Studies on *Conura torvina* (Hymenoptera: Chalcididae) Reproduction and Biology in Relation to Hosts in *Brassica* Crops.

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Dissertation submitted to the faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ENTOMOLOGY

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January 24, 1997, Blacksburg Virginia

Keywords: *Cotesia orobenae*, *Cotesia rubecula*, *Pieris rapae*, *Plutella xylostella*, development time, hyperparasite, ovaries, ovarioles, ovulation, oviposition, superparasitism, seasonality, trap plants, trap hosts.

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Abstract

Conura torvina (Cresson) (Hymenoptera: Chalcididae) is a solitary pupal endoparasite of numerous insect species. In *Brassica* crops it acts as a parasite of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and was found as a hyperparasite of *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) and several other parasitoid species. *Cotesia rubecula* was introduced into Virginia in 1987 as a biological control agent for *Pieris rapae* (L.) (Lepidoptera: Pieridae), and because *C. torvina* was thought to have eliminated this population of *C. rubecula*, studies of *C. torvina*'s reproductive biology and behavior were initiated.

A study using plants laden with "trap hosts" to detect *C. torvina* activity in the spring indicated no activity until late June, but proved trap host sampling to be an efficient and effective method of monitoring *C. torvina* activity. Studies of *C. torvina*'s ability to reproduce in *C. rubecula* pupae of different ages indicated that *C. torvina* can successfully parasitize pupae at all stages of development, but was most successful in young to middle aged pupae. Studies of *C. torvina*'s host species preference indicated the larger host species such as *P. xylostella* were preferred. Equal numbers of *P. xylostella* and *C. rubecula* were parasitized, but a greater proportion of fertile eggs were laid in *P. xylostella*. Smaller host species were often ignored.

Host dissection studies indicated that caged *C. torvina* were inefficient at host finding and oviposition. Superparasitism was common, but declined as the females gained oviposition experience. Experienced *C. torvina* produced an average of 8.25 progenies per day for a period of 12 days when provided with 13 *P. xylostella* hosts each day. *Conura torvina* produced up to 14 progenies a day when provided ≥ 26 hosts. Dissection of *C. torvina* ovaries indicated three ovarioles per ovary with a mean of 9.2 and maximum of 15 mature eggs per female. Host dissection indicated that a mean of 18 and maximum of 30 eggs could be laid per day. New eggs were produced as oviposition occurred. Significantly larger eggs were laid in *P. xylostella* than in *C. rubecula*, and significantly more eggs were laid in *C. rubecula* than in *P. xylostella.*

From these data and data from earlier studies I concluded that *C. torvina* has a poor reproductive ability and its impact as a hyperparasite is limited to the summer months. This makes *C. torvina* an unlikely cause of *C. rubecula*'s disappearance.

Acknowledgments

I would like thank Dr. L. T. Kok for giving me much freedom to design and execute my research. I have also greatly appreciated his prompt attention, guidance and sound advice in matters of research, writing and presentation. I also want to thank my committee members, Dr. R. L. Pienkowski, Dr. B. D. Opell, Dr. D. G. Pfeiffer and Dr. R. D. Fell for their advice in matters of research and writing and their careful review of my work. I also owe gratitude to: Dr. J. R. Bloomquist for research support and advice; Dr. D. E. Mullins for his support in the use of photographic and laboratory equipment; and Dr. Marvin Lentner whose class on Experimental Design and Analysis and advice on statistical software helped me design my research and get through my data.

Numerous staff members have also been of great assistance and these are: Eric Day for his support and assistance; Warren Mays and Tom McAvoy who have helped me obtain materials and prepare for research; and Keith Tignor for his assistance with computers and equipment.

I would also like to thank friends such as Jarrod Leland, Billy Van Wart, Adam Finkelstein, Mike Kirby, Tom Kuhar, Shane Evans, Cathy Knowles and Brett Marshall whose presence helped me endure the time I spent at Price Hall, and who have also participated in the enjoyment of extracurricular activities. These friends have also contributed valuable assistance at one time or another, particularly Billy whose tremendous knowledge of, and assistance with computers speeded up my work.

Finally I would like to thank my mother, Dorothy B. Gaines and father, Richard V. Gaines for their support. Their hospitality and generosity has been valuable in enabling me to escape the academic environment for brief periods of time.

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Introduction

The imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae), is an important pest of *Brassica* crops in Virginia and other areas of the United States. *Pieris rapae* is active in Virginia from May to November and may have six generations per year (Parker 1970, Gaines & Kok 1995a). In the absence of insecticides, populations of this insect is regulated by a number of parasitic Hymenopterans, the most notable of which are *Cotesia glomerata* (L.) (Hymenoptera: Braconidae), a gregarious endoparasite of the early *Pieris* instars, and *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae), a gregarious endoparasite of *Pieris* pupae. In spite of the role these natural enemies play in regulating *P. rapae*, its population reaches levels which cause economic damage to crops.

Pieris rapae originated in the Palearctic region where it's principal parasite is *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae). *Cotesia rubecula* is a solitary endoparasite of early instar of *P. rapae*, and is known to be one of the most important parasites affecting *P. rapae* in parts of Europe (Blunck 1957, Richards 1940). *Cotesia rubecula*'s superior qualities as a biological-control agent are due in part to the fact that it destroys its host before the onset of the host's fifth instar (the stage most damaging to crops). Also, *P. rapae* is unable to encapsulate *C. rubecula* eggs (Puttler *et al*. 1970). This parasite has not become fully established on this continent and can only be found in the Canadian province of British Columbia where it became established sometime during the last century (Wilkinson 1966), and in the Pacific Northwest states of Washington and Oregon (Biever 1992). It is also found in Ontario, where it was introduced in 1970 (Corrigan 1982). It's presence in the states of Washington and Oregon was discovered in 1963 when a survey was initiated to determine why *P. rapae* populations had declined to below economically important levels in unsprayed fields (Wilkinson 1966).

In the past 25 years several unsuccessful attempts have been made to import and establish *C. rubecula* in more southern areas of the United States. In 1967 and 1968, *C. rubecula*, imported from British Columbia, were released in Missouri where they showed potential to control *P. rapae*, but failed to become established for more than two seasons after its introduction (Puttler *et al.* 1970, Parker *et al.* 1971). After its introduction in Missouri, six hyperparasitic species were recovered from field collected *C. rubecula* pupae, and hyperparasitism was estimated to cause up to 72% mortality in immature *C. rubecula* during late summer in the 1968 season (Parker *et al.* 1971). One of the most commonly recovered hyperparasite species was *Tetrastichus galactopus* Ratzeburg (Hymenoptera: Eulophidae) a gregarious endoparasite of the *Cotesia* larvae found in *P. rapae*; it was originally misidentified as *Tetrastichus sinope* Walker (Puttler *et al.* 1970, Krombein *et al.* 1979). The second most commonly recovered hyperparasite was *Conura torvina* (Cresson) (Hymenoptera: Chalcididae), a solitary endoparasite of the pupae of a wide variety of insect species; it was formerly *Spilochalcis side* Walker (Parker *et al.* 1971, Krombein *et al.* 1979, Couch 1984, Delvare & Boucek 1992) (Figure 0.1). However, failure to establish the British Columbian strain of *C. rubecula* in Missouri was attributed to incompatibility of that strain's diapause behavior with the fall photoperiods and temperatures of southern latitudes (Nealis 1985). Failure of establishment was not attributed to the impact of hyperparasitism.

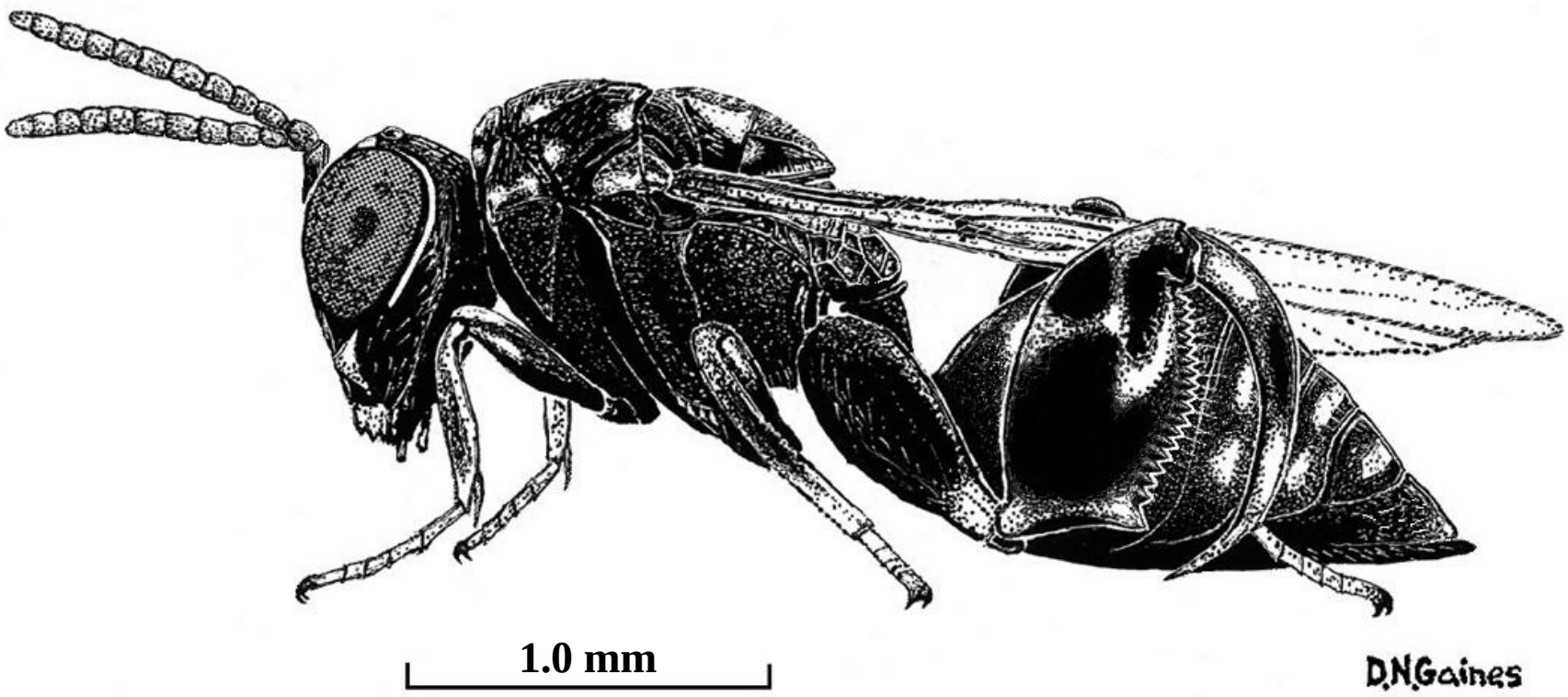

Figure 0.1: Female *Conura torvina* (Cresson) **(**Hymenoptera: Chalcididae) from a *Cotesia rubecula* host.

The most recent attempt to introduce and establish *C. rubecula*, was conducted in Montgomery County Va. and involved a Yugoslavian strain of *C. rubecula* thought to have diapause behavior more adapted to the photoperiods of southern latitudes. *Cotesia rubecula* was released in June of 1987 at the Prices Fork Research Center and the Kentland research farm at Whitethorne, Montgomery Co., Va., by McDonald (1990). *Cotesia rubecula* cocoons were recovered at both locations later that summer, indicating initial establishment, however, in late July of 1987, 50% of the recovered cocoons contained hyperparasites. As in the Missouri study by Parker *et al.* (1971) the most prominent of these hyperparasites was *Tetrastichus galactopus*. Another commonly found hyperparasite was, *Conura torvina* (McDonald 1990, McDonald & Kok 1991).

In 1988, *C. rubecula* cocoons were found only at the Whitethorne site, indicating that some of the population had successfully overwintered and adapted to the climate. During July 1988, an increasingly large percentage of the recovered cocoons were found to contain hyperparasites, and by early August, all of the 10 recovered cocoons contained hyperparasites. Almost all of the hyperparasites found in 1988, were *C. torvina*. In the fall of 1988, small numbers of *C. rubecula* cocoons, which did not contain hyperparasites, were again recovered, but in 1989, no *C. rubecula* cocoons were found in the field. Their disappearance was attributed to the activity of the hyperparasite, *C. torvina*, which had been prominent the previous year (McDonald 1990, McDonald & Kok 1991). *Cotesia rubecula* was not found during the 1990 or 1991 seasons even though its *P. rapae* hosts were abundant each year.

In North America, both *T. galactopus* and *C. torvina* have been recorded as important hyperparasites of *C. rubecula*. Richards (1940), documented the relationship between *T. galactopus* and *C. rubecula* in England, and although *T. galactopus* was found to reduce the effectiveness of *C. rubecula*, there was no record of it causing the extinction of this host. However, no extensive studies have been conducted in the United States to determine the impact that *C. torvina* and other hyperparasites have on *C. rubecula* or other beneficial species associated with *Brassica* crops. Therefore, a study was conducted by myself to determine the seasonal abundance of *T. galactopus* and *C. torvina* and investigate their impact on the beneficial species of *Cotesia* associated with *Brassica* crop pests (Gaines 1992). This study was conducted during the 1989 and 1990 crop seasons at Whitethorne Va., and included some laboratory experiments on *C. torvina* reproductive behavior.

Field studies indicated that *C. torvina* had an insignificant impact on populations of hosts. The subsequent laboratory studies reinforced these findings by indicating that *C. torvina* had a relatively low daily reproductive capacity. However, there were numerous gaps in the data on *C. torvina* biology and behavior which left unresolved questions. The following is a summary of research findings by Gaines (1992) on *C. torvina*.

Due to *C. rubecula*'s absence from the field, studies were conducted on several other *C. torvina* hosts found in *Brassica* crops. Field collections of potential hosts indicated that *C. torvina* acts as a pupal hyperparasite of: *Cotesia glomerata* (L.), *Cotesia orobenae* Forbes

(Hymenoptera: Braconidae), and *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae). *Cotesia orobenae* is a gregarious endoparasite of the cross striped cabbageworm, *Evergestis rimosalis* Guenee (Lepidoptera: Pyralidae). *Diadegma insulare* is a solitary endoparasite of late instar diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Diadegma insulare* emerges from the *P. xylostella* pupae and spins a cocoon within its hosts cocoon. *Conura torvina* was also collected as a pupal parasite of *P. xylostella*. *Plutella xylostella* occur in solitary cocoons, but the gregarious *C. orobenae* and *C. glomerata* species spin their cocoons in masses of about 10 and 25 cocoons, respectively.

Field data indicated that *C. torvina* was active from May through early October. Adult *C. torvina* ceased their reproductive activities in October, possibly in preparation for winter diapause. Most *C. torvina* activity was seen from late June through September and four generations occurred at monthly intervals during that period. Although no activity was recorded before May, *C. torvina* might be active as early as the end of March and thus, have five to six generations per year (Gaines 1992). *Conura torvina* overwinter as adults (Arthur 1958), and in early spring could be a threat to pupae of overwintering *C. rubecula*. Data on the early spring activity is lacking and would contribute much toward understanding *C. torvina*'s potential to harm the prepupae or pupae of solitary braconid parasites which overwinter in cocoons.

During its heaviest (June - August) activity period, *C. torvina* was found in <20% of its hosts cocoon masses and in <10% of the solitary hosts cocoons collected in the field. During certain weeks, *C. torvina* was found in up to 50% of the collected cocoon masses, but this only occurred when host populations were relatively low (Gaines 1992). It was not clear if the low proportion of hosts affected was due to *C. torvina*'s low reproductive capacity or some other reason. *Cotesia glomerata* cocoon masses contained *C. torvina* more frequently than *P. xylostella* cocoons even though individual *P. xylostella* cocoons were as abundant as *C. glomerata* cocoon masses. Also, many more *C. torvina* resulted from *C. glomerata* because, although only a small proportion of the cocoons in each mass were parasitized, cocoon masses are composed of numerous hosts. *Conura torvina*'s greater rate of parasitization of *C. glomerata* cocoon masses than of *P. xylostella* cocoons is probably due to the conspicuousness (large size and light color) of the cocoon masses.

Of the 41 *C. torvina* collected from *P. xylostella* during the study, 33 (80%) actually came from *Diadegma insulare* cocoons found within *P. xylostella* cocoons. This was probably due to the high (>60%) parasitization rate by *D. insulare* and the fact that parasitized *P. xylostella* cocoons sit on the plant longer and are more likely to be found by a foraging hyperparasite (Gaines 1992).

The proportion of female *C. torvina* progeny emerging from field collected *C. glomerata* and *C. orobenae* cocoons was <31%. *Diadegma insulare* and *P. xylostella* appeared to be more suitable hosts because 70% of the *C. torvina* progeny recovered from these hosts were female (Gaines 1992). In laboratory studies about 50% of the *C. torvina* emerging from *C. rubecula* cocoons were female.

Although no determination was made of the number of mature eggs *C. torvina* can hold in her ovaries or whether more than one egg is laid in each host, *C. torvina* did not appear to have a strong reproductive capacity. In the lab, *C. torvina* successfully parasitized a mean of six out of 12 *C. rubecula* hosts provided per day, and no female produced more than nine progenies daily. In the field, none of the cocoon masses affected by *C. torvina* contained more than 12 *C. torvina* progenies and the majority had less than four (Gaines 1992). The low number of hosts affected per cocoon mass may be a result of *C. torvina*'s foraging strategy, but a foraging female would probably oviposit more than just a few eggs into each cluster of hosts (cocoon mass) if it had an ample supply of eggs. *Conura torvina* may also oviposit more than one egg in each host, and since only one progeny results per host, the number of eggs oviposited per host may be as important to determine reproductive capacity as the supply of mature eggs.

During the maintenance of *C. torvina* laboratory colonies, several observations were made on *C. torvina* behavior (Gaines 1992). First, it was observed that most female *C. torvina* required a minimum of three exposures to *C. rubecula* hosts before they initiated regular oviposition behavior, and any *C. torvina* progeny which resulted from initial contacts with hosts were usually male. However, when *C. torvina* had initial contact with *P. xylostella* pupae (cocoons), oviposition behavior often occurred on the first contact and more progenies resulted from initial host contacts. Host feeding activity was also observed during initial contacts with both host species. These observations indicated that oviposition behavior either required some learning by *C. torvina ,* or females required host feeding to obtain nutrients for egg production. *Conura torvina*'s quicker oviposition response to *P. xylostella* may be either an indication of host preference or a response to the odor of frass particles which often become trapped in the web-like silk of *P. xylostella* cocoons. Frass is known to stimulate reproductive behavior in some parasitoids (Vinson 1976).

Numerous attempts were made to rear female *C. torvina* progenies from *C. glomerata* and *C. orobenae* hosts. These attempts yielded hundreds of males and only three females from these relatively small hosts. This was a considerably smaller proportion of females than was obtained from field collected hosts of the same species. Small host size is known to cause hymenopteran parasitoids to lay unfertilized eggs which result in male progeny (Clausen 1939), and the gregarious *Cotesia* species pupae are relatively small. A possible reason for the higher proportion of female progeny in field collected hosts may be due to the smaller size of the female *C. torvina* foraging in the field. Female *C. torvina* which emerged from *Cotesia* species were relatively small and because these hosts were the greatest proportion of hosts parasitized in the field, smaller females represented a large portion of the foraging females. These relatively small females may have then perceived the small *Cotesia* species they encountered as being relatively large and suitable for oviposition.

Planned studies of *C. torvina* biology and reproductive behavior were intended to determine its potential as a hyperparasite in the *Brassica* crop system. Also, because *C. torvina* and other related species are found in numerous crop systems on other species of hosts, further

knowledge of their biology and behavior could contribute to studies of *Conura* parasitism and hyperparasitism in these crops. These studies could also contribute to the general body of knowledge about the reproductive biology and behavior of generalist or opportunistic pupal parasites and hyperparasites.

The over-all objective of my study was to determine whether *C. torvina* has the capacity to eliminate an introduced population of *C. rubecula*. Specific objectives were to determine *C. torvina*'s:

(1) earliest date of spring foraging activity and its abundance in the field crop environment in the early season;
(2) response to *C. rubecula* host pupae of various ages;
(3) host preference (response to hosts of different species and sizes);
(4) daily reproductive capacity when hosts are continuously or intermittently supplied over time; and
(5) daily egg production capability, number of eggs laid, and oviposition behavior in hosts of different species supplied at different densities.

Literature Review

Conura torvina (Cresson) is one of the hyperparasites most frequently implicated in reducing introduced populations of *C. rubecula* in the United States (Parker *et al*. 1971, McDonald 1990). *Conura torvina* [formerly *Spilochalcis torvina* (Cresson)] is also frequently misidentified in the literature as *Spilochalcis side* (Walker) (Couch 1984, Delvare & Boucek 1992). As a Nearctic species, it is common throughout the United States and can be found as a primary or secondary parasite in the pupae of more than 30 species of Lepidoptera from 14 families, at least 5 species of Coleopteran hosts, and at least 13 species of Hymenoptera within the Ichneumonoidea (Krombein *et al*. 1979). Within the *Brassica* cropping system it is known as a primary parasite of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and as a hyperparasite of *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae), a parasite of *P. xylostella*. It also acts as a hyperparasite on *Cotesia glomerata* (L.) and *Cotesia orobenae* Forbes (Hymenoptera: Braconidae). *Cotesia glomerata* is a parasite of *Pieris rapae* (L.) (Lepidoptera: Pieridae), the imported cabbageworm, and *Cotesia orobenae* is a parasite of the cross-striped cabbageworm, *Evergestis rimosalis* Guenee (Lepidoptera: Pyralidae) (Krombein *et al*. 1979; Gaines & Kok 1995b).

Vickery (1929) conducted one of the earliest studies of *C. torvina*, rearing it on the pupae of *Meteorus laphygmae* (Viereck) (Hymenoptera: Braconidae) a parasite of the fall armyworm. His study was conducted in southern Texas. The duration of the development cycle from egg to adult emergence ranged from 17-19 days at a mean temperature of 22.7^{o}C and took up to 28 days at 18.9^{o}C. Adult longevity was recorded as 6.5 months for two females kept at a mean of 23.9^{o}C and fed regularly with sugar syrup. Parthenogenetic reproduction (arrhenotoky) was suspected because no mating was observed in several caged pairs, and only male progeny were produced.

Arthur (1958) reared *C. torvina* as a primary and secondary parasite on a number of Lepidopterous and hymenopterous hosts, as well as a Coleopterous host at Belleville, Ontario. All hosts were attacked in the pupal stage. A preoviposition period of from 6-8 days was determined. Oviposition times were recorded and each puncture and oviposition took at least 15 minutes. Host dissections showed that frequently up to four eggs were laid per host, but that the earliest hatching parasite larva usually killed the others. Although *C. torvina* is a solitary parasite, two adult *C. torvina* were observed emerging from a large *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) pupa. *Conura torvina* life stage sizes and development durations were determined for several different sized hosts at 22.5±1^{o}C. Eggs hatched 40 to 48 hours after deposition and measured an average of 0.75 mm in length (range 0.70 to 0.80 mm) five hours after deposition. Adults emerged 20 and 25 days after oviposition in small and large host species, respectively. Adult *C. torvina* from the smaller *Cotesia* species hosts averaged 2.43 mm in length where as those from the larger *G. mellonella* hosts averaged 4.71 mm in length. Adults fed a 10% honey water solution and misted twice a day with water lived for up to five months. Arthur (1958) also produced detailed descriptions and drawings of the immature life stages.

McNeil & Rabb (1973) reared *C. torvina* on *Cotesia congregata* (Say) taken from the tobacco hornworm, *Manduca sexta* (L.), in North Carolina. A laboratory study on life stage duration was conducted at 27±1°C with a 16L:8D photoperiod in *C. congregata* pupae. Host dissections also provided life stage duration data and measurements of *C. torvina* eggs, last instar larvae and pupae. Egg development time was from 36 to 40 hours, and mature eggs averaged 0.55 mm in length (range 0.52 to 0.59 mm). The larval development time (all instars combined) lasted from 5 to 5.4 days, and third instars averaged 3.3 mm in length (range 3.1 to 3.6 mm). The pupal stage lasted from 8 to 10 days, and pupae averaged 2.8 mm in length (range 2.5 to 3.0 mm). Development time, from oviposition to adult emergence for *C. torvina* was 14 to 16 days. The adult preovipositional period was 4-6 days. *Conura torvina* was found to overwinter in the adult stage in a state of reproductive diapause. *Conura torvina* was not seen in the tobacco field environment until July (two weeks after its host, *C. congregata* first appeared). The effects of *C. torvina* were insignificant on its host (McNeil & Rabb 1973).

Gaines (1992) observed that *C. torvina* often combine host feeding behavior with oviposition. After puncturing a host with her ovipositor, a female would then turn and feed on the host hemolymph exuding from the puncture wound. Numerous Hymenopterous parasitoid species have been known to host feed, and it is well established that this behavior provides females with nutrients needed for egg production and yolk deposition (DeBach 1964, Vinson 1985).

Cotesia rubecula is one of the principal hosts used in this reproductive study of *C. torvina*. It is a solitary endoparasite of early instar of *P. rapae.* It emerges as a third instar from the fourth instar *P. rapae*, spins a cocoon adjacent to the dying host and pupates (Richards 1940). *Cotesia rubecula* overwinters as a prepupa in its cocoon. Mean developmental times for *C. rubecula* (from egg to cocoon formation) at temperatures of 17 and 27°C were 16.6 and 7.8 days, respectively. Pupation required another 9.4 and 4.8 days at these same temperatures. Thus, the development from egg to adult requires from 12 days at 27°C to 26 days at 17°C (Richards 1940).

Two other *C. torvina* hosts used in the course of this study were *Cotesia orobenae* Forbes (Hymenoptera: Braconidae), and *Plutella xylostella* (formerly *P. maculipennis* Curtis). *Cotesia orobenae* is a gregarious endoparasite of the larval cross-striped cabbageworm, *Evergestis rimosalis* Guenee (Lepidoptera: Pyralidae) (Krombein *et al*., 1979). *Cotesia orobenae* oviposits in early instar *E. rimosalis* and the resulting larvae feed internally on the developing host, exit the last instar host, and spin cocoons in a loose mass adjacent to the dying host. The mean brood size from field collected cocoon masses varies from 8-12 cocoons per mass. *Cotesia orobenae* enter diapause in late September and overwinter in cocoons (Gaines & Kok 1995b). *Conura torvina* was one of the most frequently recovered *C. orobenae* hyperparasites during field studies in 1989, but emerged from only 32 (6%) of the 509 *C. orobenae* cocoons recovered(Gaines & Kok 1995b).

Plutella xylostella can be an important pest of *Brassica* crops in North America and is one of the three most important pests in Southwest Virginia (Harcourt 1963, Lasota & Kok

1989). *Plutella xylostella* was accidentally introduced into North America from Europe in the mid 1800's (Harcourt 1963). The biology and life history of *P. xylostella* is detailed by Marsh (1917) and Harcourt (1957). Marsh (1917) determined the seasonal history and field development times for *P. xylostella* at Rocky Ford, Colorado. Seven generations occurred in the field from early May until early October. The egg stage lasted 3 to 6 days, the larval stage 9 to 28 days and the pupal stage from 5 to 13 days at the prevailing field temperatures (no specific temperatures were mentioned). Thus, the entire development cycle from egg to adult can take from 17 to 47 days in the field. Adult females laid a mean of 287 eggs and lived as long as 14 days.

Harcourt (1957), determined that *P. xylostella* could have up to six generations per year between mid May and early October in Ottawa, Ontario. Harcourt's recorded life stage durations (in field cages) were slightly longer but very similar to those recorded by Marsh, with the entire developmental cycle lasting from 21 to 51 days. The mean development cycle duration (seasonal) was 31.6 days. Seasonal temperatures were not recorded in either Marsh's and Harcourt's studies, but the slightly longer development times observed by Harcourt may be due to lower mean temperatures in Ontario, Canada, than in Colorado. Female adults laid a mean of 159.4 eggs and lived for a mean of 16.2 days. Adults are reproductively active throughout the year in warmer areas of North America but hibernate through the winter in colder regions (Harcourt 1957).

Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae) is the most common parasite of *P. xylostella. Diadegma insulare* is a solitary endoparasite of *P. xylostella* larvae. It emerges from the *P. xylostella* pupa and then spins a cocoon within the *P. xylostella* cocoon (Harcourt 1963)

Chapter 1: Trap Plant Sampling for Early Spring *C. torvina* Activity

Introduction

Field studies (Gaines 1992) indicated that *C. torvina* is active from late May through early October when adults commence winter diapause. Most *C. torvina* activity was seen from late June through September and four generations occurred at monthly intervals during that period. Although little activity was recorded before late June (a single *C. torvina* emerged from a *C. rubecula* cocoon recovered in the field in late May, 1990), it is possible that *C. torvina* is active as early as late March, and has five to six generations per year. However, it is difficult to detect early *C. torvina* activity by sampling field crops because hosts are scarce during this period (Gaines 1992). If overwintering *C. torvina* adults were common and became reproductively active in early spring, they could adversely impact the population of *C. rubecula* which overwinter in cocoons as pre-pupae. Thus, the objective of this study was to determine whether *C. torvina* is common and active early enough in the spring to harm an overwintering population of *C. rubecula*.

General Materials and Methods

Field studies were conducted during the months of March through July to assess *C. torvina*'s activity in that period. Because hosts are scarce in the field during the spring, laboratory reared *P. xylostella* were allowed to pupate on large potted "trap plants" which were subsequently placed in the field. These pupae then served as "trap hosts" for any foraging *C. torvina* and were left in the field for a week. Each week these trap hosts were collected and replaced with new hosts. The collected trap hosts were placed in labeled 30 ml plastic cups with ventilated lids, and held at 25°±2°C. The cups were monitored daily until all hosts or *C. torvina* or other parasites had emerged.

During the early spring season when weather was relatively cool, trap plants remained in pots and were collected from the field each week along with the trap hosts. These collected plants were taken to the greenhouse where the trap hosts were removed. Plants that were removed from the field were replaced by alternate potted plants laden with fresh trap hosts. Trap plants were prepared for placement in the field by allowing fourth instar *P. xylostella* to pupate on them while in the warm greenhouse environment. This was done because the fourth instar *P. xylostella* would have taken too long to pupate had they been placed on plants in the field in the cool, early spring weather.

Laboratory colonies of *P. xylostella* were maintained at high production levels so that there were sufficient fourth instars for the trap plants each week. Preparation of trap plants commenced three days before their placement in the field. Generally , about 15-20 fourth instars were placed on each plant and allowed to pupate. Typically, up to half of the larvae on a plant were lost by dropping off or crawling away, and only about 10 pupae were present on each plant when it was placed in the field. Accurate counts of pupae on the trap plants could not be made before placing the plants in the field because cocoons or pre-pupal larvae were easily dislodged as leaves were turned during the counting process.

Adult female *Diadegma insulare* (Hymenoptera: Ichneumonidae) (a parasite of *P. xylostella* larvae) were common in the *P. xylostella* colony cages and these were allowed to remain in the rearing cages so that a large percentage of the larvae were parasitized. *Plutella xylostella* parasitized by *D. insulare* remain as hosts (pupae) on the trap plants several days longer than unparasitized *P. xylostella* because the *D. insulare* emerge later than unparasitized *P. xylostella*. The percentage of *P. xylostella* parasitized by *D. insulare* varied from week to week and ranged from 55% to 85%.

During the late spring, trap plants were removed from their pots and transplanted into the ground. This procedural change was made because the warm late spring temperatures caused the large trap plants to desiccate rapidly within their pots, and plant maintenance required daily watering. Once in the ground, the plants were able to root in the surrounding soil and obtain moisture directly from the ground. Each week, fourth instar *P. xylostella* were carried to the field to be placed on the rooted plants after all the previous week's cocoons had been removed. Due to warmer weather in the late spring, the *P. xylostella* larvae pupated within a day or two of placement on the rooted plants.

Materials and Methods
Spring 1993

Brussels sprout plants (var. Long Island Improved) were started in flats in late December 1992 so that seedlings, subsequently transplanted to 6 liter capacity, plastic pots, would be at least 40 cm tall by March. Tall plants were desired as they would stand out among the shorter early spring vegetation.

By 23 March, the potted plants were placed in the field at two locations at the VPI&SU research farm, Whitethorne, Va. The first location (Site A) was a plot within an upland field which had been planted in *Brassica* crops in fall 1992. Within this plot, there were remnant collard and kale plants which had survived the winter. The second location (Site B) was located ≈0.75 km away in a bottom-land field plot. Site B had been planted with *Brassica* crops from 1987 to 1991 and had been the site of my 1989 and 1990 field studies (Gaines 1992). Although this plot was mostly covered with thistle rosettes, there were numerous wild Brassicaceae (yellow rocket, *Barbarea vulgaris*; wild radish, *Raphanus raphanistrum*; and hedge mustard, *Sisymbrium officinale*) growing in and around the surrounding fields. At each location, five potted trap plants were set into the ground in a 16 m square array with four plants as corners and the fifth plant in the center of the square.

