cases, flies engaged in oral droplet extrusion during post-feeding behaviour. In 5 cases, they did not. Mean values are given and + S. D.

Rates of weight loss before and after feeding were compared for the 20 post-feeding bubbling flies and the 5 non-bubbling flies using a Wilcoxon Signed Rank Test (Sokal and Rohlf, 1981). The degree of association between pre- and post-feeding weight losses at the individual fly level was evaluated by a Spearman Rank Correlation test (Sokal and Rohlf, 1981). Multiple regression was used to determine the relationship of abiotic and biotic variables to bubbling and non-bubbling pre- and post-feeding weight losses. Only in the case of pre-feeding weight losses did higher order terms significantly improve the model over linear regression. The fit of observed and predicted weight loss were then plotted for each individual, together with the standardized residuals versus weight loss. For all data analysis, the

statistical software package Statistix 3.1 (Analytical Software, PO Box 130204, St. Paul, Minnesota 55113) was used.

6.3 Results

For the 5 cases in which flies did not extrude droplets orally after feeding, fly weight loss rate increased from 2.84 (+ 1.27 SD) ug/min before feeding to 4.28 (+ 2.13) ug/min after feeding, a rate that was less than twice as great but still significantly different (Wilcoxon Signed Rank Test: Ta = 15.0; P = 0.03). Correlation among these 5 flies between weight loss rates before and after feeding was not significant (Spearman Rank Correlation: Rs = 0.63; P = 0.10).

In contrast, for the 20 cases in which flies bubbled after feeding, fly weight loss rate increased from 3.57 (+ 1.26) ug/min before feeding to 37.23 (+ 7.84) ug/min after feeding, a rate that was 10 times as great, representing a highly significant difference (Wilcoxon Signed Rank Test: Ta = 210.0; P < 0.001). There was no correlation among these 20 flies between weight loss rate before feeding and that during droplet extrusion after feeding (Spearman Rank Correlation: Rs = -0.08; P = 0.36).

The average weight of liquid yeast solution ingested by flies by the time feeding ceased was 0.54 (+ 0.13) mg (n=5) for non-bubbling flies and 1.35 (+ 0.65) mg (n=20) for bubbling flies. Total average weight loss during a bout of

bubbling, which lasted from c. 5 to nearly 40 min (median = 26 min) was 0.85 (+ 0.49) mg (n=20), representing a median percent weight loss of 69.2 % of the weight of liquid food ingested by bubbling flies. This was equivalent to a median loss of 7.7 % of fly weight, including ingested liquid food. Changes in fly weight from before feeding to after bubbling of three representative flies of different size classes are shown in Fig. 6.1. On the other hand, in an extreme case a fly lost 108% of the weight of liquid food ingested in the preceding meal (up to 15.2 % of total fly weight).

Fly defecation of liquid droplets and/or oral deposition of some regurgitated droplets onto the balance platform sometimes accompanied oral droplet extrusion behaviour, thereby accelerating the rate of water evaporation and consequently fly weight loss. At the conclusion of bubbling bouts, during which flies were quiescent, flies usually became very active, initiating concentrated area movement (Bell, 1985). During such local movement, flies fed on and removed dry solutes (when present) that remained from their previous oral depositions. As described by Hendrichs et al. (1992), in cases where flies were allowed to remain bubbling near liquid food droplets upon which they had gorged earlier, they alternated several times between bouts of bubbling and further uptake of liquid food.

Weight loss of flies while bubbling was highly correlated with time engaged in bubbling behaviour ($F = 144.9$; $R2 = 0.89$; $P < 0.001$; $n = 20$) (Fig. 6.2). Stepwise

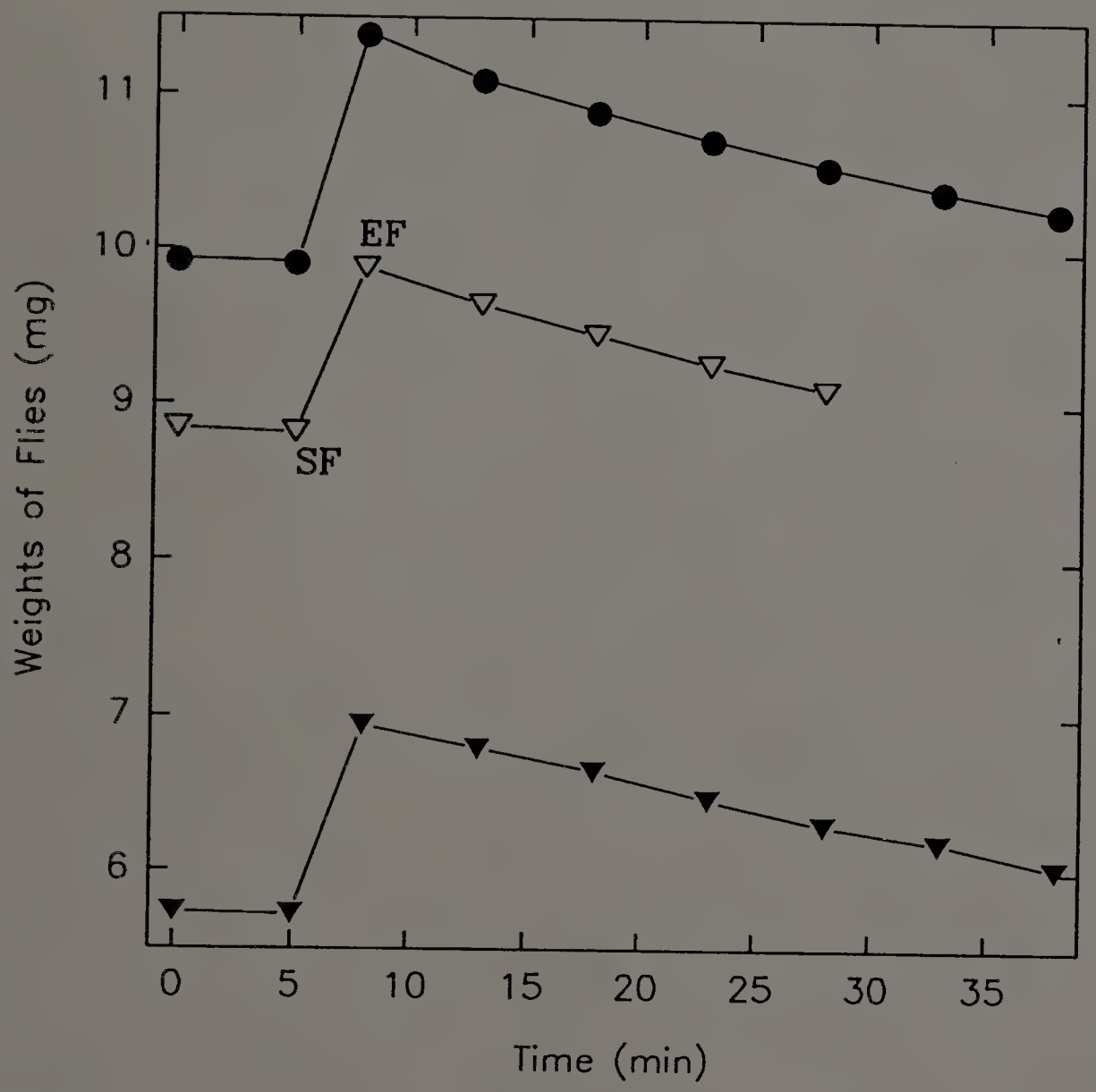

Fig. 6.1 Changes over time in the weights of three different-sized individual apple maggot flies, R. pomonella, before feeding and during bubbling or oral droplet extrusion behaviour. SF = start of feeding; EF = end of feeding.

