ON
SEASONAL INOCULATIVE
BIOLOGICAL CONTROL

Governing *Liriomyza* populations by parasitoids

Promotor: Dr. J. C. van Lenteren
Hoogleraar in de Entomologie,
in het bijzonder de Oecologie der Insekten

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On Seasonal inoculative Biological control

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Dedicated to the inventor of soft ball squash
Aan de uitvinder van squash

Contents

Stellingen

1 Bij het schatten van de effektiviteit van natuurlijke vijanden voor biologische bestrijding is, naast het bepalen van de reaktie op plaagdichtheid, ook het meten van de respons op de ruimtelijke variatie in plaagdichtheid cruciaal.

Kareiva, P. 1990. The spatial dimension in pest-enemy interactions. In: Critical Issues in Biological Control. M. Mackauer, L. E. Ehler & J. Roland (Eds.) Intercept, Andover, UK: 213-227.

2. Synchroniciteit van generaties van sluipwesp en gastheer en populatie-groeisnelheid van sluipwespen zijn biologisch relevante en meetbare kriteria om sluipwespen te selekteren in een preïntroduktie-procedure voor seizoensinokulatieve biologische bestrijding.

Dit proefschrift.

3. Het 'stopping rule' model zoals voorgesteld door Sugimoto et al. (1987) is zeer speculatief aangezien de voornaamste aanname, het deponeren en waarnemen van een merkferomoon op het blad, niet is gestaafd door resultaten uit onderzoek.

Sugimoto, T., Murakami, H. & R. Yamazaki 1987. Foraging for patchily-distributed leaf-miners by the parasitiod, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae). II. Stopping rule for host search. J. Ethol. 5: 95-103.

Sugimoto, T. & S. Tsujimoto 1988. Stopping rule of host search by the parasitiod. *Chrysocharis pentheus* (Hymenoptera: Eulophidae), in host patches. Res. Popul. Ecol. 30: 123-133.

4. De generalisatie door Ratte (1985), dat groei bij insekten een passief fysiologische proces is, is onjuist.

Ratte, H. T. 1985. Temperature and insect development. In: Environmental Physiology and Biochemistry of Insects. K. H. Hoffman (Ed.) Springer, New York: 33-66.

Taylor, M. F. J. 1988. Field measurement of the dependence of life history on plant nitrogen and temperature for a herbivorous moth. J. Anim. Ecol. 57: 873-891.

Minkenberg, O. P. J. M. & J. J. G. W. Ottenheim. 1990. Effect of leaf nitrogen content of tomato plants on preference and performance of a leafmining fly. Oecologia 83: 291-298.

5. De ontwikkeling van biologische bestrijdingstheorieën wordt ernstig beperkt, doordat projekten voornamelijk worden gefinancieerd om plaagproblemen op te lossen, en niet om te onderzoeken wat de onderliggende mechanismen zijn.

6. De bewering dat geïnduceerde resistentie in planten is ontstaan door een co-evolutie tussen plant en insekt dient nadere bewijsvoering.

   Harvell, C. D. 1990. The ecology and evolution of inducible defenses. Quart. Rev. Biol. (september 1990).

7. De traditionele, empirische methode van het selekteren van natuurlijke vijanden heeft weinig gemeen met methodieken gehanteerd in de kunst.

   Harris, P. 1973. The selection of effective agents for the biological control of weeds. Can. Ent. 105: 1495-1503.

   Van Lenteren, J. C. 1980. Evaluation of control capabilities of natural enemies: does art have to become science? Neth. J. Zool. 30: 369-381.

   Pak, G. A. 1988. Selection of *Trichogramma* for Inundative Biological Control. Doctoral thesis, Wageningen Agricultural University, the Netherlands.

8. De Nederlandse wetgeving dient zo te worden aangepast, dat overheden die van buitenlandse medewerkers eisen dat ze een huwelijksrelatie onderhouden met hun partner, niet langer mensen met een homosexuele geaardheid kunnen discrimineren.

9. De tijd die een oecoloog doorbrengt in de natuur zegt weinig over de waarde van zijn uitspraken.

10. Verandering in naam van de "desert locust" naar "dessert locust" kan helpen de aantallen sprinkhanen te verminderen.

11. Stellige meningen en menige stelling werken averechts.

Stellingen behorende bij het proefschrift "Seasonal inoculative biological control: governing Liriomyza populations by parasitoids" door O. P. J. M. Minkenberg.

Wageningen, 28 november 1990

Preface/Voorwoord

In een tijd waar de ecologische problemen wereldwijd toenemen, zien we in de landbouw een algemene tendens tot vermindering van het gebruik van vergiften voor het bestrijden van insekten. Als een van de alternatieven voor chemische bestrijding, heeft biologische bestrijding bewezen effektief en ekonomisch haalbaar te zijn. Dit heeft mede geleid tot een omwenteling in de benadering van plaagproblemen. Waar vroeger geprobeerd werd plaaginsekten uit te roeien door het vaak overvloedig gebruik van insekticiden, streven we nu naar samenleven met deze insekten, waarbij hun aantallen op aanvaardbaar nivo worden gehouden door het introduceren van natuurlijke vijanden.

U treft hierbij het verslag van een biologische bestrijdingsprojekt aan. Het voornaamste doel, een biologische bestrijdingsmethode voor mineervliegen, is bereikt mede door dit projekt. In Europa worden nu sluipwespen succesvol ingezet tegen mineerders op meer dan 500 ha, voornamelijk in groentegewassen.

In de biologische bestrijding zijn twee vragen aktueel: (1) welke mechanismen zijn werkzaam in het veld en leiden ertoe dat biologische bestrijding succesvol is? En (2) Wat zijn de kenmerken van een effektieve natuurlijke vijand? Met het beantwoorden van deze tweede vraag is onlangs een begin gemaakt. Dit onderzoek, waarvan een deel hier wordt gerapporteerd, is belangrijk omdat het kan leiden tot een efficientere methode om natuurlijke vijanden te selekteren en effektievere biologische bestrijding dan voorheen.

Het onderzoek heeft geprofiteerd van de goede ondersteuning en voorzieningen in Wageningen. Naast iedereen, die reeds elders wordt vernoemd in dit proefschrift, wil ik alle medewerkers van de bibliotheek, kassendienst, instrumentemakerij, afdeling fotografie, tekenkamer, administratie en insektekweek bedanken voor hun hulp. Ik wil alle collega's bedanken voor de fijne sfeer en de prettige vijf en een half jaar. Buiten de Binnenhaven heb ik vruchtbaar samengewerkt met Rudy Rabbinge (Vakgroep Theoretische Produktie-ecologie) en Gerard Bot (Vakgroep Natuur- en Weerkunde).

Tijdens mijn studie biologie was het prof. van Boven, die door zijn sprookjesachtige manier van college geven, me verleidde tot de wereld van de insekten en me overtuigde van het belang van biologische bestrijding. Prof. Bakker liet me na een telefoontje meeteen deel uitmaken van zijn groep. Joop van Lenteren turnde me mee naar Wageningen en wist samen met Jaap Woets mijn onderzoek gefinancieerd te krijgen. Joop "leerde" me zelfstandig onderzoek te doen, zijn voortdurende inzet was een grote stimulans voor me en hierbij wil ik je bedanken voor je begeleiding. Verder wil ik alle studenten, Aad de Vette, Louis van Vliet, Koen den Braber,

Dineke de Jager, Marjon Fredrix, Bert Mantel, Willen-Jan Sanders, Adrienne Frijters, Annemarie Heijs, Pottan Siregar, Rob Boer, Henriette Schmiermann, Aat Bal, Roelof Meijer, Paula Westerman, André Reytenbach, Ko Smidt, Marion van Rosevelt, Theo Jetten, Jo Ottenheim, Marleen Mallais, Michiel van der Haagen, Kees Groenendijk, Mees Dekker, Laurens van Miltenburg, Jan van Esch, Willem-Jan Boot, Mario Nagtsaam, Floor Arts, Niek Steegs bedanken, zonder wie ik dit projekt niet had kunnen uitvoeren. Verder wil ik Thea gedenken, die overleed kort na afronding van haar onderwerp op Entomologie.

Finally, I cordially thank Michael Parrella, whose support enabled me to write the last part of this thesis.

Allen bedankt.

Oscar Minkenberg.

Davis, 8 oktober 1990.

Summary

This doctoral thesis deals with the development of a biological control method for leafmining flies on greenhouse tomatoes. Now one can effectively control leafminers by seasonal inoculative releases of parasitoids. The influence of temperature and leaf nitrogen on the population dynamics and behavior of the flies and their parasitoids are investigated. Using leafminer parasitoids as a model, I examine four criteria to select parasitoids prior to their introduction in seasonal inoculative biological control.

Biological control is an effective tactic to regulate greenhouse pest populations below the economic injury level (Hussey and Scopes 1985, Van Lenteren and Woets 1988). Three strategies to enhance the action of natural enemies are usually distinguished in biological control: (1) conservation, (2) classical biological control and (3) augmentation. Conservation of natural enemies includes protection the natural enemies present and improve their action. Classical biological control results from the introduction and establishment of natural enemies that permanently regulate populations of originally exotic pests (Hoy 1985). Within the third strategy, augmentation, one increases the number of natural enemies within a defined area by introduction of natural enemies (King et al. 1985). Two introduction methods can be distinguished in augmentation: seasonal inoculative, where relatively small numbers are introduced, and inundative, where large numbers of natural enemies are applied. The difference between classical, seasonal inoculative and inundative biological control is best defined by their aims: effective control for many years, for one growing season, and for the duration of one pest generation, respectively.

In greenhouses, classical biological control has not been practiced because of breaks in the crop production followed by soil sterilization, which makes survival of any organism almost impossible. However, soil sterilization has become unnecessary for some crops because of the substitution of soil as substrate for rockwool, and both pests and natural enemies may survive through the next season. The first cases of natural control, i.e. control by natural enemies without intervention by man, in the greenhouse are reported (Woets and Van der Linden 1982). This suggests that classical biological control should be contemplated in future pest management programs as a possible control strategy.

In contrast, inundative biological control has been practiced on a large scale in the greenhouse, particularly with the use of *Bacillus thurgiensis* to control various caterpillars. Another example of inundative biological control can be found in cut flowers. Although low tolerance levels exist, biological control has been initiated in chrysanthemums against its key pest, the leafminer *Liriomyza trifolii* (Wardlow 1985, Jones et al. 1986, Parrella et al. 1987). Low pest densities are achieved by weekly releases of high numbers of parasitoids (*Diglyphus begini*): repeated inundative biological control (Heinz and Parrella 1990a).

Every new biological control project starts with the necessity of identifying an effective natural enemy or complex of natural enemies. Two approaches can be distinguished: (1) empirical and (2) analytical. The empirical approach in classical biological control refers to the release of all natural enemies at hand and let nature sort them out (Ehler 1990). In seasonal inoculative biological control, natural enemies are usually tested separately in an agricultural setting and the one performing best is selected. The advantage of this approach is its speed: an effective agent might be found after one trial. The analytical approach involves selection of the most promising candidates based on investigations before their introduction. The advantage of this approach is that we may gain an understanding of the biological attributes of an effective natural enemy (Van Lenteren 1980).

Selection procedures using an analytical approach are still in development. Within the analytical approach, two distinct ways are in examination (Waage 1990). First, the reductionist approach, which uses characteristics attributed to effective natural enemies to select them. Second, the holistic approach, which proceeds from theoretical notions of how natural enemies fit into the broad ecology of the pest and its other mortality factors (Miller 1983, Myers et al. 1989).

Characteristics of effective parasitoids are examined as selection criteria for possible biological control agents. The reductionist approach is well known in classical biological control and many lists of selection criteria have been published. The criteria proposed have been reevaluated for their use in seasonal inoculative release programs by Van Lenteren (1986). These criteria are further discussed below.

Parasitoids against leafminers on greenhouse tomatoes

Since its first introduction into Europe from Florida in 1975, *L. trifolii* (Diptera: Agromyzidae) has become a major pest of vegetables and ornamentals in both greenhouses and outdoor fields (Minkenberg 1988a). The economic impact of *Liriomyza* leafminers worldwide has been enormous, e.g. several hundreds million dollars in lost revenue for Californian growers has been attributed to this leafminer in the last decade. Leafminers cause economic damage through the mining activity of the larvae, reducing the photosynthetic capacity of the plants. Feeding punctures made by the adult

females can be invaded by fungi and bacteria. Further, *Liriomyza* flies may transmit viruses (for reviews on their biology, see Spencer 1973, Minkenberg and Van Lenteren 1986, Parrella 1987).