In 1993, potted plants with trap hosts were placed in the field weekly for 9 weeks (from 23 March to 29 May). After 29 May, trap plants were removed from their pots and planted in the ground (in the 16 m square arrays). These rooted plants were sampled at three 9 day intervals from 30 May to 27 June. Large numbers of fourth instar *P. xylostella* (≈25 per plant) were placed on the rooted plants on 30 May because it was thought that many would be lost due to rain, or would crawl from the trap plants to adjacent *Brassica* plants that were remnants of the 1992 fall crop. About 15 *P. xylostella* larvae were placed on each trap plant in the subsequent two sample

periods because the *P. xylostella* colony's production rate had dropped. Placement of larvae on trap plants ceased on 27 June because plants were heavily damaged by aphids and harlequin bugs.

Spring 1994

Trap plant sampling, conducted at Sites A and B between 15 April and 7 June, ended for the most part by 22 May. Trap plant procedures were similar to those in 1993 except that only 10-15 larvae were used per plant, and there was no sampling from 22 April to 6 May due to a decline in host production. As in 1993, there were five plants in a 16 m square array at each sample site. During the period of 14-22 May, one plant at Site A and two at Site B were damaged by groundhogs (*Marmota monax*). These damaged plants were replaced on 22 May, but most of the plants at Site A, and all the plants at Site B were subsequently destroyed by groundhogs. On 29 May, trap plants were replaced at Site A, but not at Site B. In spite of fencing at Site A, groundhogs heavily damaged all but one of these five plants by 7 June. Sampling was discontinued after 7 June.

Spring - Summer 1995

In the spring of 1995, Site A was not available and only Site B was sampled. Trap plant activity did not start until 15 May, and 20-30 cm tall collards plants were used in the place of the Brussels sprouts which had not grown well. Eight plants were transplanted into the ground in a 16 x 32 meter rectangular array. On 15 May, 20 fourth instar *P. xylostella* were placed on each trap plant. Plants were examined thoroughly seven days later to recover *P. xylostella* cocoons. As in 1993, fresh larvae were placed on the trap plants at the beginning of each trap plant period. Trap plant sampling was conducted between 15 May and 7 July, but a three week interruption in sampling occurred between 23 May and 12 June due to a decline in the *P. xylostella* host colony.

Results and Discussion

Spring 1993

No *C. torvina* were recovered from hosts collected at either sample site even though a relatively large population of hosts was continuously present between 23 March and 27 June (Table 1.1, Figure 1.1). The number of hosts (*P. xylostella* cocoons, including those which contained *D. insulare* cocoons) collected at any site each week was generally between 50 and 65 (about 10-13 hosts per plant). Counts lower than 50 occurred on only four occasions (Table 1.1). The distribution of hosts among plants was generally uniform at each site. Occasionally fewer hosts were recovered on a particular plant and this may have been due to differences in the attractiveness among some of the Brussels sprout trap plants. *Plutella xylostella* larvae placed on these seemingly unattractive plants during plant preparation, tended to crawl or drop off the plants and may have ended up on adjacent plants. Several low host counts occurred at Site B during each of the last two sample dates, and this was probably due to *P. xylostella* larvae crawling off several plants that had extensive aphid and harlequin bug damage.

Although *C. torvina* was not recovered from any of the trap hosts, *Conura albifrons* (Walsh) was recovered in large numbers from both sites during the month of June (Table 1.2). *Conura albifrons* has reproductive behavior similar to *C. torvina*, and its extensive host range

includes many of the same Lepidopteran and Hymenopteran host species attacked by *C. torvina* (Krombein *et al.* 1979). *Conura albifrons* appeared at the same time at Sites A and B. The only other hyperparasite collected from hosts at Whitethorne in 1993 was a single, wingless female *Gelis* sp. (Hymenoptera: Ichneumonidae) that emerged from a *D. insulare* cocoon collected at Site B on 8 June. Host mortality (dead *P. xylostella* and *D. insulare* cocoons from which nothing emerged) accounted for about 11% of all host cocoons and never exceeded 20% at any site any week. In 1993, all dead cocoons from the Whitethorne sites were dissected, and only one, collected 27 June at Site A, contained a dead shriveled hyperparasite pupa (possibly a *Conura* sp.).

Spring 1994

In 1994 the trap plant sample period at Sites A and B lasted from 15 April to 7 June, with a gap in sampling activity between 22 April and 6 May (Table 1.3). The sample period of 14-22 May yielded trap hosts from only four plants at Site A and from three plants at Site B, due to damage by groundhogs. During the 22-29 May period, groundhog feeding completely destroyed all replacement plants at Site B and heavily damaged plants at Site A. The use of site B ceased after 29 May. While plants at site A were not destroyed, they were so heavily damaged by groundhogs in the final two sampling periods that generally, only a few plants had host cocoons left on them (Table 1.3). No *C. torvina*, *C. albifrons* or other parasitoids were recovered from hosts in 1994. Host mortality in the absence of parasitism was generally less than 10% of recovered hosts.

Spring - summer 1995

In 1995 host cocoons were collected from trap plants at Site B during the third week in May, the last two weeks in June and the first week in July (Table 1.4, Figure 1.1). The cocoon recovery rate was not as good from collard plants as it had been from Brussels sprout plants in 1993, and it may be that the larvae did not find the collard plants to be attractive and crawled away to pupate elsewhere. No parasitoids emerged from the hosts collected in May, but two *C. albifrons* emerged from hosts (one from *P. xylostella* and one from *D. insulare*) collected in the third week of June (Table 1.4). Seven other parasitoids (unidentified Ichneumonoids) were also recovered from the *P. xylostella* cocoons. In the fourth week of June, 12 *C. torvina* were recovered (eight from *P. xylostella* cocoons and four from *D. insulare* cocoons). Thirteen Ichneumonoids (unidentified) were also recovered from *P. xylostella* cocoons and one *Gelis* sp. was recovered from a *D. insulare* cocoon. In the final sampling period (29 June - 7 July) only 27 trap host cocoons were recovered from the trap plants, but *C. torvina* emerged from five of these cocoons (four from *P. xylostella* and one from *D. insulare*). Also recovered were: two unidentified Ichneumonoids (from *P. xylostella*), and two *Gelis* sp. hyperparasites (from *D. insulare*).

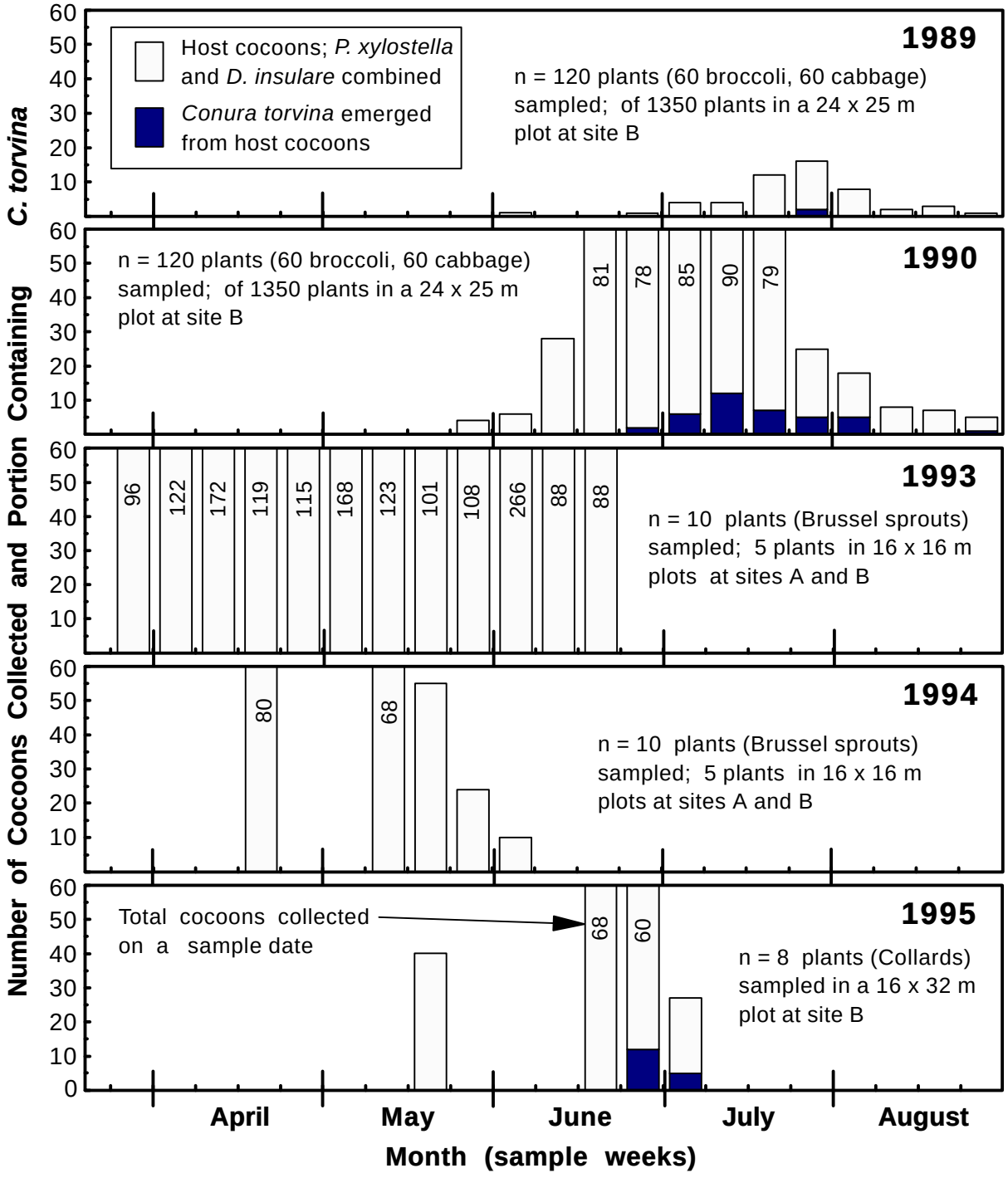

Figure 1.1: Early season occurrence of *Conura torvina* in *Plutella xylostella* and *Diadegma insulare* cocoons (combined); based on comparison of field sample data from cocoons collected from field crops at Site B in 1989 and 1990 (Gaines 1992), and from trap plants at Sites A and B in 1993, 1994, and 1995.

Table 1.1: Number of *P. xylostella* cocoons (containing both *P. xylostella* and *D. insulare* pupae) recovered from plants at Whitethorne sample sites A and B in 1993 [1].

Sample period	Site A			Site B		
	P. xylostella Recovered	*D. insulare* Recovered	**Total cocoons Recovered**	*P. xylostella* Recovered	*D. insulare* Recovered	**Total cocoons Recovered**
Mar. 23 - Mar. 30	15	30	**45**	21	30	**51**
Mar. 30 - Apr. 7	20	43	**63**	13	46	**59**
Apr. 7 - Apr. 14	22	66	**88**	30	54	**84**
Apr. 14 - Apr. 21	17	44	**61**	15	43	**58**
Apr. 21 - Apr. 28	21	35	**56**	31	28	**59**
Apr. 28 - May 8	48	44	**92**	45	31	**76**
May 8 - May 14	10	59	**69**	12	42	**54**
May 14 - May 21	14	45	**59**	10	32	**42**
May 21 - May 29	17	36	**53**	25	30	**55**
May 30 - June 8	31	100	**131**	25	110	**135**
June 8 - June 18	23	28	**51**	8	29	**37**
June 18 - June 27	14	45	**59**	12	17	**29**

[1] 5 plants per site

Table 1.2: Number of *P. xylostella* cocoons (*P. xylostella* and *D. insulare* combined) from which parasitoids were recovered, and number of dead cocoons (cocoons from which nothing emerged) in 1993.

Sample period	Site A				Site B			
	Cocoons Total	Parasitoids [1]		Dead Cocoons	Cocoons Total	Parasitoids [1]		Dead Cocoons
		Conura spp.[2]	Other			*Conura* spp.[2]	Other	
Mar. 23-30	45	0	0	6	51	0	0	2
Mar. 30 - Apr. 7	63	0	0	12	59	0	0	11
Apr. 7-14	88	0	0	14	84	0	0	6
Apr. 14-21	61	0	0	6	58	0	0	2
Apr. 21-28	56	0	0	8	59	0	0	10
Apr. 28 - May 8	92	0	0	11	76	0	0	3
May 8-14	69	0	0	12	54	0	0	5
May 14-21	59	0	0	3	42	0	0	2
May 21-29	53	0	0	2	55	0	0	8
May 30 - June 8	131	**1** [b]	0	10	135	**19** [b]	1	22
June 8-18	51	**16** [b]	0	5	37	**3** [b]	0	4
June 18-27	59	**2** [b]	0	1	29	0	0	5

[1] Parasitoids = Parasites of *P. xylostella* (excluding *D. insulare*) and hyperparasites of *D. insulare*

[2] *Conura* spp. = *Conura torvina* [a] or *Conura albifrons* [b]

Table 1.3: Number of *P. xylostella* cocoons (containing both *P. xylostella* and *D. insulare* pupae) recovered from plants at Whitethorne Sample sites A and B in 1994 [1].

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Sample period	Site A			Site B		
	P. xylostella Recovered	*D. insulare* Recovered	**Total cocoons Recovered**	*P. xylostella* Recovered	*D. insulare* Recovered	**Total cocoons Recovered**
Apr. 15-22	41	6	**47**	25	8	**33**
Apr. 22-29 [2]	*	*	*	*	*	*
Apr. 29 - May 6 [2]	*	*	*	*	*	*
May 6-14	26	2	**28**	32	8	**40**
May 14-22	21	7	**28**	19	8	**27**
May 22-29 [2]	17	7	**24**	**	**	**
May 29 - June 7 [2]	7	3	**10**	*	*	*

[1] 5 plants per site

[2] * = No trap plant activity; ** = Trap plants destroyed

Table 1.4: Number of *P. xylostella* cocoons (containing both *P. xylostella* and *D. insulare* pupae) recovered from five plants at Whitethorne Sample site B in 1995, and number of these cocoons which contained *C. torvina*, other parasitoids, or were dead (cocoons from which nothing emerged).

Sample period	Site B					
				Parasitoids [2]		
	P. xylostella	*D. insulare*	**Total cocoons**	*Conura* spp. [3]	Other	Dead Cocoons
May 15-22	32	8	**40**	0	0	3
May 22-29 [1]	*	*	*	-	-	-
May 29 - June 6[1]	*	*	*	-	-	-
June 6-13 [1]	*	*	*	-	-	-
June 13-21	35	33	**68**	**2**[b]	7	8
June 21-29	41	19	**60**	**12**[a]	14	12
June 29 - July 7	17	10	**27**	**5**[a]	4	4

[1] * = No trap plant activity.

[2] Parasitoids = Parasites of *P. xylostella* (excluding *D. insulare*) and hyperparasites of *D. insulare*

[3] *Conura* spp. = *Conura torvina* [a] or *C. albifrons* [b]

Conclusions

In 1989 and 1990, *C. torvina* were recovered from hosts collected from field crops (Gaines 1992) (Figure 1.1). In 1993 *C. albifrons* was recovered and in 1995 both *C. albifrons* and *C. torvina* were recovered from trap plant hosts. This indicates that trap plants are a viable method of monitoring hyperparasite activity. The recovery of *C. torvina* in 1989 and 1990 was accomplished by sampling 120 plants out of a field of about 1,350 plants each week, whereas *C. torvina* was recovered from sampling only eight trap plants each week in 1995. Although the 1989, 1990 and 1995 studies were conducted in the same field, there is no evidence that the actual *C. torvina* population density was the same each year. Thus, although it appears that the trap plant method requires sampling considerably fewer plants to detect *C. torvina* activity there is no metric by which to accurately compare the efficiency of these two sampling methods.

Evidence from trap plant sampling from 1993 to 1995 indicates that *Conura* spp. activity is uncommon or absent until late June even though hosts were present in large numbers throughout the spring. *Conura torvina* populations do not become abundant until after the third week in June (Figure 1.1), at which time they may occasionally be obtained from a relatively small collection of hosts. Some possible reasons for the lack of *C. torvina* activity during spring are: (a) field populations of adult *C. torvina* so small and dispersed that detection would require extensive sampling with trap hosts on trap plants, (b) the overwintering *C. torvina* adults are reproductively active in spring, but do not seek hosts in the field crop environment, and (c) overwintering *C. torvina* adults do not become reproductively active until early summer. Thus, it is unlikely that this hyperparasite could pose any danger to the pupae of overwintering parasitoids associated with *Brassica* crops, such as *C. rubecula*.

Chapter 2: Factors That Influence the Reproductive Behavior and Success for *C. torvina*

Introduction

Studies on the reproduction of *C. torvina* involve examination of environmental, behavioral and biological factors. *Conura torvina'*s reproductive behavior and success can be influenced by external factors such as the environment, host species or the biology of its hosts. Therefore, it is important to examine these factors and to quantify their effect.

Three studies were conducted to examine the effects of external factors. The first examined the effect of host age on *C. torvina* reproductive behavior and success, and included several "host age experiments" that tested *Cotesia rubecula* hosts of various ages. The second study examined the influence of three different host species (*Cotesia orobenae, C. rubecula* and *Plutella xylostella*) on *C. torvina* reproductive behavior, host preference and success. These experiments were designated as "host species choice experiments". The third study examined the effect frass from several hosts as a stimulant for *C. torvina* oviposition behavior and were designated as "frass experiments".

General Materials and Methods

Conura torvina females used in the following experiments were all kept individually in cages made from ventilated 200 ml plastic vials. Each vial had round ventilation holes 40 cm and 30 cm diameter in its bottom and side, respectively. Plastic 0.3 mm mesh screen covered these holes. The vial cages stood bottom up, and the plastic vial cap served as the base of each cage. *Conura torvina* was fed by placement of a drop of honey on the screened cage top. Honey was renewed as it was consumed. Watering was accomplished by misting the screen daily with a hand mister. Hosts were provided by scattering them on the inside of the vial cap that served as the cage bottom.

Chapter 2, Part 1: Influence of Host Age on *C. torvina* Oviposition and Progeny Survival

Introduction

As several studies have indicated that a parasitoid's acceptance of a host may be influenced by the host's age (Vinson 1976, 1985), it is possible that suitability of *C. torvina*'s pupal hosts may vary with pupal age. A female *C. torvina* may be able to detect the age of a host and avoid oviposition in an unsuitable host, or she may oviposit an unfertilized egg in the less suitable host. Understanding the effect that host age has on *C. torvina* oviposition behavior, progeny sex ratios and progeny survival rates will permit a better assessment of *C. torvina*'s reproductive potential in a particular population of hosts. Most host populations are composed of individuals of different ages, and only a small portion might be suitable as hosts at any one time.

Identifying the optimum host age allows us to provide more suitable hosts for experimental use. The elimination of unsuitable hosts from an experiment reduces that source of variation.

Host age effects were tested by providing *C. rubecula* pupae of different ages to *C. torvina*. The aim was to determine whether *C. torvina* can successfully parasitize hosts of different ages and detect host quality during oviposition. The number and sex of resulting *C. torvina* progeny, number of hosts that escaped parasitization, and number of dead hosts from which nothing emerged indicated the suitability of hosts at each age. *Cotesia rubecula* was used in the host age experiments because it is a host of major interest. Some Hymenopterous parasitoids evaluate a host's age from visual cues such as its color (Vinson 1976, 1985), but because the pupae of *C. rubecula* occur within thick cocoons, visual host age cues are hidden from *C. torvina*. By eliminating visual cues as a possible means of detecting host age, *C. torvina* had to judge its hosts only by chemosensory cues such as gustatory or olfactory cues and or cues obtained from ovipositor sensory structures. Two host age experiments were conducted, the first involved continuous host provisioning and the second involved intermittent host provisioning.

Materials and Methods

First Host Age Experiment - Continuous Host Provisioning

The first experiment was conducted in two repetitions due to the difficulty of obtaining sufficient *C. rubecula* cocoons ≤12 h in age. Single *C. torvina* females were used as replicates and 5 replicates were used per repetition. The females used in each repetition were from different cohorts, but were all about same age (about 31 and 27 days old in the first and second repetition, respectively). Each female was caged individually in a vial cage and had been mated and provided with hosts for oviposition experience before the experiment. Pre-experimental oviposition experience was provided because preliminary testing indicated that inexperienced *C. torvina* have an initially low reproductive rate that gradually increases with each day of oviposition experience, and stabilizes after three or four days. Females tested in the first repetition were given three pre-experimental host provisionings during a two week period before the experiment. Females from the second repetition were slightly more experienced, having received hosts five times in a two week period before the experiment. For each repetition, the final pre-experimental host provisioning was made two days before the start of the experimental host provisionings. The experiment was conducted in an environmental chamber at 25°C. Five host age treatments (hosts 1, 2, 3, 4, and 5 days old) were tested because the *C. rubecula* pupal (cocoon) stage lasts just over five days at 25°C. A control, consisting of *C. rubecula* cocoons not exposed to *C. torvina*, was included. Mortality of control hosts provided an indication of host quality or health.

Collection of experimental hosts commenced two days before the experiment. Cocoons were collected twice daily from the host colony so that all collected cocoons were ≤12 h old. Collected cocoons were placed directly into an environmental chamber set at about 12°C to retard pupal development until sufficient cocoons had been collected to conduct a repetition. For each repetition, cocoons were divided into 30 groups of seven. The treatments and control were each assigned five groups of seven hosts.

From the outset of the experiment all *C. torvina* and hosts were maintained in an environmental chamber set at 25°C with a 15L:9D photoperiod. On day one, each of the five *C. torvina* females was given seven cocoons that were 1 day old. After 24 h, these exposed cocoons were removed and replaced with cocoons that were 2 days old. This procedure was repeated daily so that on the fifth day females were given 5 day old hosts. After five days all the hosts except for those in the control had been exposed. Starting from day five, hosts from each age group and the control were monitored daily, and the number, sex and date of all emerging hosts and *C. torvina* were recorded.

The second repetition of the host age experiment was conducted in essentially the same manner and under the same conditions. However, a sixth *C. torvina* female was tested in this repetition. This sixth female served as a control to measure the effect of continuous oviposition activity on same age hosts over a period of five days. This sixth *C. torvina* was given seven, 1-2 day old, cocoons each day.

The design of this experiment would have been improved if the host age treatments could have been randomized. Each day, each female received hosts of a consecutively older age group, allowing the possibility that female fatigue or other effects associated with time or experience would confound the treatment effects. Treatments also lacked independence because they were each applied to the same females. Various modes of data analyses were tried for this experiment and all yielded similar results. Although the non-random order and dependence of the host age treatments pose a problem within any mode of analysis, an ANOVA for a Complete Block Design appeared to be the most appropriate. This was because each female was provided with all five host age treatments, and thus, could be considered to be a block of treatments. Therefore, an ANOVA for Randomized Complete Block Design was used, and each of the 10 females (from both repetitions combined) served as a block to which all treatments had been applied. Treatment data for the number of *C. torvina* progenies emerging, hosts emerging and dead hosts (host cocoons from which nothing emerged) were compared as mean % of the number of hosts provided. An Arcsine[$\sqrt{x}$] transformation was used on % data for analysis (Zar 1984). Treatment means for the number of female *C. torvina* emerged and the progeny development times were also compared. Post ANOVA comparison of treatment means used Tukey-Kramer's Multiple Comparison Test (α=0.05) (Hintze 1995).

Second Host Age Experiment - Intermittent Host Provisioning

This experiment was conducted to test host provisioning every second day because five continuous days of oviposition activity might have an effect on the reproductive capabilities of *C. torvina*. Thus, *C. torvina* females were each provided seven cocoons of hosts aged 1, 3, and 5 days. There were five replicates. Except for the difference in treatments, this experiment was conducted in the same manner and under the same conditions as the first experiment. Females used in the second experiment were from the same cohort, and had the same amount of pre-experimental host experience as those tested in the second repetition of the first experiment. The intermittent host provisioning plan allowed measurement of the effects of host age and also of a

one day rest period between host provisionings. It was analyzed using the same ANOVA and post ANOVA procedures and data transformations as were used in the first experiment.

Results and Discussion

A fairly consistent pattern was found in both experiments in which 3 day old host cocoons appeared to be the most suitable as they had the greatest proportion of emerging *C. torvina* progenies (Table 2.1.1, Table 2.1.2). However, the proportion of 3 day old hosts yielding *C. torvina* progenies was not significantly different (α=0.05) from that of the 1 and 2 day old hosts. Five day old hosts appeared least suitable because significantly fewer *C. torvina* progenies emerged from them in both experiments (Table 2.1.1, Table 2.1.2). Three day old hosts appeared to have the lowest proportion of host emergence (hosts that escaped parasitization), but the difference was not significant between hosts 1-4 days old. Significantly more 5 day old hosts emerged and this is an indication that *C. torvina* found them undesirable. The proportion of dead hosts (host cocoons from which nothing emerged) also appeared to be smallest in 3 day old and greatest in 5 day old hosts. The difference was not significant in the first experiment, but was significant in the second experiment (Table 2.1.1, Table 2.1.2). Three day old hosts appeared to have the lowest proportion of dead, in conjunction with the greatest proportion of emerging *C. torvina* progenies, and this indicates that they are optimal for *C. torvina* progeny survival. Conversely, a greater proportion of dead hosts with fewer *C. torvina* emerging implies failed parasitization (neither the *C. torvina* progeny or its host survive).

Dead hosts ranged from 15.7% in the 3 day old hosts to 28.5% in the 5 day old hosts. Mortality in the control hosts was the lowest, at 4.3%. Control mortality was significantly lower than that in the 1, 4 and 5 day old hosts in the first experiment (Table 2.1.1), and in the 5 day old hosts in the second experiment (Table 2.1.2). This indicates that some of the dead hosts occurring in the host age treatments resulted from *C. torvina* activity (i.e., host feeding or failed parasitism).

In the first experiment, *C. torvina* progeny development time was significantly longer in the 5 day old hosts than in the 1-3 day old hosts. Developmental time appears to be shortest in the 2 day old hosts, although there was no significant difference among 1 to 3 day old hosts. In the second experiment, developmental time was shortest in the 3 day old hosts, and longest in the 5 day old hosts, but differences between the treatments were not significant. Progeny development time may indicate host quality because developmental time was consistently longer in the oldest hosts. The slower *C. torvina* growth may be due to poor nutrient quality of the host. At 25°C, *C. rubecula* pupae ≥4 days of age could be described as pre-adults, because adult structures, coloration and formation of the exoskeleton are evident. In such a host, *C. torvina* larvae would be restricted in space and in nutrient (the amount of soft tissue to consume, its nutritional quality or its digestibility).

In both experiments, the number and proportion of *C. torvina* progenies that were female was greatest in one day old hosts and declined with increasing host age. No female *C. torvina* emerged from 5 day old hosts (Table 2.1.1, Table 2.1.2). If *C. torvina* are able to sense host

Table 2.1.1: Effect of host (*Cotesia rubecula*) cocoon age on *Conura torvina* reproductive success (n = 10 *C. torvina* females; each female was provided with seven hosts per day on days 1-5).

Response Variables	Treatments (Host age, Control hosts)							
	1 day $\bar{x}$	2 days $\bar{x}$	3 days $\bar{x}$	4 days $\bar{x}$	5 days $\bar{x}$	Control [2] $\bar{x}$	p [1]	MSE
% *C. torvina* emerging from host cocoons	61.4 bc	64.3 bc	80.0 c	60.0 b	27.1 a		<0.001	137.2
% Hosts emerging from cocoons	12.9 a	14.3 a	4.3 a	12.9 a	42.9 b	95.7 bc	<0.001	186.6
% Dead Hosts (Cocoons from which nothing emerged)	25.7 b	21.4 ab	15.7 ab	27.1 b	28.5 b	4.3 a	0.007	216.6
Development time (Days from oviposition to adult emergence)	17.1 a	16.9 a	17.2 ab	17.8 bc	18.3 c		<0.001	0.217
Female *C. torvina* progenies emerging	2.3 c	1.9 c	1.6 bc	0.5 ab	0.0 a		<0.001	1.097
Females, as a % of emerged *C. torvina*	53.5	42.2	28.6	11.9	0.0			

[1] Means within row followed by the same letter are not significantly different $\alpha = 0.05$; Tukey-Kramer Means Separation Test; ANOVA for Randomized Complete Block Design; MSE = Mean Square Error from ANOVA), (Arcsine ($\sqrt{x}$) transformation used for analysis of all % data; reported mean values are actual untransformed percentages).

[2] Control hosts not exposed to *C. torvina* (n = 10 replications, 7 hosts per replication).

Table 2.1.2: Effect of host (*Cotesia rubecula*) cocoon age on *Conura torvina* reproductive success (n = 5 *C. torvina* females, each provided with seven hosts per day on days 1, 3, and 5).

Response Variables	Treatments (Host age, Control hosts)									
	1 day $\bar{x}$		3 days $\bar{x}$		5 days $\bar{x}$		Control [2] $\bar{x}$		p [1]	MSE
% *C. torvina*, emerging from cocoons	85.7	b	97.1	b	65.7	a			0.018	177.1
% Hosts emerging from cocoons	2.9	a	0.0	a	5.7	a	91.4	b	<0.001	111.0
% Dead Hosts (Cocoons from which nothing emerged)	11.4	ab	2.9	a	28.6	b	8.5	a	0.033	162.6
Development time (Days from oviposition to adult emergence)	16.7	a	16.5	a	17.1	a			0.123	0.143
C. torvina , female progenies emerging	2.0	b	1.0	ab	0.0	a			0.032	0.917
Females, as a % of emerged *C. torvina*	33.3		14.7		0.0					

[1] Means within row followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer Means Separation Test; ANOVA for Randomized Complete Block Design; MSE = Mean Square Error from ANOVA), (Arcsine ($\sqrt{x}$) transformation used for analysis of all % data; reported mean values are actual untransformed percentages).

[2] Control hosts not exposed to *C. torvina* (n = 5 replications, 7 hosts per replication).

quality and oviposit fertile eggs in only the most suitable hosts, the proportion of female progenies would be a good indicator of host quality. Because the highest proportion of *C. torvina* females emerged from one day old hosts, these could be considered as the most optimally aged hosts for *C. torvina* reproduction. However, although the difference is not significant, data from both experiments appear to indicate that three day old hosts were more optimal than one day old hosts because 3 day old hosts had the greatest proportion successfully parasitized. Hypothetically, if the 3 day old hosts were superior, they also should have yielded the highest proportion of female *C. torvina* progenies, but this did not happen. Some possible explanations for this result are that: (1) *C. torvina* perceived one day old hosts as most suitable when actually the three day olds are superior; (2) the improved progeny survival in three day old hosts resulted from improved oviposition behavior through *C. torvina*'s additional oviposition experience; or (3) the decline in the proportion of female progenies resulted from oviposition fatigue (declining egg production).

In the second experiment, hosts were provided to *C. torvina* every second day to test the effect of a rest period between ovipositions. Results from this experiment followed the same pattern as the results from the first host age experiment. However, results appeared to indicate that *C. torvina* progeny survival was higher, across all host ages, in the second experiment than in the first experiment (Table 2.1.1, Table 2.1.2). If intermittent host provisioning had an effect on the reproductive success rate of *C. torvina* it should not show up until the second host provisioning, i.e., after one day's rest between oviposition periods. The females used in the second experiment appeared to have greater reproductive capabilities on the first day hosts were provided, than those in the first experiment. Therefore, it is likely that the higher reproductive success rate seen throughout the second experiment resulted from superior females and not from the effect of intermittent host provisioning.

In both experiments, the differences in the proportion of hosts parasitized at various host ages could be due to several factors. First, *C. torvina* may be able to detect the quality (age) of a host and choose to oviposit or not oviposit. Thus, when hosts are less suitable, the resulting proportion of emerged hosts (unparasitized hosts) should be greater. This occurred with 5 day old hosts in the first experiment (Table 2.1.1), but not in the second experiment (Table 2.1.2). Second, *C. torvina's* success may be reduced by its inability to survive in hosts of an unsuitable age and this would be indicated by a higher proportion of dead hosts. This occurred in the second experiment, where there was a significantly higher proportion of dead 5 day old hosts. These experimental results suggest that *C. torvina* can discern a host's age, but will sometimes oviposit in an unsuitable host. It is possible that when *C. torvina* is confined, it will oviposit in any host. A better test of *C. torvina*'s ability to discern host quality and oviposit selectively may require an experiment in which it can choose among hosts of various ages.

The *C. torvina* females which were provided hosts on five consecutive days showed a significant decline in their reproduction and their proportion of female progenies in the last two days of the experiment. It is possible that the decline was due to oviposition fatigue (declining egg production) instead of host age. However, some evidence does not support this idea. During this experiment a single female *C. torvina* given seven (2 day old) hosts on five consecutive days,

produced 6, 7, 5, 7 and 7 progenies from the hosts on days 1-5, respectively. The proportion of progenies that were female on days 1-5 was 50%, 85%, 20%, 14% and 14%, respectively. Thus, it shows that *C. torvina* can successfully parasitize 5-7 hosts per day on five consecutive days and produce female progenies (fertile eggs) after five consecutive days of oviposition. These results are supported by the continuous host provisioning experiments detailed in chapter 3, Part 1 (Figure 3.1.4 indicates that female progenies can be produced on each of seven consecutive days by *C. torvina* provided with *C. rubecula* hosts). Results from the continuous host provisioning experiments are additional evidence that the effects observed in the above host age experiments were due to host age.