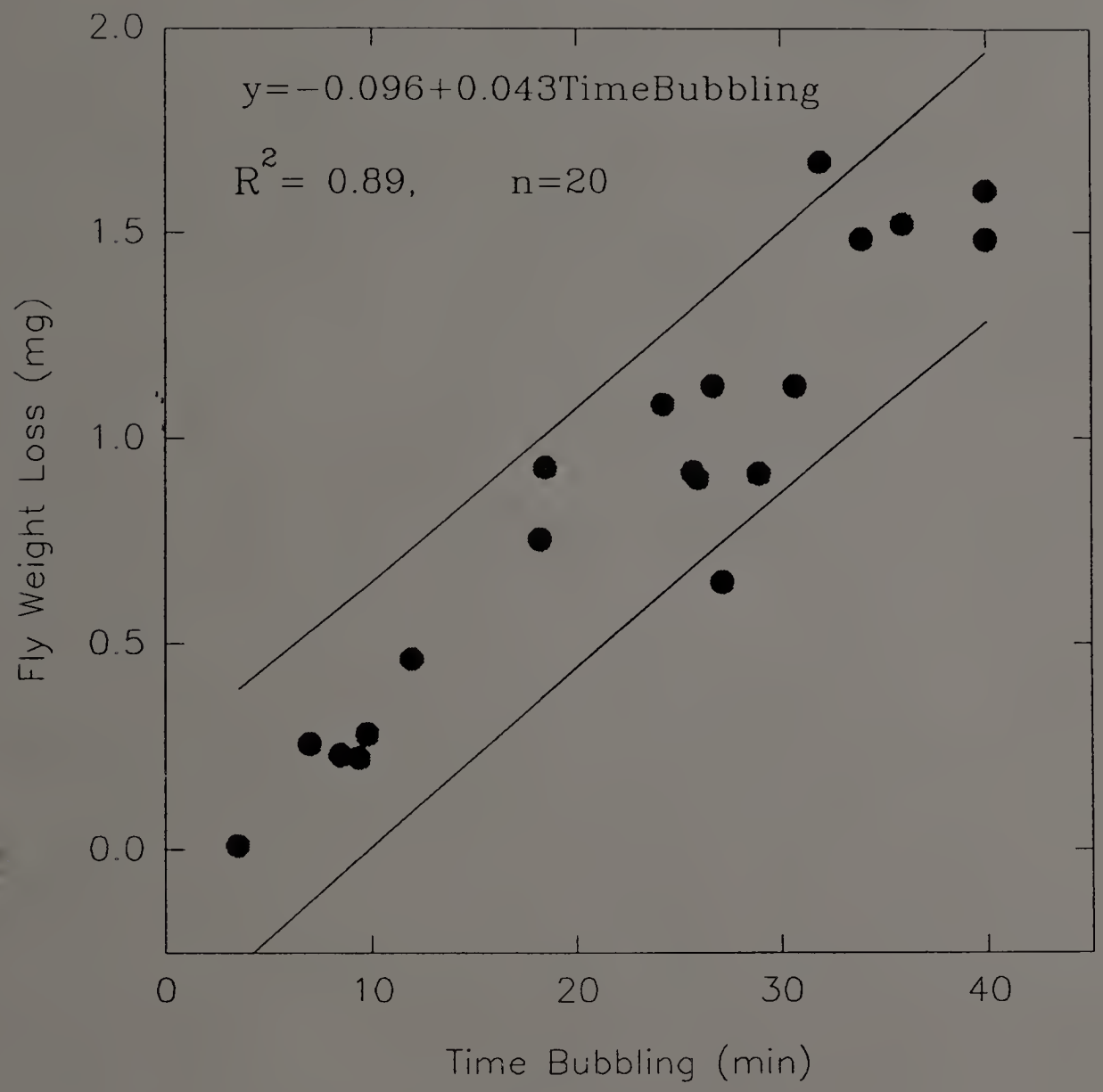

Fig. 6.2 R. pomonella post-feeding weight loss (ug) plotted against time flies engaged in bubbling (min), indicating the regression equation. Regression equation and 90% prediction intervals are given.

analysis of variance indicated that weight loss during bubbling was in addition significantly correlated with temperature and relative humidity (Table 6.1). Weight of flies before feeding, although at the margin of significance ($P = 0.08$), was also included because of its biological significance. The resulting equation for weight loss during bubbling (ug): $y = -774.3 + 42.9$ Time Bubbling $- 7.0$ Relative Humidity $+ 29.0$ Temperature $+ 47.0$ Fly Weight (Adj. $R2 = 0.95$; $P < 0.001$; $n = 20$) is based on the above parameters. Higher order terms did not improve significantly this linear equation. The close fit between observed and described fly weight loss based on the selected equation and the random distribution of standardized residuals of observed minus described bubbling weight loss are presented in Figs. 6.3A and 6.3B. However, this model describing fly weight loss during bubbling remains to be verified on a separate set of data. Other variables such as fly age, weight loss when not bubbling, and volume of food solution ingested did not significantly improve the predictive ability of the selected model (even though volume of food ingested was significantly correlated with bubbling time: $F = 16.55$; $R2 = 0.48$; $P < 0.001$; $n = 20$).

Weight loss before feeding and while not bubbling was about an order of magnitude less than weight loss during bubbling, and was less correlated with duration of time on the electrobalance platform ($F = 6.26$; $R2 = 0.26$; $P = 0.02$; $n = 20$) and with the other biotic and abiotic variables

Table 6.1. Unweighted least squares linear regression of post-feeding fly weight loss (ug) during oral droplet extrusion behaviour. ($F=93.17$; $P<0.001$; adjusted $R^2=0.95$; $n=20$). Negative Durbin-Watson Test for autocorrelation ($0.0<2.62<4.0$).