Leafminer infestations arise in three ways: (1) as naturally occurring, non-induced seasonal pests, (2) after pesticide sprays because their natural enemies have been destroyed, and (3) when adults build up on other crops or weeds and migrate to a particular plot. Under natural conditions, leafminers are usually attacked by a diverse complex of natural enemies and this may explain the often low abundance of leafminers in unsprayed crops. The use of non-selective chemicals often leads to high leafminer densities, because parasitoids are typically susceptible to these chemicals.

The leafminers *L. trifolii* and *L. bryoniae* have more than 40 parasitoid species in the families Braconidae, Eulophidae, and Pteromalidae (Chapter 1). Leafminer parasitoids have been introduced into California, Canada, Hawaii, the Netherlands, New Zealand and other countries for classical and seasonal inoculative biological control programs. Although several parasitoids are supposedly established in new areas, there are no data available on their impact on leafminer populations. Clearly, post-introduction evaluation projects are required. Several parasitoids, *Dacnusa sibirica, Diglyphus isaea* and *Diglyphus begini,* are presently used effectively in seasonal inoculative and inundative releases and are commercially produced (Minkenberg and Van Lenteren 1986, Johnson and Hara 1987, Heinz and Parrella 1990). Pest problems caused by leafminers in Europe are aggravated currently in some crops due to the importation of a new polyphagous species, *Liriomyza huidobrensis*, from America.

Research objectives

The main purpose of my project was to develop a biological control method for the imported leafminer *L. trifolii* and its native relative *L. bryoniae* on greenhouse tomato (objective 1). In addition, the project attempted to study the population dynamics of the leafminers and their parasitoids by determining the processes that lead to changes in population densities (objective 2). I also examined selection criteria to develop a preintroduction selection procedure appropriate for leafminer parasitoids applied in seasonal inoculative biological control (objective 3).

Results and discussion

1. Development of a biological control method for leafminers

In 1983, when the parasitoids *Opius pallipes, D. sibirica* and *D. isaea* were being evaluated for the biological control of *L. bryoniae* on tomato, *L. trifolii*

became a pest problem in greenhouse vegetables (Hendrikse et al. 1980, Woets and Van der Linden 1983). These parasitoids naturally occur in the greenhouse and were considered as the prime candidates with which to begin. In 1984 the parasitoid *Chrysocharis oscinidis* Ashmead (= *C. parksi* Crawford, LaSalle and Parrella, in press) was imported from California, mass-reared and tested in trials in experimental and commercial greenhouses (Woets and Van der Linden 1985, Frijters et al. 1986, Westerman & Minkenberg 1986, W.J. Ravensberg pers. com.). Using the traditional, empirical approach to evaluate these parasitoids for control of *L. trifolii,* the braconid *D. sibirica* was identified as an effective biological agent for control of leafminers infesting greenhouse vegetables in northwestern Europe. My study has extended on knowledge on the leafminers *L. trifolii* and *L. bryoniae* and two parasitoids, *D. sibirica* and *D. isaea.*

2. Study of population dynamics of leafminers and their parasitoids

Several factors, such as temperature and plant quality, were examined. Life history studies conducted between 15 and 25°C show that the optimum temperature for population growth is 25°C (Chapters 2 and 3). For *L. trifolii,* the intrinsic rate of increase becomes negative below 16°C, suggesting that *L. trifolii* populations on tomato will decrease in number below that temperature. Given the developmental rate, immature survival, adult fecundity and survival of both agromyzids at the temperatures examined, tomato appears to be a more suitable host plant for *L. bryoniae* than for *L. trifolii.*

To use data on life-history variables collected at constant temperatures in predictive models, these variables should react instantaneously to temperature changes. Development of *Liriomyza* immatures meets this condition, whereas reproduction at alternating temperatures is lower than at a comparable constant temperature. Consequently, models based on measurements at constant temperatures may overestimate population growth in the field. The reduced reproduction of the flies at variable temperatures may result from differential changes in plant quality with temperature fluctuations.

Temperature also greatly affects performance of the parasitoids (Chapters 4 and 5). Net reproduction in *D. sibirica* decreases, but the intrinsic rate of increase increases with increasing temperatures. Its oviposition rate is highest at 20°C. In contrast, optimum temperature for oviposition by *D. isaea* is greatest near 25°C. Its intrinsic rate of increase greatly increases at high temperatures compared to low temperatures. However, fecundity is independent from temperature within the range 15 to 25°C.

Percent relative humidity (% RH) only influences the pupal phase of the flies. Pupal survival positively correlates with ambient % RH. Under greenhouse conditions, both humidity and light seem minor factors in the life of these insects.

Host-plant quality in terms of leaf nitrogen content is another important factor governing *Liriomyza* population. Adult *L. trifolii* females prefer to feed and oviposit on plants with a high nitrogen content, after they have been exposed previously to plants of high nitrogen (Chapter 6). Performance is improved on the high nitrogen plants: population growth rate (r_m) doubled with an increase in nitrogen from 3.4% to 4.9%. Moreover, all of the life-history variables examined, excluding sex ratio and preoviposition period, are positively influenced by increasing leaf nitrogen content (Chapter 7). Thus, *L. trifolii* appears to be adapted in its intraspecific host plant selection because the preference will lead to an increase in overall fitness.

3. Development of a preintroduction selection procedure

Evaluation framework, selection criteria and concepts. The analytical approach should yield a procedure to select natural enemies prior to their introduction, and by which means we will have a probability of finding an effective natural enemy at least equal to that by an empirical process. To develop such a preintroduction selection procedure, criteria that will indicate effectiveness need to be formulated from ecological theory and biological control practices.

On basis of the study of the leafminer-parasitoids system, I have proposed four criteria, viz. (1) Complete Development and Offspring Quality, (2) Generation Synchrony, (3) Population Growth and (4) Searching Efficiency (Chapter 10). The preintroduction procedure should take place entirely in the laboratory. Measurements of these criteria and their significance are briefly discussed below:

Criterion 1: Complete Development and Offspring Quality. Since seasonal inoculative programs partly rely on the numerical response of the agents, i.e. the relationship between the number of parasitoids and host density over the entire season, their offspring should completely develop on the pest and be viable. Abiotic conditions under which they have to operate should allow development and reproduction and quality has to be maintained over generations of hosts.

Criterion 2: Generation Synchrony. When host generations are discrete, development of parasitoids should be synchronous with that of the pest. I introduce the term "waiting period". Waiting period is the minimum average time that a newly emerged parasitoid has to bridge before suitable hosts have become available, as a way to estimate asynchrony and hence, reduction in parasitoid impact on the pest population. On basis of a convergence to the stable age distribution, one can calculate the transitions in the relative abundance of the different stages. So, host-free periods and discrepancy with generations of hosts can be projected (Chapters 2-5).

Criterion 3: Population Growth. The usual indices for population growth are the intrinsic rate of increase, r_m (no. female offspring per female per day), and net reproduction rate, R_0 (no. female offspring per female per generation), both measured in the laboratory under specified conditions. These parameters for hosts and parasitoids may not be useful to predict effectiveness, because (1) growth of the populations of hosts and parasitoids is likely to be differently affected in each others presence, (2) growth of the pest population probably will be reduced at high densities whereas that of the parasitoid increases at high host densities, and (3) the relative densities at the moment of release will in part determine the outcome of a program. Population growth rates can be used to compare parasitoids, assuming criteria 1 and 2 to be equally met and criterion 4 to be similar.

Criterion 4: Searching Efficiency. Searching by parasitoids for hosts amounts to a complex of behavioral components and factors involved and cannot readily be simplified. Searching efficiency e.g., their ability to locate hosts at long distance or their behavior toward hosts once arrived in an area with hosts, can be measured in different ways; a standard procedure has not been developed yet. Host location at long distance can be examined in the laboratory in an olfactometer or wind tunnel. By observing individual adult female wasps their functional response, i.e. the relationship between the number of hosts parasitized per parasitoid per unit time and host density, can be estimated (Minkenberg and Parrella 1990).

Leafminer parasitoids in Europe: an evaluation of selection criteria. Dacnusa sibirica and *D. isaea* are effective parasitoids of leafminers on tomato in northwestern and southern Europe, respectively. Both agents successfully develop and reproduce in or on these hosts under conditions corresponding to a temperate greenhouse climate (criterion 1). In northwestern Europe, leafminer generations are discrete during the three to four generations after the beginning of the growing season. Generations of *D. sibirica* synchronize well with that of the leafminers and their waiting time is approximately three days. Due to fast development, *D. isaea* has a waiting time of circa twelve days. This asynchrony with the leafminer generations probably reduces its impact as control agent in northwestern Europe (criterion 2). In southern Europe leafminer generations overlap and this criterion is less likely to be important.

With respect to criterion 3, net reproduction might be a better indicator of population growth than r_m in the Northwest, because developmental time does not play a major role there and that parameter is not incorporated in R_0. At low temperatures *D. sibirica* has a higher net reproduction than *D. isaea.* However, reproduction in *D. sibirica* decreases with increasing temperatures and *D. isaea* has a higher reproduction at high temperatures. Thus, *D. sibirica* is expected, based on this criterion, to perform better in the temperate region (criterion 3).

Data on the searching efficiency of these parasitoids are limited (criterion 4). The parasitoids *D. sibirica* can distinguish plants infested by leafminers from those without any hosts at a distance. We showed that the wasps can smell the presence of *L. bryoniae*; though, visual stimuli might be important as well (Chapter 8). Further estimates of searching efficiency were derived from direct observations on *D. sibirica*'s foraging (Chapter 9). Parasitoids preferred to land initially on plants with high rather than low densities of hosts. Furthermore, parasitoids spend more time searching on the high host density plants and oviposition rates were enhanced with increasing host densities. Consequently, directly density dependent parasitism occurred at the plant level. This is probably possible because these parasitoids are informed about the distribution of the leafminers at that spatial level.

In conclusion, Generation Synchrony and Population Growth appear to explain, at least in part, the effectiveness of these parasitoids in the different regions.

A first step toward a preintroduction selection procedure. Laboratory measurements on the criteria will produce sets of values for each parasitoid. Some parasitoids can be discarded because they do not meet the qualitative standards, such as successful development on the pest insect. The parasitoids with the best set of quantitative values then should be selected from the pool to examine their effectiveness, viz. by trials in commercial greenhouses. However, there is not a single case study, in which a complete comparison of criteria has taken place. Thus, the prediction which parasitoids are likely to perform best as biological control agent needs to be verified in the greenhouse after examining a number of parasitoids in the laboratory. This is a conceivable way to evaluate selection criteria for the preintroduction selection procedure.

Future research

In the entomological literature, many publications on the development, reproduction and population growth of natural enemies are available and this information might be useful in a selection procedure. However, data are greatly lacking on the searching efficiency of natural enemies (Hopper and King 1986). A behavioral bioassay to measure searching at a range of host densities in the laboratory is in development (Minkenberg and Parrella 1990). Behavioral components that enhance the searching efficiency of natural enemies need further to be determined (Kareiva and Odell 1987).

Not only studies that evaluate the outcome of introductions of biological control agents are needed, but also specific experiments that test predictions stemming from the evaluation framework. In the case of the leafminer parasitoids, this includes (1) examination of the effectiveness of *D. sibirica* in southern Europe, (2) analysis of the impact of low reproduction in *D.*

sibirica due to high temperatures in the South. (3) further examination of the effectiveness of *D. isaea* in northwestern European greenhouses, (4) estimate of the temporal variability in leafminer density, i.e. quantification of the host free periods, in the different regions and (5) tests of the survival of *D. isaea* in the Northwest and in the South, which may be reduced due to generation asynchrony, and its impact on effectiveness.

In connection with experiments, research models need to be developed: (1) that incorporate population growth rates of natural enemies and pests, their initial densities and time of introduction, and so will allow predictions on the outcome of trials over several generations (Sabelis et al. 1983, Boot et al. 1990), (2) that incorporate behavioral components, such as probability of finding areas with hosts and the searching rate within these areas, and so will predict parasitism within one generation of the host, and (3), last but not least, that weigh the importance of the different criteria and project the values for an 'ideal' parasitoid. These models might substantially support the conclusions from an evaluation of the criteria.

Samenvatting

In dit proefschrift wordt de ontwikkeling van een biologische bestrijdingsmethode tegen mineervliegen in de tomatenteelt onder glas behandeld. Door middel van meerdere loslatingen van sluipwespen gedurende het groeiseizoen kan men nu mineerders effektief bestrijden. Temperatuur en de kwaliteit van de waardplant blijken een belangrijke rol te spelen in de populatiedynamica van de vliegen en sluipwespen. Aan de hand van het mineerder-sluipwespenwerk evalueer ik een aantal selektiekriteria voor deze vorm van biologische bestrijding, waarbij ik een analytische methode om effektieve sluipwespen te selekteren bespreek.