Host Age Experiments

Conclusions

These data indicate that host age affects the reproductive success of *C. torvina*. *Cotesia rubecula* cocoons that were reared at 25°C and used as *C. torvina* hosts, were most suitable at 1-3 days of age. These hosts enabled higher rates of parasitization, higher progeny survival and higher proportions of female *C. torvina* progenies than the 4-5 day old hosts. Thus, *C. rubecula* cocoons used in all subsequent experiments were no more than three days old (i.e., pupal stage was $\leq 3/5$ or 60% complete).

Conura torvina is able to develop in older pupae but its survival rate and the proportion of female progenies which emerge is reduced. *Conura torvina*'s reduced reproductive rate in older *C. rubecula* pupae, may be due to both to its ability to discern poor host quality and to the effects of host quality on progeny survival.

Chapter 2, Part 2: *Conura torvina* Host Species Choice Experiments

Introduction

Conura torvina is categorized as a generalist parasitoid because it parasitizes pupae of more than 50 host species in the Orders Lepidoptera, Coleoptera and Hymenoptera. Some generalist parasitoids can have a preferred host, but may casually oviposit in any other species of host encountered while foraging (Vinson 1976, 1985). A generalist parasitoid may also be solely opportunistic (have no specific host preference), but may oviposit in any suitable host within a preferred environment. It is not known whether *C. torvina* has any host preferences. Host preference may be based on factors such as host size, smell, taste or movement (DeBach 1964, Vinson 1976, 1985). A parasitoid may also be conditioned to the odor of the host species from which it emerged and, thus, prefer that species (Vinson 1976, 1985). Host preference may be indicated by how frequently one host species is chosen relative to other available species or, if the parasitoid oviposits in all host species present, by the outcome of the oviposition (a parasitoid may lay more fertile eggs in preferred hosts than in non-preferred hosts).

General Materials and Methods

Two "host choice" experiments were conducted to determine whether *C. torvina* had preference for any host species. The three host species tested (*P. xylostella, Cotesia orobenae* and *C. rubecula*) occur in the *Brassica* crop environment, but differ either in size or appearance. *Plutella xylostella* and *C. rubecula* differ considerably in appearance, but are of similar size (*P. xylostella* cocoons are slightly larger than those of *C. rubecula*). *Cotesia rubecula* and *C. orobenae* are of similar appearance, but *C. orobenae* cocoons are less than half as large as those of *C. rubecula*. The female *C. torvina* used in these experiments were between one and three weeks old and had no previous oviposition experience or exposure to hosts. Experienced females were not used as they might show some preference toward the hosts to which they were last exposed.

The influence of *C. torvina*'s "host origin" (host from which it emerged) on its subsequent host preference was also tested. Equal numbers of *C. torvina* females originating from two different host species (*C. rubecula* and *P. xylostella*) were used in the mixed host treatments. The only notable difference between female *C. torvina* originating from these two hosts was size; those from *C. rubecula* and *P. xylostella* averaged 3.3±0.2 mm and 3.6±0.3 mm in length (frons to abdomen tip), respectively ($\bar{x}$ ±sd; n=20 for each group). An attempt was made to include female *C. torvina* originating from *C. glomerata* or *C. orobenae* hosts because these females would have been comparatively small (<3 mm) and their smaller size may have improved their acceptance of smaller hosts. However, efforts to obtain female *C. torvina* progenies from these hosts were unsuccessful; all but a few of the progenies resulting from hundreds of these parasitized hosts were male.

First Host Choice Experiment - Three Host Species

Materials and Methods

In this experiment the hosts *C. rubecula*, *P. xylostella* and *C. orobenae* were tested. Female *C. torvina* originating from either *P. xylostella* or *C. rubecula* were divided into two groups (based on their original host) and each group was assigned a mixture of hosts or its original host species. Treatments and groups were as follows:

Treat. 1	A mixture of nine cocoons (3 *C. rubecula*, 3 *P. xylostella* and 3 *C. orobenae*) provided to each female each provisioning.	n = 10
Group 1	*C. torvina* originating from *C. rubecula* hosts.	n = 5
Group 2	*C. torvina* originating from *P. xylostella* hosts.	n = 5
Treat. 2	Nine *P. xylostella* cocoons provided to each female each provisioning; *C. torvina* originating from *P. xylostella* hosts.	n = 5
Treat. 3	Nine *C. rubecula* cocoons provided to each female each provisioning; *C. torvina* originating from *C. rubecula* hosts.	n = 5

where n = the number of replications (female *C. torvina*) in each treatment or group within a treatment.

Host cocoons were provided to each female *C. torvina* every three days for 21 days (8 times). Due to an initial shortage of hosts, only six hosts (two of each species in the mixed treatments) were provided per female on the first day. This probably had little restrictive effect on the number of hosts parasitized because various preliminary experiments showed that naive (inexperienced) *C. torvina* generally affect fewer than six hosts on their first encounter.

Before the experiment, a male was introduced into each female's vial cage for mating purposes. Females were assumed to be fertile although not all were known to have mated; within an hour of being paired, mating was observed in >70% of the cages. Females which had been observed in copula were distributed as evenly as possible among the treatments and groups within treatments. Host cocoons were provided by random placement on the floor (inverted vial lid) of the cage. Cages from all treatments were held in an environmental chamber set at 25°C, with a 14L:10D photoperiod. After each host provisioning the cages were placed randomly on trays within the chamber. All exposed hosts were maintained in this environmental chamber after removal from *C. torvina*'s cages.

Although hosts were provided to females every three days, the duration of the host provisioning changed as the experiment progressed. During the first three provisionings, hosts were provided for 14 h periods. During the second three provisionings hosts were provided for 7 h periods and the last two provisionings only lasted 3.5 h each. Long exposures were used at the beginning of the experiment to stimulate *C. torvina* oviposition behavior. By the fourth host exposure, parasitization had been confirmed in most of the treatment replicates and the host

exposure period was reduced to seven hours. It was thought that if preferred hosts were selected first (in the "mixed host" treatments), the reduced time would restrict any further host selection, revealing a host preference. After three seven hour host provisionings, there was no detectable decrease in the number of hosts parasitized, so the exposure duration was reduced to 3.5 h for the remaining two provisionings. The experiment ceased after eight provisionings.

All hosts provided were less than two days old. Although neither *P. xylostella* nor *Cotesia orobenae* had been tested in a host age experiment, I assumed that pupae aged <3 days old would be optimal for *C. torvina,* because the development period for these pupae is about five days at 25°C (similar to that of *C. rubecula*). Hosts collected two to three days before provisioning were placed in containers and held at 10°C to retard their development. On each host provisioning day, a control of nine randomly selected cocoons of each host species were placed in vials with no *C. torvina*. This control served as a measure of host viability.

The number, sex and emergence date were recorded for all *C. torvina* progenies emerging from different hosts. The number of dead hosts was also recorded. All dead hosts were dissected to check for remnants of un-emerged *C. torvina*.

Data from the fourth through sixth host provisionings were used for statistical analysis. Data from the first three provisionings were not included in the analysis because the response to hosts by inexperienced *C. torvina* females was highly variable. Their reproductive rate had not stabilized. No analysis was done on data from the last two (3.5 hour) provisionings because several females failed to produce progenies in their hosts. Females which produced no progenies during the experiment were considered defective and data from these females were excluded from analysis. Data from the mixed host treatment were first compared between females from each "host origin" treatment group. Because each female in the mixed treatment received all three host species, she was considered to be a block to which three treatments (host species) were applied. Data for *C. torvina* emergence, host emergence, and % female *C. torvina*, were compared. To test the difference between the females of different host origin to different host species, these means were averaged across the fourth through sixth provisioning for each host species. Comparison was based on a Two way ANOVA for a Randomized Complete Block Design (RCBD). Next, data from females in both treatment groups were combined (n=10 females) to compare the overall reaction of females to each host species within the mixed host treatment. This analysis used data from each of the fourth through sixth host provisioning in a Repeated Measures ANOVA for RCBD. The Repeated Measures ANOVA provides a more precise comparison of treatment effects than an analysis of the average of daily measurements, because it accounts for variation among each day (Norman & Streiner 1994). Finally, the totals (all hosts species combined) from the mixed host treatment were compared with totals from females in the *P. xylostella* and *C. rubecula* host treatments. A standard Repeated Measures ANOVA was used for this analysis. All ANOVA means were compared using the Tukey-Kramer means separation test (α=0.05). An Arcsine($\sqrt{x}$) transformation was used on all proportion data before analysis (Zar 1984, Hintze 1995).

Results and discussion

The first 14 hour host provisioning stimulated oviposition activity with all but one female in the *C. rubecula* host treatment, and this female failed to oviposit throughout the experiment. Thus, the number of replicates in the *C. rubecula* treatment was reduced to four (Table 2.2.1). Typically fewer than three *C. torvina* progenies resulted from the hosts provided on the first exposure although one female in the *C. rubecula* host treatment successfully parasitized all six of the provided hosts. The mean reproductive rate for females in all treatments increased from the first through third provisioning and then leveled off. This progressive increase in the number progenies per female indicates that naive *C. torvina* females needed at least three host provisionings before their oviposition behavior stabilized (Figure 2.2.1). From the fourth through the sixth host provisioning, the treatment means for the number of *C. torvina* progenies per female were relatively stable (ranging between 5.5 and 7.5 per female) (Figure 2.2.1). There was a slight reduction in the mean number of progenies produced per female in the last two host provisionings and this was due, in part, to reproduction having declined or ceased in several females, and in part, to the shorter (3.5 h) duration of the host provisioning period. The reproductive decline was most notable in a female from the *P. xylostella* treatment and its cause is unknown.

In the mixed host treatments, no significant differences (α=0.05) could be seen in the response to hosts by female *C. torvina* of different host origins. A female's host origin did not affect either the mean number of *C. torvina* progenies per female (p=1.00) or the mean % female progenies emerging (p=1.00). The interaction of the host species and host origin factors was also not significant for mean number of *C. torvina* progenies (p=0.362) and mean % female progenies (p=0.730). Thus, *C. torvina*'s host origin has no apparent effect on its subsequent host preference.

The mixed host treatment was analyzed with data from n=10 females (both original host groups combined) (Table 2.2.1). There was no significant difference in the number of *C. torvina* progenies emerging from *P. xylostella* or *C. rubecula*, but significantly fewer *C. torvina* emerged from *C. orobenae*. The pattern is similar for the number of hosts emerging; significantly higher numbers of *C. orobenae* emerged than the other two hosts. This response indicates that *C. torvina* did not oviposit in many of the relatively small *C. orobenae* hosts. No female *C. torvina* emerged from *C. orobenae*, and a significantly higher proportion of females emerged from *P. xylostella* (58%) than from *C. rubecula* (30%). Although nearly equal numbers of *C. rubecula* and *P. xylostella* were parasitized, *P. xylostella* appears to be the favored host because of the higher proportion of fertile eggs laid in it. Typically, most of the three *C. rubecula* and three *P. xylostella* provided for each female were successfully parasitized, but the *C. orobenae* were ignored. Thus, the presence of *C. orobenae* essentially reduced the number of desirable hosts available from nine to six, lowering the mean number progenies per *C. torvina* female in the mixed host treatment.

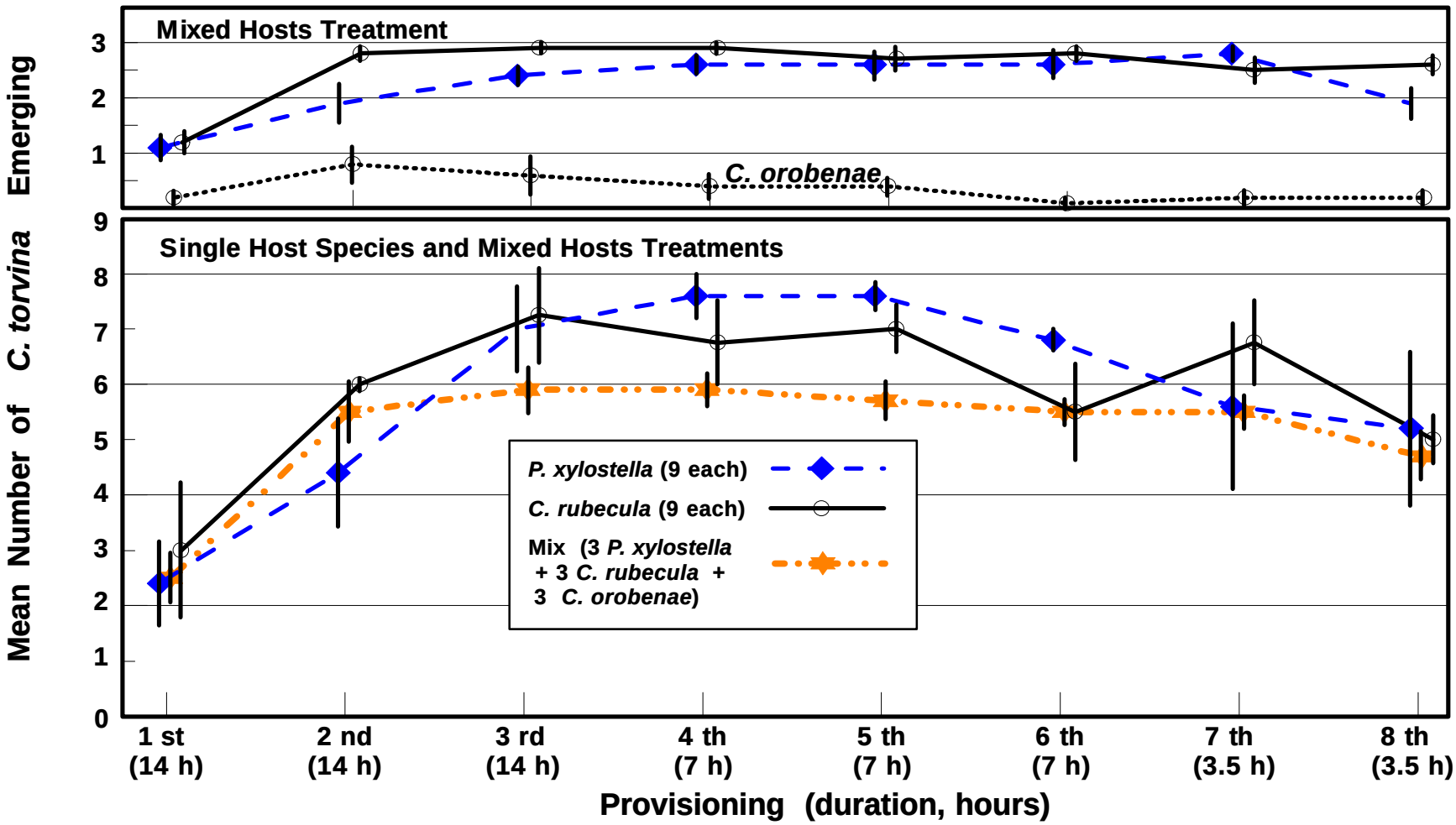

Figure 2.2.1: Effect of three host species on *Conura torvina* reproductive success. Comparison of treatments in which *C. torvina* females were provided with nine *Plutella xylostella* or nine *Cotesia rubecula* cocoons or were provided with a mixture of three host species (three each of *P. xylostella*, *C. rubecula* and *Cotesia orobenae*) (error bars = mean ± SE).

Table 2.2.1: Effect of three host species on *Conura torvina* host selection and reproductive success [1].

TREATMENT (Host species and number provided per day)	**n**[1]	Mean Emerging *C. torvina* Adults	Mean Emerging Hosts (non parasitized)	*C. torvina* Emerging (Mean % Female)
Mixture of hosts (9 per provisioning)	**10**			
P. xylostella (3 each)		2.60 a [2]*	0.17 b [2]*	58.0 a [2]**
C. rubecula (3 each)		2.80 a	0.07 b	30.0 b
C. orobenae (3 each)		0.30 b	2.67 a	0.0 c
		MSE[1] = 0.419 p <0.001	MSE = 0.285 p <0.001	MSE = 179.1 p <0.001
Mixture of hosts (9 per provisioning: 3 *P. xylostella* + 3 *C. rubecula* + 3 *C. Orobenae*)	**10**	5.70 b [3]*	2.90 a [3]*	35.5 a [3]**
P. xylostella hosts (9 per provisioning)	**5**	7.33 a	0.53 b	38.9 a
C. rubecula hosts (9 per provisioning)	**4**	6.42 ab	1.41 b	26.7 a
		MSE = 0.993 p <0.001	MSE = 1.08 p <0.001	MSE = 240.0 p = 0.288

[1] Hosts, single species or a mixture of species, provided to *C. torvina* females for periods of 7 h in the 4th - 6th host provisionings periods; **n** = number of female *C. torvina* which produced progenies (responded to their hosts). MSE = Mean Square Error from the ANOVA

[2] Means within column followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer means separation test; *Repeated measures ANOVA for Randomized Block Design (RBD); ** ANOVA for RCBD, Arcsine; ($\sqrt{x}$) transformation used on % data).

[3] Means within column followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer means separation test; *Repeated measures ANOVA; **one way ANOVA, Arcsine ($\sqrt{x}$) transformation used on % data).

Dead hosts (pupae from which nothing emerged) means were 0.03, 0.13 and 0.23 for *C. orobenae, C. rubecula* and *P. xylostella* hosts, respectively in the mixed host treatments. The mean number of dead hosts was not significantly different between host species (p=0.100). From 1-8% of the hosts in these treatments were counted as dead, whereas among the control hosts (72 of each species), the proportion of dead hosts was 0% of *P. xylostella,* 1.4% of *C. rubecula* and 1.6% of *C. orobenae*. Thus, when a greater proportion of dead hosts occurred among hosts exposed to *C. torvina* the additional mortality was probably due to *C. torvina* activity (host feeding or failed parasitism). Dissection of dead hosts revealed failed parasitism (remains of dead *C. torvina*) in only 8.1% and 7.5% of the dead *C. rubecula* and *P. xylostella* hosts, respectively. During the experiment some host feeding activity was observed and it is probable that the majority of the dead hosts resulted from host feeding activity.

When the number of hosts affected in the mixed host treatment (all species combined) is compared with that from the *P. xylostella* and *C. rubecula* hosts treatments, the mixed host treatment shows significantly fewer hosts affected (Table 2.2.1). This indicates that even though *C. torvina* females had sufficient eggs and time to lay them, they generally chose not to lay them in the *C. orobenae* hosts of the mixed host treatment. Among treatments where females received either *P. xylostella* or *C. rubecula* hosts, an apparently higher proportion of female *C. torvina* emerged from *P. xylostella,* but the difference was not significant.

Second Host Choice Experiment - Two Host Species

Materials and Methods

Conditions in the second host choice experiment were similar to those of the first experiment. The notable exceptions were: (1) only *C. rubecula* and *P. xylostella* were tested in the mixed treatments; (2) eight hosts were provided per female; (3) hosts were provided every three days, but only six provisionings were made and; (4) the duration of host provisionings was reduced to only two hours in the first and 1.5 h in the remaining five provisionings. The seven hour and 3.5 hour host provisioning periods used in the first host choice experiment did not appear to restrict reproduction to a significant degree. I felt that a further reduction in host exposure time would insure that if preferred hosts were selected first (in the mixed treatments), little time would be left for less desirable hosts. The treatments were as follows:

Treat. 1	A mixture of eight cocoons (4 *C. rubecula* and 4 *P. xylostella*) provided for each female each provisioning.	n = 10
Group 1	*C. torvina* originating from *C. rubecula* hosts.	n = 5
Group 2	*C. torvina* originating from *P. xylostella* hosts.	n = 5
Treat. 2	Eight *P. xylostella* cocoons provided for each female each provisioning; *C. torvina* originating from *P. xylostella* hosts.	n = 5
Treat. 3	Eight *C. rubecula* cocoons provided for each female each provisioning; *C. torvina* originating from *C. rubecula* hosts.	n = 5

where n = the number of replications (female *C. torvina*) in each treatment or group within treatment.

Females which produced no progenies during the experiment were considered defective and data from these females were excluded from analysis. In the mixed host treatment, a Two-way ANOVA for RCBD was used to compare the response to different host species by females from the different host origin groups, averaged across the fourth through sixth provisionings. Data from the fourth through sixth host provisionings were used because the reproductive rate had not stabilized until then. Data from mixed host treatment females in both treatment groups were combined (n=9 females) to compare the overall reaction of females to each host species. This analysis used data from the fourth through sixth host provisioning in a repeated measures ANOVA for RCBD. The response variables (from both host species combined) in the mixed host treatments were also compared with those from *C. torvina* provided with either *P. xylostella* or *C. rubecula* hosts. A standard, repeated measures ANOVA was used for this analysis. Post ANOVA means comparison used the Tukey-Kramer means separation test (α=0.05). The proportion of female *C. torvina* progenies resulting from each treatment was calculated as a proportion of the sum of all progenies in a treatment. This was because proportional data were not normal according to the Skewness, Kurtosis, and Omnibus Normality tests (Hintze 1995). Proportion female data were characterized by numerous zero values from replicates where no females were produced, and there were great inequalities in the number of progenies between replicates. Treatment proportions for emerged female *C. torvina* were compared using a multiproportion comparison (chi square) test and the two proportion, Fishers Exact (Z) test (a=0.05) (Zar 1984, Hintze 1995).

Females that failed to respond to any of the provided hosts during the experiment were subjected to further testing. An oviposition test using all the non responding females was conducted to determine if they would oviposit when given enough stimulus. In this test each female was given five *P. xylostella* hosts (for a 14 h period) on a cage base coated with *P. xylostella* frass as an oviposition stimulus. Host pupae were held until the host or *C. torvina* progenies emerged.

Results and discussion

It was evident by the third host provisioning that the initial two hour host provisioning was not sufficient to stimulate all females into oviposition activity. During the first host provisioning, there was little oviposition activity in the *P. xylostella* host treatment, less in the mixed host treatment and none in the *C. rubecula* host treatment. The five subsequent 1½ hour long host provisionings did not stimulate these non responding females into action. Only two of five females responded to hosts in the *C. rubecula* host treatment. The response was better in the treatments which included *P. xylostella*; four of five females responded in the *P. xylostella* host treatment and nine of ten females responded in the mixed host treatment (Table 2.2.2). The non responding female from the mixed host treatment was from the *P. xylostella* host origin group. Of the females which did respond to hosts, those receiving *P. xylostella* were able to successfully parasitize a mean of six hosts per provisioning during the last three provisionings (Figure 2.2.2). These provisionings lasted only 1.5 h each, yet some *C. torvina* successfully parasitized all eight hosts in that period. This is an indication that host handling time is not an important factor limiting *C. torvina*'s daily reproductive rate.

Odor from *P. xylostella* frass particles may have stimulated more oviposition behavior in treatments where *P. xylostella* hosts were included. Close examination of *P. xylostella* cocoons revealed that the loose net-like silk cocoons can trap and hold small particles of frass. Most of the *P. xylostella* cocoons used in the experiment had small particles of frass stuck to them. The *P. xylostella* host colonies consisted of large populations of larvae maintained on plants in several small cages. Frass from larvae feeding in upper plant leaves fell on and stuck to the cocoons of *P. xylostella* which had pupated below. Although *C. rubecula* was also reared in crowded cages, the silk from its cocoons is tightly woven and the relatively large frass particles from its *P. rapae* host do not easily adhere to its surface.

As in the first host choice experiment, the reproductive rate of naive *C. torvina* (from the mixed host and *P. xylostella* treatments) increased progressively until the third host provisioning and then number of progenies per female leveled off (Figure 2.2.2). Those females which did reproduce in the *C. rubecula* host treatment responded poorly during the first three host provisionings, and did not equal the reproductive rate of females receiving *P. xylostella* hosts until the fifth host provisioning.

There was no significant difference between the females from the two original host groups in the mixed host treatment ($p=1.00$ for the host origin effect on the mean number of *C. torvina* progenies). However, the interaction of the host species and host origin factors was significant ($p=0.033$) and this was due to females from *C. rubecula* having more progenies from *P. xylostella* hosts, and females from *P. xylostella* having more progenies from *C. rubecula* hosts. There is no obvious reason for this result and it does not follow the same pattern of the interaction results from the first host choice experiment.

Within the mixed host treatment, there was no significant difference between the number of *C. torvina* progenies from *P. xylostella* or *C. rubecula* hosts (Table 2.2.2). However, a significant difference was seen between these two hosts in the proportion of female *C. torvina* progenies that emerged. As with the first host choice experiment, a significantly higher proportion of female *C. torvina* emerged from *P. xylostella* than from *C. rubecula*.

An apparently greater number of *C. torvina* emerged from hosts in the *P. xylostella* and *C. rubecula* host treatments than from hosts (*P. xylostella* and *C. rubecula* combined) in the mixed host treatment (Table 2.2.2). This difference is not significant, but may be due to the low number of replicate *C. torvina* females tested in the *P. xylostella* and *C. rubecula* host treatments (replicates = 4, and 2, , respectively).

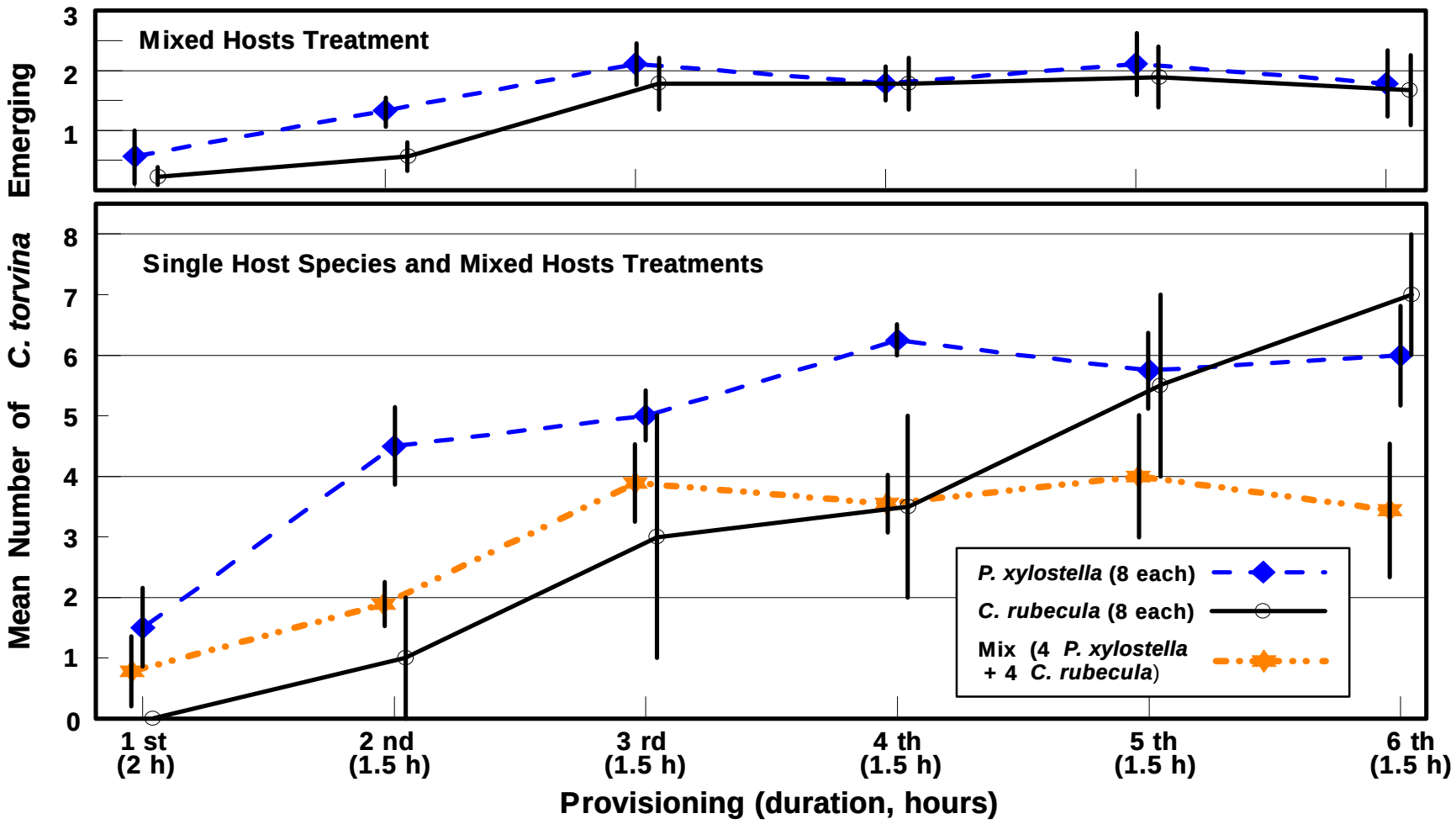

Figure 2.2.2: Effect of two host species on *Conura torvina* reproductive success. Comparison of treatments in which *C. torvina* females were provided with eight *Plutella xylostella* or eight *Cotesia rubecula* cocoons, or were provided with a mixture of two host species (four each of *P. xylostella* and *C. rubecula*) (error bars = mean ± SE)

Table 2.2.2: Effect of two host species on *Conura torvina* host selection and reproductive success [1].

Treatment (Host species and number provided per day)	**n**[1]	Mean Emerging *C. torvina* adults	Mean Emerging Hosts (non parasitized)	*C. torvina* Emerging (Mean % Females)
Mixture of hosts (8 per provisioning)	**9**			
P. xylostella (4 each)		1.89 a [2]*	1.81 a [2]*	48.0 a [2]**
C. rubecula (4 each)		1.78 a	1.96 a	18.8 b
		MSE[1] = 0.667 p = 0.631	MSE = 0.796 p = 0.559	Z = 3.06 p = 0.003
Mixture of hosts (8 per provisioning: 4 *P. xylostella* + 4 *C. rubecula*)	**9**	3.67 a [3]*	3.77 a [3]*	33.7 a [3]**
P. xylostella hosts (8 per provisioning)	**4**	6.00 a	1.67 a	25.0 a
C. rubecula hosts (8 per provisioning)	**2**	5.33 a	2.17 a	44.4 a
		MSE = 12.5 p = 0.178	MSE = 10.4 p = 0.177	χ^2 = 4.13 0.10 < P < 0.25

[1] Hosts of a single species or a mixture of species, provided to *C. torvina* females for periods of 1.5 hours in the 4th - 6th provisioning periods; **n** = number of female *C. torvina* which produced progenies (responded to their hosts); MSE = Mean Square Error from the ANOVA

[2] Means within column followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer means separation test; *Repeated measures ANOVA for Randomized Complete Block Design (RCBD), ** Fishers Exact Test (Zar, 1984).

[3] Means within column followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey-Kramer means separation test; *Repeated measures ANOVA; **Chi Square test for multiple proportions (Zar, 1984).

Because several *C. torvina* (in each of the three treatments) had sufficient eggs and time to successfully parasitize all eight hosts within a 1.5 h provisioning period, it is unclear why an average of less than half the available hosts were parasitized in the mixed host treatment (Figure 2.2.2). Even though fewer than half the mixed hosts were parasitized, nearly equal numbers of both species were affected.

In the mixed host treatment, there was no significant difference ($p=0.760$) between host species for the number of dead hosts (pupae from which nothing emerged). Means were 0.259 and 0.296 for *C. rubecula* and *P. xylostella* hosts, respectively. Between 6% and 7% of the hosts in the mixed host treatment were counted as dead whereas in the control hosts (48 of each host species) there was no mortality. The absence of mortality in the controls indicates that dead hosts from the mixed host treatment resulted from *C. torvina* oviposition or host feeding activity. Dissection of dead hosts did not reveal failed parasitism (dead *C. torvina*) in any of the *C. rubecula* hosts, but found it in 9.1% of the dead and *P. xylostella* hosts. This difference may be due the difficulty of distinguishing the remnants of dead *C. torvina* from the flesh of dead, shriveled *C. rubecula* hosts.

After the second host choice experiment, an oviposition test was conducted on the five experimental *C. torvina* females which had not responded to hosts. All five of the non responding females successfully parasitized their *P. xylostella* hosts during their first post experimental host provisioning. This indicated that these non responding females were normal (capable of reproductive behavior) and would respond to hosts when given enough time and the right stimulus. In this case the stimulus may have been the *P. xylostella* frass.

Host Species Choice Experiments

Conclusions

Both host selection experiments indicated that the host of origin for a *C. torvina* female had no effect on her subsequent host selection. There was also no difference in the preference for *C. rubecula* and *P. xylostella*. *Cotesia orobenae* was probably a non preferred host because of its much smaller size. However, *C. orobenae* and the similarly sized *Cotesia glomerata* frequently serve as *C. torvina* hosts in the field. The frequent parsitization of these small hosts in the field might be due to the smaller size (<3.0 mm) of the *C. torvina* that forage in the field. Female *C. torvina* in the field were probably smaller because a large proportion of them had emerged from *C. glomerata* hosts (Gaines 1992). *Cotesia orobenae* may have been parasitized more in the mixed host treatments if relatively small *C. torvina* females originating from smaller hosts were used.