Variable	Coefficient	S. E.	Student's t	P
Constant	-774.34	413.96	-1.87	0.0810
Time Bubbling	42.94	2.30	18.65	0.0000
RH	-7.05	2.82	-2.50	0.0244
TempoC	29.00	12.58	2.31	0.0358
Pre-Feeding Fly Weight	47.02	24.97	1.88	0.0792

measured (even though higher order terms improved the fit significantly) than was post-feeding weight loss during bubbling. Stepwise analysis of variance showed that in addition to correlation with duration of time on the balance platform, pre-feeding weight loss (ug) was significantly correlated with temperature, subsequent volume of liquid food ingested and age of flies (Table 6.2). The resulting equation: -38.8 + 1.0 (Time)2 + 1.2 Temperature - 0.5 (Volume Ingested)3 + 1.0 Fly Age (Adj. R2 = 0.53; P = 0.003; n = 20) is based on these parameters. The fit between observed and described pre-feeding weight loss of flies is shown in Fig. 6.4A. However, standardized residuals of observed and described pre-feeding fly weight loss based on this model are less randomly distributed (Fig. 6.4B). They tend to increase with increasing weight loss, indicating presence of another factor not included in the selected model to account for fly weight loss while not bubbling. All other recorded variables, such as fly weight and relative humidity, did not significantly improve the predictive ability of the selected model. A variable not recorded, however, that may have influenced prefeeding fly weight loss was proboscis pumping. Possibly weight loss was greater when flies extended the proboscis fully and moved it up and down in a type of 'panting' behaviour (similar to proboscis movement within a regurgitation droplet while bubbling). Because non-bubbling behaviours were lumped together while recording weight loss, no data are available to substantiate

Table 6.2. Unweighted least squares linear regression of pre-feeding fly weight loss (ug) while not engaging in oral droplet extrusion behaviour (F=6.44; P=0.003; adjusted R2=0.53; n=20). Negative Durbin-Watson Test for auto-correlation (0.0<1.47<4.0).

Variable	Coefficient	S. E.	Student's t	P
Constant	-38.83	15.53	-2.50	0.025
(Time)2	0.99	0.27	3.63	0.003
TempoC	1.20	0.50	2.39	0.030
(Volume)3*	-0.47	0.22	-2.10	0.053
Age of Flies	0.91	0.44	2.05	0.058

* Liquid food volume ingested immediately after the pre-feeding weight loss measurement.

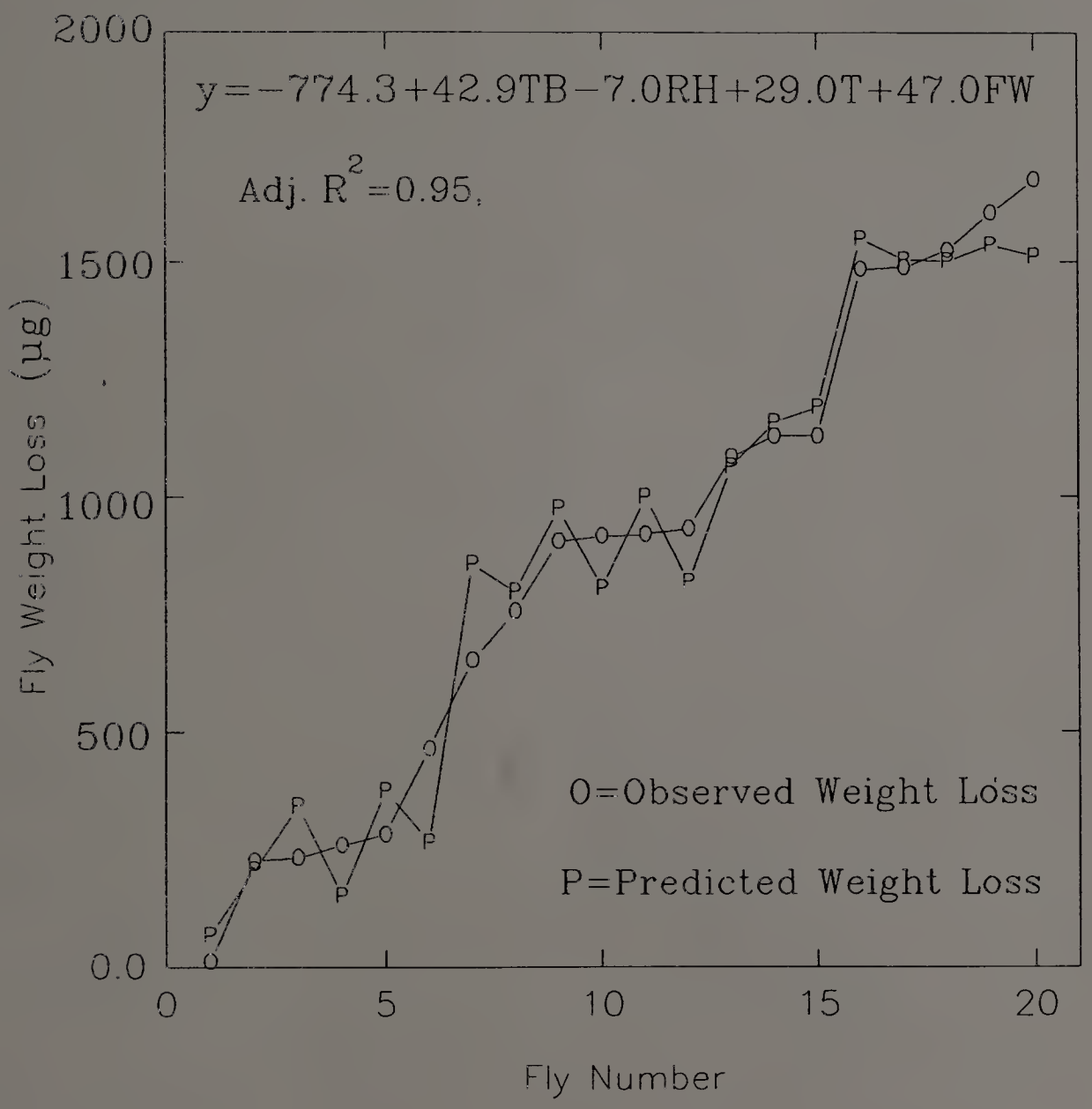

Fig. 6.3A. Observed and predicted post-feeding weight losses (mg) plotted for each individual R. pomonella fly. The regression model on which the predictions are based is also given (Fig. 6.3A) and which incorporates Standardized residuals of observed minus predicted weight losses plotted against observed fly weight loss (mg) (Fig. 6.4B).