Biologische bestrijding kan een effektieve methode zijn om plaagpopulaties in kassen onder de schadedrempel te houden. Ieder plaaginsekt heeft meestal een heel scala van natuurlijke vijanden, die mogelijk te gebruiken zijn. Bij biologische bestrijding kunnen drie toepassingsstrategiën worden onderscheiden: (1) behoud en beheer van natuurlijke vijanden, (2) eenmalige introduktie, i.e. klassieke biologische bestrijding and (3) herhaalde introdukties, i.e. seizoensinokulatieve en inundatieve biologische bestrijding. Behoud en beheer omvat alle maatregelingen die genomen worden om de aanwezige natuurlijke vijanden te beschermen en hun effektiviteit te vergroten. Klassieke biologische bestrijding dient populaties van insekten te reguleren die van elders afkomstig zijn. Deze 'exotische' plaaginsekten zijn vaak ingevoerd zonder hun natuurlijke vijanden en men bestrijdt ze door natuurlijke vijanden in te voeren vanuit het gebied van herkomst. De derde strategie, seizoensinokulatieve en inundatieve introdukties, bestaat uit het verhogen van de aantallen natuurlijke vijanden binnen een bepaald gebied door ze daar los te laten. Bij seizoensinoculatieve biologische bestrijding worden relatief lage aantallen en bij inundatieve biologische bestrijding relatief hoge aantallen natuurlijke vijanden geintroduceerd. Het verschil tussen klassieke, seizoensinokulatieve en inundatieve biologische bestrijding kan het best worden aangegeven door hun respectievelijke doelstellingen: effektieve bestrijding voor vele jaren, voor een groeiseizoen en voor de duur van een plaaggeneratie.

Elk nieuw biologische bestrijdingsprojekt start met de noodzaak een effektieve natuurlijke vijand of een complex van natuurlijke vijanden te vinden. Twee benaderingen zijn mogelijk, namelijk een empirische of een analytische. De empirische benadering bestaat uit het loslaten van natuurlijke vijanden in praktijkomstandigheden, waarna de meest effektieve wordt

gekozen. Deze aanpak kan vrij snel tot resultaat leiden. De analytische methode streeft ernaar de meest belovende kandidaten te selekteren op basis van onderzoek voordat er toetsen in de praktijk plaatsvinden. Deze laatste benaderingswijze geeft meer inzicht in de redenen of oorzaken die ten grondslag liggen aan effektiviteit.

Selektieprocedures waarbij men analytisch te werk gaat, zijn in onderzoek. Een ontwikkeling gaat uit van het idee dat effektieve natuurlijke vijanden algemene kenmerken vertonen, die gebruikt kunnen worden als selektiekriteria. Deze reductionische benadering heb ik gevolgd. Een tweede ontwikkeling hanteert een holistische benadering. Deze gaat uit van theoretische concepten hoe natuurlijke vijanden passen binnen de oekologie van een plaaginsekt en zijn sterftefaktoren.

Sluipwespen tegen mineervliegen op tomatenplanten onder glas

De mineervlieg *Liriomyza trifolii* is ingevoerd in Nederland vanuit Florida. Dit insekt veroorzaakt plagen in groenten- en siergewassen in kassen en in de vollegrond. De geleden verliezen zijn gigantisch: verscheidene honderden millioenen dollars alleen al in Californië in de afgelopen tien jaar. De vliegen en hun larven veroorzaken beide schade. De larven eten gangen door het bladmoes, "mineren", waarbij ze de fotosynthetische kapaciteit van de plant reduceren. De volwassen vliegevrouwtjes maken openingen in het blad om zich te voeden, de zogenaamde voedingsstippen. Deze stippen zijn invalspoorten voor schimmels en bacteriën. Verder brengen de vliegen plantenvirussen over.

Bladmineerders veroorzaken in drie situaties plaagproblemen: (1) als natuurlijk voorkomende, niet-geïntroduceerde seizoensplagen, (2) nadat pesticiden hun natuurlijke vijanden hebben gedood en (3) wannneer de adulten in aantal toenemen op andere gewassen of op onkruiden en immigreren. Deze plaaginsekten worden onder natuurlijke omstandigheden aangevallen door een complex van natuurlijke vijanden. De lage aantallen bladmineerders in onbespoten gewassen kunnen hierdoor worden verklaard. Het gebruik van breedwerkende pesticiden mag mede worden beschouwd als een oorzaak van hoge aantallen mineerders, omdat natuurlijke vijanden en met name sluipwespen zeer gevoelig zijn voor deze chemicaliën.

De bladmineerders *L. trifolii* en *L. bryoniae* hebben zover bekend meer dan 40 soorten sluipwespen uit de families der Braconidae, Eulophidae en Pteromalidae als parasiet (hoofdstuk 1). Mineerder-sluipwespen zijn geïntroduceerd in Californië, Canada, Hawaï, Nederland, Nieuw Zeeland en andere landen voor klassieke en seizoensinokulative biologische bestrijdingsprogramma's. Hoewel we mogen veronderstellen dat sommige soorten zich gevestigd hebben in de verschillende landen, is er weinig onderzoek gedaan aan de invloed van deze loslatingen op de aanwezige mineerderpopulaties.

Het moge duidelijk zijn dat post-introduktie evaluatieprogramma's noodzakelijk zijn. De sluipwespen *Dacnusa sibirica, Diglyphus isaea* en *Diglyphus begini* worden momenteel gebruikt in seizoensinokulatieve en inundatieve loslatingen. Ze worden kommerciëel geproduceerd door een aantal firma's. Mineerders kunnen nu effektief worden bestreden. In sommige gewassen zijn plaagproblemen door bladmineerders verergerd door de invoering van een nieuwe, polyfage soort, *Liriomyza huidobrensis.*

Probleemstellingen van het onderzoeksprojekt

De voornaamste doelstelling was de ontwikkeling en integratie van een biologische bestrijdingsmethode voor de ingevoerde bladmineerder *L. trifolii* en zijn inheemse soortgenoot *L. bryoniae* voor de teelt van kastomaten (punt 1). Daarvoor werd het noodzakelijk gevonden inzichten te verwerven in de populatiedynamica van bladmineerders en hun sluipwespen (punt 2); oftewel, welke processen spelen een belangrijke rol in het bepalen van de populatiedichtheden van deze plaaginsekten? Een andere vraagstelling van mijn projekt was: welke kriteria zijn waardevol voor het selekteren van mineerdersluipwespen voor seizoensinokulatieve biologische bestrijding (punt 3). De achterliggende bedoeling was het ontwikkelen van een preïntroduktie selektiemethode oftewel een methode om sluipwespen te selekteren alvorens men overgaat tot introduktie in kommerciële kassen.

Resultaten en diskussie

1. Ontwikkeling van een biologische bestrijdingsmethode voor bladmineerders

Toen in 1983 de sluipwespen *Opius pallipes, Dacnusa sibirica* en *Diglyphus isaea* werden geëvalueerd door J. Woets en A. van der Linden (Proefstation voor Tuinbouw onder Glas te Naaldwijk) in samenwerking met A. Hendrikse (Rijksuniversiteit te Leiden) voor biologische bestrijding van *L. bryoniae*, werd ook *L. trifolii* een plaag in de tomatenteelt onder glas. Deze sluipwespen kwamen van nature voor in kassen en werden als voor de hand liggende kandidaten beschouw. In 1984 voerde men daarbij de sluipwesp *Chrysocharis oscinidis* in vanuit Californië. Massakweken werden opgezet en we deden toetsen in experimentele en kommerciële kassen. Door gebruik te maken van de traditionele, empirische selektieprocedure werd *D. sibirica* geïdentificeerd als de meest effektieve sluipwesp voor de bestrijding van bladmineerders. Ik richtte mijn studie, aan de Landbouwuniversiteit te Wageningen, verder op de bladmineerders, *L. trifolii* en *L. bryoniae,* en twee van hun sluipwespen, *D. sibirica* en *D. isaea.*

2. Studie van de populatiedynamica van bladmineerders en hun sluipwespen

Belangrijke faktoren in de populatiedynamica van insekten zijn temperatuur en de kwaliteit van de waardplant als voedsel. In het onderzochte temperatuurstrajekt 15 tot 25°C is de groeisnelheid van de vliegenpopulaties het hoogst bij 25°C (hoofdstuk 2 en 3). De populatiegroeisnelheid van *L. trifolii* wordt negatief onder 16°C, hetgeen aangeeft dat *L. trifolii* populaties in de tomatenteelt in aantal zullen afnemen bij lage temperaturen. Gegeven de ontwikkelingssnelheid, de overleving van de pre-adulte en adulte stadia en de eilegkapaciteit van beide soorten mineervliegen, blijkt de tomatenplant een geschiktere waard voor *L. bryoniae* dan voor *L. trifolii*. De *life-history* gegevens kunnen verder gebruikt worden in beschrijvende populatiedynamische modellen. Om gegevens die verzameld zijn bij konstante temperaturen te gebruiken in modellen, dienen de variabelen momentaan te reageren op temperatuursveranderingen, dwz. veranderingen in de temperatuur dienen direkt (lineaire) veranderingen in deze variabelen te veroorzaken. De ontwikkelingsnelheid van *Liriomyza* reageert momentaan met temperatuur. Echter de reproduktie bij wisselende temperaturen is lager dan die bij een vergelijkbare konstante temperatuur. Dit zou het gevolg kunnen zijn van niet-momentane veranderingen in waardplantkwaliteit. Het gevolg is dat modellen die gebaseerd zijn op metingen bij konstante temperatuur, de populatiegroei in het veld zullen overschatten, indien men hiermee geen rekening houdt.

Temperatuur beinvloedt ook sterk de sluipwespen (hoofdstuk 4 en 5). Reproduktie van *D. sibirica* vermindert sterk bij hogere temperaturen. Eileg is het hoogst bij 20°C. Omdat de ontwikkelingsduur sterk afneemt, neemt de populatiegroei toe bij stijgende temperaturen. De populatiegroei voor *D. isaea* neemt ook toe bij stijgende temperaturen en is het hoogst bij 25°C. Eileg door *D. isaea* blijkt onafhankelijk te zijn van temperatuur binnen het bekeken temperatuurstrajekt.

Luchtvochtigheid (% RH) is alleen van belang gedurende het popstadium van de vliegen. Overleving van de vliegepoppen korreleert positief met de luchtvochtigheid. Onder kasomstandigheden lijken zowel luchtvochtigheid als licht relatief onbelangrijke faktoren te zijn in het leven van deze insekten.

Waardplant-kwaliteit in termen van hoeveelheiden bladstikstof is een belangrijke faktor in de ontwikkeling van *Liriomyza* populaties. Volwassen *L. trifolii* vrouwtjes hebben een voorkeur voor planten met een hoog stikstofgehalte om te voeden en eieren te leggen. Deze preferentie vertonen ze pas nadat ze een ervaring hebben gehad met planten van een hoog stikstofgehalte (hoofdstuk 6). Overleving en andere life-history variabelen zijn hoger op de planten met een hoog stikstofgehalte: de populatiegroeisnelheid (r_m) verdubbelt met een toename in bladstikstof van 3,4 tot 4,9% (hoofdstuk 7). De voorkeur van deze vlieg voor tomatenplanten met een hoog stikstofgehalte leidt tot een maximalisatie van ontwikkeling en reproduktie.

3. Ontwikkeling van een preïntroduktie selektieprocedure

Evaluatieschema, selektiekriteria en koncepten. De analytische benadering dient een procedure op te leveren, waarmee men sluipwespen kan evalueren voor hun introduktie in kommerciële kassen. Deze procedure zal even goed moeten zijn als de empirische methode: de kans dat men een effektieve natuurlijke vijand identificeert met deze procedure zal minstens even groot moeten zijn. Om zo'n preïntroduktie selektieprocedure te ontwikkelen, moeten kriteria die effektiviteit aanduiden worden geformuleerd vanuit de oekologische theorie en de biologische bestrijdingspraktijk.

Er blijken vier hoofdkriteria te zijn voor het mineerder-sluipwespensysteem, te weten: (1) Volledige Ontwikkeling en Kwaliteit van de Nakomelingen, (2) Synchroniciteit tussen Generaties, (3) Populatiegroei en (4) Zoekefficiëntie (hoofdstuk 10). De preïntroduktie procedure dient bij voorkeur volledig plaats te vinden in het lab. De betekenis van deze kriteria, en hoe ze eventueel gekwantificeerd kunnen worden, wordt hieronder in het kort bediskussieerd.