Dead hosts accounted for up to 20% of the total hosts affected. Host feeding activities probably accounted for most of the dead hosts and failed parasitism caused <10% of dead hosts. Host mortality in the controls was <2%.

Host species had a significant influence on the proportion of female progenies produced; host species size may be the influencing factor. A higher proportion of female *C. torvina*

progenies (generally more than twice as many) was obtained from *P. xylostella* than from *C. rubecula*. Host species had little influence on *C. torvina* developmental times as they were nearly equal in all three hosts. The developmental time from egg to adult was 13 days at approximately 25°C.

Conura torvina which responded to hosts had relatively low reproductive rates in initial host provisionings. The reproductive rate improved with each provisioning and reached its maximum level by the third or fourth exposure to hosts. Long duration initial host provisioning periods stimulated *C. torvina* oviposition activity better than short duration periods. When the first host provisioning period was short, as in the second experiment, more females appeared to respond when *P. xylostella* hosts were provided. The reason *P. xylostella* hosts provided a much stronger oviposition stimulus than *C. rubecula* on the initial host contact may be due to frass adhering to the *P. xylostella* cocoons. Thus, increased frequency or duration of host encounters is necessary to stimulate oviposition activity when no olfactory cues such as frass are present.

Duration of host exposure had little influence on the number of hosts successfully hyperparasitized. As many as eight hosts were successfully hyperparasitized in a 1.5 h period. This indicates that host handling time is not an important factor limiting the number of hosts which *C. torvina* can successfully parasitize per day.

Chapter 2, Part 3: Influence of Frass on *C. torvina* Ovipositional Behavior

Introduction

Early in this study, I noticed that inexperienced *C. torvina* showed little interest in *C. rubecula* hosts until they had been in close contact with the hosts for several days. However, when *P. xylostella* was used as a host, there was usually some oviposition activity on the first day. An examination of *P. xylostella* cocoons revealed that the net-like cocoon structure would easily trap and hold small particles of frass which had fallen from *P. xylostella* larvae feeding above. *Plutella xylostella* hosts used in these experiments were reared in cages at high population densities, and most cocoons from these cages had bits of frass stuck in them. Conversely, *C. rubecula* cocoons are made of tightly woven silk and the relatively large particles of frass from *Pieris rapae* (*C. rubecula*'s host) do not stick to them easily. I decided to study the effects of frass on *C. torvina* because host frass is known as an ovipositional behavior stimulant (DeBach 1964) in other hymenopteran parasitoid species.

Two experiments were conducted to confirm the hypothesis that frass is an important stimulus to *C. torvina* oviposition behavior. Experiments were designed to measure the influence of frass from *P. xylostella* and *Pieris rapae* on the reproductive rate of *C. torvina* which had no previous experience with hosts. Although *P. rapae* is not a host to *C. torvina,* it is a host to *C. rubecula* and *C. glomerata,* both of which may serve as *C. torvina* hosts. Thus, *P. rapae* frass was considered as a possible stimulant. The principal host used in these experiments was *C. rubecula* because inexperienced *C. torvina* do not usually respond to this host readily. Thus, if frass has a stimulatory effect, it will be easily detected.

Materials and Methods

Dried frass pellets from *P. rapae* and *P. xylostella* were collected separately from the bottoms of rearing cages with a mechanical aspirator and screened of soil and plant debris. At the beginning of the experiment, clean frass from either species was mixed with enough water to make a paste. Pea sized portions of this paste were smeared inside each cage bottom (inverted vial lid). The frass paste was allowed to dry before replacing the cage bottom. Host cocoons were subsequently placed on the dry frass.

Two experiments were conducted in an environmental chamber set at 25°C with a 14L: 10D photoperiod. The first experiment used 15 female *C. torvina* originating from *C. rubecula* hosts. Experimental females were 2-3 weeks old and had never been exposed to hosts before. Each female was held in a screened vial cage and was mated. Five *C. torvina* females were tested for each treatment. The first experiment had three treatments which consisted of:

(1) *C. rubecula* provided on *P. xylostella* frass
(2) *C. rubecula* provided on *P. rapae* frass and;
(3) *C. rubecula* provided in the absence of frass.

The third treatment served as a control for the frass treatments. Three different host provisionings were made, each three days apart, and five hosts were provided to each female at each provisioning. The first provisioning lasted 14 hours (the duration of one day's photoperiod), and the subsequent two host provisionings lasted only two hours each. The short duration provisionings were meant to reduce the possibility that continued exposure to hosts rather than frass stimulated oviposition activity. It was assumed that females which had been stimulated by the presence of frass during the first host provisioning would be stimulated enough to parasitize hosts during the shorter (2 h) provisionings which followed. All host cocoons used were less than three days old.

A second frass experiment was conducted because females in the first experiment responded poorly to the hosts. In the second experiment, methods and conditions were nearly identical as in the first. The major difference between experiments was that an additional (fourth) treatment was included in which *P. xylostella* hosts were tested. Another difference was that female *C. torvina* in all treatments originated from a mixture of *C. rubecula* and *P. xylostella* hosts, and six *C. torvina* were tested per treatment. Treatments were as follows:

(1) *C. rubecula* provided on *P. xylostella* frass
(2) *C. rubecula* provided on *P. rapae* frass
(3) *C. rubecula* provided in the absence of frass and;
(4) *P. xylostella* provided on *P. xylostella* frass.

The number of host provisionings, days between each provisioning, the duration of each provisioning, and number of hosts provided per female were the same as in the first experiment..

In both experiments, data from treatments where females failed to respond to hosts had skewed distributions. This required a nonparametric analysis. In each experiment, there was no increase in the number of responding females after the first provisioning, but treatment variances increased in later host provisionings (possibly because the shorter provisioning duration amplified differences between females within a treatment). Therefore, treatment means were only compared for the first provisioning. A Kruskal-Wallis One Way ANOVA on Ranks, and a Kruskal-Wallis Multiple Comparison (Z-Value) Test were used for analysis (Hintze 1995).

Results and discussion

Conura torvina's response to hosts in all treatments of the first frass experiment was poor despite the first provisioning period's 14 h duration. During the three host provisionings, only 2/5 of the females in each of the frass treatments oviposited in hosts and none oviposited in hosts provided without frass. No explanation could be found for the generally poor response to hosts in this experiment. However, it is notable that the responding females were only in treatments where frass was used as a stimulus (Table 2.3.1, Figure 2.3.1). In the treatments having frass as a stimulus, *C. torvina* appeared to have a higher reproductive rate in the presence of *P. xylostella* frass. There was no significant difference (α=0.05) between treatments in the first experiment (Table 2.3.1), possibly because of the small number of *C. torvina* which oviposited.

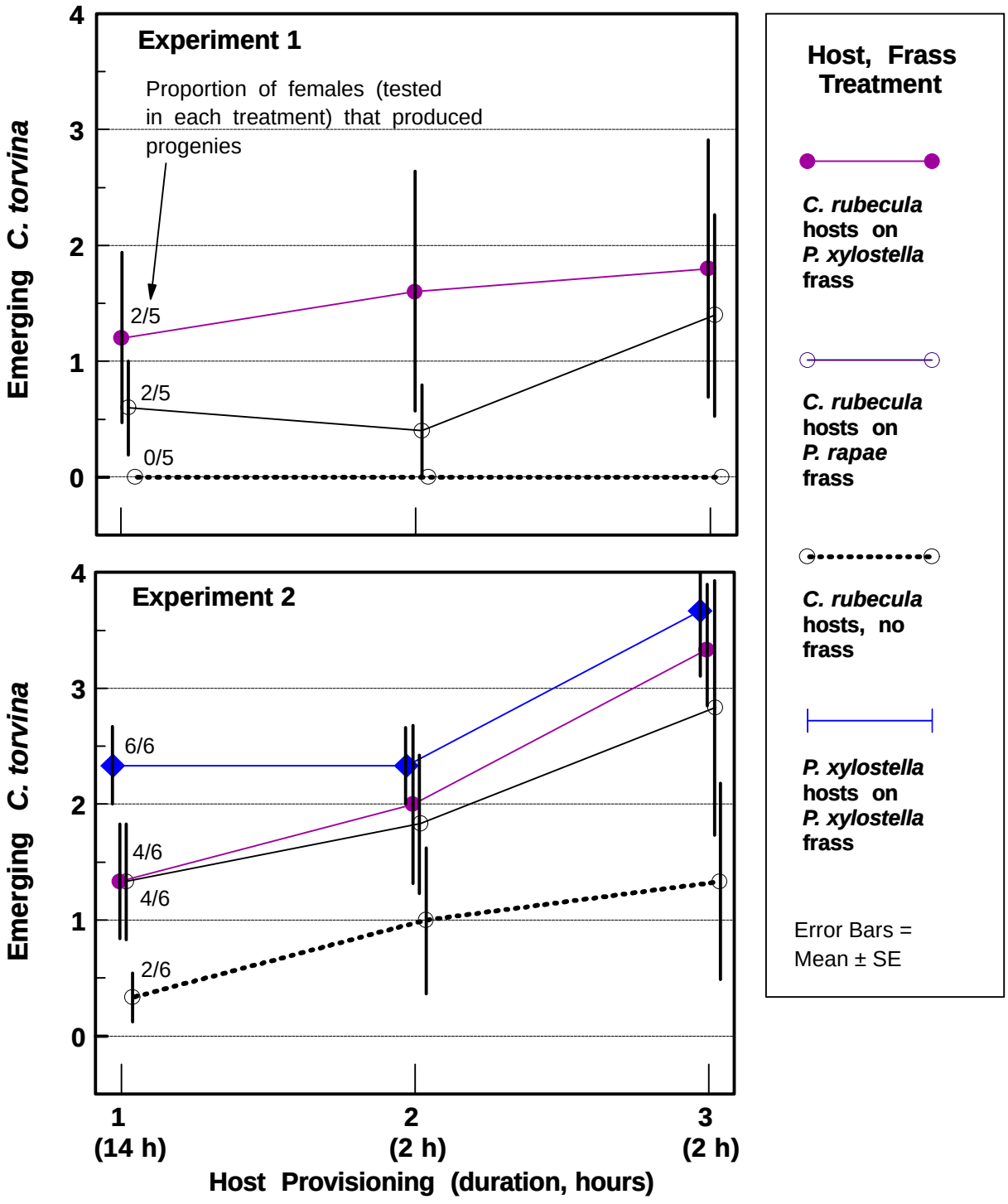

Figure 2.3.1: Effect of frass on oviposition behavior of *Conura torvina* with no previous oviposition experience (n = 5 and 6 *C. torvina* tested per treatment in Experiment 1 and Experiment 2, respectively).

Table 2.3.1: Mean number of progenies per *Conura torvina* females from the first host provisioning of *Plutella xylostella* on *P. xylostella* frass, or of *Cotesia rubecula* hosts on *P. Xylostella* frass, *Pieris rapae* frass or no frass at all (n = number of *C. torvina* females tested; five hosts provided per female).

Treatment; Host, Frass Combination	Experiment 1 n = 5 $\bar{x}$	Experiment 2 n = 6 $\bar{x}$
P. xylostella Hosts on *P. xylostella* Frass	- [1]	2.33 **a**[2]
C. rubecula Hosts on *P. xylostella* Frass	1.2 a[2]	1.33 ab
C. rubecula Hosts on *Pieris rapae* Frass	0.6 a	1.33 ab
C. rubecula Hosts Without Frass	0.0 a	0.33 **b**
	p = 0.271	p = 0.029
	χ^2 = 2.61	χ^2 = 9.02

[1] No *P. xylostella* hosts on *P. xylostella* frass treatment in Experiment 1.

[2] Means within columns are not significantly different (α=0.05) if followed by the same letter (Kruskal-Wallis One Way ANOVA on Ranks [χ^2 = Chi square statistic]; Kruskal-Wallis Multiple Comparison Z-Value Test).

The response of *C. torvina* to hosts in the second frass experiment was stronger than in the first experiment. The proportion of *C. torvina* which reproduced was highest (6/6) in the *P. xylostella* hosts on *P. xylostella* frass treatment, and lowest (2/6) in the *C. rubecula* without frass treatment (Figure 2.3.1). As in the first experiment, it appears that the treatments which included frass had a higher proportion of ovipositing females. *Conura torvina* provided with *P. xylostella* hosts on *P. xylostella* frass had a significantly higher reproductive rate than those which received *C. rubecula* hosts without frass (Table 2.3.1). Initially there was no apparent difference between the reproductive rates of *C. torvina* which received *C. rubecula* on *P. xylostella* frass and *C. rubecula* on *P. rapae* frass treatments, but females in the former treatment appeared to have a slightly higher rate of reproduction in subsequent provisionings (Figure 2.3.1). Although this difference is not significant, it follows the same pattern seen in the first frass experiment.

In both experiments, *C. torvina* which oviposited during the first host provisioning continued to do so in the subsequent provisionings. On average, these *C. torvina* were also able to increase their reproductive rate with each host provisioning even though the provisionings were of shorter duration.

Conclusions

Although there was a significant difference between the frass treatments of *P. xylostella* hosts on *P. xylostella* frass and *C. rubecula* hosts without frass, the comparison is between two different host species. The possibility exists that *P. xylostella* in itself may provide some important additional stimulus for oviposition behavior. Results from both frass experiments appear to indicate that *C. torvina* oviposition behavior is stimulated by the presence of frass and that *P. xylostella* frass is a stronger stimulant than *P. rapae* frass. In nature, the presence of *P. rapae* frass does not guarantee the presence of Braconid hosts such as *Cotesia* spp. pupae, but when *P. xylostella* frass is present it is likely that *P. xylostella* pupae are also present. Increasing the number of *C. torvina* (replicates) may show statistical differences between the various frass treatments.

Chapter 3: Reproductive Capacity of *C. torvina*

Introduction

Initial studies on *C. torvina* examined factors which might influence reproductive behavior and success. Results indicated that *C. torvina* females with no previous oviposition experience or exposure to hosts can be stimulated to initiate oviposition behavior by the presence of frass, and that *C. torvina*'s reproductive rate improves after several days of oviposition experience. Experiments also indicated that host acceptability, determined by the age and species of a host, influenced *C. torvina*'s reproductive success. As these experiments did not clearly indicate the maximum reproductive rate for *C. torvina*, I conducted some experiments to measure the limits of *C. torvina*'s reproductive ability.

Three different series of experiments were used to test the reproductive capacity of *C. torvina*. In the first series of experiments called "continuous host provisioning experiments", *C. torvina* females were each provided with 13 hosts per day for periods of a week or more to determine whether they could maintain a steady rate of reproduction over time. Only thirteen hosts were provided to each female per day because hosts were a limited commodity and previous experimentation (Gaines 1992) had indicated that *C. torvina* females could not produce more than 13 progenies in a day. The second experiment series designated as "abundant supply of hosts experiments" tested *C. torvina*'s reproductive rate when each female was provided with 20 or more hosts per day, and was conducted to determine whether the limited number of hosts provided in earlier experiments had restricted the daily reproductive rate of *C. torvina*. The third series were "dissection experiments" designed to study *C. torvina*'s egg laying capacity and behavior. This was done by dissecting *C. torvina* ovaries to count the number of mature eggs that could be carried, and by dissecting parasitized hosts to count the number of eggs laid.

Chapter 3, Part 1: Continuous Host Provisioning Experiments

Introduction

My studies on the daily reproductive capacity of *C. torvina* (Gaines 1992), indicated that some females could produce 12 progenies per day, but that the average was about 9 progenies per day. However, the daily reproductive rate for *C. torvina* over a continuous period of days had not been tested. I thought that continuous daily provisioning of hosts to *C. torvina* females would test their reproductive limits by taxing their reproductive system and their supply of nutrients required for production of mature eggs. After several days, females would reach a reproductive rate limited by the maximum number of eggs they could produce daily.

Determining the daily number of eggs a female could lay would require dissection of parasitized hosts, but this would not necessarily indicate how many progenies she could produce. This is because she may lay several eggs per host, and there is no guarantee that any of these eggs would survive, and once a host is dissected, it is impossible to tell if an egg would have survived.

Therefore, the object of this experiment was to determine the reproductive rate for *C. torvina* (i.e., how many progenies could be produced per day over time).

General Materials and Methods

One component of the continuous host provisioning experiments tested the intermittent versus continuous provisioning of hosts. The object of comparing these two methods was to determine whether continuous host provisioning taxed the reproductive capacity of *C. torvina*. My hypothesis was that a high rate of continuous daily oviposition would tax a female's energy (nutrient) reserves more than that of a female which had several days of rest between each bout of oviposition. Stress would be indicated if females that received hosts on intermittent days had better reproductive success per host than those which were given hosts daily.

Another component of the continuous host provisioning experiments was to compare host species because the host species might also influence the reproductive success (progeny survival) of *C. torvina*. If host species affected *C. torvina*'s reproductive rate, it might become more apparent under the stress of continuous host provisioning. The hosts compared in these experiments were *P. xylostella* and *C. rubecula*.

Four continuous host provisioning experiments were conducted. In the first experiment, intermittent and continuous host provisioning were compared using only *P. xylostella* as the host species. This experiment ran for 13 days. Difficulties in the production of experimental hosts limited the duration of the three remaining experiments to a week or less. The second experiment, which tested *P. xylostella* versus *C. rubecula* hosts and continuous versus intermittent provisioning of *C. rubecula* hosts, ran for seven days. The third and fourth experiments which tested continuous provisioning of *P. xylostella* and *C. rubecula* hosts, ran for six days and for four days, respectively. Response variables examined in all experiments were: (1) number of progenies per female, (2) number of hosts surviving exposure to females; (3) number of hosts which died without producing *C. torvina*; (4) proportion of female *C. torvina* progenies; and (5) developmental time for *C. torvina* progenies (days from oviposition to adult emergence).

The female *C. torvina* tested in these experiments were caged individually in 200 ml vial cages. Before an experiment, several male *C. torvina* were introduced into each female's cage to provide a mating opportunity. Cages were maintained in environmental chambers set at 25°C with a 14L: 10D photoperiod. Host provisioning was accomplished by scattering hosts on the floor of each cage. Experimental host provisionings were made just as the previous day's hosts were removed from the cages. This was done several hours after the photoperiod ended, and required interrupting the scotophase for a short period each day, about 8-9 h before the commencement of the 14 hour photoperiod. All hosts used were ≤3 days old, and 13 hosts were provided to each *C. torvina* female per day. Exposed hosts were held in covered, 30 ml, plastic cups in the environmental chamber until all *C. torvina* had emerged. Dead hosts (those from which nothing emerged) were dissected to determine whether they contained the remains of *C. torvina*.

In experiments where there was an ample supply of hosts, a random selection of "control" hosts was made from each day's pool of experimental hosts. One or more groups of 13 control hosts were then placed in cages without *C. torvina* and were subsequently handled in the same manner as hosts which had been provided to *C. torvina*. Control hosts served as a measure of host mortality in the absence of parasitism.

After the first experiment, a Campbell Scientific, Inc., Model 21X, Data Logger was set up, and temperature probes from the data logger were placed in the center of each environmental chamber to monitor the temperature throughout each experiment. Temperature measurements were made every 30 seconds and averages were logged for each hour of a day. The average temperature for a particular host provisioning was determined by averaging all the hourly averages from the day of the host provisioning until the day of emergence of the last *C. torvina*.

All females used in the continuous provisioning experiments were given pre-experimental oviposition experience because *C. torvina* females with little or no oviposition experience have relatively low reproductive rates. Females gained oviposition experience through at least three separate host provisionings starting one to two weeks before the experiment. An oviposition stimulus in the form of a 1 cm square of paper impregnated with frass odor was placed in each cage along with the hosts in initial host provisionings. Frass odor impregnated paper squares were cut from a paper towel that had been soaked in a wet *P. rapae* frass paste and then dried.

Continuous Versus Intermittent Provisioning of *P. xylostella*

Materials and methods

Fourteen *C. torvina* females were randomly selected from a cohort of >100 females which were about 2½ months old and had been maintained in a cool room at 15°C. These 14 females were caged individually and were given three pre-experimental host provisionings for oviposition experience over a period of two weeks. In the initial host provisioning each female received four hosts, and hosts were left in the cages for a period of five days. In the second and third host provisionings six hosts were given to each female for about three days. Each female was watered daily and fed with honey throughout the experiment.

The 14 experienced *C. torvina* females were divided into two groups of seven. Experimental host provisioning started two days after the end of their third pre-experimental host provisioning. Females in one group were provided with *P. xylostella* hosts every day for 13 days, and those in the other group were provided with *P. xylostella* hosts every third day (on days 1, 4, 7, 10, and 13) of the experiment. All exposed hosts except those from the first experimental host provisioning (day 1) were held in the environmental chamber (25°C) until all *C. torvina* had emerged. Hosts from the first day of provisioning were separated to be dissected for egg counts, and were placed in a refrigerator (at 10°C), but a busy schedule prevented their dissection and after two weeks they were returned to the 25°C chamber. All dead hosts were dissected to determine whether they contained remnants of *C. torvina*.

Response data from the two host provisioning methods were compared on host provisioning days 4, 7, 10 and 13 (data from day 1 were not be used because effects of intermittent host provisioning would not be evident on the first day). Treatments were compared on these days using a Repeated Measures ANOVA because it is the most appropriate analysis when repeated measurements are taken on the same experimental subjects. The Repeated Measures ANOVA provides a more precise comparison of treatment effects than an analysis of the average of daily measurements because it accounts for daily variation (Norman & Streiner 1994). Replicate means for % female progenies were unbalanced because on a given day, the proportion from each replicate was calculated from a different number of progenies. To minimize this imbalance, replicate data for the number of female *C. torvina*, and number of progenies were averaged across provisioning days 4, 7, 10 and 13. Proportions (% females) from a replicate were calculated using the four day averages. Due to skewness of the data, treatment means for % females were then compared with a non-parametric, two sample, Mann-Whitney, rank sum test (Hintze 1995).

Following the last experimental host provisioning (day 13), an additional host provisioning was made with females from these two treatment groups receiving a large number of hosts (26 each). Results from this provisioning will be discussed in the section concerning the "abundant supply of hosts experiments" (Chapter 3, Part 2).

Results and Discussion

Experimental results showed a consistent difference between the two host provisioning treatments. *Conura torvina* in the intermittent provisioning treatment group (hosts provided every third day) consistently produced slightly more progenies (but not significantly more; $\alpha > 0.05$) than those in the daily provisioning group (Table 3.1.1, Figure 3.1.1). A possible cause for this is that consistently fewer (but not significantly fewer) hosts from the intermittent provisioning group died without producing *C. torvina* (Table 3.1.1, Figure 3.1.1). *Conura torvina* from both treatment groups appeared to affect the same number of hosts because the number of emerging hosts from each group is nearly the same. Dissection revealed that 18 of 74 dead hosts (24.3%) from the daily provisionings, and 9 of 44 dead hosts (20.4%) from the intermittent host provisionings contained dead *C. torvina* remnants. A Fishers exact test, used for comparison of two proportions, indicated no significant difference between these two treatment groups ($p=0.659$) (Zar 1984). Although the daily provisioning method appeared to result in higher host mortality, it had no apparent effect on the proportion of dead *C. torvina* found in the hosts. Unfortunately, dissection of dead hosts only revealed dead *C. torvina* as late instars, pupae or un-emerged adults. If dead *C. torvina* eggs or early instars were present, they could not be distinguished from the flesh of the dead hosts and thus, any difference in *C. torvina* egg mortality between treatments is not known.

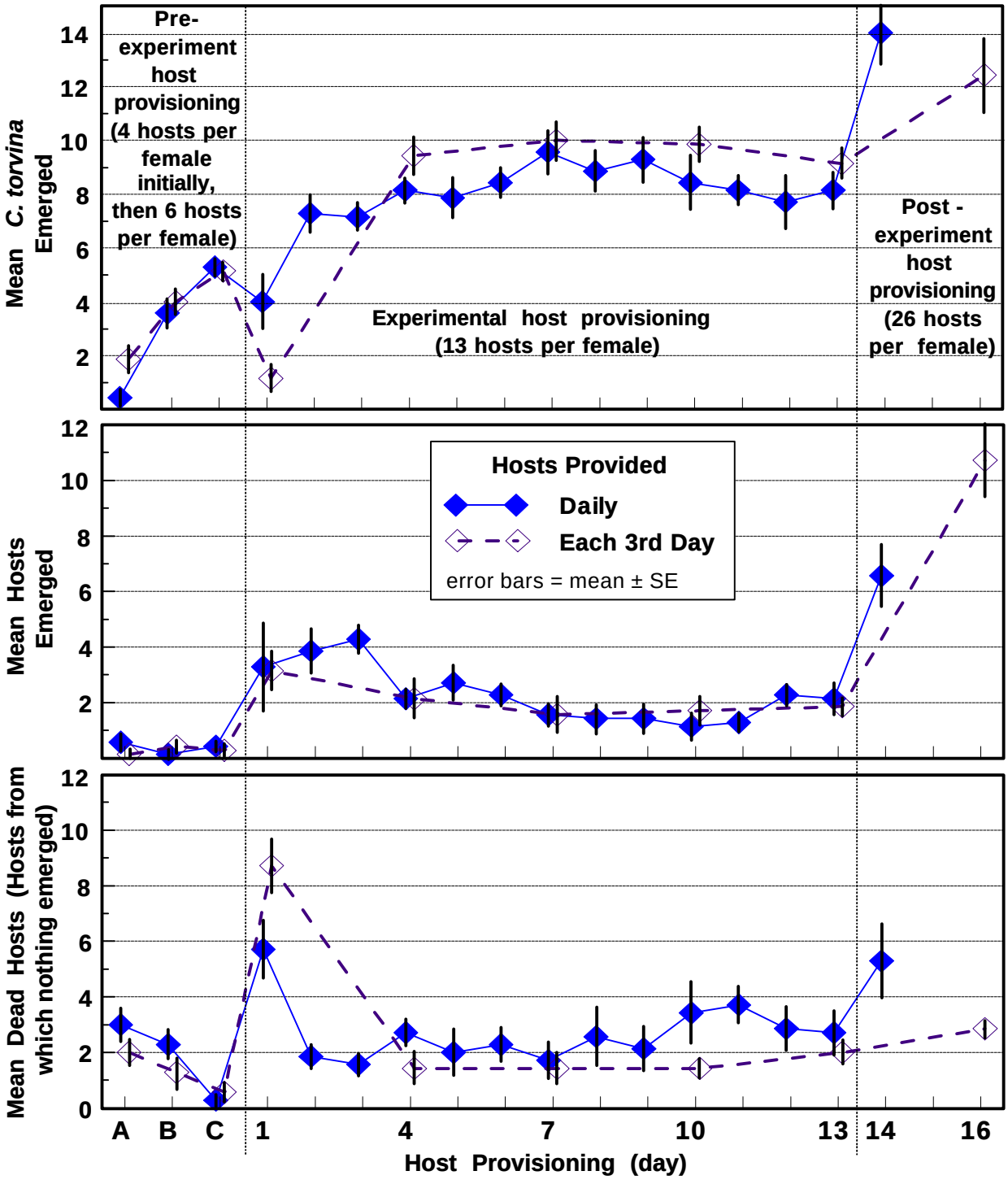

Figure 3.1.1: Comparison between *Conura torvina* females provided with *Plutella xylostella* hosts daily or every third day (n = 7 females tested per treatment).

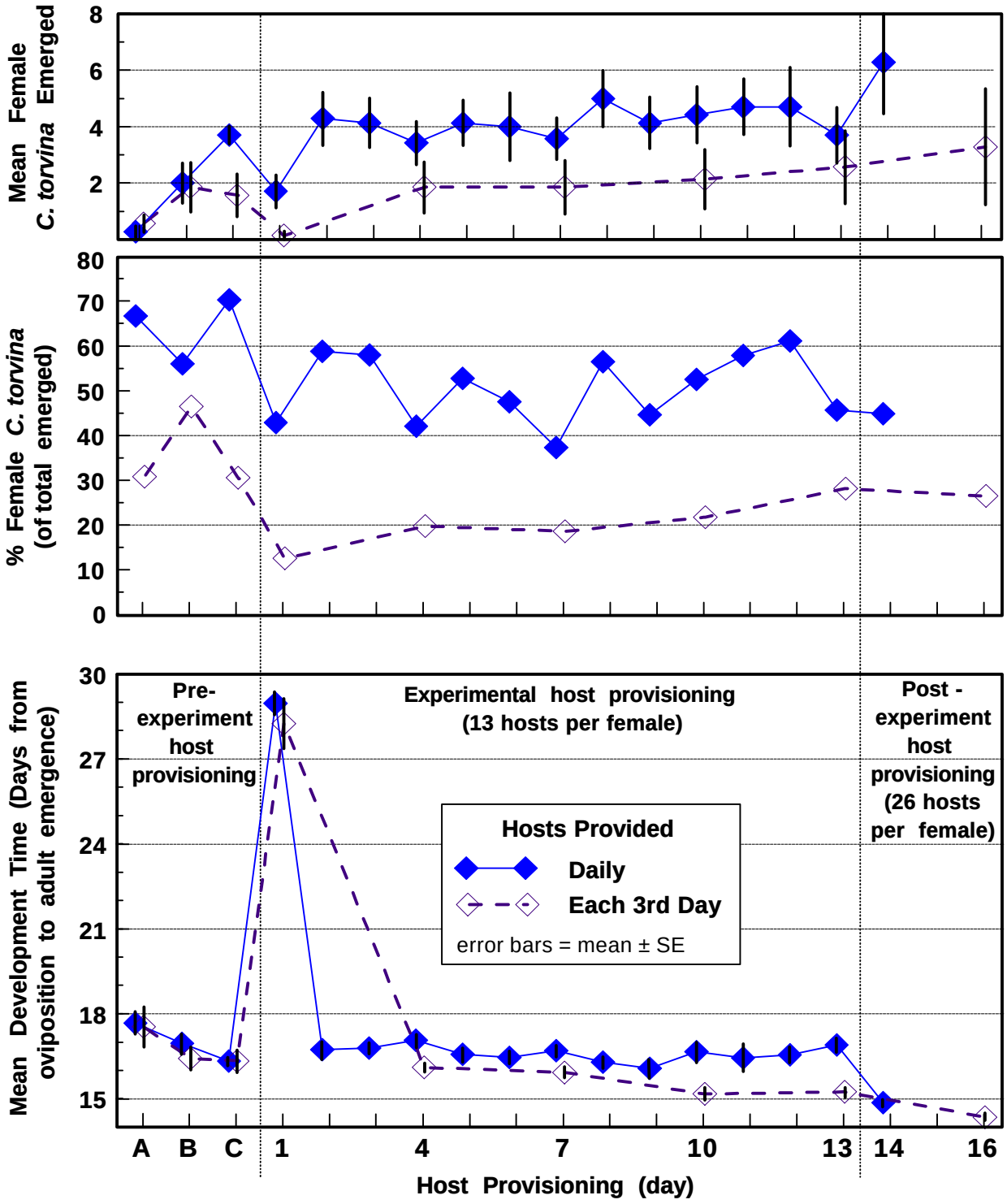

Figure 3.1.2: Comparison between *Conura torvina* females provided with *Plutella xylostella* hosts daily or every third day (n = 7 females tested per treatment).

Table 3.1.1: Comparison of *Conura torvina* reproductive success when *Plutella xylostella* hosts are provided either daily or every three days (n = 7 *C. torvina* females tested per treatment).

Response Variables	Hosts Provided Daily	Hosts Provided Every 3rd Day			
	$\bar{x}$	$\bar{x}$	p	Z	MSE[2]
Progeny per *C. torvina* per day [1]	8.57 a	9.61 a	0.176		7.27
Hosts emerging per day [1]	1.75 a	1.82 a	0.892		3.69
Dead hosts per *C. torvina* per day [1]	2.64 a	1.57 a	0.119		5.69
% Female *C. torvina* emerge [3]	44.4 a	22.2 a	0.193	1.37*	
C. torvina development time [1] (Days from oviposition to adult emergence)	17.0 **a**	15.6 **b**	<0.000		0.680

[1] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Repeated Measures ANOVA).
[2] MSE = Mean Square Error (Standard Error of the difference, from the ANOVA).
[3] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Nonparametric Mann-Whitney (rank sum) test; * Z-value)

Conura torvina in the intermittent provisioning group appeared to produce fewer females than those which received hosts daily. However, the difference is not significant (Table 3.1.1, Figure 3.1.2). This result is probably due to the fact that only three of the seven females in the intermittent group produced any female progenies. This contrasts with six of the seven females from the daily provision group which did produce female progenies. It is possible that the difference in progeny sex ratios is due to differences between the *C. torvina* females tested rather than to an effect of the treatment. Other "unpublished" data from my research have shown that some *C. torvina* females in any group, including ones which have had numerous mating opportunities, fail to produce female progenies. If both host provisioning methods are compared using only data from *C. torvina* which produced female progenies, females accounted for 44.2% and 50.0% of progenies in the daily and intermittent provisioning groups, respectively. The same comparison for other response variables (i.e. *C. torvina* emerging = 8.7 vs. 9.5; hosts emerging = 1.63 vs. 1.33; dead hosts = 2.8 vs. 2.2; and development time = 17.1 vs. 15.6), is similar to that when all females in both treatments are compared.