TB= time bubbling; RH= relative humidity; T= temperature in ^{o}C; FW= fly weight before feeding;

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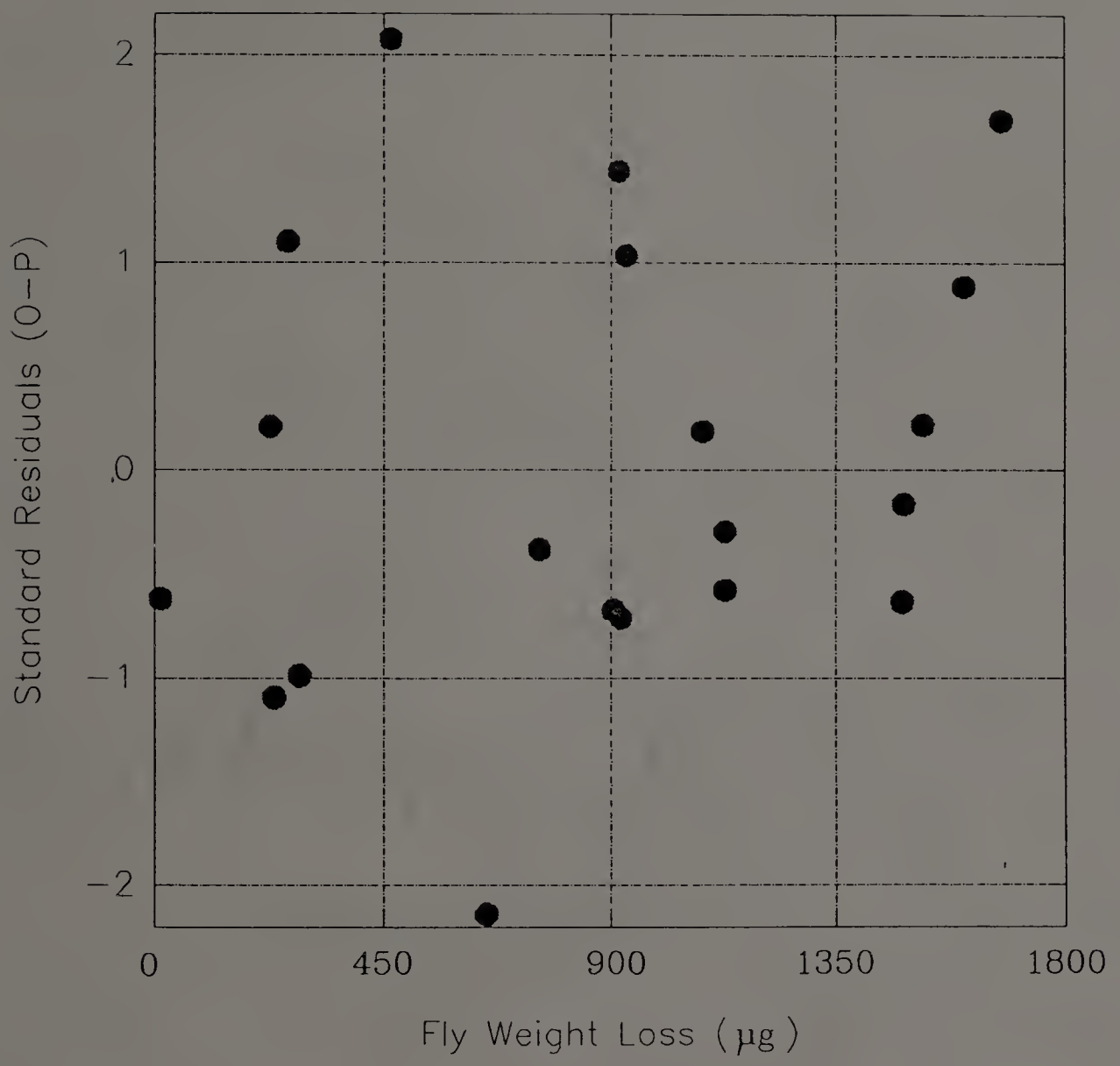
Standard Residuals (O-P)
2
1
0
-1
-2
0
450
900
1350
1800
Fly Weight Loss (µg)

Fig. 6.3B.

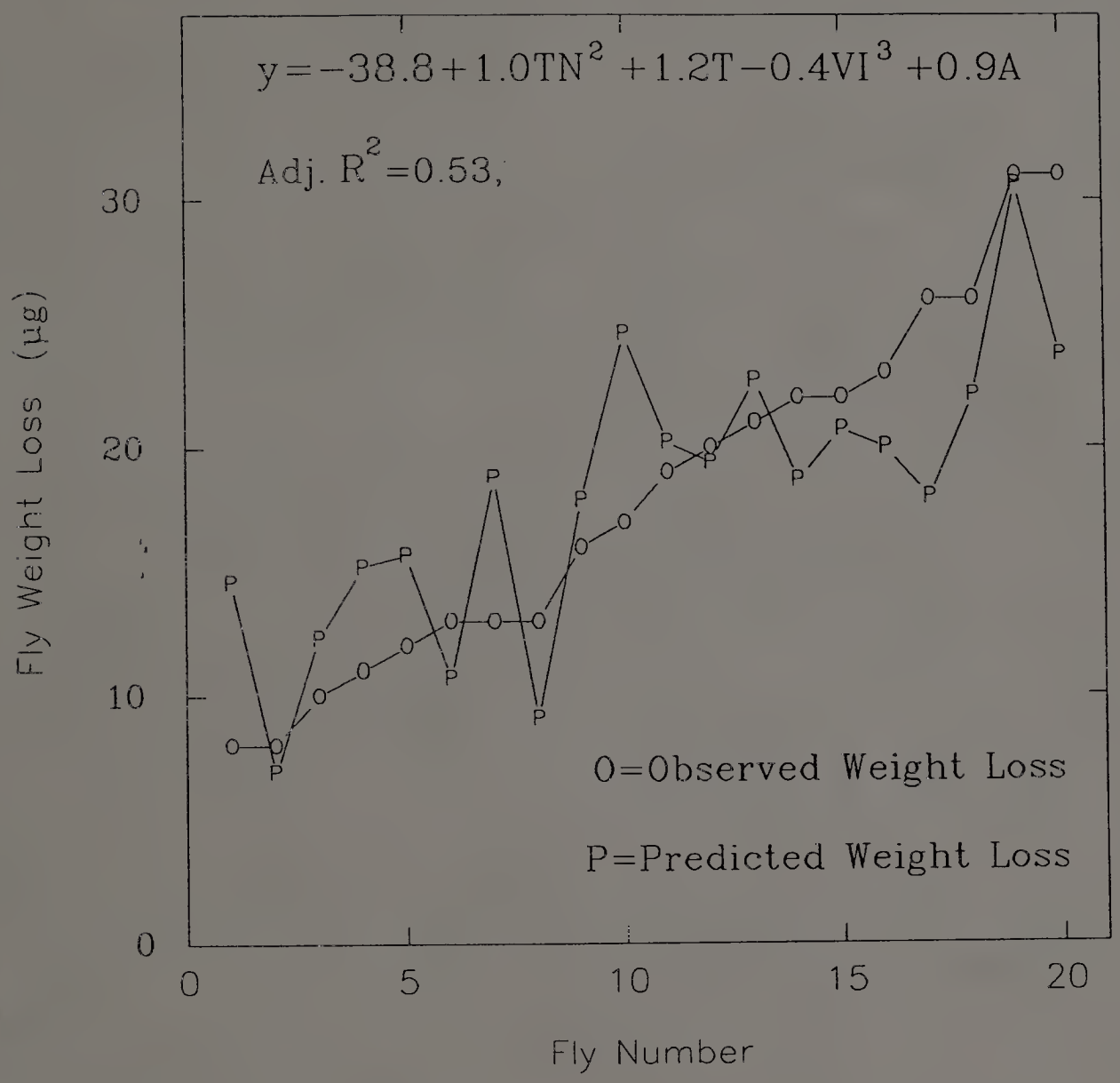

Fig. 6.4A. Observed and predicted pre-feeding weight losses (ug) plotted for each individual R. pomonella fly. The regression model on which predictions are based is also given (Fig. 6.4A), and Standardized residuals of observed minus predicted weight losses plotted against observed fly weight loss (mg) (Fig. 6.4B).