Kriterium 1: Volledige Ontwikkeling en Kwaliteit van de Nakomelingen. Omdat seizoensinokulative programma's gedeeltelijk vertrouwen op een numerieke toename van de sluipwespen over het gehele groeiseizoen, dienen de nakomelingen van de losgelaten sluipwespen zich volledig te ontwikkelen en zich te reproduceren op het plaaginsekt. Onder de abiotische faktoren die van invloed zijn onder praktijkomstandigheden, moeten ontwikkeling en reproduktie van de sluipwespen mogelijk zijn; hun kwaliteit dient gehandhaafd te blijven over generaties van gastheren.

Kriterium 2: Synchroniciteit van Generaties. Als de generaties van het plaaginsekt gescheiden zijn, dient de ontwikkeling van de sluipwespenpopulaties synchroon te zijn met die van het plaaginsekt. Ik introduceer de term "wachtperiode", die als volgt is gedefinieerd: een wachtperiode is de gemiddelde periode die een sluipwesp minimaal moet kunnen overbruggen voordat geschikte stadia van de gastheer beschikbaar komen. Op deze manier kan een indruk worden verkregen van asynchroniciteit en een eventuele vermindering van de invloed van de sluipwespenpopulatie op de plaag. De wachttijden worden berekend op basis van *life-history* studies (hoofdstuk 2-5).

Kriterium 3: Populatiegroei. De gebruikelijke indices voor populatiegroei zijn intrinsieke groeisnelheid, r_m (aantal vrouwelijke nakomelingen per vrouwtje per dag), en netto reproduktie snelheid, R_0 (aantal vrouwelijke nakomelingen per vrouwtje per generatie), die beiden kunnen worden bepaald in het lab onder gespecificeerde omstandigheden. Deze parameters voor gastheren en sluipwespen zijn op zichzelf onvoldoende om de effektiviteit van de sluipwespen te voorspellen, omdat (1) de groei van de populaties van gastheren en sluipwespen verschillend van elkaar worden beïnvloed in

elkaars aanwezigheid, (2) de groei van de plaagpopulaties af zal nemen bij hogere dichtheden, terwijl die van de sluipwespen juist zal toenemen bij hogere plaagdichtheden en (3) de relatieve dichtheden op het moment van loslating gedeeltelijk de uitkomst van een bestrijdingsprogramma zal bepalen. Populatiegroei-snelheden kunnen wel worden gebruikt om sluipwespen onderling te vergelijken, aangenomen dat alle andere eigenschappen gelijk blijven (zie kriteria 1, 2 en 4).

Kriterium 4: Zoekefficiëntie. Het zoeken van sluipwespen naar gastheren behelst een complex van gedragingen en faktoren en kan moeilijk worden gesimplificeerd tot een getal. De zoekefficiëntie van sluipwespen kan bijvoorbeeld afhankelijk zijn van hun kapaciteit om gastheren te localiseren op afstand of van hun gedrag als ze eenmaal aangekomen zijn op een plek met gastheren. Dit kan gemeten worden op verschillende manieren; een standaardprocedure is nog niet ontwikkeld. Gastheerlokalisatie van een afstand kan in het lab worden onderzocht met een olfaktometer of een windtunnel. Sluipwespen kunnen worden geobserveerd om hun funktionele respons, i.e. de relatie tussen het aantal gastheren dat wordt geparasiteerd en de aangeboden gastheerdichtheid, te bepalen.

Mineerder-sluipwespen in Europa: een evaluatie van selektiekriteria. De sluipwespen *D. sibirica* en *D. isaea* zijn effektief tegen mineerders in respectievelijk Noordwest en Zuid Europa. Beide ontwikkelen en reproduceren in of op deze gastheren onder omstandigheden vergelijkbaar met een gematigd kasklimaat (kriterium 1). In noordwestelijk Europa zijn na de start van het groeiseizoen de mineerdergeneraties gescheiden, dwz. dat een populatie op elk gegeven moment grotendeels uit één stadium (ei, larve, pop of vlieg) bestaat, gedurende de eerste drie of vier generaties. De generaties van *D. sibirica* synchroniseren bijna perfekt met die van de gastheren en zijn wachttijd is ruwweg gemiddeld drie dagen. Daarentegen vertoont *D. isaea* een snellere ontwikkeling tot adult en de wachttijd voor deze sluipwesp is twaalf dagen. De asynchroniciteit tussen generaties van *D. isaea* en mineerders vermindert bij deze soort de mogelijkheden voor de biologische bestrijding in Noordwest Europa (kriterium 2). In Zuid Europa waar de generaties overlappen speelt dit kriterium minder een rol.

Vanwege de gescheiden generaties in het Noordwesten speelt ontwikkelingsduur daar geen grote rol. Dit betekent voor kriterium 3 dat netto reproduktie hoogstwaarschijnlijk een betere parameter voor populatiegroei is dan r_m, omdat ontwikkelingsduur niet is geinkorporeerd in R_0. Bij lage temperaturen is de netto reproductie van *D. sibirica* hoger dan die van *D. isaea*. De reproduktie van *D. sibirica* vermindert echter bij hogere temperaturen, terwijl *D. isaea* een hoge reproduktie heeft bij hoge temperaturen. Op basis van dit kriterium kunnen we verwachten dat *D. sibirica* minder effektief zal zijn in de warme zone (kriterium 3). Gegevens over zoekefficiëntie van deze sluipwespen zijn beperkt voorhanden

(kriterium 4). De sluipwesp *D. sibirica* is bekeken in een windtunnel. Deze soort kan vanaf een halve meter planten met mineerders onderscheiden van planten waar er geen inzitten. We toonden aan dat zij de aanwezigheid van gastheren kunnen ruiken, vermoedelijk met hun antennen, alhoewel visuele stimuli ook een rol kunnen spelen (hoofdstuk 8). Verder informatie over het zoekgedrag van *D. sibirica* werd verkregen door direkte observaties (hoofdstuk 9). De sluipwespen landden vaker op planten met een hogere mineerderdichtheden en verbleven langer op de planten met hogere gastheerdichtheden dan op planten met lagere dichtheden. Verder nam de parasiteringssnelheid proportioneel toe bij hogere dichtheden van gastheren. Dit had tot gevolg dat de parasitering dichtheidsafhankelijk was op plantnivo. Dit is waarschijnlijk gerelateerd aan hun vermogen om op deze schaal de verdeling van de mineerders over planten waar te nemen.

Synchroniciteit van Generaties en Populatiegroei verklaren gedeeltelijk de effektiviteit van elk van de sluipwespen in een verschillende regio.

Een eerste stap naar een preïntroductie selektieprocedure. Labmetingen aan de kriteria zal een set van waarden produceren voor elke sluipwesp. Sommige sluipwespen kunnen worden afgewezen omdat ze niet voldoen aan de kwalitatieve voorwaarden, zoals een volledige ontwikkeling op het plaaginsekt. De sluipwespen met de beste set van kwantitatieve waarden zullen worden geselekteerd om hun effektiviteit te bepalen. Helaas is er nog geen enkele studie gedaan met een komplete vergelijking van kriteria. De voorspelling, welke sluipwespen het meest effektief zullen zijn, dient dus geverifieerd te worden door meerdere sluipwespen te toetsen. Dit is een manier waarop de selektiekriteria kunnen worden geëvalueerd.

Toekomstig onderzoek

Er zijn in de entomologische literatuur vele publikaties over de ontwikkeling, reproduktie en populatiegroei van natuurlijke vijanden beschikbaar en deze informatie kan zeer bruikbaar zijn in een selektieprocedure. Gegevens over de zoekefficiëntie van natuurlijke vijanden ontbreken echter in vele gevallen. Een gedragsbiotoets om het zoeken van sluipwespen te meten bij verschillende gastheerdichtheden is in ontwikkeling. Gedragskomponenten die de zoekefficiëntie van een natuurlijke vijand verhogen, dienen verder onderzocht te worden.

Niet alleen studies die de uitkomst van loslatingen van sluipwespen evalueren zijn van belang, maar ook specifieke experimenten waarmee we voorspellingen van het evaluatieschema kunnen toetsen. In het geval van de mineerder-sluipwespen houdt dit in: (1) onderzoek naar de effektiviteit van *D. sibirica* in zuidelijk Europa, (2) analyse van de invloed van een lage reproduktie van *D. sibirica* veroorzaakt door hogere temperaturen in het Zuiden, (3) verder onderzoek naar de effektiviteit van *D. isaea* in kassen in

noordwestelijk Europa, (4) schatting van variatie in mineerderdichtheden over de tijd oftewel kwantificatie van de gastheer-vrije perioden, in de twee regio's en (5) toetsing van de overleving van *D. isaea* in het Noordwesten en het Zuiden en de invloed van asynchroniciteit tussen generaties op zijn effektiviteit.

In samenhang met experimenten moeten onderzoeksmodellen worden ontwikkeld: (1) waarin populatiegroeisnelheden van natuurlijke vijanden en hun plaaginsekten, hun begindichtheden en het moment van introduktie worden ingebouwd, en we zo de uitkomst van proeven kunnen voorspellen over meerdere generaties, (2) die gedragskomponenten, zoals de kans dat een gebied met gastheren wordt gevonden en de zoeksnelheid binnen deze gebieden, inkorporeren en zo parasitering binnen een generatie voorspellen en (3), *last but not least*, die het belang van de verschillende kriteria afwegen en zo de eigenschappen van de 'ideale' sluipwesp voorspellen. Deze modellen kunnen substantieel de konklusies van studies aan de evaluatie van selektiekriteria ondersteunen.

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Scientific publications

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Resume

I was born on September 5, 1957 at Roermond (the Netherlands), where I graduated from the Bisschoppelijk College Schödeln in 1976. In the same year I started my study of biology at the University of Leuven (Belgium). The 'kandidaatsexam' (comparable to a Bachelor's degree) was passed in 1979 and was followed by the 'licentiaatsexam' (comparable with a Master of Science) in Zoology with honours, with a major in Animal Ecology under the supervision of Prof. Dr. J.K.A. van Boven, in 1981.

From 1982 to 1983 I worked as guest scientist with Prof. Dr. K. Bakker at the University of Leiden in the Department of Animal Ecology (the Netherlands). In 1983 I moved with Prof. Dr. J.C. van Lenteren to the Department of Entomology at the Wageningen Agricultural Unversity, where I was appointed to a temporary position as a researcher. The doctoral study described herein was initiated under his supervision. Since April 1989, I have been employed as a researcher at the Department of Entomology, University of California, at Davis (USA), where I am continuing to conduct research into biological control in cooperation with Dr. M.P. Parrella.

Kort levensoverzicht

Op 5 september 1957 werd ik geboren te Roermond als oudste zoon van een boomkweker/tuinarchitekt. Ik behaalde het diploma Gymnasium B aan het Bischoppelijk College Schödeln aldaar in 1976. In hetzelfde jaar begon ik mijn studie Biologie aan de Katholieke Universiteit van Leuven (België). Het kandidaatsexamen haalde ik in 1979 en dat werd in 1981 gevolgd door een licentiaat in de Dierkunde met onderscheiding, met als hoofdvak Dieroecologie onder begeleiding van Prof. Dr. J.K.A. van Boven.

Van 1982 tot 1983 werkte ik als wetenschappelijk gastmedewerker aan de toenmalige vakgroep Dieroecologie van de Rijksuniversiteit te Leiden onder begeleiding van Prof. Dr. K. Bakker. In 1983 verhuisde ik samen met Prof. Dr. J.C. van Lenteren naar de vakgroep Entomologie aan de Landbouwuniversiteit te Wageningen. Daar werd ik aangesteld als wetenschappelijk medewerker en het promotie-onderzoek onder zijn begeleiding startte. In 1989 nam ik een betrekking aan bij het Department of Entomology van de University of California te Davis (USA), waar ik het onderzoek aan biologische bestrijding voortzet in samenwerking met Dr. M.P. Parrella.

1

The leafminers *Liriomyza trifolii* and *L. bryoniae*, their parasitoids and host plants: a review[1]

Abstract

The leafminer *Liriomyza trifolii* has become an important pest organism in ornamentals and vegetables throughout the world. This leafminer poses a threat to pest control system in greenhouse vegetables in the Netherlands that employs parasitic wasps, predators and selective chemicals. Chemical control of *L. trifolii* is problematic due to the rapid development of resistance against insecticides. Application of biological control may help to overcome both the difficulty of control of *L. trifolii* and integration problems with other biological pest control methods. Ideally, a biological control method for *L. trifolii* should also be effective against an other leafminer species, *L. bryoniae,* which also occurs as a pest in greenhouses. The current situation with regard to control of *L. bryoniae* and *L. trifolii* is evaluated in this paper. The relationships between these leafminer species, their parasitoids, their host plants, and factors influencing these relationships are reviewed. A list of parasitoids and predators is provided with some notes on their biology. Finally, procedures for evaluation of the effectiveness of parasitoids are discussed.