The one significant difference between the two treatment groups was progeny development time (Table 3.1.1, Figure 3.1.2). Progenies from the intermittently provisioned *C. torvina* had a significantly shorter development time. A possible reason for this difference is that females in the intermittently provisioned group had more time to mature eggs between ovipositions. Thus, their eggs were slightly more developed and the progenies that hatched out of these eggs had a shorter development time. Also, if intermittently provisioned females laid eggs that were more mature, this might explain the better survival of their progenies. Longer development time in the daily provisioned females could be an indication that the eggs being laid were not fully developed, and could indicate that these females were producing eggs at or close to their maximum capacity.

The main conclusion from this experiment was that progenies from females supplied intermittently with hosts have a shorter development time. This experiment also showed that *C. torvina* females, when provided with 13 hosts daily, can continuously produce at least eight progenies per day (Figure 3.1.1); some females in each treatment group produced as many as 12 progenies per day on several different days. During the 13 days of continuous host provisioning, generally >40% progenies each day were females (Figure 3.1.2).

Continuous Provisioning of *P. xylostella* and *C. rubecula* , and Intermittent Provisioning of *C. rubecula*

Materials and Methods

The second continuous host provisioning experiment was conducted under similar conditions and using the same procedures as in the first experiment. Two host species, *P. xylostella* or *C. rubecula* were tested, and the response of *C. torvina* when provided daily with either host species was compared. This experiment also compared daily and intermittent (every third day) host provisioning using *C. rubecula* as a host. Limited host availability allowed for only three intermittent provisioning days (days 1, 4, and 7).

Twenty females were selected from a cohort of three month old *C. torvina* that had been held at 15°C since emergence. These 20 females were mated and divided into three groups (two groups of seven and one group of six). The two groups of seven females were tested in each of the daily *P. xylostella* and *C. rubecula* provisioning treatments, and six females were tested in the *C. rubecula* intermittent host provisioning treatment. Three pre-experimental host provisionings were made during a seven day period. Six hosts were provided to each female for three days on the first pre-experimental provisioning and ten hosts were provided to each female for a day on the subsequent two provisionings. Experimental host provisioning began five days after the last pre-experimental provisioning.

Groups of 13 control hosts, randomly selected from the host supply, were collected on all but the first pre-experimental provisioning day. One control group of each host species was tested on the second and third pre-experimental provisionings, three control groups of each host species were tested on days 1, 4 and 7 of the experiment, and two control groups of each species were tested on days 2, 3, 5 and 6* of the experiment (*no *C. rubecula* controls were tested on day 6).

Response data from the daily *P. xylostella* and *C. rubecula* host treatments were compared for host provisioning days 1-7 using a Repeated Measures ANOVA. Because one female in the daily *C. rubecula* provisioning group failed to reproduce throughout the experiment her data were excluded from the analysis, leaving only six replicates of *C. torvina* for that treatment. Response data from *C. rubecula* hosts used in the daily and intermittent host provisioning treatments were compared on host provisioning days 4 and 7, using a Repeated Measures ANOVA. Treatment means for % females (averaged across treatment days) were compared with a non-parametric, two sample, Mann-Whitney, rank sum test.

Results and Discussion

There was no significant difference (α=0.05) between *C. torvina* provided with either host species for the number of *C. torvina* progenies emerging, number of hosts emerging, or for the number of dead hosts (Table 3.1.2, Figure 3.1.3). The difference between the proportion of the female *C. torvina* that emerged from *P. xylostella* or *C. rubecula* was not significant, but it appeared that the proportion of females from *P. xylostella* was consistently higher across all provisioning days (Table 3.1.2, Figure 3.1.4). *Conura torvina* progenies also appeared to have

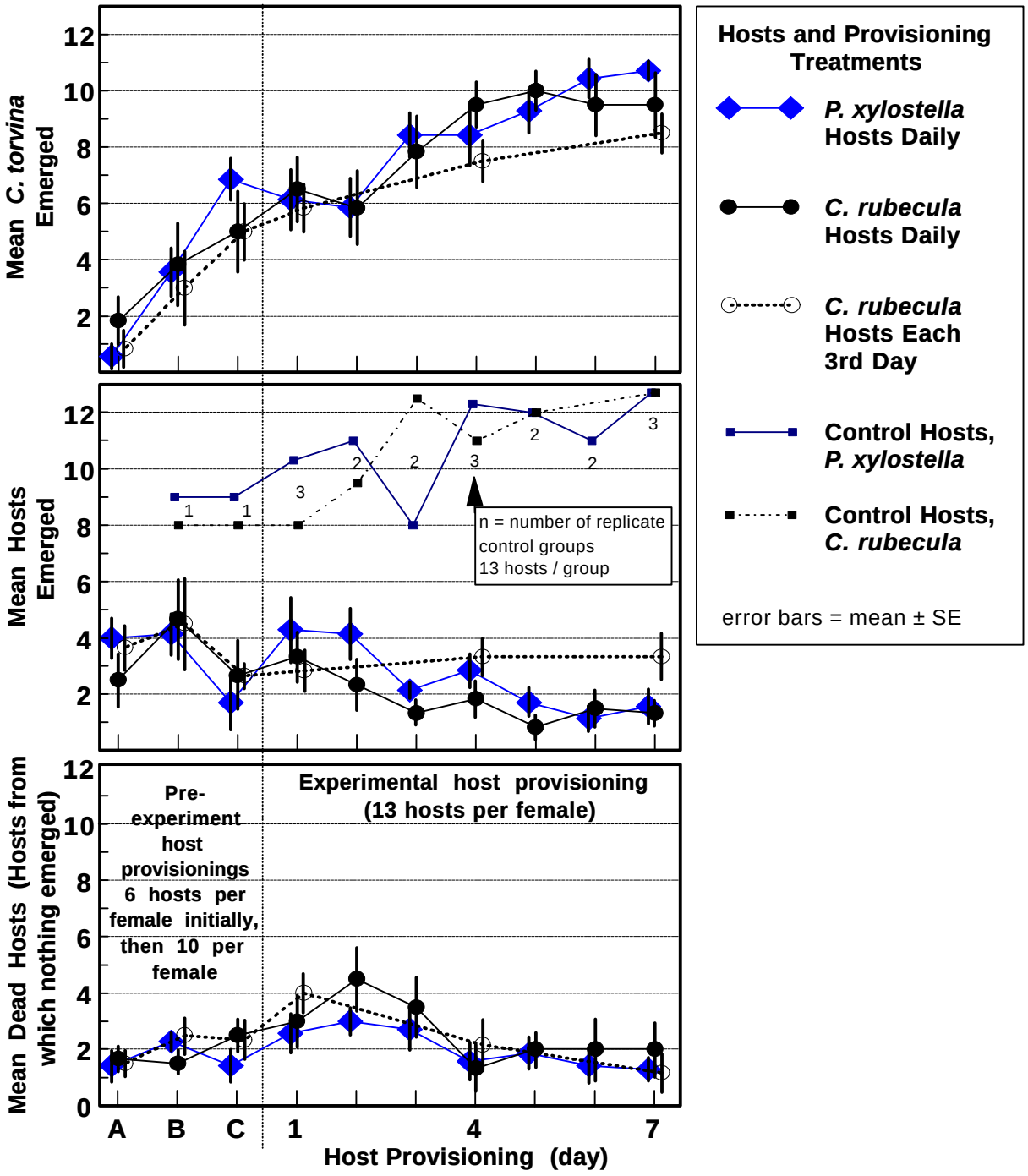

Figure 3.1.3: Comparison between *Conura torvina* females provided with *Plutella xylostella* or *Cotesia rubecula* hosts daily or with *C. rubecula* every third day (n = 7, 6 and 6 female *C. torvina* tested with *P. xylostella*, *C. rubecula* [daily] and *C. rubecula* [each 3rd day], respectively).

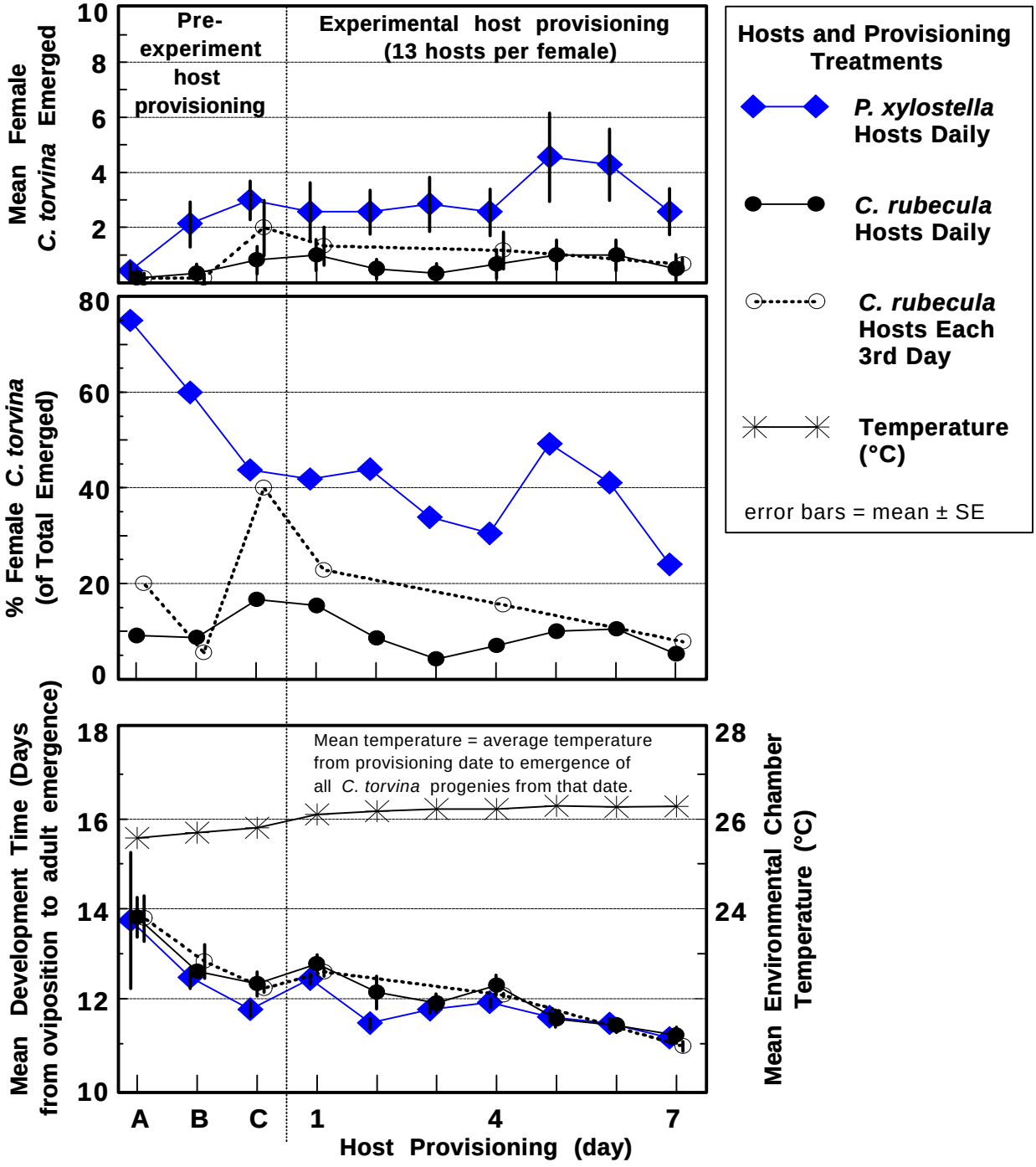

Figure 3.1.4: Comparison between *Conura torvina* females provided with *Plutella xylostella* or *Cotesia rubecula* hosts daily or with *C. rubecula* every third day (n = 7, 6 and 6 female *C. torvina* tested with *P. xylostella*, *C. rubecula* [daily] and *C. rubecula* [each 3rd day], respectively).

Table 3.1.2: Comparison of *Conura torvina* reproductive success with *Plutella xylostella* or *Cotesia rubecula* provided daily for seven days (n = number of *C. torvina* replicates).

Response Variables	*P. xylostella* Hosts Provided Daily (n=7)	*C. rubecula* Hosts Provided daily (n=6)			
	$\bar{x}$	$\bar{x}$	p	Z	MSE[2]
Progeny per *C. torvina* per day [1]	8.46 a	8.38 a	0.936		26.40
Hosts emerging per day [1]	2.55 a	1.78 a	0.252		9.04
Dead hosts per *C. torvina* per day [1]	2.06 a	2.61 a	0.530		16.76
% Female *C. torvina* emerge [3]	35.0 a	8.7 a	0.151	-1.43*	
C. torvina development time [1] (Days from oviposition to adult emergence; mean temperature = 26.3 ± 0.1°C [4])	11.7 a	11.9 a	0.144		0.659

[1] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Repeated Measures ANOVA).
[2] MSE = Mean Square Error (Standard Error of the difference, from the ANOVA).
[3] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Nonparametric Mann-Whitney (rank sum) test; * Z-value)
[4] Experimental mean temperature ± 95% confidence level; determined from average of provisioning day means (mean temperature from day of host provisioning to emergence of all *C. torvina* from those hosts).

Table 3.1.3: Comparison of *Conura torvina* reproductive success when *C. rubecula* hosts are provided either daily or every three days (n= *C. torvina* replicates).

Response Variables	*C. rubecula* Hosts Provided Daily (n=6)	*C. rubecula* Hosts Provided Every 3rd Day (n=6)			
	$\bar{x}$	$\bar{x}$	p	Z	MSE[2]
Progeny per *C. torvina* per day [1]	9.50 a	8.00 a	0.198		7.10
Hosts emerging per day [1]	1.58 a	3.33 a	0.055		3.91
Dead hosts per *C. torvina* per day [1]	1.67 a	1.67 a	1.000		7.73
% Female *C. torvina* emerge [3]	6.1 a	11.7 a	0.310	-1.01*	
C. torvina development time [1] (Days from oviposition to adult emergence; mean temperature = 26.3 ± 0.3°C [4])	11.8 a	11.5 a	0.222		0.23

[1] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Repeated Measures ANOVA).
[2] MSE = Mean Square Error (Standard Error of the difference, from the ANOVA).
[3] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Nonparametric Mann-Whitney (rank sum) test; * Z-value)
[4] Experimental mean temperature ± 95% confidence level; determined from average of provisioning day means (mean temperature from day of host provisioning to emergence of all *C. torvina* from those hosts).

a slightly shorter (but not significantly shorter) development time in *P. xylostella*. *Conura torvina* provided with *C. rubecula* or *P. xylostella* hosts daily produced 8.4 to 8.5 progenies per day, and this is close to the 8.6 progenies per day from *P. xylostella* hosts in the first experiment (Table 3.1.1).

Plutella xylostella and *C. rubecula* host quality appeared to be relatively low during the initial three experimental host provisionings. Control host survival was ≤11/13 (84%) for *P. xylostella* in the first three provisionings and ≤9.5/13 (73%) for *C. rubecula* in the first two provisionings. This may have contributed to a slightly higher host mortality and a corresponding reduced reproductive rate for *C. torvina* in the first days of the experiment (Figure 3.1.3).Control host survival for both host species was ≥11/13 (84%) after the third host provisioning. *Conura torvina*'s low reproductive rate early in the experiment may have also been due to reduced oviposition activity, indicated by slightly more unparasitized hosts (host emergence) (Figure 3.1.3). Mortality of the control hosts during the experiment was 14.0% for *P. xylostella* hosts and 15.2% for *C. rubecula* hosts.

There was no significant difference between *C. torvina* provided daily or intermittently with *C. rubecula* hosts (Table 3.1.3, Figure 3.1.4). It appeared that more progenies were produced by the daily provisioned females than by those provisioned intermittently, and this result is contrary to results from the first continuous host provisioning experiment in which *P. xylostella* hosts were used. However, there are notable differences between these two experiments. First, different hosts were used and *C. torvina*'s reproductive behavior is slightly different for *C. rubecula* hosts than for *P. xylostella* hosts (e.g., generally more female progenies are produced in *P. xylostella* hosts). These slight differences might affect the outcome of a provisioning method. Second, provisioning methods were compared on four intermittent provisioning days (days 4, 7, 10, and 13) in the first experiment, but on only two days (days 4, and 7) in the second experiment. Seven days of host provisioning may not be sufficient time to show a difference between these two methods because *C. torvina* may not immediately show the stress of daily host provisioning. The intermittently provisioned females in the second experiment had a lower reproductive rate because they parasitized fewer hosts; the number of hosts emerging was greater from this group, and the difference was nearly significant ($p=0.055$) at $\alpha = 0.05$ (Table 3.1.3). The proportion of female progenies appears to be higher from the intermittently provisioned group, and as in the first experiment, development time appeared to be shorter. However, the difference is not significant in either case. Females from the intermittently provisioned group appeared to be producing a higher proportion of female progenies before the provisioning method could have had any effect (before and during the first the first experimental provisioning).

Re-testing of Continuous Daily Provisioning of *P. xylostella* and *C. rubecula* Hosts

Materials and Methods

The third and fourth continuous provisioning experiments re-tested *C. torvina* when provided daily with *P. xylostella* or *C. rubecula* hosts. In the third experiment, 13 females were selected from a cohort of five week old *C. torvina*. Females were caged, mated and divided into two groups (one group of six and one group of seven). Female *C. torvina* in the group of six received *P. xylostella* hosts and those in the group of seven were provided with *C. rubecula* hosts. All females were given six pre-experimental host provisionings during a six week period before the experiment. The last pre-experimental provisioning was made four days before the first of six experimental host provisionings. On the first day of the experiment one of the six females being provided with *P. xylostella* hosts escaped, reducing the number tested to five. Control hosts (one group of *P. xylostella* and two groups of *C. rubecula* per day) were collected for the first two days of the experiment.

In the fourth experiment, 24 females were selected from a cohort of two month old *C. torvina*. These females were caged and mated. In the pre-experimental provisionings, twelve were provided with *P. xylostella* hosts and twelve were provided with *C. rubecula* hosts. Three pre-experimental host provisionings were made in a two week period, with the last being made six days before the first experimental provisioning. Six females out of each group of twelve were used in the experiment, and were provided with the host species they had in the pre-experimental provisionings. Experimental provisioning of *P. xylostella* or *C. rubecula* hosts lasted for four days. One female in each group of six died on the second day of host provisioning and data from these females were excluded from analysis. This reduced the number of replicate females tested for each treatment to five. A control group of each host species was collected on each day hosts were provided.

Response data from the daily *P. xylostella* and *C. rubecula* host treatments in the third and fourth experiment were compared across host provisioning days using a Repeated Measures ANOVA. Data for mean % females emerging from each host species (averaged across provisioning days) were compared using a non-parametric, two sample, Mann-Whitney, rank sum test.

Results and Discussion

Conura torvina provided with *C. rubecula* hosts had significantly more progenies (α=0.05) than those provided with *P. xylostella* hosts (Tables 3.1.4 and 3.1.5). The lower reproductive rate in *C. torvina* provided with *P. xylostella* hosts was due to reduced oviposition activity. In both experiments, the number of emerging *P. xylostella* (hosts which escaped parasitization) was significantly higher than the number of emerging *C. rubecula* (Tables 3.1.4 and 3.1.5). These results differ from that seen in the second experiment, where *C. torvina*'s reproductive rate in both host species was equal. In third and fourth experiments *C. torvina'*s mean reproductive rate in *P. xylostella* was 5.77 and 6.45 progenies per female per day, respectively. This appeared to be considerably lower than the 8.6 and 8.5 progenies per female per day from *P. xylostella* in the first and second experiments. No reason could be found for the consistently poor performance by the females provided with *P. xylostella* hosts in these last two experiments. The un-characteristically low reproductive rate in *P. xylostella* hosts suggests that there was a problem with these particular *C. torvina* females or with their hosts.

Data for control host mortality did not indicate that *P. xylostella* hosts were of a poorer quality than *C. rubecula* hosts. Control mortality was 1/26 (3.8%) for *P. xylostella* and 11/52 (21.2%) for *C. rubecula.* In the fourth experiment, control hosts were collected on each day of the experiment and overall mortality was 2/52 (3.8%) for *P. xylostella* and 5/52 (9.6%) for *C. rubecula*. It appears that the general health of the hosts had little influence on whether *C. torvina* selected them for oviposition.

In the third experiment as in the second, an apparently higher (but not significantly higher) proportion of female *C. torvina* emerged from *P. xylostella* than from *C. rubecula* hosts (Table 3.1.4). There was no apparent sex ratio difference from different hosts in the fourth experiment (Table 3.1.5), but the proportion of female *C. torvina* which emerged from *P. xylostella* seemed to be uncharacteristically low (11.1%) when compared with the higher proportion of females (>30%) resulting in other experiments where *P. xylostella* is the host.

In both the third and fourth experiments, the development time for *C. torvina* appears to be slightly shorter in *P. xylostella* than in *C. rubecula*, but the difference is not significant (Tables 3.1.4 and 3.1.5). This result is similar to that seen in the second experiment (Table 3.1.2).

Table 3.1.4: Comparison of *Conura torvina* reproductive success when *Plutella xylostella* or *Cotesia rubecula* are provided daily for six days (n = number of *C. torvina* replicates).

Response Variables	*P. xylostella* Hosts Provided Daily (n=5)	*C. rubecula* Hosts Provided daily (n=7)			
	$\bar{x}$	$\bar{x}$	p	Z	MSE[2]
Progeny per *C. torvina* per day [1]	5.77 **b**	7.64 **a**	0.017		7.46
Hosts emerging per day [1]	4.87 **a**	2.62 **b**	0.016		10.43
Dead hosts per *C. torvina* per day [1]	2.36 a	2.69 a	0.666		9.26
% Female *C. torvina* emerge [3]	29.0 a	17.5 a	0.222	1.22*	
C. torvina development time [1] (Days from oviposition to adult emergence; mean temperature = 25.3 ± 0.1°C [4])	12.9 a	13.4 a	0.152		1.54.

[1] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Repeated Measures ANOVA).
[2] MSE = Mean Square Error (Standard Error of the difference, from the ANOVA).
[3] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Nonparametric Mann-Whitney (rank sum) test; * Z-value)
[4] Experimental mean temperature ± 95% confidence level; determined from average of provisioning day means (mean temperature from day of host provisioning to emergence of all *C. torvina* from those hosts).

Table 3.1.5: Comparison of *Conura torvina* reproductive success when *P. xylostella* or *C. rubecula* are provided daily for four days (n = number of *C. torvina* replicates).

Response Variables	*P. xylostella* Hosts Provided Daily (n=5)		*C. rubecula* Hosts Provided daily (n=5)				
	$\bar{x}$		$\bar{x}$		p	Z	MSE[2]
Progeny per *C. torvina* per day [1]	6.45	**b**	8.75	**a**	0.008		4.28
Hosts emerging per day [1]	5.06	**a**	2.40	**b**	0.002		3.25
Dead hosts per *C. torvina* per day [1]	1.50	a	1.85	a	0.170		0.54
% Female *C. torvina* emerge [3]	11.1	a	10.8	a	0.916	0.11*	
C. torvina development time [1] (Days from oviposition to adult emergence; mean temperature = 24.1 ± 0.1°C [4])	17.1	a	17.4	a	0.415		1.55

[1] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Repeated Measures ANOVA).
[2] MSE = Mean Square Error (Standard Error of the difference, from the ANOVA).
[3] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Nonparametric Mann-Whitney (rank sum) test; * Z-value)
[4] Experimental mean temperature ± 95% confidence level; determined from average of provisioning day means (mean temperature from day of host provisioning to emergence of all *C. torvina* from those hosts).

Continuous Provisioning Experiments

Conclusions

The main conclusions from these experiments are that *C. torvina* can maintain a relatively high reproductive rate and fairly consistent proportion of female progenies over a period of up to two weeks. When 13 hosts were provided per day, *C. torvina* could produce ≥8 progenies per day. *Conura torvina'*s reproductive rate did not appear to differ much between *P. xylostella* and *C. rubecula* hosts, but there was a consistently higher proportion of female progenies produced from *P. xylostella* hosts. Although this difference was not significant, similar results were seen in the "host species choice" experiments (Chapter 2, Part 2, Table 2.2.1 and Table 2.2.2) and "provisioning an abundant supply of hosts" experiments (Chapter 3, Part 2, Table 3.2.1). The development time for *C. torvina* also appeared to be consistently shorter (from 0.2 to 0.5 days shorter) in *P. xylostella* hosts. This difference was not significant, but was consistent across three different experiments. The shorter development time in *P. xylostella* hosts may result from this host's slightly larger size, allowing more nutrient per progeny.

Other conclusions from these experiments are that the development time for *C. torvina* progenies is greater when hosts are provided daily rather than intermittently. The difference was seen in both *P. xylostella* and *C. rubecula* hosts but was only significant in *P. xylostella* hosts. A possible explanation for this difference is that when *C. torvina* oviposits daily, there is less time for eggs to mature between bouts of oviposition and nutrient reserves are more heavily taxed. Thus, they lay smaller, less-developed eggs than *C. torvina* which have two days to recuperate between periods of oviposition.

From the poor performance of *C. torvina* provided with *P. xylostella* hosts in the third and fourth experiments, one might conclude that laboratory selection was having a detrimental effect on the population of experimental *C. torvina* reared from *P. xylostella.* This assumption would carry some weight if the chronology of the experiments were the same as their first through fourth designations. However, these designations are based on the similarity of the experiments rather than their chronology. The four experiments were conducted in a 9 month period and the third and fourth experiments came after the first, but before the second experiment.

The results of these experiments support conclusions from the host age experiments (Chapter 2, Part 1), that the observed decline in the reproductive rate and proportion of female progenies during five consecutive (daily) host provisionings was due to the increasing age of the provided hosts rather than from reproductive fatigue. It is evident that *C. torvina* can maintain a relatively high rate of reproduction with a consistent proportion of female progenies over a period of more than a week.

Chapter 3, Part 2: Provisioning With an Abundant Host Supply

Introduction

Most previous host provisioning experiments provided ≤13 hosts per female per day. This was partly due to a limited supply of hosts, and because more *C. torvina* females (replications) could be tested if fewer hosts were provided per female. However, there was a possibility that the potential reproductive rate for *C. torvina* (maximum number of progenies that can be produced per day) was not being reached because a female provided with ≤13 hosts may visit and oviposit in some hosts more than once. This would be particularly true if *C. torvina* did not recognize hosts it had already oviposited in and few hosts were available. I thought that by increasing the number of hosts available to a female, I would reduce the probability of her visiting the same host twice, thus, increasing the number of parasitized hosts. There was also the possibility that *C. torvina* lays multiple eggs during each visit to a host, and the presence of a relatively large number of hosts would induce females to distribute their eggs more uniformly among the available hosts.

Materials and Methods

Several experiments were conducted in which ≥20 hosts were provided daily to each *C. torvina*. The first experiment was conducted at the conclusion to the first continuous provisioning experiment where *C. torvina* was tested with a daily and intermittent supply of *P. xylostella*. In that experiment, each female from the daily and intermittent host provisioning treatments was provided with 26 *P. xylostella* hosts. Those *C. torvina* from the daily provisioning group were given 26 hosts each on the fourteenth day of the experiment, and females which had been provided hosts intermittently, received 26 hosts each on the sixteenth day of the experiment.

The second abundant host supply experiment tested nine females from the same *C. torvina* cohort used in the fourth continuous provisioning experiment. These *C. torvina* had gained experience from three pre-experimental host provisionings before the fourth continuous provisioning experiment, but were not used in that experiment. Of the nine females, five had received *P. xylostella* hosts and four had received *C. rubecula* hosts. Because these females had been maintained at 25°C for nearly a month without receiving hosts, they were given one additional pre-experimental host provisioning to prime them for oviposition behavior before the commencement of the abundant host supply experiment. Pre-experimental and experimental host provisionings were made with the same host species as females had originally received one month earlier. In the pre-experimental provisioning each female received 16 hosts for a day. Six subsequent experimental host provisionings were made, starting two days after the pre-experimental provisioning. Hosts were provided every second day for a day (14 h photoperiod), and each female was provided 20 hosts on the first two provisionings, 26 hosts on the next two provisionings and 32 hosts on the last two provisionings. Two groups of control hosts (one group of each species, 32 hosts per group) were collected on the final day of the experiment.

A repeated measures ANOVA was used for each pair of days in which the same number of hosts was provided. Replicate data for mean % females emerging were averaged across both provisioning days for each number of hosts provided. The proportion of female *C. torvina* progenies from each host species were compared using a non-parametric, two sample, Mann-Whitney, rank sum test.

Results and Discussion

The provisioning of 26 hosts per female at the conclusion of the first continuous provisioning experiment resulted in a dramatic increase in the number of progenies per *C. torvina* per day (Figure 3.1.1). When 13 hosts were provided to *C. torvina*, the mean number of progenies per day had been 8.6 and 9.6 in thedaily and intermittent treatments, respectively (Table 3.1.1). When 26 hosts were provided, the corresponding mean number of progenies per day increased to 14 and 12.6, respectively (Figure 3.1.1). A maximum of 17 progenies were produced by one female and three of the *C. torvina* females produced 15 progenies.

In the second abundant hosts experiment, *C. torvina* appeared to respond poorly to the relatively large number of hosts in the pre-experimental host provisioning (16 hosts per female) and in the first experimental host provisioning (20 hosts per female) (Figure 3.2.1). The poor response to hosts in the initial host provisioning may have resulted from *C. torvina* being held for nearly a month without host provisioning and only honey to eat. It is possible that *C. torvina* may lose some of its reproductive drive or have a reduced egg load due to egg resorption after a long period without oviposition and host feeding opportunities. When 20 hosts were provided per day, *C. torvina* produced a mean of 9.9 and 9.6 progenies per female (*P. xylostella* and *C. rubecula* hosts, respectively), and there was no difference in the response to either host species (Table 3.2.1). On the third experimental host provisioning (26 hosts per female) *C. torvina* appears to reach its maximum reproductive rate in each host species (means of 11 and 14 progenies per day in *P. xylostella* and *C. rubecula* hosts, respectively). The mean number of progenies per female remained approximately the same or declined slightly after this provisioning (Figure 3.2.1). Significantly more progenies (α=0.05) were produced in *C. rubecula* than in *P. xylostella* hosts when 26 hosts were provided per female (Table 3.2.1). The difference appears to be due to reduced oviposition by the females provided with *P. xylostella* hosts because the mean host emergence was significantly greater for *P. xylostella* (11.6) than for *C. rubecula* (8.4) ($p=0.016$, MSE=4.68). During the 20 host and 26 host provisionings there was no difference in the mortality for *P. xylostella* or *C. rubecula* (dead hosts from which no *C. torvina* emerge) (Figure 3.2.1). When 32 hosts were provided per day, there was no significant difference in the mean number of progenies per female from *C. rubecula* or *P. xylostella* hosts (Table 3.2.1).

There appeared to be a slight decline in the number of *C. torvina* progenies from *C. rubecula* when 32 hosts were provided per female and this may be due to higher *C. rubecula* mortality (dead hosts) (Figure 3.2.1). At 32 hosts per female, the mean 8.5 dead *C. rubecula* is significantly greater than the 4.2 dead *P. xylostella* ($p<0.001$, MSE=1.09), and is more than double the mean *C. rubecula* mortality (3.4 dead) seen when 26 hosts were provided per female.