TN= time not bubbling; T=temperature in oC; VE= weight of volume eaten of liquid food; A= age of flies.

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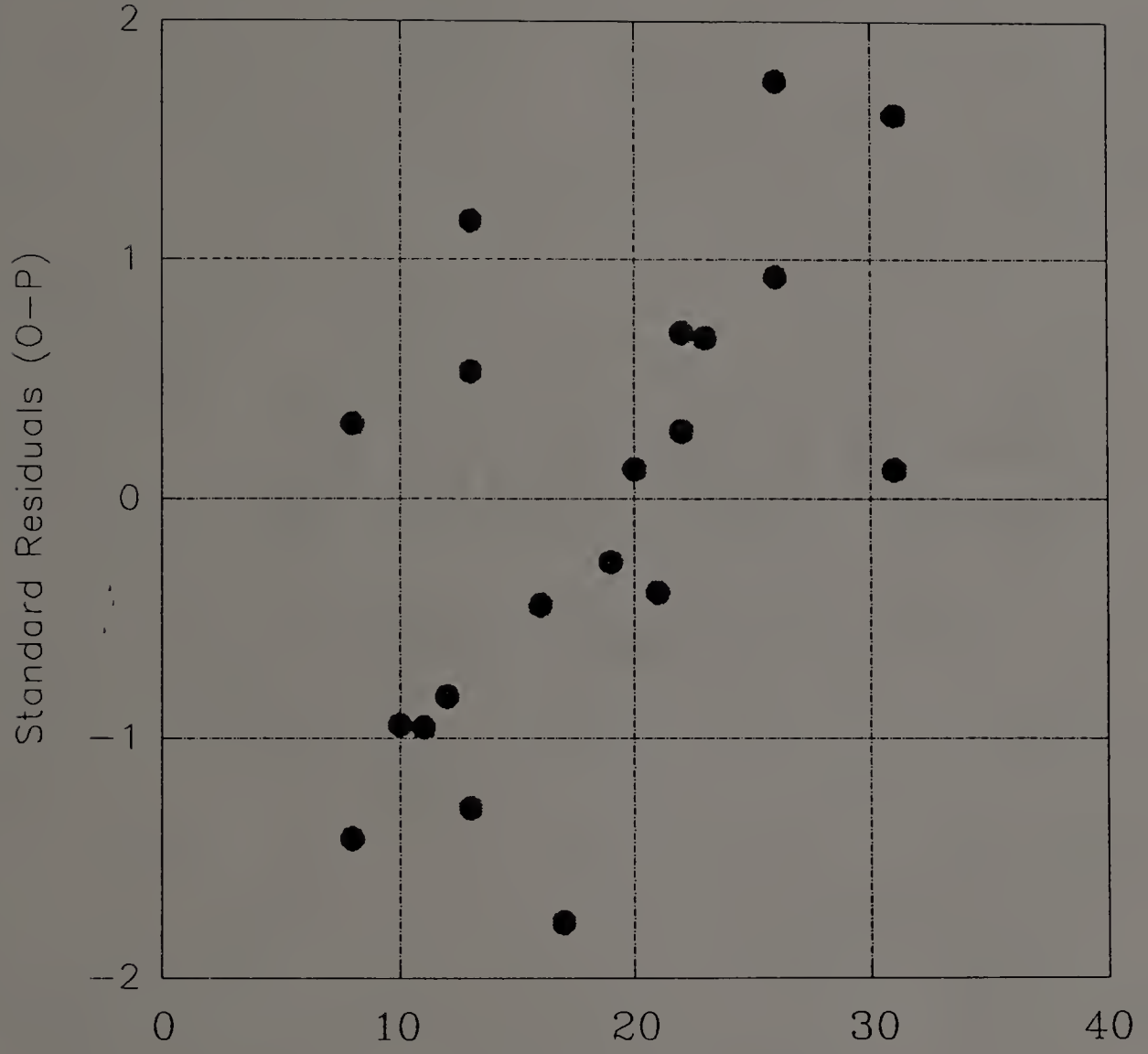

Fig. 6.4B.

this speculation. It therefore remains to be shown quantitatively that prefeeding weight loss differs depending on proboscis position and movement.

Finally, some flies were dissected within minutes after they ingested 1 % enzymatic yeast hydrolysate solution containing 1 % of a red or blue food dye (Durkee-French Foods, Inc., 07470 Wayne, New York), as well as after they had been exposed to such a solution for several hours. In all cases, crop content colour changed from very light (nearly translucent) red or blue within minutes after ingestion to a more dense red or blue colour hours or days after ingestion, with accompanying increased viscosity of crop content.

6.4 Discussion

The results of this study confirmed our hypothesis that oral droplet extrusion behaviour or bubbling is a post-feeding mechanism by which R. pomonella flies, and possibly other Diptera, eliminate excess water through evaporation. During the relatively long post-feeding bubbling periods of c. 30 min, during which flies are quiescent, gorged flies increase ten-fold the pre-feeding rate of water loss, thereby eliminating a large proportion of the water ingested in their most recent meal of liquid food.

In nature, one of the primary food sources of frugivorous tephritid adults is juices oozing from fruit that contain nutrients in dilution (Hendrichs and Hendrichs,

1990; Hendrichs et al., 1991a). Also, frugivorous tephritids sometimes complement their diet with plant surface leachates often suspended in dew or guttation liquids (Hendrichs et al., 1991b). Flower-infesting tephritid adults feed on sap at oviposition wounds or on flower nectar (Foote, 1967; Headrick and Goeden, 1990). Other Diptera in which bubbling has been observed, as for example in screwworm flies, also feed in nature on floral and extra-floral nectaries as well as host wound exudates (Thomas, 1991).