Introduction

Agromyzid leafminers cause serious pest problems in ornamentals and vegetables throughout the world. The larvae feed in leaf mesophyll tissue, which may cause reductions in crop value or yield (Spencer 1973).

The polyphagous leafminers, *Liriomyza trifolii* (Burgess) and *L. bryoniae* (Kaltenbach), are important greenhouse pests in the Netherlands. *Liriomyza bryoniae* is long known as a pest of tomato and *L. trifolii* was reported for the first time in 1976 (Van Frankenhuyzen and Van de Bund 1979).

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Liriomyza trifolii has become a cosmopolitan species and is reported as a pest of chrysanthemum, gerbera, gypsophila, celery, tomato, cowpea, bean and potato (Genung and Janes 1975, d'Aguilar et al. 1980, Lindquist et al. 1980, Singh and Merrett 1980, Vercambre 1980, Leibee 1981a, Parrella et al. 1981a, Price 1981, Trumble 1981, Broadbent 1983, Fagoonee and Toory 1983, Price and Stanley 1983, Grill 1984, Zehnder and Trumble 1984).

Under natural conditions, agromyzid larvae are heavily parasitized by a diverse parasitoid-complex, e.g. 15 species parasitize *Phytomyza ranunculi* Schrank and 21 species parasitize *Chromatomyia syngenesiae* Hardy (Sugimoto et al. 1983b, Cornelius and Godfray 1984). This may explain the often low abundance of leafminers in sprayed crops (Hills and Taylor 1951, Michelbacher et al. 1951, Frick 1952, Lange et al. 1957, Oatman 1959, Harding 1965, Oatman and Platner 1969, Bragg 1974, Hafez et al. 1974, Genung and Janes 1975, Greathead 1975, Musgrave et al. 1975a, b, 1976, Price and Poe 1976, Hendrickson 1980, Johnson et al. 1980a, Trumble 1981, Chandler 1982, MacCollum et al. 1982, Price and Dunstan 1983). The use of non-selective chemicals is generally considered as the main cause of leafminer damage, because parasitoids of leafminers are very susceptible to these chemicals (Speyer and Parr 1948, Wene 1955, Getzin 1960, Wolfenbarger 1962, Shorey and Hall 1963, Jensen and Koehler 1970, Bragg 1974, Genung and Janes 1975, Musgrave et al. 1975b, Johnson et al. 1980b,c, Lange et al. 1980, Waddill 1978, Falcon et al. 1983). Oatman and Kennedy (1976) clearly demonstrated the elimination of beneficial parasitoids by broad spectrum insecticides. The combined effect of elimination of natural enemies and the development of resistance of leafminers against commonly used insecticides leads to increasing control problems.

This paper reviews the current situation with regard to the control of *Liriomyza spp.* To obtain a good insight into the pest problems and possibilities for biological control, the relationships between leafminers, parasitoids and host plants, and factors influencing these relationships are reviewed.

The leafminer problem in the Netherlands

The area of heated greenhouses in the Netherlands comprised ca. 8000 ha in 1985, viz. 4109 ha with ornamentals such as rose, chrysanthemum, gerbera and gypsophila, and 3868 ha with vegetables such as lettuce, tomato, cucumber and sweet pepper. Tomato covered 1959 ha, of which 45% is rockwool cultivation. The main vegetable cropping period for tomato starts in mid winter, December or January, and extends over 6 to 10 months.

For more than a decade, the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), is being controlled successfully with the parasitic wasp *Encarsia formosa* Gahan in 25-30% of the total acreage of greenhouse tomatoes (Van Lenteren et al. 1980). Against other insect or mite pests,

control methods compatible with this biological control method are available (Ravensberg et al. 1983). *Liriomyza bryoniae* was reported as a pest in the Netherlands in 1965 and 1966 (De Brouwer and Van Offeren 1967), but since 1976 *L. bryoniae* occurs in large numbers on tomato. A biological control program against the leafminer did not exist so growers had to apply chemicals. As a consequence, biological control of the whitefly was disturbed. This prompted research into the possibilities of controlling *L. bryoniae* biologically. The parasitoid *Opius pallipes* Wesmael proved to be the most promising candidate (Hendrikse et al. 1980) and trials in commercial greenhouses were reasonably successful (Woets and Van der Linden 1983). However, since 1980 a second leafminer species, *L. trifolii,* appeared as a pest in vegetables. Biological control by *O. pallipes* is ineffective against this leafminer species because the parasitoid eggs are encapsulated when deposited in *L. trifolii* larvae (Woets and Van der Linden 1982a). Chemical control of *L. trifolii* requires pyrethroids, methomyl and other compounds, the use of which upsets the current system of integrated control (Ravensberg et al. 1983). Leafminers of both species are common on greenhouse tomato, although *L. trifolii* thrives only in the summer. Leafminers are able to flourish due to the year-round cultivation and the use of rockwool as substrate, which does not require soil disinfection. Another contributing factor to fast population development is immigration with young plants in winter and spring, and during summer via open windows because most of the greenhouses are located in close vicinity to each other.

For cutflower producers, *L. trifolii* has presented a recurrent problem on chrysanthemum, gerbera and gypsophila (Van de Vrie and Dirkse 1982). In 1982 an intensive information campaign was started by the Extension Services at Wageningen, the Glasshouse Crops Research and Experiment Station at Naaldwijk and Research Station for Floriculture at Aalsmeer to improve control of *L. trifolii.* Eradication was impossible. Finland prohibited the importation of several host plants of *L. trifolii* from the Netherlands in 1980 and British plant health authorities demanded more stringent inspections. In 1981 a pre-export inspection system for cutflowers, the "green corner", was started. For export to be allowed it requires that the registered nursery is completely free from *L. trifolii* infestation and not a single mine is allowed in the greenhouses of participating growers. This has resulted in an intensive chemical control program in ornamentals with one to two chemical applications per week.

Damage

The damage caused by *Liriomyza* spp. can be divided into two categories: (1) Direct damage. The most serious damage is caused by larval feeding. The mining activity of larvae can reduce the photosynthetic capacity of the plant. Heavy infestation will cause desiccation and premature fall of leaves. In (sub)tropical areas this can lead to sunburning of fruits, e.g. of melons and

tomatoes (Michelbacher et al. 1951, Musgrave et al. 1975b). Feeding punctures made by the adult females can also cause damage. Total destruction of seedlings and young plants has been reported; and (2) Indirect damage. The feeding punctures can be invaded by fungi and bacteria. Price and Harbaugh (1981) observed an increase of bacterial leafspot disease, probably *Pseudomonas cichorii* (Swing) Stapp, on chrysanthemum infested by *L. trifolii*. This aggravates the conditions of the mined leaves remarkably. Transmission of viruses, such as tobacco, soybean, celery and watermelon mosaic virus, by *Liriomyza* flies has been demonstrated by Costa et al. (1958) and Zitter and Tsai (1977).

Surprisingly little research has been done to determine injury levels, especially when one considers the many decisions on control measures, which are regularly taken. Accurate assessment of economic thresholds in vegetables is difficult. The relation between the density of a leafminer population, leaf injury and reduction of yield are influenced by a complex of factors, e.g., among others, season, cultivation method and host-plant quality. Especially the effect of low infestations, on yield when complete compensation by the host plant may be expected, is unknown.

Researchers have not been able to correlate leafminer injury with yield loss (Wolfenbarger and Wolfenbarger 1966, Levins et al. 1975, Schuster et al. 1976). In tomato, the position of the leaf with leafminer injury in relation to the developmental stage of the trusses adjacent to that leaf seems important. The economic injury level was assessed to be 15 mines of *L. bryoniae* per leaf, if the leaves were adjacent to fruit at an early to mid stage of swelling (Ledieu and Helyer 1982a). Wyatt et al. (1984) found the highest correlation between the yield of a truss and *L. bryoniae* infestations on the six leaves surrounding that truss, when the fruit is half grown. The loss was directly proportional to the number of mines (30 mines/leaf: 10% loss; 60 mines/leaf: 20% loss). Defoliation experiments showed that removal of lower leaves hardly affected yield. Also removing a quarter of each leaflet or one leaf between each truss caused no loss. Thus, the tomato plant seems to tolerate at least 25% defoliation before any loss occurs (Stacey 1983). Nevertheless, Ledieu and Helyer (1985) stress that picking off the lower leaves too early will result in loss of yield. Johnson et al. (1983) and Trumble et al. (1985) proved that photosynthesis rates in leaves mined by *Liriomyza* spp. are greatly reduced. An increase of the net photosynthesis in the remaining leaves after removal of a leaf from a tomato plant was shown by Wolk et al. (1983).

Control measures

Cultural control methods such as good sanitation, removal of weeds, manual removing of mined leaves, specific substrates, film mulches or intercropping usually will not control a leafminer population sufficiently (Wolfenbarger and Moore 1968, Price and Poe 1976, Chalfant et al. 1977, Price and Harbaugh 1981, Oetting 1983, Price 1983, Schuster et al. 1983, Herbert et al. 1984). Some authors reported on the usefulness of yellow sticky traps in greenhouses to control leafminers (McClanahan 1983, Nucifora et al. 1983, Herbert et al. 1984, Van de Veire and Vacante 1984). But this method has not shown to be sufficiently reliable and is not commercially feasible yet.

Chemical control. The history of chemical control of agromyzid leafminers has been described by Spencer (1973, p. 2-4, p. 342-350). He concludes: "In view of the greater toxicity to hymenopterous parasitoids of many of the chlorinated hydrocarbons and organophosphorous compounds currently recommended for use against leafminers, the search for effective selective insecticides clearly demands the highest priority". Broad spectrum chemicals are most commonly used for leafminer control while research efforts for new insecticides are continuing.

Many authors report the development of resistance in *Liriomyza* populations against insecticides, including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Genung 1957, Wolfenbarger 1958, Genung and Harris 1961, Wolfenbarger and Getzin 1963, Janes and Genung 1975, Musgrave et al. 1975b, Parrella et al. 1981a). In a review on chemical control of leafminers, Leibee (1981b) points out the shortening of 'effective life' of insecticides till approximately 3 years. To minimize this problem a change of insecticide-use policy is necessary, e.g. rotating insecticides (Keil and Parrella 1983, Trumble 1985a).

In guided chemical control programmes where pesticide use is based on monitoring the leafminer population and economic thresholds, the levels of infestation were reduced for gypsophila, celery, tomato and bean (Genung et al. 1978, Pohronezny and Waddill 1978, Johnson et al. 1980d, Price et al. 1980a,b, Schuster et al. 1980, Waddill et al. 1981, Van Steenwijk and Toscano 1982, Price and Stanley 1983, Trumble 1983). The effect of an insecticide on the parasitoid complex of the leafminer should be examined and taken into account before a chemical is used (Poe et al. 1978, Waddill 1978, Trumble and Toscano 1983, Trumble 1985b).

Aqueous neem extracts from leaves of the neem tree *Azadirachta indica* A. Juss. are quite effective in controlling *L. trifolii* (Fagoonee and Toory 1984). Webb et al. (1983) showed that neem seed solutions had an anti-ovipositional effect on *L. trifolii* females in contrast with *Liriomyza sativae* Blanchard. Larval mortality of both species was high: 100% for *L. trifolii* and 98% for *L. sativae.* These effects on *L. trifolii* were confirmed by Stein and Parrella (1985). Neem seed extract used as a soil drench to chrysanthemum proved also to be effective against *L. trifolii with* 98% mor-

tality of pupae (Larew et al. 1985). But neem extract was also toxic to the parasitoid *Hemiptarsenus semialbiclava* (Girault) (Fagoonee and Toory 1984).

Insect growth regulators (IGR's) have a more selective insecticidal action than broad-spectrum insecticides. Two IGR's effective against *Liriomyza* spp., triprene ZR-619 and kinoprene ZR-777, were found to be harmful to the important parasitoid *Opius dimidiatus* (Ashmead) (Poe 1974, Lema and Poe 1978). Parrella et al. (1983a) and Robb and Parrella (1984) found that two IGR's, cyromazine 75W and Ro 13-5223 1E, provided more than 80% control of *L. trifolii* and they are compatible with *Chrysocharis parksi* Crawford under specific conditions. The results of these compounds are promising, but tests on the whole parasitoid complex are necessary to determine whether the beneficial impact of natural control is affected.

Biological control. The biological control of agromyzid leafminers was reviewed by Spencer (1973, p. 350-354). Natural control of *Liriomyza* populations by a complex of parasitoids is regularly observed to occur in agriculture, which may indicate a high probability that effective parasitoids for biological control of *Liriomyza* pests will be found.