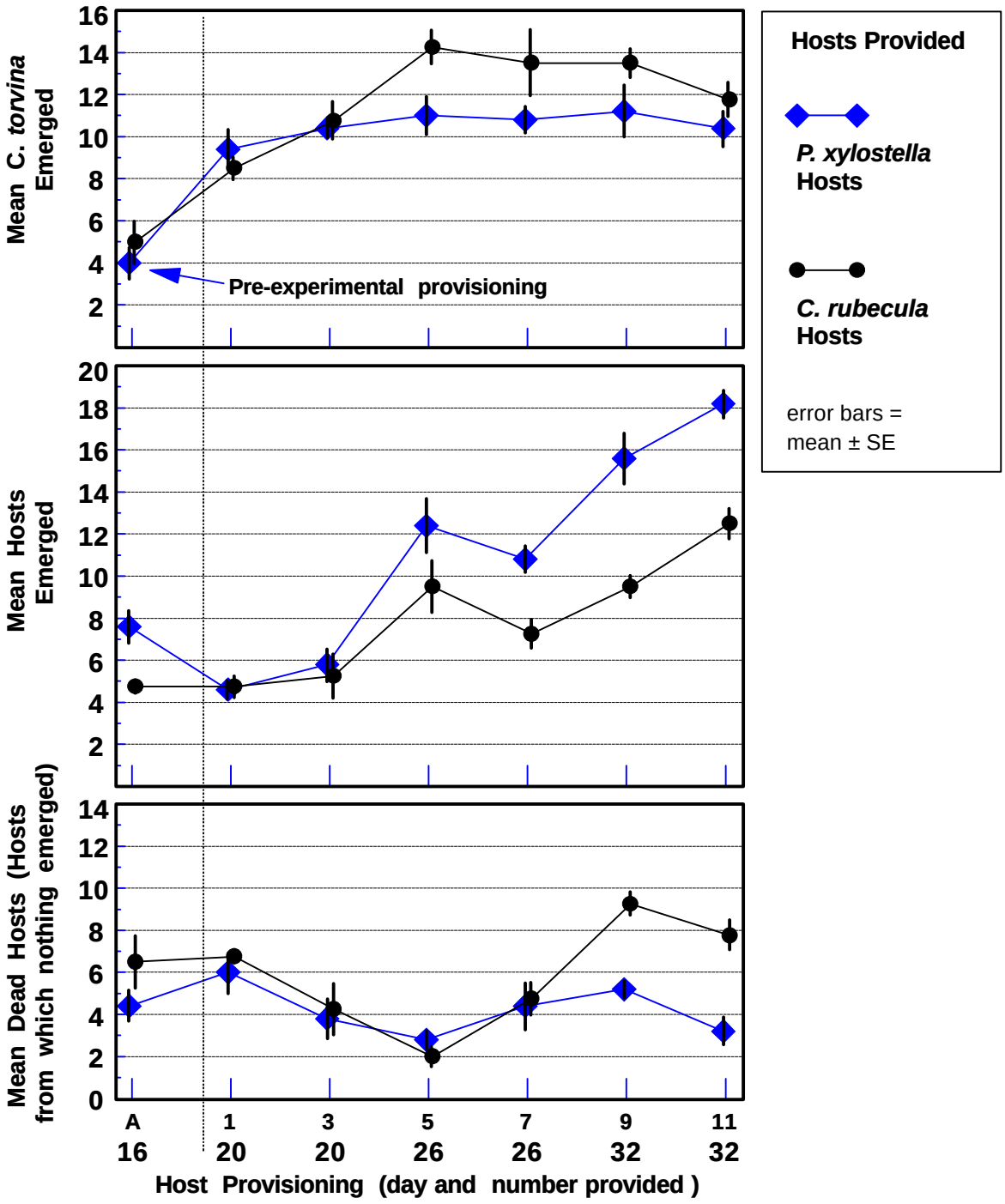

Figure 3.2.1: Comparison between *Conura torvina* provided with *Plutella. xylostella* or *Cotesia rubecula* hosts in increasing abundance (20, 26 and then 32 hosts per female, two provisioning days at each level, hosts provided every other day for an 11 day period) (n = 5 and 4 *C. torvina* tested in the *P. xylostella* and *C. rubecula* treatments, respectively).

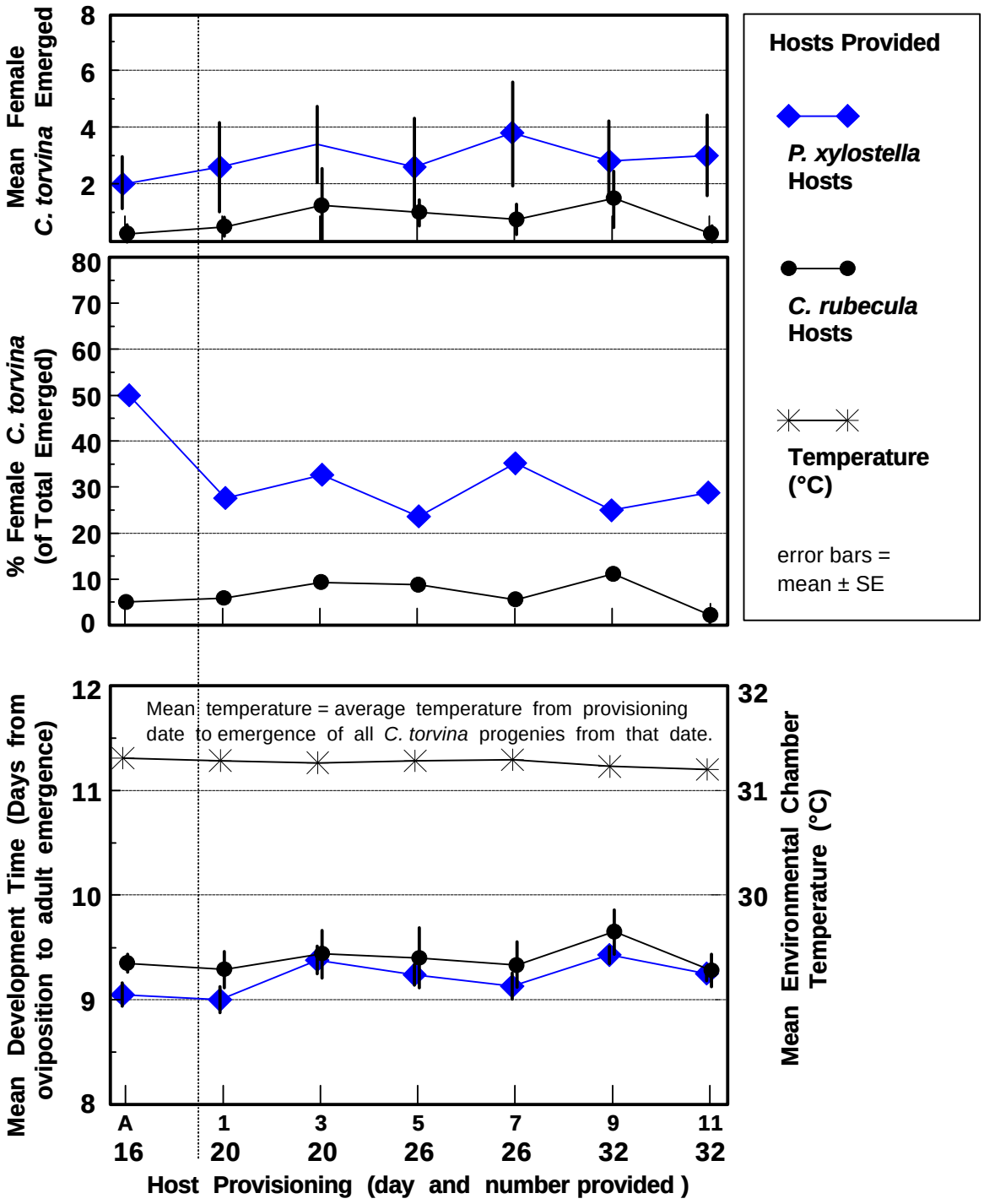

Figure 3.2.2: Comparison between *Conura torvina* provided with *Plutella. xylostella* or *Cotesia rubecula* hosts in increasing abundance (20, 26 and then 32 hosts per female, two provisioning days at each level, hosts provided every other day for an 11 day period) (n = 5 and 4 *C. torvina* tested in the *P. xylostella* and *C. rubecula* treatments, respectively).

Table 3.2.1: Comparison of *Conura torvina* reproductive success when *Plutella xylostella* or *Cotesia rubecula* were provided in increasing abundance (20, 26 and then 32 hosts per female, two provisioning days at each level, host provisionings made every other day for 11 days) (n = number of *C. torvina* replicates).

Response Variables		*C. torvina* Provided *P. xylostella* Hosts (n=5)	*C. torvina* Provided *C. rubecula* Hosts (n=4)			
		$\bar{x}$ [1]	$\bar{x}$ [1]	p	Z	MSE[2]
20 Hosts per *C. torvina* per day	Progeny per *C. torvina* per day	9.9 a	9.6 a	0.702		2.11
	% Female *C. torvina* emerge [3]	29.5 a	7.2 a	0.214	-1.24*	
26 Hosts per *C. torvina* per day	Progeny per *C. torvina* per day	10.9 **b**	13.9 **a**	0.035		5.83
	% Female *C. torvina* emerge [3]	29.0 a	7.6 a	0.456	-0.74*	
32 Hosts per *C. torvina* per day	Progeny per *C. torvina* per day	10.8 a	12.6 a	0.133		5.13
	% Female *C. torvina* emerge [3]	31.0 a	8.9 a	0.456	-0.74*	

[1] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Repeated Measures ANOVA).

[2] MSE = Mean Square Error (Standard Error of the difference, from the ANOVA).

[3] Means within row followed by same letter are not significantly different ($\alpha = 0.05$; Nonparametric Mann-Whitney [rank sum] test; * Z-value)

The increase in *C. rubecula* mortality during the last two host provisionings may be due to poor host quality. Mortality in control hosts (collected on the last provisioning day only) was only 1/32 (3.1%) for *P. xylostella*, but 13/32 (40.6%) for *C. rubecula*.

Although the mean number of *C. torvina* progenies appeared to be lower from *P. xylostella* than from *C. rubecula*, this difference may not reflect *C. torvina*'s real reproductive capability in *P. xylostella*. The means of 12.6 and 14.0 *C. torvina* progenies from *P. xylostella* hosts in the first abundant supply of hosts experiment prove that *C. torvina* can produce more than the maximum of 11.2 progenies from *P. xylostella* seen in the second experiment. It appears that the *C. torvina* used in the second experiment responded abnormally to *P. xylostella* and may have been defective in some way. These *C. torvina* came from the same cohort as those used in the fourth continuous provisioning experiment where the reproductive rate in *P. xylostella* was also uncharacteristically low (Table 3.1.5).

Conura torvina appeared to reach its maximum reproductive rate when provided with 26 hosts; the number of progenies per female does not increase when 32 hosts are provided. Two females produced as many as 16 progenies per day and one produced 15 (all from *C. rubecula* hosts). The pattern of host emergence and host mortality (dead hosts) appears to be the same for both host species (Figure 3.2.1). The similarity of host emergence patterns may be partly due to the increase in the number of unparasitized hosts when a greater number of hosts is provided. The similar pattern of mortality (dead hosts) in both host species must be due mostly to activity by *C. torvina* because the host species are completely unrelated, and environmental conditions were stable during the experiment.

The proportion of female *C. torvina* progenies ranged from 29 to 31% from *P. xylostella* hosts and from 7.2 to 8.9 % from *C. rubecula* hosts (Table 3.2.1, Figure 3.2.2). Although there was no significant difference between the proportion of female *C. torvina* progenies from either host species, *C. torvina* appeared to consistently produce a higher proportion of female progenies in *P. xylostella* hosts. This experiment had too few replications of each treatment to prove significance. The higher proportion of female *C. torvina* from *P. xylostella* hosts is consistent with results from most other experiments.

Although the development time for *C. torvina* was not significantly, shorter in *P. xylostella* than in *C. rubecula* hosts, it appeared to be consistently, shorter(Figure 3.2.2). The difference of ≤0.3 days is similar to that seen in other host provisioning experiments.

Conclusions

The major conclusions from the abundant supply of hosts experiments are that *C. torvina* females can produce a mean of 14 progenies per day when 26 hosts are provided per female, and that the number of progenies per day does not increase when more hosts are provided. Individual *C. torvina* occasionally produced 15 progenies per day and the maximum seen from any female was 17.

Results from this experiment appear to indicate that caged *C. torvina* do not mark their hosts or select hosts in a systematic manner. *Conura torvina* were able to successfully parasitize a mean of 14 hosts per day, but usually parasitized far fewer than that in other experiments where ≤13 hosts were placed in a cage; some hosts are completely missed, and it is probable others are visited and oviposited in more than once. When provided with ≥20 hosts, females had a better chance of distributing their eggs among many hosts. The apparent lack of host marking and apparently poor host searching behavior may actually be an artifact of the relatively small volume of the cages. Caged females might mark their hosts, but the marking chemical may saturate the air in the cage, making the female: (1) unable to distinguish which hosts were marked; or (2) fail to respond to marked hosts by habituating the female to the chemical.

Chapter 3, Part 3: *Conura torvina*'s Egg Production Capacity and Egg Laying Behavior

Introduction

Host provisioning experiments indicated that *C. torvina* females typically produced no more than 14 progenies per day, even when provided with up to 32 hosts. Although *C. torvina*'s observed reproductive capacity appeared to be relatively low, the actual number of eggs it could lay was not known because it may lay more than one egg per host. Arthur (1958) reported that *C. torvina* will lay multiple eggs per host. Pupal parasitoids generally have a lower egg production capacity than the parasitoids of the earlier host developmental stages (Price 1973a, 1973b), and since *C. torvina* is a pupal parasite, its reproductive system might be expected to conform to these findings. Price (1973a, 1973b) also indicated that a good estimation of the egg laying capacity of a hymenopteran parasitoid species can be made by counting the number of ovarioles per ovary, and the number of mature oocytes stored within the ovarioles or oviducts. Parasitoid species with relatively few ovarioles typically carry only one mature oocyte per ovariole, and this makes determination of the egg carrying capacity quite simple (Price 1973b). Thus, dissection of *C. torvina* should provide an accurate estimate of its egg carrying capacity. An estimate of *C. torvina*'s egg production capacity could be obtained by dissecting all the hosts *C. torvina* parasitized in one day and counting the eggs laid in those hosts. Host dissections would also provide an estimate of how many eggs are laid by *C. torvina* in each host.

Two dissection experiments were conducted. The first examined the effect of *C. torvina*'s level of oviposition experience on oviposition capacity and behavior (i.e., the number of eggs that can be laid and the way eggs are distributed among hosts). The second experiment examined *C. torvina*'s daily egg production capacity as influenced by factors such as host species and host abundance.

Effect of *C. torvina*'s Oviposition Experience on its Oviposition Behavior

Materials and Methods

Two groups of *C. torvina* females were randomly selected, six from among a group of females which had oviposition experience, and six others from a group of females which had little experience. The experienced females were about 11 weeks old and had been given seven host provisionings in the previous two months. The inexperienced females were about seven weeks old and were given three consecutive provisionings of *P. xylostella* hosts to stimulate oviposition behavior. As the experienced females had not had any hosts for two weeks they were given another host provisioning to stimulate reproductive behavior before the start of the experiment. This provisioning coincided with the third pre-experimental host provisioning for the less experienced females. One day after the third pre-experimental host provisioning, each group was given hosts which were dissected after exposure. In this experimental provisioning, each female was provided with six 2-3 day old *P. xylostella* pupae for a day (14 h photoperiod). The exposed *P. xylostella* were then removed from cages and held in 30 ml plastic diet cups at ambient laboratory temperatures (30 ± 2°C) for approximately 27 h to allow some development of the

C. torvina eggs. Hosts were then held in a refrigerator (at 10°C) until dissection. Host dissection commenced three days after refrigeration and was completed in four days. When possible, equal numbers of hosts from each treatment group were dissected each day.

Host dissections were conducted in insect (Ringers) saline solution under a dissecting microscope. Before each dissection the host was examined and the number of oviposition puncture wounds was counted. Egg counts from each host were then correlated with the number of oviposition wounds to obtain a correlation coefficient (Zar 1984, Hintze 1995). *Conura torvina* eggs and larvae found during dissection were counted and measured with an ocular micrometer.

There were initially six females used in each treatment group, but data from one female in the experienced group were excluded from the analysis because host dissection revealed that she had not laid any eggs. A review of her past oviposition history indicated that she had not produced progenies in her seven prior host provisionings. Counts of eggs and larvae per female and means for egg and larva size were compared between treatments using two-sample t-tests (Hintze 1995).

Results and Discussion

Dissection of hosts yielded both eggs and larvae of *C. torvina*. This indicates that *C. torvina* eggs hatched before host refrigeration, and within 41 hours after oviposition at 30°C (14 h light during the provision period + 27 h host storage = 41 h). The egg development time of <41 h is comparable to the 36-40 hour development time observed by McNeil & Rabb (1973) at 27°C. Larvae from eggs that hatched before refrigeration may have continued development and growth after refrigeration at 10°C because a few second instars (larvae ≥1.66 mm in length) (Arthur 1958) were found in hosts which were dissected after seven days storage. Live larvae dissected from hosts on the first dissection day averaged 1.18 mm in length, whereas those found on the third day averaged 1.35 mm, but a two sample t-test ($\alpha = 0.05$) found no significant size difference ($p = 0.116$) between both groups of larvae (larvae compared were progenies of experienced females only; n = 11 larvae dissected from hosts on first and on the third day; the hosts [from a female in the experienced group] dissected on the fourth day contained no larvae or eggs).

Larval length ranged from 0.6 mm to 1.7 mm, and larvae found in hosts from experienced females were significantly larger ($\alpha=0.05$) than those from the less experienced females (Table 3.3.1). Many of the *C. torvina* eggs that were found in hosts after 5 days refrigeration were brownish in color and appeared to be dead. This might indicate that eggs stored at 10°C will not hatch. Egg size ranged from 0.45 mm to 0.60 mm in length, and was not significantly different between experienced and inexperienced *C. torvina*. *Conura torvina* larval mortality was as high as 50% in some replicates. Dead larvae appeared to occur more frequently in hosts containing multiple larvae, and the dead larvae were usually smaller than the live larvae found with them. The mean size for dead and live larvae was 0.69 mm and 1.07 mm, respectively. Larval mortality

was probably due to a larger larva killing the other smaller larvae within a host because some dead larvae appeared to have wounds. Arthur (1958) noted that larger first instar *C. torvina* will generally kill all their smaller rivals within a host and also observed wounds on the dead larvae.

No *C. torvina* eggs or larvae were observed in hosts without oviposition puncture wounds. The number of oviposition wounds was significantly higher in hosts parasitized by the less experienced than the experienced females. The number of eggs laid per host (rate of superparasitism) was also significantly higher for the less experienced females (Table 3.3.1). The number of puncture wounds was typically larger than the number of eggs laid. Only 1 of 27 hosts and 1 of 34 hosts dissected from the experienced and less experienced groups, respectively, contained more eggs than puncture wounds (one wound for two eggs in each case). The number of puncture wounds equaled the number of eggs found in 8 of 27 hosts and 3 of 34 hosts dissected from the experienced and less experienced groups, respectively. There was a significant correlation (α=0.05, $p = 0.005$) between the number of oviposition wounds and the number of eggs laid per host for the less experienced females, but no correlation ($p = 0.606$) for experienced females; correlation coefficients were 0.54 for less experienced and 0.16 for experienced females (1 = strong positive correlation, 0 = no correlation and -1 = strong negative correlation) (Zar 1984, Hintze 1995). The number of oviposition wounds per host ranged from 1-20 from the less experienced females and 1-7 from experienced females. The number of eggs laid per host ranged from 1-14 from the less experienced females and 1-4 from experienced females. Eggs and larvae were found in the thorax, abdomen and head of the *P. xylostella* pupae; slightly more eggs were found in the thorax than in the abdomen, but larvae were found mostly in the abdomen. Only 2 of 56 eggs were found in the head. On average, host dissection required 20 minutes per host.

Although test females came from two different cohorts and differed in age and oviposition experience, there was no difference between each group for the mean number of parasitized hosts (Table 3.3.1). A major difference between groups was seen in the number of progenies (eggs laid) per female. The less experienced females laid significantly more eggs than the experienced females, and a result of this was that they also laid significantly more eggs per host. Significant differences are also seen in the number of eggs and larvae dissected from hosts in each group (Table 3.3.1). The experienced females laid fewer eggs than the less experienced females and 79% of eggs laid by experienced females had hatched and were in the larval stage whereas only 54% of the eggs laid by less experienced females had hatched. Also, the larvae dissected from the experienced female's hosts were significantly larger than those dissected from the hosts of less experienced females (Table 3.3.1). This difference is probably due to the effect of superparasitism by the less experienced females. When there are too many eggs laid per host, the competition for nutrients may result in slower egg hatch and larval development. Another possible explanation is that since experienced females laid fewer eggs, the eggs being laid were more developed and consequently hatched sooner.

Table 3.3.1: Number of eggs laid in *Plutella xylostella* hosts by inexperienced and experienced *Conura torvina* females (six hosts provided per female; n = number of *C. torvina* tested)

Response Variables	Inexperienced *C. torvina* (n = 6)		Experienced *C. torvina* (n = 5)		p	df
	$\bar{x} \pm SD$		$\bar{x} \pm SD$			
Hosts Parasitized per *C. torvina* [1]	5.7 ± 0.5	a	5.2 ± 0.4	a	0.283	9
Progenies (eggs + larvae) per *C. torvina* replicate [1]	18.0 ± 5.0	**a**	8.4 ± 1.8	**b**	0.003	9
Eggs Dissected from hosts per replicate [1]	8.2 ± 4.4	**a**	1.8 ± 1.1	**b**	0.015	9
Larvae Dissected from hosts per replicate [1]	9.8 ± 2.8	**a**	6.6 ± 1.6	**b**	0.050	9
Progenies (eggs + larvae) per Host per replicate [1]	3.1 ± 0.8	**a**	1.6 ± 0.3	**b**	0.003	9
Oviposition Puncture Wounds per Host per replicate [2]	7.0 ± 3.3	**a**	3.3 ± 0.6	**b**	0.006	9
Egg size (mm) [1]	0.53 ± 0.04	a	0.52 ± 0.03	a	0.964	56
Larva size (mm) [1]	0.85 ± 0.23	**a**	1.15 ± 0.32	**b**	<0.001	90

[1] Means within row followed by the same letter are not significantly different ($\alpha = 0.05$; Two-sample t-test)
[2] Means within row followed by the same letter are not significantly different ($\alpha = 0.05$; Mann-Whitney non-parametric Rank -Sum Test)

Among the experienced females, no female produced more than 10 eggs in the 14 hour period of light during which they could oviposit. However, one of the females from the less experienced group laid 25 eggs in this period (14 of these progenies [eggs + larvae] were found in a single host). This indicates that *C. torvina* with little oviposition experience can and will lay a relatively large number of eggs, and that a large proportion of her eggs may be wasted through superparasitism.

Conclusions

The main conclusions from this experiment are that *C. torvina* with little oviposition experience do not use their eggs as efficiently as more experienced females. Inefficient use of eggs in the form of superparasitism, resulted in apparently slower development of eggs or the larvae that hatched from them, and mortality of the extra larvae in each host. Experienced *C. torvina* tended to conserve their eggs and distribute them more evenly among the available hosts. The *C. torvina* with little oviposition experience laid an average of 18 eggs in a day (14 hour photoperiod), and one laid 25 eggs in the same period. This indicated that average egg production can be higher than the maximum average of 14 progenies produced per female per day (in the abundant supply of hosts experiment), and can be almost 50% greater than the maximum of 17 progenies per day produced by a female.

Effect of Host Species and Abundance on *C. torvina*'s Egg Production Capacity and Oviposition Behavior

Introduction

The main objective of this experiment was to determine *C. torvina*'s daily egg production and to examine the influence of factors such as host species and host abundance on the number of eggs laid per host. *Conura torvina* was tested with *C. rubecula* or *P. xylostella* hosts in an ample or restricted supply. *Conura torvina* females were dissected to determine the number of mature eggs found in an ovary both before and after host feeding opportunities and oviposition.

Materials and Methods

This experiment was conducted in an environmental chamber at 26 ± 1.0°C ($\overline{x}$ ±SD) with a 14 L:10 D photoperiod. The experiment involved four host provisionings, three to provide oviposition experience and host feeding opportunities to females, and the fourth to test the effects of oviposition and host supply on egg production and oviposition behavior. For this experiment, a cohort of 40, ten-day-old female *C. torvina* reared from *P. xylostella* hosts was used. Each female was caged in a 50 ml plastic vial with 3 cm diameter ventilation opening the bottom which was covered with 0.30 mm mesh, plastic screen. These 50 ml vial cages were considerably smaller than the 200 ml cages employed in all previous experiments. Smaller cages were used so that the large number of *C. torvina* replicates (females) tested in this experiment would all fit in one environmental chamber. Females were each provided a mating opportunity by exposure to two males for a day.

The 40 females were randomly divided into two groups of 15 females and one group of 10 females (Figure 3.3.1). The females in the group of ten were not given hosts, but were held until 10 h after females from the other groups had their third host provisioning. They were then sacrificed for dissection; these females had not had any host feeding opportunity and were only fed honey.

Females in both groups of 15 were each provided with five hosts every other day over a period of five days. To ensure that females were stimulated by the hosts and had ample opportunity to practice oviposition during the initial host provisioning, hosts were provided for 36 h. All subsequent host provisionings were limited to the 14 h photoperiod. A dry, 1 cm, square piece of paper towel which had been soaked in *P. rapae* frass paste was placed in each cage during the first host provisioning to stimulate oviposition behavior. Females in one group of 15 received *C. rubecula* pupae as hosts, and those of the other group of 15 received *P. xylostella* pupae. On the morning of the sixth day (10 h after the end of their third host provisioning), five females from each group of 15 were randomly selected and sacrificed for dissection. Sacrificed females were placed in 75% ethanol and stored at 10°C until dissection. The sacrificed females had each had oviposition and host feeding opportunities and a full night (10 hours) to regenerate mature eggs to replace those eggs which had been used in oviposition the previous day (see experimental plan, Figure 3.3.1). The hosts from the third provisioning were collected immediately after the end of the provisioning period and sacrificed for dissection (placed in 75% ethanol and stored at 10°C until dissection). The exposed hosts from the initial two host

provisionings were not dissected, but were maintained in the environmental chamber until all adult *C. torvina* progenies emerged. The number, sex, and date of emergence was then recorded for the emerging progenies of each female.

Ten hours after the end of the third host provisioning, the remaining 10 females in each host species treatment group were divided into two groups of five, and each group received either 5 or 15 hosts for the fourth (14 hour) host provisioning period. At the end of this fourth host provisioning period, these females were sacrificed for dissection. Hosts from this fourth provisioning were also sacrificed immediately for dissection.

On the second, third and fourth host provisionings a small number of control hosts (10 of each species) were randomly selected from the hosts that were being provided. These hosts were held in a vial until they had emerged, and served as an indication of host quality by providing an estimate of host mortality in the absence of *C. torvina*.

Female *C. torvina* and hosts (sacrificed by aspiration into 70% ethanol and held at 10°C) were dissected under insect (Ringers) saline solution. In dissecting *C. torvina* females, the reproductive tracts (ovaries plus oviducts) were teased out, placed in a drop of saline solution on a glass microscope slide with spacers and a cover slip, and examined under a compound microscope. The number of ovarioles were recorded for each female, and the mature eggs (eggs >0.4 mm in length) visible in each ovariole were counted and measured with an ocular micrometer. Host dissection was conducted under a dissecting scope and *C. torvina* eggs were measured with an ocular micrometer.

No dissections were made after the first two host provisionings, and *C. torvina* progenies were allowed to develop and emerge. The number of progenies per *C. torvina* and progeny development time were compared between host species and provisioning days using a Repeated Measures ANOVA.

For the third and fourth host provisionings, analysis was first carried out for the mean size and number of mature eggs per dissected *C. torvina* from each treatment group. The same analysis was carried out for the mean number and size of eggs found in hosts, per female, from each treatment group. The third host provisioning tested only one factor (female's response to either *P. xylostella* or *C. rubecula* hosts), and dissection data from *C. torvina* females and hosts in each treatment were compared with Two-sample t-tests. Because *C. torvina* dissected after the fourth provisioning were from treatments in which two host species and two host densities were tested, a Two-way ANOVA was used for analysis. Treatment means for egg size and egg number from females dissected after the third host provisioning were pooled. Pooling of data was possible because there were no significant differences between the means or variances for females in each treatment group. A comparison was made between pooled females from the third host provisioning and the control females (which had not been given hosts) because females in both groups had been sacrificed for dissection at the same time.

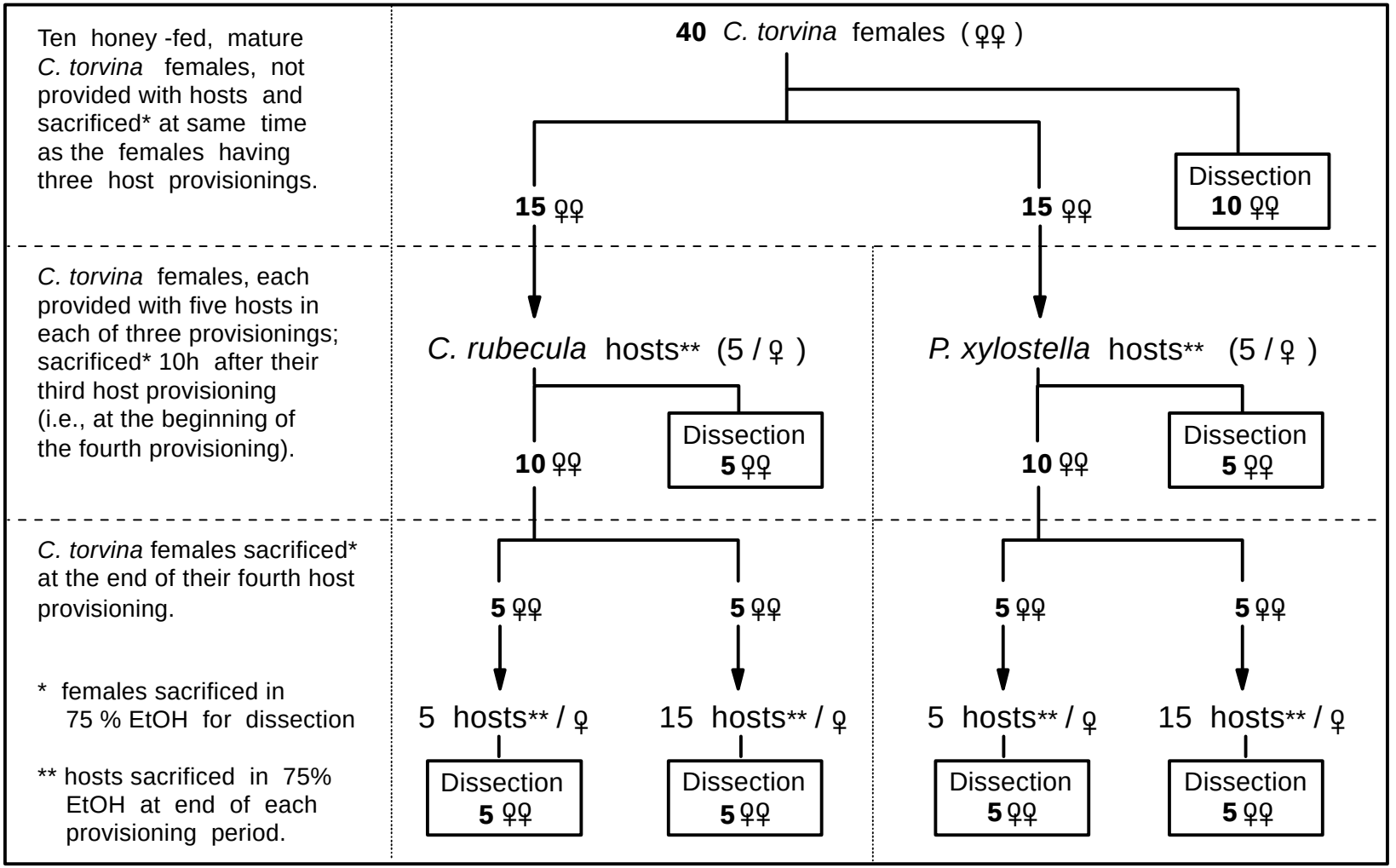

Figure 3.3.1: Dissection experiment plan to determine *Conura torvina's* ovariole number, rate of egg production, egg size, number of eggs laid per host, and size of eggs laid in *Cotesia rubecula* and *Plutella xylostella* hosts.

Results and Discussion

An unusually large proportion (13 of 15) of *C. torvina* in each treatment group produced progenies on the first host provisioning, and 15 of 15 females produced progenies in each host treatment group during the second provisioning. The high proportion of responding *C. torvina* may have been due to use of 50 ml cages instead of the usual 200 ml cages because all other conditions were essentially the same as in most initial host provisionings in previous experiments. The smaller cages confined the *C. torvina* females in close proximity to the hosts and to the frass impregnated paper placed alongside of the hosts, and this may have stimulated oviposition behavior sooner. The mean number of progenies per female was significantly greater (α= 0.05) in the second than in the first host provisioning, but there was no difference between *C. torvina*'s reproductive rate in either host species (Figure 3.3.2, Table 3.3.2). The significant increase in *C. torvina*'s reproductive rate from the first to the second host provisioning was not unexpected. It fits the pattern observed in the initial host provisionings of numerous other experiments (i.e., reproductive success increases up until the third or fourth provisioning after the initial provisioning). Although hosts from the third and fourth host provisioning were dissected, Figure 3.3.2 shows the potential number of progenies that would be obtained by the number of hosts which contained *C. torvina* eggs.