Fluid-feeders, in general, appear to possess specialized mechanisms for concentrating nutrients by removal of water. However, none appears to be similar to the mechanism described here. Oral elimination of excess water to facilitate immediate re-initiation of feeding thereafter and/or to unload water has, to our knowledge, not been shown previously in Diptera. While transpiration is by far the most prominent mechanism of water loss in terrestrial insects, aquatic insects and even terrestrial fluid feeders often eliminate excess water via the anus (Wharton, 1985). Plant feeding insects that ingest nectar, sap or cell contents accomplish elimination of excess water via filter-chambers that pass water directly from the anterior midgut into Malpighian tubules, which in turn carry it to the rectum, where solutes are actively removed. Blood-feeding insects likewise possess specialized mechanisms for discharging excess water following a blood meal. For example, Rhodnius pass excess water from the midgut to the

haemolymph and then eliminate it quickly via the Malpighian tubules to the rectum (Maddrell 1980). Argasid ticks have specialized coxal glands through which they achieve water loss thereby concentrating a blood meal (Kaufman et al., 1982). Blood-feeding tsetse flies maintain their spiracles open after large blood meals, allowing an increased rate of water transpiration (Gee, 1975; Lester and Lloyd, 1928; Moloo and Kutuza, 1970). In addition, through buzzing (beating of wings), tsetse flies produce heat by endothermy, thereby accelerating the rate of shedding excess water from the blood meal through the spiracles and through diuresis (Howe and Lehane, 1986). Only in ixodid ticks has a mechanism been demonstrated (Gregson, 1957) that is somewhat analogous to that found here: after passing water from a blood meal into the haemolymph, ixodid salivary glands remove the water from the hemolymph and return it orally to the host.

Many insects conserve water. This is achieved when excretory products are converted to uric acid which, being almost insoluble in water and non-toxic in form, can be eliminated through defecation without necessity of using water as a solvent. Unlike many insects, however, tephritids and most muscoid flies defecate in the form of liquid urine (Dethier, 1976). This mechanism, in the context of a fluid-feeding habit, appears not to oblige flies "to drink often to counterbalance fluid loss" (pp. 338, Dethier, 1976). Rather, it appears to function as an additional means of

facilitating elimination of excess water. Similarly, 'squirting' of copious dilute urine occurs during flight in honeybees (Pasedach-Poeverlein, 1941), and in particular in the larger bumblebees (Bertsch, 1984) and carpenter bees (Nicolson, 1990) in which, in addition, metabolic water production during flight is much higher. In reality, in many fluid-feeders, the act of ingesting liquid food itself stimulates release of a diuretic hormone from neurosecretory cells. In Rhodnius, for example, diuresis begins 30 s after initiation of feeding (Highnam and Hill, 1977). In many mosquitoes, rapid diuresis follows soon after a meal of vertebrate blood (Nijhout and Carrow, 1978; Plawner et al., 1991). Furthermore, fully gorged blood-feeding insects appear to accommodate ingestion of voluminous meals by distension of the abdomen through stretching intersegmental membranes. Both in Rhodnius and the tick Boophilus, which take only one large blood meal during each larval stadium, within minutes of the start of feeding a reversible plasticization of the abdominal cuticle occurs due to a lowering of the haemolymph pH (Hackman, 1975). This results in a thinning of the cuticle and a four-fold increase of abdominal surface area.

There are a number of behaviours during which insects extrude salivary, body or excretory fluids via non-anal routes. These behaviours are mostly either defensive (such as reflex bleeding of secretions or enteric discharges (Mathews and Mathews, 1978)), take place within specific

feeding contexts (such as trophallaxis in social or subsocial insects), occur within the context of courtship (such as regurgitated crop contents or production of frothy masses by males as nuptial gifts on which females feed during mating (Kessel, 1955; Foote, 1967; Pritchard, 1967; Steele, 1986a, 1986b; Headrick and Goeden, 1990)), or are adopted only when insects are heat-stressed to allow for emergency evaporative cooling. Of all these behaviours, only in the last one is water loss involved.

For a majority of insects, water reserves are generally too low to allow for routine use of this type of thermoregulation (May, 1985). To illustrate, dragonflies and locusts open their spiracles and accelerate their ventilation rate under heat-stress (Willmer, 1982). Some desert tenebrionids extrude their moist genitalia as an emergency measure under extreme heat stress (Bolwig, 1957). Sawfly larvae elevate the abdomen and extrude fluids over their posterior surfaces at high temperatures (Seymour, 1974). In contrast, fluid feeding insects that ingest abundant water with their food may rely routinely on evaporation to prevent overheating. Some aphids engage in 'honeydew panting' to maintain body temperature below an upper critical level (Paul, 1975). Blood-feeding tsetse flies open their spiracles fully when temperatures approach 40oC, allowing them to lower body temperature 2oC below ambient temperature in dry air (Edney and Barass, 1962). In nectar-feeders, such as some species of sphingids, a droplet

of fluid is extruded from the proboscis and spread over the thorax when thorax temperatures exceed 40oC (Adams and Heath, 1964). Honeybees extend their tongues ('tongue-lashing'), extrude a droplet of fluid from the honeycrop, manipulate it with the tongue and withdraw it (Lindauer, 1954; Lensky, 1964; Heinrich, 1980). However, bees exhibit this behaviour at elevated temperatures (rarely below 35oC) to cool the body, often smearing the droplet over the thorax, enabling bees to fly at high ambient temperatures (Esch, 1976).

Unlike the evaporative cooling behaviours referred to above, post-feeding bubbling behaviour described for R. pomonella in this study occurs not only at high temperatures, but in moderate to cool conditions as well (Hendrichs et al., 1992). Consequently, droplet extrusion in R. pomonella appears to be primarily a mechanism of shedding excess water. Of course simultaneous cooling occurs whenever temperature and humidity allow evaporation to take place. In this event, however, thermoregulation would occur as a secondary effect. Although there is indication that food dilution thresholds triggering droplet extrusion in R. pomonella decrease somewhat with increasing temperatures (Hendrichs et al., 1992), we have observed regurgitation behaviour at temperatures as low as 17oC. Similarly, Bertsch (1984), describing bumblebee "tongue-lashing" at 20oC air temperature, concludes that this behaviour can only be understood as a means of eliminating surplus water.

There may also be other potential secondary benefits resulting from bubbling behaviour. For example, Hewitt (1912) suggested that in Musca flies, extrusion of crop contents may be concerned primarily with extra-intestinal digestion of food. By mixing liquid food with salivary enzymes, hydrolysis may be initiated in the crop, an apparently non-secretory organ (Ribeiro, 1987; Terra, 1988, 1990). Another possible benefit is mixing of ingested liquid food with internal bacteria (which may provide an adult food source) to facilitate bacterial growth. Finally, as suggested by Drew and Lloyd (1987) for B. tryoni, deposition of regurgitate onto host plant substrates, besides being of primary value in accelerating evaporation of excess water from regurgitated droplets, may inadvertently result in bacterial inoculation or spread onto plant surfaces upon which adults later feed.