Parasitoids can be used in different ways in biological control programs (Van Lenteren 1983): (1) Inoculative release method or classical biological control. Parasitoids are collected in an exploration area and introduced in the area where the pest occurs. Only a limited number of parasitoids is released. The aim is a long-term control effect. A good example is the control of the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), which has accidentally been imported into North America, by *Dacnusa dryas* (Nixon) and *Chrysocharis punctifacies* Delucchi (Hendrickson and Plummer 1983). Introductions of different species of parasitoids were made for control of *Liriomyza* spp. in Hawaii (Lai et al. 1982, Lai and Funasaki 1985); (2) Seasonal inoculative release method. Parasitoids are released periodically in short-term crops, i.e. 6-9 months, where multivoltine pests occur. A relatively large number of parasitoids is released to obtain both an immediate control effect and also a build-up of a parasitoid population for control later during the same growing season. This introduction method seems most suitable for biological control of *Liriomyza* spp. in greenhouse vegetables; and (3) Inundative release method. Parasitoids are collected, mass reared and periodically released in large numbers to obtain an immediate control effect. This method is usually applied against univoltine pests in annual crops. This type of release may be used to force extreme low pest numbers, e.g. *L. trifolii* on greenhouse chrysanthemums (Heinz and Parrella 1990b).

Biological control is only successful when the economic threshold is not exceeded during the entire growing season. But as mentioned earlier, few data on injury levels are available and decisions by growers are taken more on a psychological basis than by criteria based on research data. Successful control was defined by de Lara (1981) as at least 90% parasitization with

less than one mine per plant on chrysanthemums and no mines on tomato from next generation of the pest, which normally occurs 4-6 leaves up the plant.

It is evident that before biological control of leafminers can be applied, the use of broad spectrum pesticides has to be discarded and no harmful residues should be present. In the Netherlands, several parasitoid species have been tested from 1977 to 1985 in experimental and commercial tomato greenhouses (Zucchi and Van Lenteren 1978, Hendrikse et al. 1980, Woets and Van der Linden 1982b, 1983, 1985, Hendrikse 1983). It was found that: (1) in most greenhouses, ca. 60%, *L. bryoniae* is present and can cause problems, (2) natural control of *L. bryoniae* by *O. pallipes* and/or *Dacnusa sibirica* Telenga may occur, if sufficient overwintering parasitized pupae are present in the greenhouse, (3) when there are no parasitoids present or parasitism is too low, e.g. 30% in April depending on the leafminer density, it is necessary to release parasitoids, (4) introduction of parasitoids should be made at regular intervals instead of only once, and as soon as possible after appearance of leafminers in the spring, (5) *O. pallipes* shows a slightly faster population increase than *D. sibirica* and *O. pallipes* was observed to control *L. bryoniae* faster population increase than *D. sibirica. Chrysocharis parksi* gave some promising results in experimental greenhouses, (6) a leafminer infestation appearing after mid May can be controlled by naturally occurring parasitoids, mainly *Diglyphus isaea* (Walker), which invade the greenhouse in May or June.

Dacnusa sibirica can be effective against *L. trifolii* (W.J. Ravensberg pers. comm.). Since 1980 biological control of *Liriomyza* spp. has been practiced in the Netherlands on ca. 30 ha. per year. Nowadays, *D. sibirica* is introduced in commercial greenhouses under integrated control. This method was used on 60 ha in 1985 using a total of 10,000 to 20,000 parasitoids per ha, which roughly equals one female parasitoid per four plants. This number of parasitoids proved to be sufficient to control *Liriomyza* spp. during the growing season.

In the UK and Sweden, bad control results were reported when using *D. sibirica* as a control agent (De Lara 1981, Nedstam 1983, Wardlow 1983, 1984a). This is probably due to, among many other possible factors, immigration of flies, incorrect timing of release of parasitoids, insufficient introduction rates or bad quality of the parasitoids. In Ohio in the USA, *O. dimidiatus,* which is cited as *Opius bruneipes* Gahan, was unable to control *Liriomyza* spp., despite a high introduction rate of 4.5 parasitoid per plant (Lindquist and Casey 1983). Poor results were also obtained with *O. dimidiatus* in Ontario in Canada, but *Diglyphus begini* (Ashmead) was promising (McClanahan 1980). In the Rhône delta in France, *D. isaea* was mass-reared and used successfully on a large scale and *L. trifolii* is no longer a pest problem in this area (J.P. Lyon pers. comm.). Positive results were obtained in English greenhouses using *D. sibirica* or *O. pallipes* combined with *D. isaea* later on, against *C. syngenesiae* on chrysanthemum (Ledieu and Helyer 1982b, Wardlow 1983, Cross et al. 1983). In 1985 biological

control of *Liriomyza* spp. with parasitoids has been carried out in greenhouses in Belgium, Denmark, France, the UK, West Germany, the Netherlands, Sweden and the USA on an estimated area of 460 ha.

In floricultural crops, biological control of leafminers by parasitoids is presently not feasible because of the zero tolerance level for leafminer symptoms and the frequent applications of non-selective insecticides. In gerbera where only the flowers are harvested, are possibilities for biological control of leafminers by inundative releases of parasitoids.

Integrated control. The aim of integrated control of insects is to minimize disturbance of the control effect of natural components of the agroecosystems (Levins and Wilson 1980, Altieri et al. 1983). Integration of chemicals with biological control can be achieved by the use of selective chemicals and of chemicals with short-term residual activity, or by choosing the appropiate moment or place of application. The use of chemicals can be decreased further by selecting insect resistent host plants and by including cultural control methods.

An IPM program for Dutch greenhouse tomatoes consists of the following control measures: *E. formosa* against greenhouse whiteflies, fenbutatinoxide, a selective insecticide, or the predatory mites *Phytoseiulus persimilis* Athias-Henriot against two-spotted spider mite, *Tetranychus urticae* Koch, the selective chemical pirimicarb against aphids, *D. sibirica* against *Liriomyza* spp. and *Bacillus thuringiensis Berliner* against caterpillars (Woets and Van der Linden 1982, Ravensberg et al. 1983). An IPM program for greenhouse tomatoes recommended in the U.K. is given by Wardlow (1984b).

Liriomyza trifolii

Systematics

Liriomyza trifolii (Burgess 1880) was originally described as *Oscinis trifolii* and collected from white clover, *Trifolium repens* L. The holotype got lost, which led to confusion about the status of this species and related ones in the genus *Liriomyza* Mik. Spencer (1965) designated a neotype and clarified the situation by distinguishing species by the structure of the male genitalia. Despite of this, the identification in this genus remained confused partly due to the occurrence of mixtures of species on the same host plant species, e.g. in Lindquist and Casey (1983), Poe and Montz (1981a) and Chandler (1985). Diagnosis of *L. trifolii* and other *Liriomyza* spp. is possible by gel electrophoresis of the flies or the larvae and pupae (Zehnder et al. 1983, Menken and Ulenberg 1983, 1986). Morphological characteristics of the female genitalia are useful in identification (Knodel-Montz and Poe 1983). Synonyms of *L. trifolii*, are *Oscinis trifolii* Burgess (1880), *L. trifolii* de Meijere (1925) and *L. alliovora* Frick (1955) (Spencer 1973, p. 226).

Origin and distribution

Originally, *L. trifolii* is a nearctic and neotropical species but now it is cosmopolitan. Florida is thought to be its endemic focus (Spencer 1965). The population range has extended northwards through the eastern United States as far as Ontario in Canada and southward the Bahamas, Guyana and Venezuela. According to Spencer (1973): "This species can survive in areas where the winters are invariably severe with sub-zero temperatures for extended periods, but it only thrives in subtropical and tropical conditions". In 1968 the Colombian flower industry began importing chrysanthemum cuttings from Florida and the leafminer was first noticed there in 1974-1975. The first severe outbreak of *L. trifolii* occurred in Colombia in 1977 (Price 1983). In 1975-1976 this species was imported on plant material from Florida into California (Parrella 1982), the Netherlands (Van Frankenhuyzen and Van de Bund 1979) and Kenya (De Lima 1979). Augmentation of chrysanthemum cuttings was initiated in Kenya and in 1977 cuttings were exported to the UK, Germany, Denmark and the Netherlands (Anon. 1978). The farms on the Canary Islands and Malta could also be an indirect source of *L. trifolii.* Since 1973 *L. trifolii* occurs on the Canary Islands (Pena Estevez and Rodriquez 1983). In the U.K. it was first seen in 1977 at a nursery where chrysanthemum were being grown from cuttings imported from Kenya and Malta. In 1978 infestations were found originating on chrysanthemum cuttings from Kenya and Canary Islands and on gerbera from the Netherlands (Bartlett and Powell 1981). In Canada, it was also introduced on chrysanthemum cuttings from Florida (McClanahan 1983). In Japan, *L. trifolii* is recorded for the first time in 1949 and still occurs primarily on leguminous plants (Nakazawa pers. comm.). A world distribution map of *L. trifolii* is given in Fig. 1-1, showing the dispersion of the leafminer *L. trifolii* throughout the world by the flower industry. This is mainly due to failure of quarantine procedures and misidentification of species, which facilitated establishment (Lindquist 1983, Parrella and Keil 1984). Several countries have eradication campaigns whenever the pest is found, e.g. the UK and Finland. Now it seems that *L. trifolii* has not been established outside the greenhouses in northern and eastern Europe and Canada and its significance as a pest has decreased in these areas (McClanahan, Hansen and Nedstam pers. comm., Pehzes 1983). In southern Europe and Israel, *L. trifolii* occurs outdoors during all seasons and is still an important pest (Berlinger et al. 1983, J.P. Lyon pers. comm.; for a review on quarantine and policy, see Minkenberg 1988a).

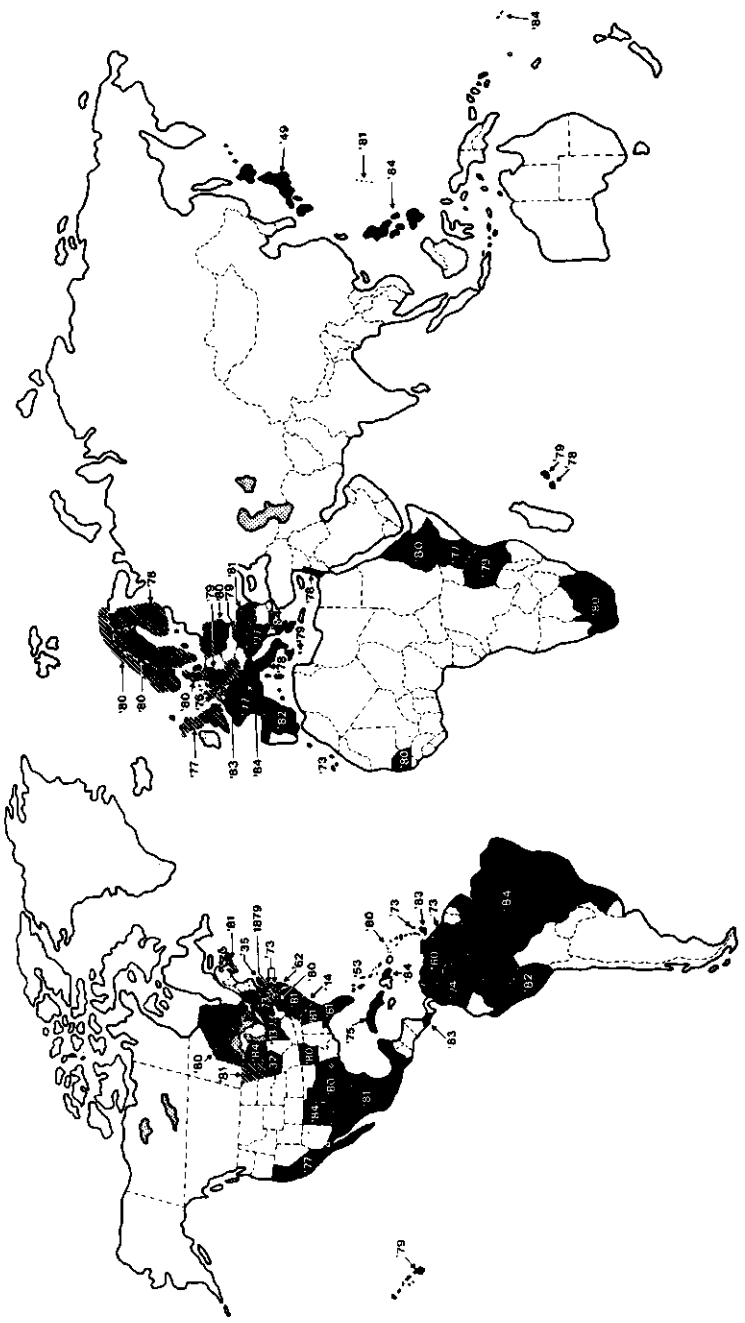

Fig. 1-1. The world distribution of *Liriomyza trifolii*. Years of first record are indicated and shaded areas are records only from greenhouses. Further details and bibliographic references are given in Minkenberg (1988a). Note that *L. trifolii* has now been eradicated in a few European countries.