There was an obvious difference in the *C. torvina* progeny development times, measured as days from oviposition to adult emergence, between the host species treatments, and between the first and second host provisionings (Figure 3.3.2, Table 3.3.3). Development time was significantly shorter in *P. xylostella* than in *C. rubecula* hosts, and for progenies from the second than from the first host provisioning (Table 3.3.3). Although other experiments had consistently indicated that *C. torvina* development was slightly shorter in *P. xylostella* hosts than in *C. rubecula*, the difference had usually been too small to show significance at α= 0.05. However, initial host provisioning data from several other experiments (e.g., Chapter 4, Part 1, Figure 4.1, Table 4.1) do indicate that *C. torvina* progeny development time in the second host provisioning is significantly shorter than in the first provisioning. These results indicate that eggs from inexperienced females (on their first host encounter) are different, or lack some important nutrient for rapid development, and that *P. xylostella* hosts allow for more rapid progeny development than *C. rubecula* hosts. Eggs of some parasitoid species are known to obtain nutrients from host hemolymph through the egg chorion (King *et al*. 1971, Rotheram 1973).

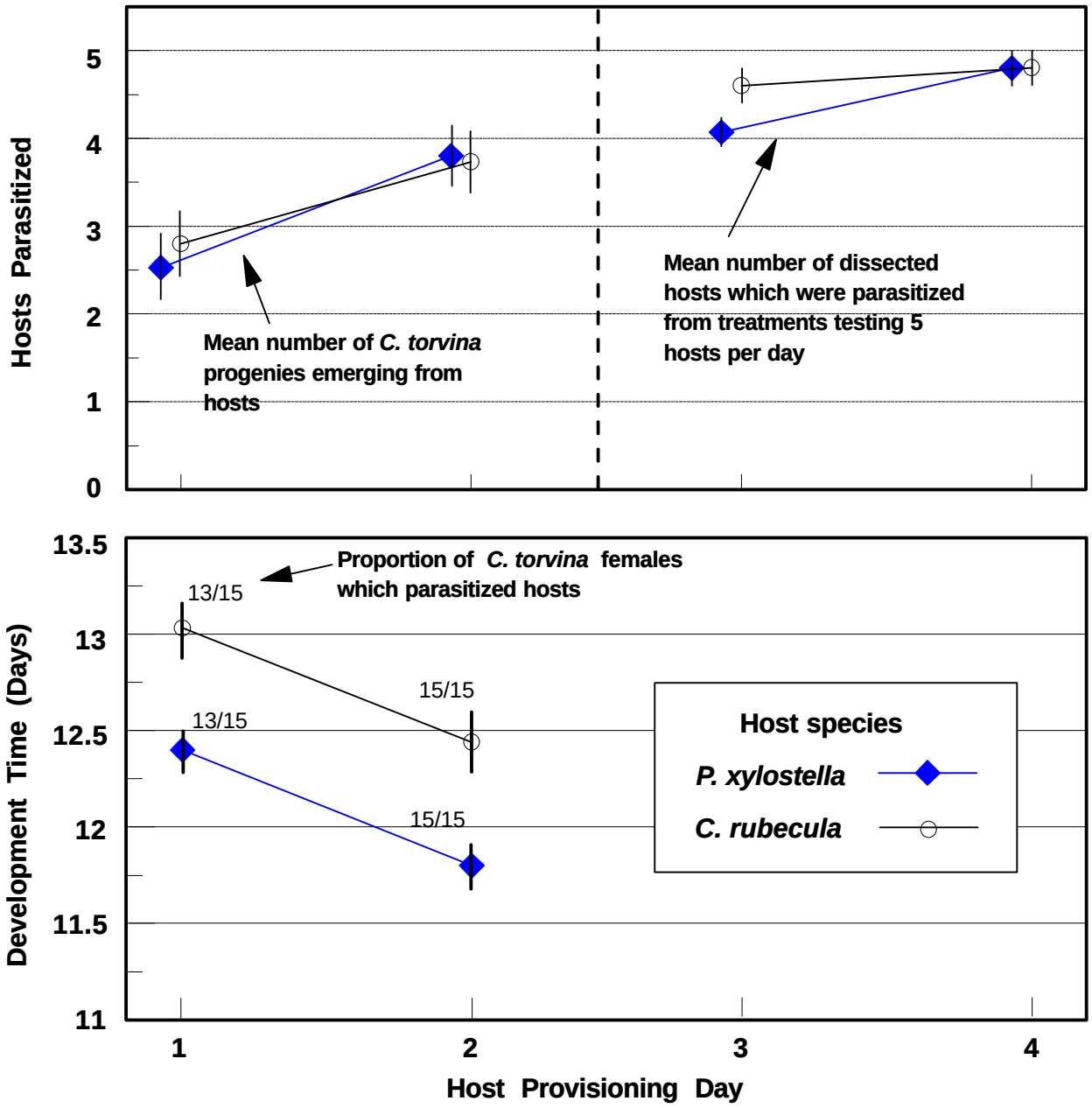

Figure 3.3.2: Mean number of progenies per *Conura torvina* and mean progeny development time (days) in the first and second provisionings of *Plutella xylostella* and *Cotesia rubecula* hosts; mean number of parasitized hosts (dissected hosts which contained *C. torvina* eggs) from the third and fourth host provisionings.

Table 3.3.2: Number of progenies per *Conura torvina* from *Plutella xylostella* or *Cotesia rubecula* hosts in the first and second host provisionings of the Dissection Experiment (n = 15 *C. torvina* replicates per host species).

Response Variable	Host Species					
	P. xylostella as host $\bar{x}$ [1]		*C. rubecula* as host $\bar{x}$ [1]		p	MSE
Progenies per *C. torvina* (across host provisionings)	3.17	a	3.27	a	0.828	3.126
	Provisioning Day					
	First Provisioning $\bar{x}$ [1]		Second provisioning $\bar{x}$ [1]		p	MSE
Progenies per *C. torvina* (across host species)	2.7	**a**	3.8	**b**	<0.001	0.712

Comparison of progenies per *C. torvina* from individual treatment effects (Repeated Measures ANOVA)

	Progenies per *C. torvina* $\bar{x}$ [2]	
P. xylostella, First Provisioning	2.53	**a**
C. rubecula, First Provisioning	2.80	**a**
P. xylostella, Second Provisioning	3.80	**b**
C. rubecula, Second Provisioning	3.73	**b**

[1] Means within rows followed by same letter are not significantly different (α=0.05; Repeated Measures ANOVA).
[2] Means within column followed by the same letter are not significantly different (α=0.05; Tukey-Kramer, second order comparison)

Table 3.3.3: *Conura torvina* progeny development time (days from oviposition to adult emergence) from the *Plutella xylostella* and *Cotesia rubecula* hosts in the first and second host provisionings of the Dissection Experiment (n = 13 *C. torvina* tested per host species).

Response Variable	Host Species					
	P. xylostella as host $\bar{x}$ [1]		*C. rubecula* as host $\bar{x}$ [1]		p	MSE
Development time (Days) (across host provisionings)	12.1	**a**	12.8	**b**	<0.001	0.325
	Provisioning Day					
	First Provisioning $\bar{x}$ [1]		Second provisioning $\bar{x}$ [1]		p	MSE
Development time (Days) (across host species)	12.8	**a**	12.2	**b**	<0.001	0.093

Comparison of progeny development times from individual treatment effects (Repeated Measures ANOVA)

	Development time (Days) $\bar{x}$ [2]	
C. rubecula, First Provisioning	13.1	**a**
C. rubecula, Second Provisioning	12.5	**b**
P. xylostella, First Provisioning	12.4	**b**
P. xylostella, Second Provisioning	11.8	**c**

[1] Means within rows followed by same letter are not significantly different (α=0.05; Repeated Measures ANOVA).
[2] Means within column followed by the same letter are not significantly different (α=0.05; Tukey-Kramer, second order comparison)

Results from dissection of *C. torvina* females indicated that each of the paired ovaries consists of three ovarioles, for a total of six ovarioles per female (Figure 3.3.3). Ovaries from females which had not been provided with hosts, had no more than one mature egg per ovariole, and in most cases some ovarioles did not contain any mature eggs. Thus, inexperienced females contained ≤6 mature eggs in their ovaries (Figure 3.3.3). Mature eggs were distinguished as those eggs >0.4 mm in length. Oocytes which were <0.4 mm in length were not considered mature because they were usually connected to obvious trophocyte cell bundles (Figure 3.3.3, Figure 3.3.4). Ovaries from females which had recent oviposition experience often had one and sometimes two mature eggs per ovariole (Figure 3.3.4). A few experienced females averaged slightly more than two mature eggs per ovariole (the maximum seen was 15 mature eggs per female). Thus, a typical female with a full complement of mature eggs should generally contain from 6 to 12 eggs. Distribution of mature eggs was not uniform and often an individual ovariole would have two mature eggs while others would have one or none.

Conura torvina females had been divided into two groups, each receiving a different host species. When some females from each group were dissected after the third host provisioning, egg count and egg size means were not significantly different between groups (Table 3.3.4). This indicated that the host species provided to *C. torvina* had no apparent effect on her egg production, and may indicate that host feeding from either host species (*P. xylostella* or *C. rubecula*) provides females with equivalent nutrition.

Egg count and egg size data from these two groups were pooled and compared to the data for dissected females which had not been provided with hosts (Table 3.3.5). Results indicated that the mean number of mature eggs per ovariole from females dissected after three host provisionings was significantly greater (three times greater) than that from females which had not been provided hosts. Mean egg length was not significantly different between both groups. These results indicate that exposure to hosts is important as a stimulus or source of nutrients for egg production, and that although inexperienced females have fewer mature eggs in their ovarioles, the mature eggs are generally the same size as those in experienced females.

Data from hosts dissected after the third host provisioning indicate that a female in the *P. xylostella* host treatment failed to lay any eggs. Data from this female was excluded from analysis of the host dissection data, reducing the number of replicates for the *P. xylostella* treatment from 15 to 14. The mean number of eggs laid per female and the mean number of eggs laid per host appear to be higher in *C. rubecula* hosts than in *P. xylostella* hosts (Table 3.3.6), but the differences were not significant at α=0.05. Eggs dissected from *C. rubecula* hosts were significantly shorter than those dissected from *P. xylostella*. The smaller egg size found in *C. rubecula* hosts may indicate that *C. torvina* lays smaller (less mature) eggs in *C. rubecula* hosts. If female progenies are the result of a *C. torvina* female only fertilizing the largest or most mature eggs that she laid, such behavior would explain why there are fewer female progenies produced from *C. rubecula* hosts. *Conura torvina* eggs dissected from *C. rubecula* ranged from 0.43 to 0.65 mm in length and those dissected from *P. xylostella* ranged from 0.48 to 0.65 mm long.

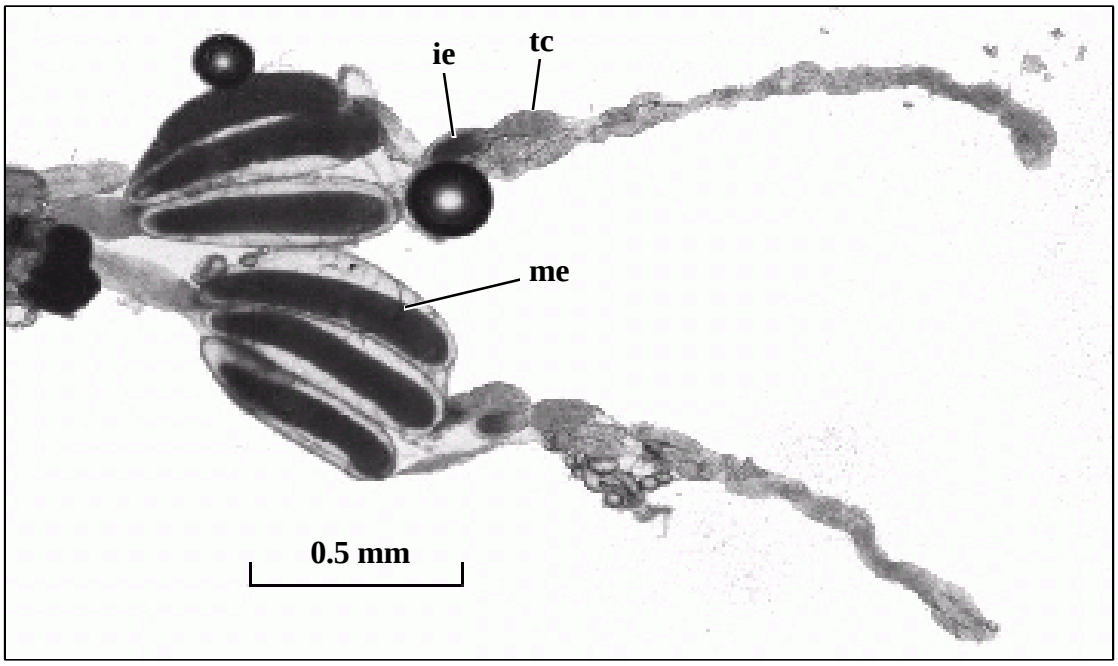

Figure 3.3.3: *Conura torvina* ovaries from a female with no oviposition experience, showing three ovarioles per ovary and one mature egg per ovariole. Some immature eggs and their associated bundles of trophocyte cells are also evident; **me**, mature egg; **ie**, immature egg; **tc**, trophocyte cells.

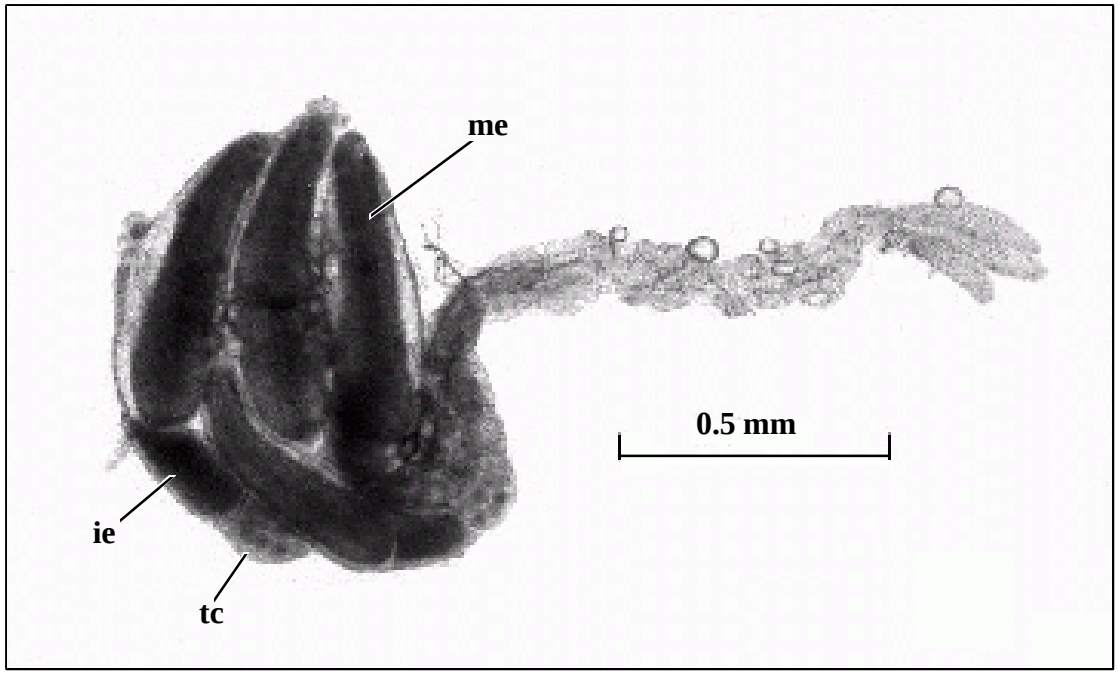

Figure 3.3.4: *Conura torvina* ovary from a female with oviposition experience, showing three ovarioles, one with two mature eggs. Several large immature eggs and their associated trophocyte cells are also evident.

Table 3.3.4: Egg number and size in *Conura torvina* females dissected 10 hours after the end of their third provisioning, from either *Cotesia rubecula* or *Plutella xylostella* hosts [1].

Response Variables	*C. rubecula* as hosts $\bar{x}$		*P. xylostella* as hosts $\bar{x}$		p [2]	df
Eggs (≥ 0.4 mm long) per dissected female	8.8	a	9.6	a	0.737	8
Egg length (mm) for eggs ≥0.4 mm long	0.54	a	0.54	a	0.816	8

[1] Five host pupae were provided per female (n = 5 *C. torvina* females dissected from each treatment).
[2] Means within rows followed by same letter are not significantly different (α=0.05; Two-sample t- test).

Table 3.3.5: Egg number and size from honey-fed, inexperienced "control" females and from females with experience from three separate oviposition / host feeding opportunities [1].

Response Variables	Inexperienced Females (No Hosts Provided) $\bar{x}$		Experienced Females (Three Host Provisionings) $\bar{x}$		p [2]	df
Eggs (≥0.4 mm long) per dissected female	3.1	**a**	9.2	**b**	<0.001	18
Egg length (mm) for eggs ≥0.4 mm long	0.56	a	0.54	a	0.309	18

[1] Data from *C. rubecula* and *P. xylostella* hosts were pooled for experienced females (n = 10 *C. torvina* females dissected from each treatment).
[2] Means within rows followed by same letter are not significantly different (α=0.05; Two-sample t- test).

Table 3.3.6: Egg number and size laid by *Conura torvina* in *Cotesia rubecula* or *Plutella xylostella* hosts during the third provisioning [1].

Response Variables	*C. rubecula* as hosts $\bar{x}$		*P. xylostella* as hosts $\bar{x}$		p [2]	df
Eggs laid / female	12.5	a	9.5	a	0.081	27
Egg length (mm) laid / female	0.52	**a**	0.54	**b**	0.015	27
Eggs laid / parasitized host	2.7	a	2.3	a	0.296	27

[1] Five host pupae were provided per female (n = 15 and 14 *C. torvina* females tested for *C. rubecula* and *P. xylostella* hosts respectively).

[2] Means within rows followed by same letter are not significantly different (α=0.05; Two-sample t- test).

Conura torvina females provided with either host species and dissected immediately after the fourth host provisioning showed no significant differences in the size or number of mature eggs they contained (Table 3.3.7). Although the difference in the number of mature eggs was not significant at α= 0.05 (p=0.084), it appeared that more eggs were found in the females that were provided with *C. rubecula* hosts than in those provided with *P. xylostella*. A larger sample size than n = 5 females might show a difference. Host density (either 5 or 15 hosts provided per female) caused no significant difference in the size or number of the eggs in found *C. torvina* ovaries.

Host dissections after the fourth provisioning revealed that one *C. torvina* female from the *P. xylostella* hosts / 15 hosts per female treatment combination failed to lay any eggs. This was the same female which failed to lay eggs in *P. xylostella* hosts in the third host provisioning. Host dissection data from this female were excluded from analysis, reducing the number of replicates for this treatment combination from five to four.

Results from host dissections after the fourth host provisioning fit the same pattern as was seen in the host dissections from the third provisioning (Table 3.3.8), but with significant differences. The mean number of eggs laid per female was significantly greater and mean egg length significantly less in *C. rubecula* hosts. These results are further evidence that *C. torvina's* egg laying behavior is different for *C. rubecula* and *P. xylostella* hosts; a behavioral difference that was already suspected due to the difference in the sex ratios of the progenies emerging from these hosts.

Table 3.3.7: Egg number and size in *Conura torvina* females dissected immediately after the end of their fourth host provisioning [1].

Response Variables	Host Species					
	C. rubecula as hosts $\bar{x}$ [3]		*P. xylostella* as hosts $\bar{x}$ [3]		p [2]	MSE
Eggs (≥0.4 mm long) per dissected female	6.7	a	4.7	a	0.084	6.07
Egg length (mm) for eggs ≥0.4 mm long	0.47	a	0.47	a	0.739	0.0005
	Host Density					
	5 hosts per female $\bar{x}$ [4]		15 hosts per female $\bar{x}$ [4]		p [2]	MSE
Eggs (≥0.4 mm long) per dissected female	6.2	a	5.2	a	0.378	6.07
Egg length (mm) for eggs ≥0.4 mm long	0.47	a	0.47	a	0.608	0.0005

Individual Effects	Treatment	5 hosts per female	15 hosts per female	
Eggs (≥0.4 mm long) per dissected female	*C. rubecula* hosts	7.2	6.2	
	P. xylostella hosts	5.2	4.2	
Egg length (mm) for eggs ≥0.4 mm long	*C. rubecula* hosts	0.48	0.46	
	P. xylostella hosts	0.46	0.47	

[1] *Conura torvina* was provided *C. rubecula* or *P. xylostella* hosts at densities of 5 or 15 hosts per female (n = 5 *C. torvina* females dissected from each treatment combination).

[2] Means within rows followed by same letter are not significantly different (α=0.05; Two-way ANOVA; there were no significant treatment interactions for the two response variables tested).

[3] Host species means are for responses across host density.

[4] Host density means are for responses across host species.

Table 3.3.8: *Conura torvina* egg number and size in hosts dissected immediately after the end of their fourth host provisioning [1].

Response Variables	Host Species.					
	C. rubecula as hosts $\bar{x}$ [3]		*P. xylostella* as hosts $\bar{x}$ [3]		p [2]	MSE
Eggs laid / female	16.4	**a**	10.0	**b**	0.016	25.9
Egg length (mm) laid / female	0.53	**b**	0.55	**a**	0.036	0.0005
Eggs laid / parasitized host	2.5	a	1.7	a	0.057	0.711
	Host Density					
	5 hosts per female $\bar{x}$ [4]		15 hosts per female $\bar{x}$ [4]		p [2]	MSE
Eggs laid / female	13.4	a	13.0	a	0.867	25.9
Egg length (mm) laid / female	0.54	a	0.54	a	0.800	0.0005
Eggs laid / parasitized host	2.7	**a**	1.4	**b**	0.004	0.711

Individual Effects	Treatment	5 hosts per female	15 hosts per female
Eggs laid / female	*C. rubecula* hosts	16.8	16.0
	P. xylostella hosts	10.0	10.0
Egg length (mm) laid / female	*C. rubecula* hosts	0.53	0.53
	P. xylostella hosts	0.56	0.55
Eggs laid / parasitized host	*C. rubecula* hosts	3.4	1.6
	P. xylostella hosts	2.1	1.2

[1] *Cotesia rubecula* or *P. xylostella* hosts were provided at densities of 5 or 15 hosts per female (n = 5; hosts from 5 females tested in each *C. rubecula* host density combination and in the *P. xylostella* low density combination: n = 4; hosts from 4 females tested in the *P. xylostella* high density combination).

[2] Means within rows followed by same letter are not significantly different (α=0.05; Two-way ANOVA; there were no significant treatment interactions for any of the response variables tested).

[3] Host species means are for responses across host density.

[4] Host density means are for responses across host species.

Although significantly more eggs were laid by females given *C. torvina* as hosts, than females given *P. xylostella* during the fourth provisioning period, the females which had laid more eggs did not appear to have taxed their resources any more than those females which had laid fewer eggs. Dissection of these females had indicated that there was no significant difference in the number or size of eggs they had in their ovaries. In fact, the females which had laid significantly more eggs actually appeared to have more eggs in their ovaries and the difference ($p=0.084$) would have been significant if tested at the significance level of $\alpha = 0.10$. These results would then indicate that host species has an effect on a female's oogenesis (production of eggs).

The number of hosts provided to a female (host density) had no effect on the number of eggs she laid because the mean number of eggs laid per female was not significantly different at each host density (Table 3.3.8). These results seem to indicate that each female will dispose of all her mature eggs in a day, regardless of how many hosts are available. The effect of this is that the rate of superparasitism (number of eggs laid per host) is significantly greater when fewer hosts are available.

Data for *C. torvina* dissections after the third and fourth host provisioning indicate that the mean number of eggs and mean egg length (for eggs >0.4 mm long) per female, appear to decline from the third to the fourth host provisioning (Figure 3.3.5). This result would be expected because females from the third host provisioning had ≥10 hours to regenerate eggs before dissection whereas females that were dissected immediately after the end of the fourth provisioning had little time to regenerate eggs. Females dissected 10 hours after the third provisioning period (the beginning of a fourth host provisioning period) had an average of 9.2 mature eggs which were 0.54 mm in length (combined average from both host species treatment groups, Table 3.3.4). It can be assumed that the dissected females carried a similar egg load as their cohorts which were not dissected and that these "live" females had an average of 9.2 mature eggs in their ovarioles as they entered the fourth provisioning period. Host dissections after the fourth host provisioning indicated that an average of 13.2 eggs were laid by each female (combined average from both host species treatments, Table 3.3.8). Thus, females produced and laid an average of four additional mature eggs during the 14 hour host provisioning period, indicating that *C. torvina* females produce eggs continuously as they lay them. Females sacrificed immediately after the fourth host provisioning period, during which they had laid a mean of 13.2 eggs each, still had a mean of 5.7 mature eggs in their ovarioles (combined average from both host species treatments, Table 3.3.7). These eggs were relatively small with a mean length of 0.47 mm. Although a *C. torvina* female dissected before the fourth host provisioning had as many as 15 mature eggs in her ovaries, one female was found to have laid as many as 30 eggs in the fourth provisioning period and another laid 20 eggs. Results from host dissections from the third and fourth provisioning periods are indicated in Figure 3.3.6, and show the effect that the two host species had on the number and size of eggs laid in them. There appears to be an overall trend towards an increase in the reproductive ability of *C. torvina* from the third to the fourth host provisioning. However, the difference between provisioning periods does not appear to be significant.

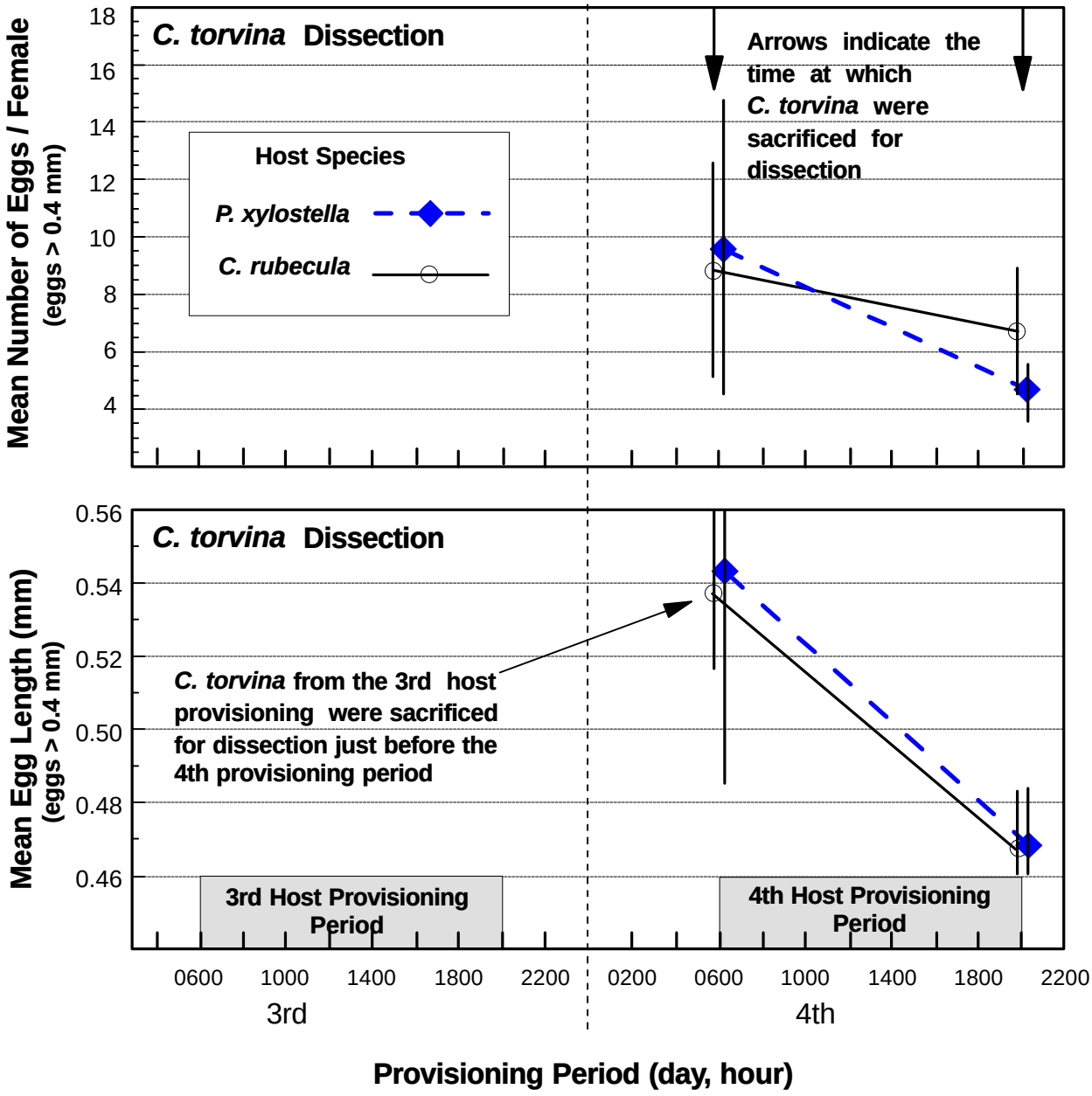

Figure 3.3.5: Counts and measurements of eggs dissected from *Conura torvina* after the third and fourth host provisioning (error bars = mean ± SE).

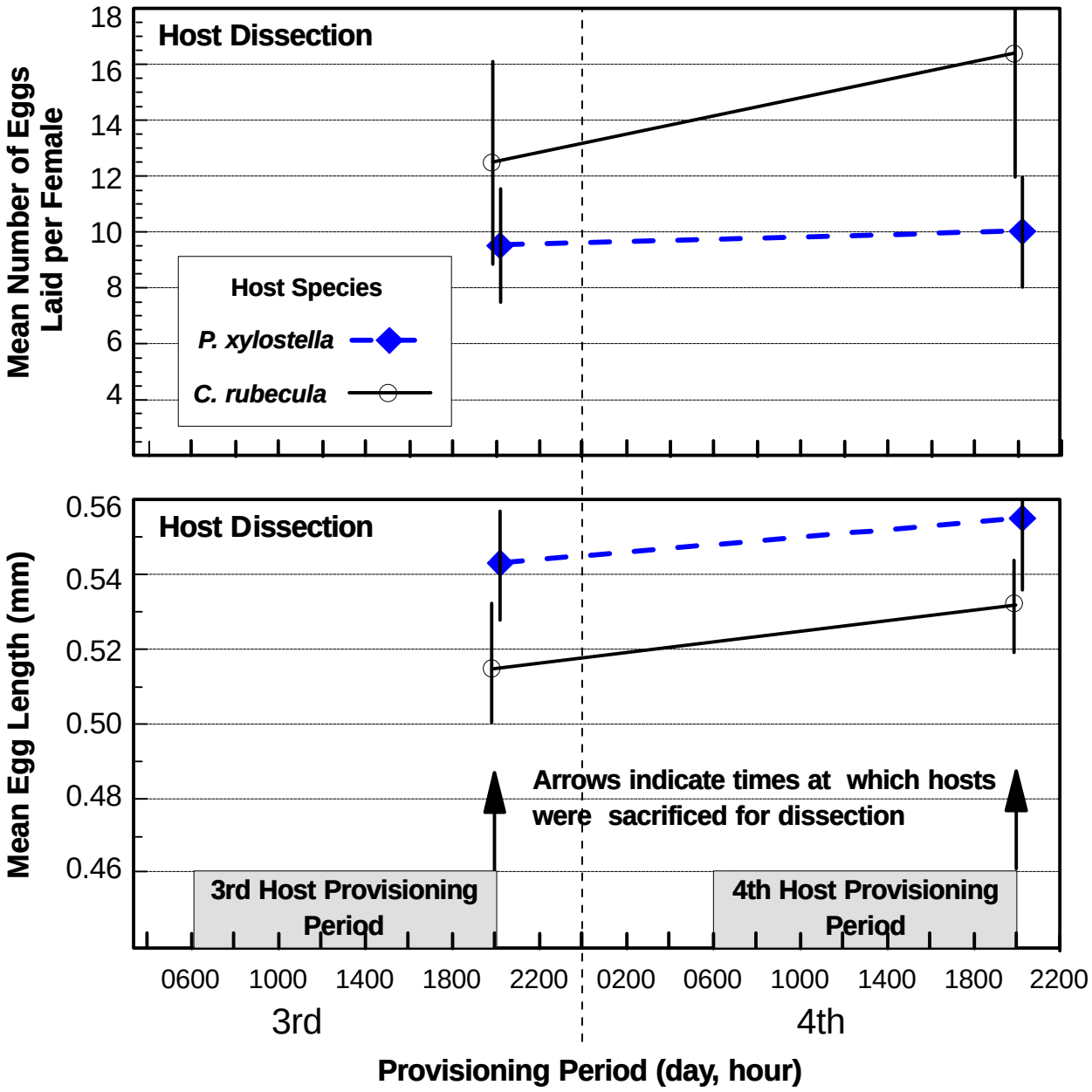

Figure 3.3.6: Counts and measurements of *Conura torvina* eggs dissected from *Cotesia rubecula* and *Plutella xylostella* hosts after the third and fourth host provisionings.