Evidence from this study suggests that R. pomonella flies are able to regulate water loss in more subtle ways once they have eliminated the bulk of excess water through oral droplet extrusion, deposition of regurgitate, or liquid defecation. Although to a much lower degree than in bubbling flies, weight loss also increased after feeding in non-bubbling flies. Flies may accomplish water balance at a finer level by spiracle control and by neuroendocrine regulation of integumentary water loss. Such regulation of integumentary water loss has been described in Periplaneta americana, where the brain appears to release a water-loss

promoting factor and a water-loss restricting factor, depending on the physiological state of the cockroach and the environmental conditions (Treherne and Willmer, 1975; Noble-Nesbitt and Al-Shukur, 1987, 1988). The inverse relationship we found between pre-feeding weight loss and subsequent liquid volume ingested, possibly is also related to such a finer water loss modulation. On the other hand, the direct relationship we found between pre-feeding weight loss and fly age may be related to increased permeability of the integument with fly age. Possibly the most important factor, however, that may explain variation among non-bubbling flies in weight loss during pre- and post-feeding is 'pumping' or 'panting' behaviour, (i. e., continuous extension and retraction of the proboscis without visible extrusion of droplets). Such pulsating of the proboscis, although not quantified, was observed in several flies before and after feeding, and even after bubbling. It is likely that this behaviour, together with any possible additional control through spiracular and integumentary water loss, allows flies to modulate the elimination of water to a finer degree than through bubbling. In addition, during 'panting' behaviour, flies may actually be engaging (above certain temperatures) in evaporative cooling.

In conclusion, our findings suggest that bubbling behavior enables such fluid feeders as tephritids, and possibly other (non-blood consuming) Diptera, to take up nutrients from liquid food solutions in repeated fashion

over a relatively short time period. Through this post-feeding mechanism, in which a diuretic hormone may be involved, engorged flies eliminate from their crop a large proportion of excess water ingested in their most recent meal. Furthermore, by minimizing the overall water load during subsequent activity, it allows flies not only to release space for metabolic water produced during flight, but also to reduce the cost of post-feeding movement and in particular the risk of predation due to increased flight speed and maneuverability.

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CHAPTER 7

CONCLUSIONS, IMPLICATIONS AND FUTURE STUDIES

7.1 Introduction

Foraging for food is a resource foraging behavior that has never been examined in a systematic way under natural or semi-natural conditions in any tephritid species. This dissertation was therefore intended to lay a foundation of knowledge and questions upon which to proceed in future studies in a number of theoretical and applied directions. Furthermore, the information to be gained has a direct bearing on the design and execution of strategies and tactics for managing the two major fruit fly pests selected as study animals: the apple maggot fly (Rhagoletis pomonella Walsh) and the Mediterranean fruit fly (Ceratitis capitata Wiedemann).

This concluding chapter is divided into sections based on the five research chapters of this dissertation: (2+3) quantitative assessment of fly feeding sites and activities over time and space in nature; (3+4) collection of substrates identified from feeding sites and assessment of their contribution to fly maintenance and fecundity; (5) field cage assessment of fly intra-tree food-foraging as affected by food quality, quantity and form; and (6) laboratory analysis of bubbling behavior to understand its significance in a post-feeding context. In each section,

results and conclusions are presented, implications are discussed and possibilities for future studies are explored.

7.2 Feeding Sites and Activities in Nature

The first research study on C. capitata in nature (Chapter 2) was carried out under high population densities in a semi-isolated orchard and surroundings in southern Egypt. Another investigation of medfly food foraging in nature (Chapter 3), conducted under low population densities, took place in an orange grove and surroundings on the island of Chios in Greece. Sites and sources of adult food foraging activities over time and space were assessed through systematic quantitative observations. In addition to feeding behavior, the overall natural history of flies was determined by recording locations and diel patterns of all other fly activities in nature. In both studies, flies were found at dawn to be resting in upper sunlit parts of tree canopies. Here, females primarily initiated feeding and males pheromone release and calling activities. With increasing temperature and light, flies moved progressively to lower, more shaded areas of the canopy. There were diel shifts in male and female location. Females required a substantial and varied diet to realize peak fecundity. This diet was acquired or complemented away from the primary host, orange. Foraging for food throughout most of the day on host and non-host foliage (including feeding on bird droppings) as well as on juice oozing from wounds in ripe

fruit such as guavas, oranges, grapes and figs, females dispersed and fed more than males. Throughout most of the day, males aggregated in leks within the inner canopy of the primary host, orange. In the case of the high fly densities in Egypt, there was some overflow of calling males to other nearby host trees. Visits to displaying males during the warmest hours of the day by receptive females, followed by pair formation, reinforced the lek mating system on host foliage. Preferred sites for lek formation were the illuminated areas of tree canopies which were on or near fruiting host trees, and were protected by dense foliage from intense predation by Odonata and wasps. The greatest number of calling males, bouts of male-male competition, leks, and mating pairs were found on fruiting citrus trees. Female attraction to calling males and formation of mating pairs peaked in midmorning and again after the hot midday temperatures. In the afternoon, females shifted to host fruit, where they suffered from high predation mortality while ovipositing. Soon after, males also shifted there and attempted matings with ovipositing females. Male feeding on fruit occurred late in the day, a time when they were least likely to find a mate. The high level of predation of females on fruit was proposed as an explanation for the origin of lek formation on foliage.

Comparison of these medfly studies with a similar earlier study of apple maggot fly food foraging in an abandoned apple orchard and surroundings in Massachusetts

(Hendrichs and Prokopy 1990) indicates that, for both species, females disperse and feed more than males and daily invest considerable time and energy foraging for food away from fruiting host plants. For both species, fly populations were sustained by host trees and surrounding plants that mostly harbored an apparent paucity of insect honeydew, even though honeydew has been widely considered the normal source of nutrients of adult tephritid fruit flies in nature. Flies of both species seem to obtain or even require nutrients from other sources, possibly even multiple sorts of natural food. Females scavenge for any available nitrogenous sources on foliage, where bird droppings constituted an important feeding site for both species. The main difference in food foraging found between the two species was that whereas feeding on host fruit was common in medflies, it was relatively rare in apple maggot flies, which fed mostly on leaf surfaces of host and non-host vegetation. In terms of mating systems, apple maggot males remained mostly on fruiting host trees where they fed on leaf surfaces and guarded fruit to mate with females arriving to oviposit. In contrast, medfly males shifted daily between forming leks on host foliage and feeding on fruit, accompanied by attempted matings on fruit with unreceptive females.

Practical implications of these findings are numerous. One is that under mixed host conditions in a natural setting, flies adjust their food foraging activities in response to dynamic changes in the spatial, temporal and

seasonal distribution of food resources and host phenologies. Under commercial orchard monoculture conditions, fly food foraging is probably less complex and more predictable. Measures such as harvesting fruit before maturity, removing wounded or fallen ripe fruit, or adjusting pruning practices to discourage formation of fresh water sprouts and attendant buildup of aphids would maintain plantations or orchards comparatively free of some important natural food resources. Furthermore, discouraging flocks of birds from entering orchards through the use of Scare-Eye balloons would result in fewer wounded fruit and less bird droppings as sources of food for adults. In such food-scarce commercial plantations or orchards, one could expect that immigrating flies might remain largely in the perimeter rows of trees because of their need to move regularly back and forth to the surrounding vegetation to obtain food. Such an obligatory movement would increase many-fold the effectiveness of food-baited interception traps placed around orchards. Possibly as important, the widely used ground or aerial insecticide bait sprays would become more effective in the face of reduced competition from natural food. In addition, it is possible that sprays could be confined specifically to the perimeters of food scarce orchards, thereby contributing successfully to a more environmentally oriented management of these frugivorous pests. Future experiments should be designed to test the effects of these applied measures on fruit fly control.