Host plants

Liriomyza trifolii is a polyphagous species, attacking ornamentals, vegetables and weeds. Stegmaier (1966) has listed forty-seven plant genera in ten families in which the leafminer has been observed. Among the vegetable crops are melon, cucumber, squash, bean, pea, onion, pepper, tomato, eggplant, potato, celery, lettuce and carrot and among the ornamentals are chrysanthemum, gerbera, gypsophila and marigold. The number of host plants recorded is still increasing and now about 120 species in 21 families are known (Stegmaier 1968, Genung and Janes 1975, Powell 1981, Spencer 1981). Host plants are found in 27 genera of the Compositae, which is almost 40% of the total number of host plant genera. The family ranked next are the Leguminoseae, in which 10 genera or almost 15% of the total number of genera contain host plants of *L. trifolii.*

Life history

The adult *L. trifolii* is a small fly and is about 2 mm long. The head is yellow with plum-red eyes. The thorax and abdomen are grayish-black with a noticeable yellow patch at the hind end of the mesonotum. The underside and legs are mostly pale yellow. Peak emergence of the adults occurs before midday (Charlton and Allen 1981). Mating usually takes place within the day of emergence. Unfertilized females are unable to produce fertile eggs. Female flies feed by cutting the leaf epidermis with their ovipositor and sucking the content of the macerated mesophyll cells (for description of ovipositor see Hendel 1938, Knodel-Montz and Poe 1983). The size of feeding punctures is 0.15 by 0.3 mm. Spencer (1973, p. 19) writes: "The feeding by the adult female appears to have a threefold function, firstly to confirm that the host plant is correct, second to ingest proteins specific to the host plant which are necessary for maturation of the eggs and without which they are unable to oviposit, and finally actual feeding on the available carbohydrates". Charlton and Allen (1981) found that feeding and oviposition occurs throughout the daylight hours but *L. trifolii* feeds and oviposits most frequently around midday. Males are unable to puncture leaves and have been observed feeding at punctures produced by females. Both males and females take nectar from flowers or honeydew and feed in the laboratory on diluted honey. The punctures also serve as sites for oviposition, i.e. egg punctures. The eggs are oval, originally translucent and later become creamy. Their size is ca. 0.2 by 0.1 mm and they are inserted just below the epidermis. The number of feeding punctures and eggs varies considerably. The larva feeds in the leaf on the mesophyll layer producing a contorting mine. In chrysanthemum, *L. trifolii* larvae prefer to feed the palisade mesophyll (Parrella et al. 1985). Shape and form of the mine are variable and depend on the host-plant species and cultivar. The larva, which is initially colorless, darkens to yellow as it matures. There are three larval

stages. The third instar leaves the mine by cutting an opening at the end of the mine. The larval emergence from leaves occurs primarily in the morning (Charlton and Allen 1981). The opening can be in the upper or lower surface of leaves. The larva drops on the ground and normally pupates in the soil or in the darkest accessible area. The pupa is orange-yellow and turns brown as it gets older. Both the larva and pupa have an anterior and posterior pair of distinctively shaped tricorn spiracles. The duration of the life cycle is strongly variable. This species is not known to enter diapause. The above description of the life history is mainly based on information given by Spencer (1973, p. 226), Anonymous (1984b), Bartlett and Powell (1981) and Fagoonee and Toory (1984). The males of *L. trifolii* live only a few days, 2.3 d, whereas females live longer, 7.2 d, if they have access to host plants (Charlton and Allen 1981). Zehnder and Trumble (1983) found that an fifty-fifty sex ratio existed in the field. Adult flies of *L. trifolii* can cover distances of 100 m within a few hours (Van de Vrie and Dirkse 1982).

Liriomyza bryoniae

Systematics

Liriomyza bryoniae (Kaltenbach 1858) was originally described as *Agromyza bryoniae* and belongs to the genus *Liriomyza* Mik. Synonyms are *L. solani* Hering 1927 and *L. citrulli* Rohdendorf 1950 (Spencer 1973, p. 209).

Origin and distribution

The fly is a palaearctic species and commonly occurs outdoors in southern Europe, whereas in the rest of Europe it is only found in greenhouses (Spencer 1973). In the Netherlands, *L. bryoniae* was never found outdoors. Reports outside Europe are from Egypt, Israel and Japan (Abul-Nasr and Assem 1961, Kamijo 1978, Berlinger et al. 1983).

Host plants

Liriomyza bryoniae is a polyphagous species attacking tomato, cucumber, lettuce, melon and other vegetables. Polyphagy was defined by Spencer (1964) as the indiscriminate feeding on a number of plant species belonging to different plant orders. Only a few agromyzid species are polyphagous. This species has been reared from many host plant genera in almost 35 families (Buhr 1954 in Spencer 1973, p. 210).

Table 1-1. Parasitoids reared from *Liriomyza* spp. in the nearctic region.

	Host	*L. trifolii*								*L. trifolii/sativae*[a]							*L. sativae*					
Parasitoid	Reference	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Dacnusa sp.[c]			+																				
D. maculipes[d]			+																				
Chorebus misella[e]			+																				
Oenonnogastra microrhopalae[f]										*									+				
Opius sp.		+	+								*		+	+		+		*					+
O. arides																		*					
O. dimidiatus		+	+		+	*	+	+		*		*			*			*	*	+	*		
O. dissitus									+														
Cothonaspis sp.														+									
Ganaspidium sp.[g]																+		*					
Mirzagrammosoma lineaticeps		+	+																				
Zagrammosoma americanum														+		+			?				
Pnigalio flavipes																			*				
Diglyphus sp.		+	+															*	*				
D. begini				+									+				*	*	*		*	*	
D. intermedius		+	+	+	+		+	+			*		+	+		+		*	+	+		*	
D. pulchripes		+				*				*					*				*				
D. websteri			+																				
Eulophidae (genus unknown)																						*	
Chrysocharis sp.		+	+													+		*				*	+
C. ainslei													+	+			*	*				*	
C. mallochi																			+				
C. parksi				+							*		+				*	*					
C. viridis																			*				
Chrysonotomyia sp.[h]		+	+				+	+						+		+							+
C. formosa[i]		+	+		+			+			*				+					+			
C. punctiventris[j]		+	+										+				*	*				*	
Closterocerus sp.																+							
C. cinctipennis		+	+											+									+
C. utahensis																		*				*	
Tetrastichus sp.																		*				*	
Halticoptera sp.											*						*						
H. circulus			+		+					*			+	+	+	+		*		+			
H. patellana[k]		+	+																*			*	+
Total number of species		12	16	3	4	2	3	4	1	4	5	1	7	8	4	8	5	14	10	4	2	9	5
Place of sampling[l]		F	F	C	F	O	F	F	F	O	F	F	C	T	O	T	C	COn		FOn		C	F

Life history

The following description of *L. bryoniae* is based on Spencer (1973, p. 209-211) and details of its life history originate from Hendrikse (1983). The adult is a small fly. Mean length of males is 1.5 mm and of females 2.0 to 2.3 mm. They have a shining black mesonotum, largely yellow femora, and

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Footnote (Table 1-1, continued).

[a] Probably a mixture of species (see Poe and Montz 1981a, Lindquist and Casey 1983, Chandler 1985).

[b] References: 1. Stegmaier (1966a, 1972), 2. Poe and Montz 1981b, 3. Parrella et al. 1982, 4. Genung and Janes (1975), 5. Lindquist and Casey 1983, 6. Price and Stanley 1983, 7. Price 1981, 8. Murphy 1984, 9. Fogg 1981, 10. Poe et al. 1978, 11. Poe 1974, 12. Trumble and Nakakihara 1983, 13. Chandler 1982, 14. Lindquist et al. 1979, 1980, 15. Chandler 1984, 16. Johnson et al. 1980a, 17. Oatman and Johnson 1981, 18. McClanahan 1977, 19. Genung et al. 1978, 20. McClanahan 1975, 21. Oatman and Kennedy 1976, 22. Stegmaier 1966b.

ad 5: the parasitoid species cited is *Opius bruneipes* Gahan. Specimens were identified as *Opius dimidiatus* by Van Achterberg (Van der Linden pers. com.) and by Wharton (Lindquist pers. com.).

ad 13 and 15: leafminer species mentioned as *L. sativae* is a mixture of *L. trifolii* (more than 95%) and *L. sativae* (Chandler 1985).

ad 22. the leafminer species mentioned as *L. munda* is presumably *L. sativae* (Spencer 1973, p. 221).

[c] + means present, * means present with tomato as host plant, and ? means presence is doubtful.

[d] Formerly *Rhizarcha* (Fitton et al. 1978).

[e] *Chorebus misella* is presumably a misidentification; its distribution is palaeartic (Shenefelt 1974, p. 1056).

[f] Mentioned as parasitoid of *L. trifolii* on greenhouse chrysanthemums in Georgia (Oetting and Bodri 1984).

[g] Possibly *Disorygma* sp.; this generic group including *Ganaspidium* sp. and *Disorygma* sp. has not been revised taxonomically (Chandler 1984).

[h] Formerly *Achrysocharella* sp. and *Derostenus* sp. (Yoshimoto 1978).

[i] Formerly *Derostenus variipes* (Yoshimoto 1978).

[j] Formerly *Derostenus arizonensis* and *Derostenus agromyzae* (Yoshimoto 1978).

[k] Formerly *Halticoptera aenea*; *H. patellana* and *H. aenea* only occur in Europe; Canada and U.S.A. records of *H. aenea* presumably refer to *H. circulus* (Graham 1969, p. 165). The palaeartic species of *Halticoptera* have been revised by Askew (1972).

[l] A = Arizona, C = California, F = Florida, H = Hawaii, O = Ohio, On = Ontario, T = Texas.

both vertical bristles on yellow background. The flies can easily be sexed by the clearly visible, larger and more pointed black abdominal tip, i.e. last three segments of the female. Rearing data revealed a fifty-fifty sex ratio with males to females was 518 to 493. Ca. 30% of the males emerged one day before most females emerged. Before mating both female and male are rhythmically bending their legs and vibrating their wings. Then the male mounts the abdomen of the female, separates her wings and grasps the thorax. The abdomen of the male is brought forward to connect the copulatory organs. During copulation the female stands still. Copulation takes about a quarter of an hour (6-31 minutes; N = 10). Adult females feed on leaf mesophyll. They scrape openings in the leaf with their ovipositor and ingest the fluids. These feeding punctures resemble egg punctures, but feeding punctures are round and egg punctures are oval shaped. Egg punctures contain one egg each The eggs are opaque and ellipsoidal with a size of 0.15 by 0.25 mm. There are three larval stages in *Liriomyza*, which can be distinguished by the size of the sclerotized mouth hooks (Oatman and Michelbacher 1958). The first larval stage of *L. bryoniae* is ca. 0.57 mm with a mean length of mouth hooks of 0.095 mm (0.075-0.113 mm; N = 15), second larval stage measures ca. 1.55 mm with mouth hooks of 0.188 mm (0.150-0.250 mm; N = 15) and the last larval instar is ca. 2.50 mm with mouth hooks of 0.323 mm (0.300-0.350 mm; N = 15) on tomato. Their posterior spiracles have each an eclipse of 7-12 pores. The older larvae have a yellow front part and a white back part, by which they are quite different from the entirely yellow *L. trifolii* larvae. If a leaf is not large enough to provide sufficient food, larvae can move up in the stem into a second leaf. The larva is unable to penetrate leaves from the outside. The third instar larva cuts a characteristic semi-circular exit slit in the epidermis of the mine prior to pupation. Then the third larval instar leaves the mine to pupate in the soil down to a depth of 5 cm and forms a puparium, which is ca. 0.9 by 2 mm. The color of the pupae varies from gold-yellow to darkbrown-black. Under rearing conditions ca. 10% of the pupae were found attached to mines, leaves or stems. The longevity of the males was less than 3 days and females lived for more than one week. On alternative food, e.g. sugar water, honey and flowers of the tomato plant, none of the females lived longer than 3 days. During autumn and winter only few flies emerged. Low temperatures rather than short day circumstances appeared responsible. It is not yet clear whether this is diapause or a retarded development.

Natural enemies of *Liriomyza* spp.