Conclusions

The development time (oviposition to adult emergence) for progenies of *C. torvina* which have no prior oviposition experience is significantly longer than that for progenies of the same females after one day's oviposition experience. During the first two host provisionings, the progeny development time is significantly longer in *C. rubecula* hosts than in *P. xylostella* hosts.

Dissections of *C. torvina* show that: (1) *C. torvina* females have two ovaries, each consisting of three ovarioles; (2) eggs without visible trophocytes were generally >0.40 mm in length; (3) no female carried more than 15 mature eggs (eggs >0.40 mm in length), and the number was typically about nine mature eggs per female; (4) females without oviposition experience had significantly fewer eggs in their ovaries than experienced females, but there was no difference in the size of the mature eggs carried by inexperienced and experienced females.

Host dissections indicated that: (1) *C. torvina* laid eggs ranging from 0.43 to 0.65 mm in length; (2) *C. torvina* may lay more eggs in a day than they can carry in their ovaries; females produce eggs as they are laid; (3) *C. torvina* typically superparasitize their hosts (lay more than one egg per host); and (4) the average number of eggs laid per female per day ranged from 11 to 13 (both host species combined, third and fourth provisioning, respectively), but one female was able to lay 30 eggs per day.

Host density (the number of hosts provided per female per day) had no measurable effect on *C. torvina*, oogenesis, ovulation or oviposition. *Conura torvina* dissections indicated that the number of hosts that had been provided to a female had no effect on her oogenesis or ovulation (the number or size of eggs she produced). Host dissections indicated that host density also had no effect on the number or size of eggs laid by a female.

Host species had some effect on *C. torvina* oogenesis, ovulation and oviposition, but the exact causes are unclear. Female dissections indicated that host species had no effect on the size of eggs in a female's ovaries (i.e., no effect on oogenesis). Female dissections also indicated that host species had no significant effect on the number of eggs carried by a female, but results of analysis from the fourth host provisioning period ($p= 0.084$) leave some question as to whether further testing (a fifth or sixth host provisioning) would show a difference between females provided either host species (i.e., that host species would have some effect on oogenesis or the number of mature eggs carried by a female. Host dissections indicated that significantly larger eggs were laid in *P. xylostella* than in *C. rubecula*, and significantly more eggs were laid in *C. rubecula* than in *P. xylostella*. This suggests that *C. torvina* controls its ovulation (the size of mature eggs released into the oviduct) and oviposition (the number of eggs laid) based on the host species.

Chapter 4: Factors Affecting *C. torvina* Development Time

Introduction

Experimental data indicated that *C. torvina*'s development time varied with host age, host species, oviposition experience and temperature. Results from the dissection experiments (Table 3.3.1, Figure 3.3.2, and Table 3.3.3) indicated that *C. torvina*'s ovipositional experience affected its progeny development rate; progenies from inexperienced females had a significantly longer development times than those from females with some oviposition experience. Results from this experiment also indicated that development time was significantly shorter in *P. xylostella* than in *C. rubecula* hosts. In other experiments, the difference in development time between host species was consistent between experiments, but generally too small to be significantly different. As temperature is an important factor for development, data from experiments where temperature was monitored were used to examine the effects of oviposition experience, host species and temperature on *C. torvina* development time.

Effect of *C. torvina* Oviposition Experience on Progeny Development Time in *C. rubecula* and *P. xylostella* hosts

Materials and methods

Data used in this analysis were obtained from the three initial host provisionings before the second continuous host provisioning experiment (Figure 3.1.4). These data were from pre-experimental host provisionings that were conducted in a controlled environment where temperatures were monitored. In these provisionings, six *C. torvina* were provided with *P. xylostella,* and 14 were provided with *C. rubecula* hosts. The first provisioning lasted three days and subsequent host provisionings lasted one day each. The second and third provisioning were made two days and five days after the first. Six hosts were provided to each female in the first provisioning and 10 hosts were provided to each female in the second and third provisionings. Development times for the progenies of each female were calculated as the average number of days from oviposition. Because the first provisioning lasted three days, it was not possible to determine the exact day when *C. torvina* started oviposition activity. Thus, the last day of that period was chosen as the starting date. Comparison of development time in *C. rubecula* hosts between provisioning days was carried out. A single factor Repeated Measures ANOVA was used to compare between days (Norman & Streiner 1994). Analysis only included data for progenies from the six females that oviposited on the first provisioning day and continued to parasitize hosts in the subsequent provisionings. A Repeated Measures ANOVA was also used to compare development time in *C. rubecula* and *P. xylostella* hosts between the second and third provisioning day. Data were used from the nine females which parasitized *C. rubecula* hosts and the six females which parasitized *P. xylostella* hosts on the second host provisioning. All of these females also parasitized their respective hosts on the third provisioning.

Results and Discussion

Progeny development times declined progressively with each consecutive provisioning (Figure 4.1). The decline in development time follows the same pattern in both host species. This pattern was seen in initial host provisionings from other experiments (e.g. Figure 3.1.2), but

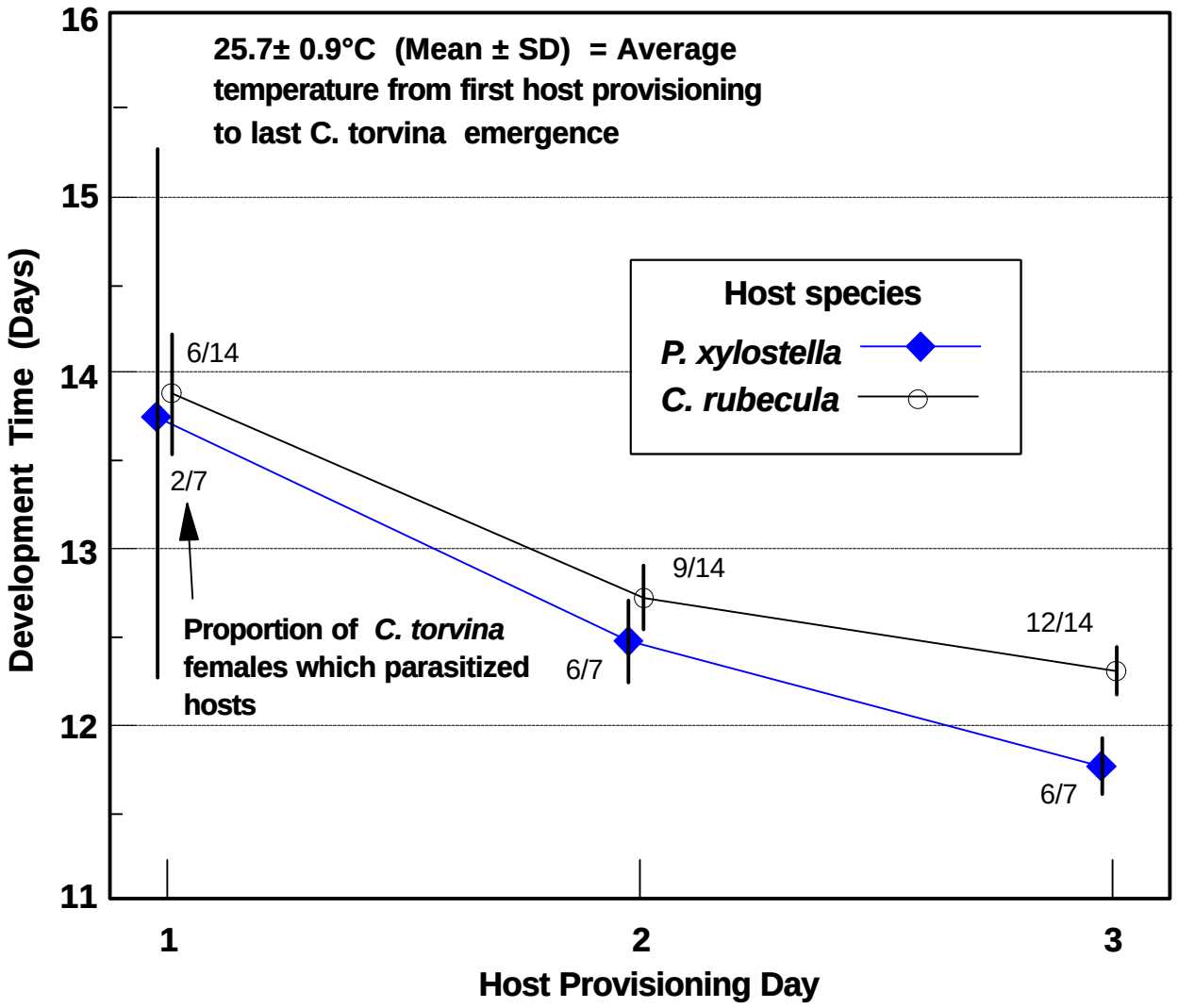

Figure 4.1: Effect of *Conura torvina* experience on progeny development time (days from oviposition to adult emergence) after the first three host provisionings of *Plutella xylostella* and *Cotesia rubecula* hosts to inexperienced females (Error bars = Mean ± SE).

Table 4.1: Effect of *Conura torvina* oviposition experience on progeny development time (days) from oviposition to adult emergence) in *Cotesia rubecula* hosts [1].

Response Variable	Provisioning Day (n = 6 *C. torvina* replicates)				
	First $\bar{x}$	Second $\bar{x}$	Third $\bar{x}$	p [2]	MSE
Development time (Days from oviposition to adult emergence)	13.9 **a**	12.6 **b**	12.4 **b**	<0.001	0.146

[1] n = 6 *C. torvina* tested for progeny development time (temperature = 25.0 ± 1.0°C, $\bar{x} \pm$ SD).

[2] Means followed by same letter are not significantly different (α=0.05; Tukey-Kramer means separation test; one factor Repeated Measures ANOVA).

environmental conditions were not controlled or monitored during those provisionings, and I could not be sure if the effect was due to temperature change. Development time in *C. rubecula* was significantly longer (>1 day longer) in the first provisioning than in the second provisioning (Table 4.1). Although the development time for the third host provisioning appears to be even shorter, it was not significantly different from that of the second provisioning. Progeny development time from the first provisioning may be even longer than was estimated because that provisioning lasted three days and development time was only counted from the third day.

When the *C. torvina* progeny development time is compared in *C. rubecula* and *P. xylostella* hosts between the second and third host provisioning period (Figure 4.1), there was a significant difference (α = 0.05) between host species, with *C. torvina* progenies from *P. xylostella* hosts having the fastest development. There was also a significant difference between provisioning days indicating that progenies from females on their second day of oviposition have longer development times than those from females on their third day of oviposition. Unlike the analysis from Table 4.1, this analysis shows the difference between the second and third days for progenies in *C. rubecula* hosts because data from more females (replicates) are used. Results from these analyses support the findings, from the first two host provisionings of the dissection experiment, that *C. torvina* progeny development time decreases with female experience and is shorter in *P. xylostella* hosts.

The cause of a longer development time for *C. torvina*'s first progenies is not known. It may be that naive females lack an important nutrient used in egg production (yolk deposition) and this nutrient is probably obtained through host feeding. Another possibility is that naive females lay too many eggs in each host and the crowding and competition for nutrients slows the progeny's development rate.

Table 4.2: Effect of *Conura torvina* oviposition experience on progeny development time (days) from oviposition to adult emergence) in *Cotesia rubecula* and *Plutella xylostella* hosts in their second and third host provisionings (n = 9 and 6 *C. torvina* females tested for *C. rubecula* and *P. xylostella* hosts, respectively).

Response Variable	Host Species			
	P. xylostella as host $\bar{x}$ [1]	*C. rubecula* as host $\bar{x}$ [1]	p	MSE
Development time (across host provisionings)	12.21 **b**	12.58 **a**	0.035	0.200
	Provisioning Day			
	Second Provisioning $\bar{x}$ [1]	Third provisioning $\bar{x}$ [1]	p	MSE
Development time (across host species)	12.73 **a**	12.08 **b**	<0.001	0.120

Comparison of *C. torvina* development time from individual treatment effects (Repeated Measures ANOVA)

	Development Time $\bar{x}$ [2]	
C. rubecula, Second Provisioning	12.87	**a**
P. xylostella, Second Provisioning	12.59	**ab**
C. rubecula, Third Provisioning	12.31	**bc**
P. xylostella, Third Provisioning	11.84	**c**

[1] Means within rows followed by same letter are not significantly different (α=0.05; Repeated Measures ANOVA).
[2] Means within column followed by the same letter are not significantly different (α=0.05; Tukey-Kramer, second order comparison)

Effect of Temperature on *C. torvina* Development Time

Materials and Methods

A data logger with temperature probes was set up to monitor the environmental chambers because chambers used in various experiments did not run at their exact set temperatures and tended to vary over time. Data logger readings gave an accurate temperature measurement ($T \pm 0.5°C$) and detected any extreme or abnormal fluctuations of temperature in the chambers. Temperature readings were made at 30 second intervals and averaged for each hour of the day. The temperature for an experiment was determined by averaging hourly temperatures for the duration of the experiment (from first host provisioning to the last *C. torvina* emergence)

Development time data were obtained from a number of experiments that were conducted at temperatures ranging from 15 to 35°C (Table 4.2). Data for the lowest temperature (15°C) were obtained from a single provisioning of *P. xylostella* hosts to eight experienced *C. torvina*. Because this provisioning was conducted before the data logger was set up, the 15°C temperature that was registered by the environmental chamber's digital display may not be accurate. Data for the highest temperature also came from a single provisioning of hosts to 23 slightly experienced females (this host provisioning was only the second time these females had seen hosts; 12 females received *P. xylostella* and 11 received *C. rubecula* hosts). Data from temperatures ranging from 24 to 32°C were from replicated experiments where repeated provisionings of *C. rubecula* and *P. xylostella* host species were made to experienced *C. torvina* females. The number of repetitions (provisionings) and replicates (female *C. torvina* tested) for each host species are indicated in Table 4.3. The development rate (inverse of development time) was calculated for *C. torvina* progenies from both host species and was plotted against temperature. A linear regression on data for the *P. xylostella* hosts was used to obtain a model for development rate at temperatures between 15 and 35°C.

Results and discussion

. Development rates for *C. torvina* in *P. xylostella* and *C. rubecula* hosts were similar for each temperature (Figure 4.2). However, progenies from *P. xylostella* hosts appear to consistently have a slightly higher rate of development (shorter development time) (Table 4.3, Figure 4.2). The linear regression on data from *P. xylostella* hosts had an R^2 of 0.974. The estimated model from the regression (Figure 4.2) provides an estimate of *C. torvina* development rate within the tested temperature range. If additional data for the lower temperature range had been obtained, they would have contributed to the accuracy of this model, particularly at lower temperatures.

Predicted development times from this model are comparable to those reported by Vickery (1929) at 22.9°C and 18.9°C. However, development times predicted by this model are shorter than those reported by Arthur (1958) at 22.5°C, and McNeil and Rabb (1973) at 27°C. Host species, host age, female experience or other unknown factors may cause the differences between the developmental times predicted by this model and those reported in other studies.

Table 4.3: Experiments used for temperature and progeny development time data in development rate regression model.

Experiment - Mean temperature *(actual mean temperature in environmental chamber)		Number of host provisionings	Number of replicates (*C. torvina*) tested with *P. xylostella*	Mean development time (days) in *P. xylostella* hosts	Number of replicates (*C. torvina*) tested with *C. rubecula*	Mean Development time (days) in *C. rubecula* hosts
Test of *C. torvina* development time at 15°C	15°C [1]	1	8	56.2	-	-
Fourth continuous provisioning experiment	*24.0°C	4	5	17.1	5	17.5
Third continuous provisioning experiment	*25.3°C	6	5	12.8	7	13.4
Second continuous provisioning experiment	*26.3°C	7	7	11.7	7	11.9
Abundant host supply experiment	*31.3°C	7	5	9.3	4	9.4
Test of *C. torvina* development time at 35°C	*34.3°C	1	12	8.6	11	8.7

[1] Temperature reported by environmental chamber digital readout (not verified by data logger measurements).

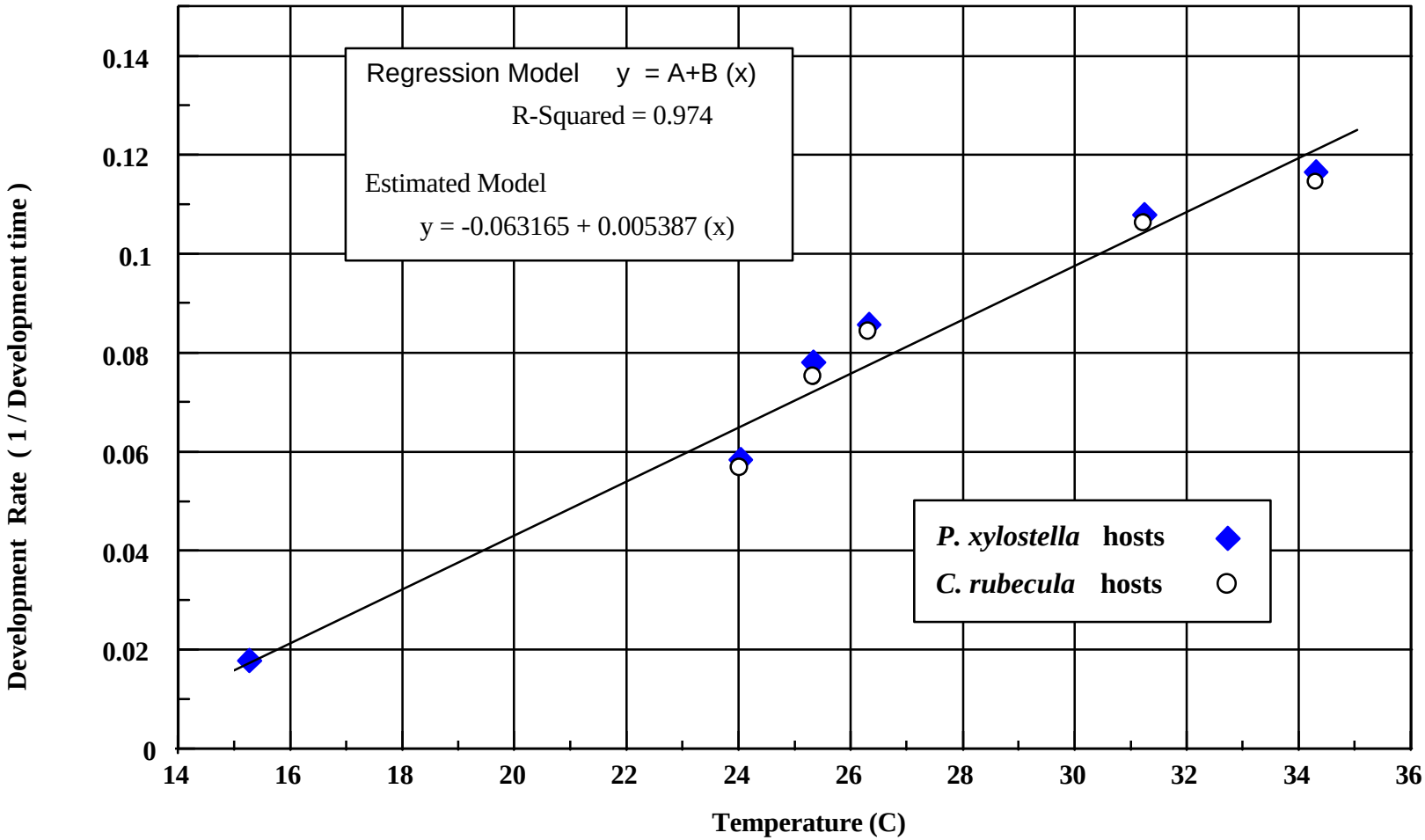

Figure 4.2: Temperature effect on *Conura torvina* development rate (inverse of development time from oviposition to adult emergence) in *P. xylostella* and *C. rubecula* hosts.

Summary

Conura torvina was active only during the summer, as none were recovered earlier than the last week in June in three years of spring time trap plant sampling. These results were similar to those of an earlier study (Gaines 1992) where almost no *C. torvina* activity was detected before the end of June even though large numbers of hosts were collected starting two weeks before its detection. The earliest field record of *C. torvina* activity was in late May, 1989, when a single wasp was recovered from a *C. rubecula* cocoon (Gaines 1992). *Conura torvina*'s rarity or absence from the *Brassica* field crop environment before early summer makes it an unlikely threat as a hyperparasite of beneficial parasitoids associated with these crops. Its presence in mid summer is also of little concern because it typically affects <10% of the available hosts (pupae) and has not been found to affect >50% of its hosts at any time. The sampling of trap hosts on trap plants appeared to be a more effective method of monitoring for *C. torvina* activity, and required considerably less effort and input, than the sampling of wild host populations in field crops.

Studies on the effect of host age on *C. torvina*'s reproduction indicated that reproductive success was best on *C. rubecula* pupae which were middle aged or younger. This finding is not surprising, as pupal hosts begin to develop adult structures about half way through the pupal stadium. The onset of histogenesis causes the easily consumed soft tissues such as fat body and hemolymph to develop into harder tissues such as muscle, organs and appendages which may be less easily consumed by *C. torvina* larvae. Differentiation of the cuticle into appendages and tagmata also restricts movement and development space for *C. torvina* larvae. The oldest hosts suffered higher mortality than the younger hosts and this may have been due to failed parasitism by *C. torvina*. *Conura torvina* emerged from some of these older hosts, but a higher proportion of the older hosts than younger hosts escaped parasitization, indicating that *C. torvina* did not readily accept the older hosts for oviposition. Although *C. torvina* oviposited in some unsuitable hosts, this behavior may be an artifact of being confined to a small cage.

In host preference studies, *P. xylostella* was the most preferred and *C. orobenae* the least preferred. The assessment of host preference was based both on the proportion of hosts parasitized and the proportion of female progenies resulting from parasitization. When equal numbers of hosts were provided, the proportion of *C. rubecula* and *P. xylostella* that were parasitized by *C. torvina* was equal, but a significantly higher proportion of female *C. torvina* emerged from *P. xylostella* hosts. A number of other experiments in which *P. xylostella* and *C. rubecula* were tested confirmed this result. A significantly smaller proportion of *C. orobenae* was parasitized and none of the resulting progenies was female. Host preference appears to be based on host size, as the preference was greatest for the largest host species and least for the smallest. This study did not identify other possible factors influencing *C. torvina*'s host preference.

Conura torvina with no previous exposure to hosts was more attracted to and commenced oviposition activity sooner in *P. xylostella* than *C. rubecula* hosts. One factor which may have stimulated this attraction and oviposition activity was host frass adhering to the net-like *P. xylostella* cocoons. Two experiments were conducted where *C. rubecula* hosts were provided to inexperienced *C. torvina,* on a surface smeared with frass from *P. rapae,* or on a surface with no frass. Although results were not significant, more females in each experiment commenced oviposition activity when hosts were provided with frass. Frass from *P. xylostella* was also tested with *C. rubecula* hosts, and appeared to have a stronger stimulatory effect than frass from *P. rapae*. However, the effect of frass was significantly different from the "no frass" treatment, only when *P. xylostella* hosts were provided along with their own frass. Thus, frass appears to be an important stimulus for oviposition behavior, but other unidentified factors associated with host species may also play a role.

Studies on *C. torvina*'s reproductive capacity indicated that it can maintain a relatively high daily reproductive rate for two weeks. Progenies of *C. torvina* which were provided hosts intermittently developed significantly faster than those from daily provisioned females. Progeny survival also appeared to be higher and host mortality appeared to be lower (fewer hosts died without producing *C. torvina*) when hosts were provided intermittently. This indicated that continuous daily provisioning of hosts may tax a female's reproductive system. Females that laid eggs daily had little time to replace the eggs they had laid before the next bout of oviposition. Females that were provided hosts intermittently had more time to regenerate eggs between bouts of oviposition and probably laid well nourished, healthier eggs than those from females that were provided with hosts continuously. Females provided 13 hosts daily during a 12 day period, produced an average of 8.25 progenies per female per day (range 5-12 per female). There was no apparent reduction in the reproductive capacity over that period, indicating longer periods of continuous reproduction are possible. *Conura torvina* could have produced a larger number of progenies per day if it had been provided with more hosts daily. Studies in which females were provided ≥26 hosts per day indicated that *C. torvina* could produce a mean of 14 progenies per day. One *C. torvina* produced 17 progenies in one day. There was no apparent difference between the reproductive rates (progenies per female per day) of females provided daily for seven days with either *P. xylostella* or *C. rubecula*.

Studies on the provisioning of an abundant supply of hosts indicated that *C. torvina*'s reproductive rate does not improve significantly when more than 26 hosts are provided in a one day period. Although *C. torvina* females can produce a mean of 14 progenies per day, numerous experiments indicated that when ≤13 hosts are provided per female, some go unparasitized. Some females occasionally produced 12 progenies from 13 hosts, but generally <9 progenies were produced per female. In the continuous host supply experiment which ran for two weeks, *C. torvina* appeared to use up her daily supply of eggs each day without parasitizing all 13 hosts. This is an indication that *C. torvina* does not parasitize its hosts in a systematic manner. *Conura torvina* may oviposit more than once in some hosts and completely miss other hosts. Results from field studies (Gaines 1992) also indicated possible inefficiency in *C. torvina*'s oviposition behavior in *C. glomerata* cocoon masses. Although cocoon masses consist of 20 to 30 cocoons,

generally <4 of the cocoons in each affected *C. glomerata* cocoon mass were parasitized. However, *C. torvina*'s apparently inefficient reproductive behavior in the lab may actually be an artifact of the artificial environment (i.e., small cages), and more studies would be needed to conclude that *C. torvina* is an inefficient forager and parasite.

Conura torvina typically had a poor reproductive rate on its initial encounter with hosts. Its reproductive rate improved with each subsequent host provisioning until the third or fourth provisioning, when it stabilized at close to the maximum rate permitted by the number of hosts being provided. Dissections of *C. torvina*'s hosts revealed that *C. torvina* females with little oviposition experience lay more eggs per host than experienced females. Females with only three days of oviposition experience laid an average of 18 eggs per day (3 eggs per host) when provided with six hosts. Experienced females (7 days of experience) laid an average of 8.4 eggs for the same number of hosts. One of the less experienced females laid 25 eggs in one day, and 14 of these eggs were laid in a single host. These results indicate that oviposition behavior is at least partly learned because its efficiency improves with experience.

Dissections of *C. torvina* ovaries revealed that each ovary consists of three ovarioles. Females with no previous oviposition experience contained an average of three mature eggs (i.e., only half of their ovarioles contained a mature egg). Mature eggs (those without attached trophocyte cells) were >0.4 mm in length; host dissections indicated that *C. torvina* laid eggs ranging from 0.43 to 0.65 mm in length. Females provided with three days of oviposition experience contained an average of nine mature eggs in their ovaries or about 1.5 mature eggs per ovariole. The maximum number of mature eggs observed in any female was 15. It is possible that inexperienced females carry few eggs and have low initial reproductive rates because they lack nutrients that must be obtained from host feeding during oviposition. Although the number of eggs found in inexperienced and experienced females was different, there was no difference in the mean length (size) of the mature eggs found.

Progenies from *C. torvina*'s first, second and third oviposition had significantly different development times from each other. Progeny development time decreased as a female gained more oviposition experience. The relationship between progeny development time and a female's level of oviposition experience might be based on the number of host feeding opportunities a female has had. Females that have had several host feeding opportunities may have acquired the nutrient reserves needed to improve the nutritional status of their eggs (i.e., provide the egg's yolk with some important nutrient(s) which facilitates embryonic development). Poorly nourished eggs laid by inexperienced females may have to obtain the needed nutrient(s) directly from the host. Eggs of some parasitoid species are known to obtain nutrients from host hemolymph through the egg chorion (King *et al*. 1971, Rotheram 1973).

Conura torvina provided with *P. xylostella* or *C. rubecula* hosts had no apparent differences in the number or size of mature eggs in their ovaries after the third or fourth host provisionings. However, *C. torvina* appeared to have a different ovulation and oviposition strategy for each host species. Host dissections revealed that *C. torvina* lays significantly more

eggs per host in *C. rubecula* than in *P. xylostella* and the eggs laid in *P. xylostella* were significantly larger. It is possible that when *C. torvina* is provided with *P. xylostella* hosts, she typically lays larger, more fully nourished eggs, and in conserving her eggs until they are fully nourished, she lays fewer eggs. Conversely, when *C. torvina* oviposits in *C. rubecula* hosts, she lays the eggs she has at hand, and because the majority of the eggs she laid were average sized or smaller, she is able to produce and lay more eggs. *Conura torvina* appears to lay its full complement of eggs no matter how many hosts are at hand.

Host dissections indicated that *C. torvina* laid the same number of eggs whether provided with five or fifteen hosts. This behavior resulted in significantly more eggs laid per host (superparasitism) when there were fewer hosts. It is also further evidence that *C. torvina* will dispose of all the eggs she has regardless of the number of hosts available, and supports the conclusion that females in the daily, continuous provisioning experiment were taxing their reproductive nutrient supply each day.

Conura torvina is able to produce eggs as she lays them. Females dissected after the third host provisioning (just before the fourth provisioning) contained an average of 9.2 mature eggs, but females from this same group which were not dissected laid an average of 13.1 eggs each during the fourth host provisioning. This indicates a net gain of nearly four mature eggs during the fourth provisioning period. These females, dissected immediately after the fourth host provisioning, had an average of 5.7 mature eggs in their ovaries. These eggs were relatively small (average 0.47 mm long). Thus, it appears that *C. torvina* was able to produce nearly 10 mature eggs during a 14 hour host provisioning period. Although no more than 15 mature eggs were counted in any female dissected before the fourth host provisioning, one female laid 30 eggs in her *C. rubecula* hosts during the fourth provisioning period.

In summary, *C. torvina* is most active during the summer months. It is an opportunistic parasitoid which will lay eggs in any suitable host it encounters. Some host species are preferred over others and this preference may be indicated by the proportion of female progenies produced. Ovulation, egg fertilization and oviposition behavior appear to be controlled by the female in response to its host species. *Conura torvina* requires an oviposition stimulus such as host frass or possibly host odor, and requires nutrients from host feeding for normal egg production. Oviposition behavior is partly a learned behavior which becomes more efficient with experience. Females often superparasitize their hosts, but the occurrence of superparasitism declines with an increase in oviposition experience. Host searching and oviposition behavior (at least under laboratory conditions) appear to be relatively inefficient, and females with as many as seven separate days of oviposition experience will superparasitize some of their hosts. *Conura torvina* can lay an average of 18 eggs per day and exceptional *C. torvina* may lay as many as 30 eggs in a day, but no female produced more than 17 progenies per day. An average of 14 progenies may be produced per day in *P. xylostella* hosts when the supply of hosts is ample.

Conura torvina's reproductive behavior may be somewhat different in the field than under laboratory conditions, but results from this study generally support an earlier study (Gaines 1992) which found an apparently low reproductive rate in the field. It is possible that females use their eggs more efficiently in the field than when caged (i.e., they do not oviposit in unsuitably aged hosts or superparasitize her hosts). However, in the field, *C. torvina* might have a lower reproductive capability than in the laboratory because caged females are not forced to expend much energy foraging for hosts, and thus, have more energy available for egg production.

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Vita: David N. Gaines

David N. Gaines was born July 16, 1953 in Joplin Missouri, and moved shortly after that to Grand Junction, Colorado. His years in grade school were spent in Mexico City, Mexico where he learned to speak Spanish fluently. He acquired an early interest in nature from the numerous trips he took with his father, a geologist, into the Mexican wilderness. In 1967 his family moved to southeastern Pennsylvania and he finished high school in 1972 at the Miquon Upper School in Germantown, Pennsylvania. For five years after high school, he traveled and gained skills and work experience from employment in various parts of the United States. This work included positions as: a roughneck on oil-field drilling rigs, a factory assembly-line worker, a construction worker, a farm worker, and as an independent contractor for carpentry, masonry and home repair. In 1975, while studying solar energy design and urban garden ecosystems at the Farrallones Institute in Berkely, California, he became interested in entomology. In 1977 he entered Penn State University to get a B.S. in Entomology. Shortly after graduation in 1981, he was employed as a sales manager and entomologist by an insecticide company in Pittsburgh Pa**.** This work gave him a background in structural pest control, insecticide usage and consulting. In 1986 he joined the Peace Corps and spent two years in The Gambia, West Africa, working as an agricultural research assistant for an agricultural development project. His work in Africa dealt primarily with cultural practice and variety trials of grain crops and peanuts. On his return from Africa he settled in Virginia, and enrolled in a Masters degree program with the Entomology Department at VPI & SU under the guidance of Dr. L. T. Kok. His masters thesis concerned the impact of hyperparasites on beneficial species associated with *Brassica* crops. Thesis research required the maintenance of field crops and laboratory colonies of various hosts and parasitoid species. He completed his M.S. in 1992 and immediately started his Ph.D. program in entomology, again under Dr. Kok at VPI&SU. The Ph.D. dissertation concerned the reproductive biology and behavior of an opportunistic, facultative hyperparasitoid associated with hosts in *Brassica* crops, and was completed and successfully defended in January of 1997. In the future, Mr. Gaines is hoping to work as a research entomologist on applied topics in the realm of biological control of pests, possibly in relation to agricultural development in the third world.