Examination of fly food foraging in other important agroecosystems favored by flies, for example coffee plantations in the case of medflies, should also be an objective of future studies.

7.3 Assessment of Nutritive Value of Natural Foods

Assessment of foods identified for their contribution to egg laying and energetic maintenance was carried out for medfly in the laboratory (Chapter 3) and for the apple maggot fly both in the laboratory and in field cages with potted host trees (Chapter 4). Results indicate that fruit such as grapes did not support egg development in medflies, contributing only to longevity. Fig fruit, however, with a higher content of proteins than most fruit, sustained both longevity and fecundity. Bird feces alone supported neither egg production nor longevity. However, when added to a diet of figs, bird feces significantly increased fly fecundity. Male survival did not differ among the natural diets evaluated.

For apple maggot flies, results indicate that fly survival can be sustained by carbohydrates obtained from host foliage surfaces apparently in the form of plant leachates. This would explain the oft-observed extensive "grazing" of flies (in the absence of insect honeydew) on non-visible substances on host plant surfaces. Apple maggot fly fecundity was not sustained by host foliage leachates. Also, preparations of leaf surface bacteria, pollen, insect

frass, and uric acid did not support any significant egg-development, whereas bird droppings, aphid honeydew and to a lesser extent hawthorn fruit did sustain egg development, though at a level significantly below that of laboratory food (enzymatic yeast hydrolysate).

One can conclude from these studies that flies feed on a variety of substrates in nature, some of which have a higher nutritive content than others and some of which provide nutrients only for survival or only for egg development. Future studies to assess the nutritive contribution of natural foods to fly fecundity should therefore allow for diet balancing, i. e. self-selection by flies from a combination of identified natural food sources. In addition, one of the implications from the results obtained, supported by field observations, is that flies probably feed on substances of low nutrient value only in the absence of more readily available substances of higher nutrient value. The fact that females respond to ammonia and probably many other food associated odors and move away from host trees in search of more concentrated or complementing sources of odor-emitting food probably allows flies to reduce time and energy spent foraging for food and to increase time available foraging for fruit in which to oviposit. An important priority for future studies would therefore be identification and development of formulations of female attractants from such natural sources of food volatiles as bird droppings or insect honeydews.

7.4 Intra-Tree Foraging as Affected by Food Quality and Quantity

Studies of intra-tree foraging behavior of apple maggot flies in relation to different type, quality and quantity of food under field cage conditions are presented in Chapter 5. Feeding and post-feeding behaviors were recorded after flies were presented with yeast hydrolysate or sucrose droplets, varying either in concentration, amount of food solute or total droplet volume. Our objectives were (a) to establish, at a constant level of previous food deprivation, food ingestion thresholds in relation to food quality and quantity, and (b) to study the effect of initial food quantity and quality on food handling time and subsequent food foraging behavior.

We found that for both carbohydrate and protein substrates, fly foraging time (termed giving up time) on a tree branchlet was positively related to total amount of food solute previously encountered on a leaf surface, though largely independent of food volume or concentration. The volume and state of concentration of food presented, however, affected significantly food "handling" and "processing" time and therefore foraging time available following consumption. In fact, total patch (branchlet) residence time was more closely linked to food handling and processing time than to foraging time. Less time was needed for uptake of liquid than dry food, the latter requiring

liquification by salivary secretion and eliciting considerable intermittent cleaning of mouthparts by feeding flies. Similar to other fluid feeders, uptake time in R. pomonella decreased with increasing dilution, although below a threshold of 30% concentration of solute, rate of nutrient intake decreased rapidly. When the level of dilution and total volume of food ingested was great enough, engorged flies entered extended quiescent post-feeding periods (termed food processing time) during which they engaged in oral extrusion of droplets of liquid crop contents (termed "bubbling"). After this they reinitiated feeding, followed by more bubbling and feeding bouts.

Particularly important from an applied point of view would be follow-up intra-tree and inter-tree studies where food foraging flies are tracked as they search for food under circumstances where artificial foods such as are used in bait-insecticide sprays or food trap baits are in competition with natural foods such as bird droppings or insect honeydew. The methodology developed and knowledge gained here should also facilitate subsequent analyses of the dynamics of fly foraging behavior under interactive food, mate, and oviposition site resource conditions. This could serve as a model for future work on multiple-type resource foraging behavior.

7.5 Significance of Bubbling Behavior

When one considers that regurgitation behaviors have been observed in several species of insects, it is surprising that no attempt has been made to understand their proximate or ultimate significance. Multivariate logistic regression analysis, carried out as part of intra-tree foraging studies under Chapter 5, suggested that bubbling behavior is determined by liquid food volume, degree of food dilution, fly hunger (manifest by extent of food consumption, as well as feeding and foraging times) and environmental temperature. Although thresholds triggering bubbling decreased with increasing temperature, higher temperature by itself did not result in bubbling behavior. The conclusion is that bubbling is not primarily a mechanism to achieve evaporative cooling, but rather a behavior to eliminate excess water, thereby enabling engorged flies to continue feeding on diluted food sources.

In Chapter 6, the hypothesis was investigated that through bubbling fully gorged apple maggot flies eliminate excess water by evaporation and thereby concentrate nutrients. Fly weights were measured continuously during pre- and post-feeding periods and in relation to occurrence of regurgitation behaviours. Fly weight losses during pre-feeding were an order of magnitude lower than post-feeding weight losses when flies regurgitated liquid crop contents. During a bout of droplet extrusion, lasting on average 23 min, weight loss averaged 66 % of the weight of liquid

ingested by a fly in the preceding meal. Fly weight loss while bubbling was significantly correlated with duration of bubbling, temperature and relative humidity during post-feeding and to initial fly weight (Adj.R^2 = 0.95). Fly age, volume of liquid ingested and rate of pre-feeding weight loss did not significantly improve predicted weight loss through bubbling.

These results confirmed the stated hypothesis that post-feeding bubbling allows fluid feeders primarily to take up nutrients from liquid solutions in repeated fashion and also to minimize the water load during subsequent resource foraging. At the same time flies should be expected to prefer food solutions that not only minimize food handling costs but also maximize the rate of nutrient intake. Implications of these findings are not only theoretical. For example, knowledge of food foraging and handling is required in efforts to develop non-sticky traps that incorporate a slow-release feeding stimulant with a toxicant. To be effective, food type and quantity on such traps not only must arrest and stimulate fly feeding, but also the form of presentation of food should maximize food handling time. Thereby, flies would be exposed for a sufficiently long period (through contact or ingestion) to the pesticide, permitting use of the lowest doses of toxicant. Disease transmission due to regurgitation behaviors in dipteran species of medical importance is another potential application. By gaining an understanding of the significance

of regurgitation behaviors in the biology of the regurgitating insect, transmission of some diseases could be addressed more properly.

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