Parasitoids of Liriomyza trifolii

Many authors have made surveys of parasitoids of *Liriomyza* spp. The parasitoid species differ for the various crops and geographical areas. The parasitoids which have been recorded in different states of the nearctic region are mentioned in Table 1-1. Sixteen hymenopteran species in the families Braconidae (Alysiinae and Opiinae), Eulophidae (Elachertinae, Eulophinae, Entedontinae and Tetrastichinae) and Pteromalidae parasitize *L. trifolii.* With the exception of two species, they were all found in Florida in the USA: *Chorebus misella* (Marshall), Florida, *Dacnusa maculipes* (Ashmead), Florida, *Oenonogastra microrhopalae* (Ashmead), Georgia, Ohio and Ontario, *Opius dimidiatus* (Ashmead), Florida, California, Ohio and Ontario, *Opius dissitus* Muesebeck, Florida, *Mirzagrammosoma lineaticeps* Girault, Florida, *Diglyphus begini* (Ashmead), California and Ontario, *Diglyphus intermedius* (Girault), Florida, California, Texas and Ontario, *Diglyphus pulchripes* (Crawford), Florida, Ohio and Ontario, *Diglyphus websteri* (Crawford), Florida, *Chrysocharis parksi* Crawford, Florida and California, *Chrysonotomyia formosa* (Westwood), Florida and Ohio, *Chrysonotomyia punctiventris* (Crawford), Florida and California, *Closterocerus cinctipennis (*Ashmead), Florida and Texas, *Halticoptera circulus* (Walker), Florida, Texas, California and Ohio, and *Halticoptera patellana* (Dalman), Florida, California and Ontario.

In the neotropical region surveys were made by Prieto and Chacó de Ulloa (1982), Velez Angel et al. (1982) and Murphy (1984): *D. begini,* Colombia/Trinidad, *Diaulinopsis callichroma* Crawford, Trinidad, *Chrysocharis caribea* Boucek, Trinidad, *Closterocerus purpureus* (Howard), Trinidad, and *H. circulus,* Trinidad.

In the Ethiopian Region, Vercambre and Thiery (1983a) and Bourdouxhe (1982) sampled five parasitoid species of *L. trifolii* in Reunion and two species in Senegal. The only identified species was the eulophid *Hemiptarsenus semialbiclava* Girault. In Israel, Freidberg and Gijswijt (1983) made a survey in greenhouse ornamentals infested with *L. trifolii* and found nine eulophids and one braconid parasitoid. The completely identified species were: *Diglyphus isaea* (Walker), *Diglyphus crassinervis* Erdös, *Ratzeburgiola incompleta* Boucek, *Hemiptarsenus dropion* (Walker), *Pnigalio soemius* (Walker), *Chrysocharis pentheus* (Walker) and *Chrysonotomyia formosa* (Westwood). In the Netherlands, three species were found to parasitize *L. trifolii* in greenhouses, namely *Dacnusa sibirica Telenga, Diglyphus isaea* (Walker) and *Halticoptera crius* (Walker) (Van der Linden and Gijswijt pers. comm.). The British species of *Halticoptera* are revised by Askew (1972).

A total 28 parasitoid species of *L. trifolii* have been identified. The most important literature on nearctic parasitoids is for the Braconidae for Alysiinae given by Wharton (1980, 1984), for Chalcidoidea with a key to

subfamilies by Yoshimoto (1984), for Eulophidae by Peck (1963) and by Boucek (1977), for *Diglyphus* by Gordh and Hendrickson (1979), for *Zagrammosoma* by Gordh (1978), for *Chrysonotomyia* by Yoshimoto (1978), for *Pnigalio* by Yoshimoto (1983), for *Chrysocharis by* Yoshimoto (1973a) and for *Pediobus by* Peck (1985). Detailed biological studies have been conducted on some species only. Results of these studies are summarized below.

The parasitoid Diglyphus begini (Ashmead). Diglyphus begini (Ashmead 1904) is nearctic and neotropical. It is numerically the most abundant species of the genus in Canada. In the USA, it appears to be more common in the western states than elsewhere (Gordh and Hendrickson 1979). This wasp is reported on *Liriomyza* spp. from Ontario, Ohio, Arizona, California, Puerto Rico and Colombia (Perez Perez 1973, Prieto and Chacó de Ulloa 1982; Table 1-1). Doutt (1957) mentions that the host range of *D. begini* include 19 species in five genera of Agromyzidae. This species is mentioned as parasitoid of *L. trifolii* on chrysanthemum and of *L. trifolii* and *L. sativae* on tomato and celery (Allen and Charlton 1981, Prieto and Chaco de Ulloa 1982, Parrella et al. 1982, Zehnder and Trumble 1984).

Life history studies were done by Hills and Taylor (1951), Doutt (1957), Allen and Charlton (1981). *Diglyphus begini* is facultative gregarious. Developmental time, longevity and fecundity of *D. begini* are given in Tables 1-5 and 1-6. Longevity is greatly increased when parasitoids are provided with honey. The adults could be kept for months at 5°C (Allen and Charlton 1981). The amount of larvae killed by host feeding is impressive, from a total of 716 larvae killed, 448 were host fed upon (Allen and Charlton 1981).

In field studies of *Liriomyza* spp., *D. begini* is often found to be quite abundant in relation to other parasitoid species. Hills and Taylor (1951) mentioned *D. begini* as numerically one of the most important parasitoids of leafminers infesting cantaloupes and lettuce in Arizona, besides *H. aenea* and *C. punctiventris*. Oatman (1959) in a study of natural control of *Liriomyza* sp., which is probably *L. sativae* (Spencer 1973, p. 203), on melon reported that parasitization was low early in the year but increased rapidly, and that the two most numerous species were *D. begini* and *H. patellana*. Natural control of *L. brassicae* on cabbage in Southern California is important. Oatman and Platner (1969) reported that the leaf injury caused by this leafminer was insignificant and that the parasitization varied from 26.7% in January to 84.1% in October. *Diglyphus begini* was mentioned as by far the most common larval parasitoid and *H. patellana* as dominant pupal parasitoid. Populations of *C. syngenesiae* in artichokes in California remained consistently below economic damage levels due to parasitization by *C. ainslei, D. websteri* and *D. begini* in order of importance (Bragg 1974). On alfalfa infested by *Liriomyza* spp. in California, *D. begini* was present in moderate numbers throughout the season (Jensen and Koehler 1970). Parasitization of *L. sativae* on tomato averaged 62.8% from mid June until September and *D. begini* was far the most numerous species, representing

80.7% of the total number of parasitoids reared (Oatman and Kennedy 1976). Johnson et al. (1980c) reported that *D. begini* and *C. punctiventris* were the most abundant parasitoids reared from tomato. Zehnder and Trumble (1984) found that *D. begini* was the most numerous species from *L. sativae* and *L. trifolii* on tomato, whereas in celery *D. begini* and *D. intermedius* were the commonest parasitoids.

In Canadian greenhouses, *D. begini* is an important parasitoid of *L. sativae* on tomatoes later in the year. This parasitoid caused up to 67% parasitism in 1973, together with the less numerous *O. dimidiatus*. In 1974 *D. begini* was not abundant and only one individual of *O. dimidiatus* was recorded (McClanahan 1975). McClanahan (1980) mentions successful biological control of *L. sativae* in a greenhouse experiment in autumn using *D. begini*, whereas the released *O. dimidiatus* and *Chrysocharis viridis* (Provancher) failed to control the leafminers.

The parasitoid Diglyphus intermedius (Girault). *Diglyphus intermedius* (Girault 1916) is nearctic and neotropical. It is reported on *Liriomyza* spp. from Ontario, Texas, Florida and California (Table 1-1). This wasp is introduced in Hawaii (Nakao and Funasaki 1979). Eight host species in three genera of Agromyzidae are mentioned by Gordh and Hendrickson (1979).

Although usually solitary, *D. intermedius* is probably occasionally gregarious (Hendrickson and Barth 1978). Biological notes for *D. intermedius* are provided by Hendrickson and Barth (1978) and Patel and Schuster (1983). This parasitoid uses all three larval instars of its host for host feeding and prefers third instar larvae for oviposition. Complete development of *D. intermedius* immatures on second larval instars of *L. trifoliearum* was not possible, probably due to inadequate food supply. The parasitoid has three larval stages, distinguishable by the length of their mouth hook. The shortest developmental time, ca. 9 days, occurs at 27 °C (Table 1-5).

Diglyphus intermedius was the dominant parasitoid of *Liriomyza* sp. on tomato in California besides *H. patellana* (Shorey and Hall 1963). Also on melon, *D. intermedius* was reported as the dominant parasitoid of *Liriomyza* sp. (Michelbacher et al. 1951). In north-eastern states of the USA, *D. intermedius* was the commonest parasitoid of *A. frontella* on alfalfa (Hendrickson and Barth 1978). In Florida this parasitoid was the most abundant one on *L. sativae* on tomato (Poe et al. 1978, Schuster et al. 1979) or on *L. sativae* on celery together with *C. formosa* (Tryon and Poe 1981). In California *D. intermedius* was later found to be less abundant on tomato than on celery (Oatman and Kennedy 1976, Zehnder and Trumble 1984).

The parasitoid Diglyphus pulchripes (Crawford). *Diglyphus pulchripes* (Crawford 1912) is nearctic and it shows a morphology similar to the palaearctic species *D. crassinervis* (Gordh and Hendrickson 1979). This parasitoid is recorded on *Liriomyza* spp. from Ontario, Ohio, California, Florida and Massachusetts (Miller and Jensen 1970; Table 1-1). It is

introduced in Hawaii (Nakao and Funasaki 1979). It is known on seven species in five genera of Agromyzidae (Gordh and Hendrickson 1979).

Diglyphus pulchripes is reported as the most abundant parasitoid on leafminers together with *O. dimidiatus* in greenhouses in Ohio (Lindquist et al. 1979). In autumn *L. trifolii* on greenhouse tomato could be controlled by *D. pulchripes* when the parasitoids were either introduced or invaded the greenhouse naturally (Lindquist and Casey 1983).

The parasitoid Diglyphus websteri (Crawford). Diglyphus websteri (Crawford 1912) is nearctic and neotropical (Gordh and Hendrickson 1979). It is recorded on *Liriomyza* spp. from Florida, Texas, Arizona, California and Peru (Campos 1982; Table 1-1).

Campos (1982) reported that *Liriomyza huidobrensis* (Blanchard) on potato was held in check by *D. websteri* and other parasitoids early in the season. *Diglyphus websteri* was reported as parasitoid of *C. syngenesiae* in artichokes in addition to *D. begini* and *C. ainslei* (Bragg 1974).

The parasitoid Chrysocharis parksi Crawford. Chrysocharis parksi Crawford (1912) belongs to the Entedontinae of the family Eulophidae. This subfamily also includes the genera *Pediobus, Chrysonotomyia* and *Closterocerus.* The latter genus is probably a synonym of *Chrysonotomyia* (Askew 1979). The subfamily contains both endo- and ectoparasitoids. *Chrysocharis* spp. are primary and rarely secondary endoparasitoids of larvae and pupae (Boucek and Askew 1968). The North American species of this genus have been revised by Yoshimoto (1973a,b). *Chrysocharis parksi* is nearctic. This parasitoid has been introduced into the Netherlands and France (Woets and Van der Linden 1986, W.J. Ravensberg pers. comm.). Yoshimoto (1973a) lists nine host species in six genera of Agromyzidae. It is reported on *Liriomyza* spp. from California and Florida and is introduced in Hawaii (Lai and Funasaki 1985; Table 1-5). The female has a blue-green color and is ca. 1.4 to 1.5 mm long. The legs beyond the coxae are pale except terminal tarsal segment, which is brown. The males, which are ca. 1.3 to 1.4 mm long, can be distinguished by the form of the abdomen. The male abdomen is shrunken and triangular in form, whereas the abdomens of females are round.

Chrysocharis parksi is an endoparasitoid. The host larvae are usually able to pupate and the parasitoids emerge from the puparia formed by the agromyzid larva. *Chrysocharis parksi* parasitizes the late third instars of *L. sativae* (Johnson et al. 1980a). Parasitized *L. trifolii* pupae can be separated from those unparasitized because of a difference in color. *Chrysocharis* females feed on hosts (Parrella et al. 1982).

On *Liriomyza* spp. in alfalfa in California, *C. parksi* occurred in moderate numbers and only during the early part of the season (Jensen and Koehler 1970). In tomatoes on *L. sativae, C. punctiventris* (60%) and *D. begini* (24%) were the dominant parasitoids, whereas *C. parksi* had only a minor part in parasitism (7.2%) (Johnson et al. 1980a). Also in Florida on

L. sativae in tomato, *C. parksi* was found to be insignificant (Poe et al. 1978). However, Zehnder and Trumble (1984) found that *C. parksi* was the predominant parasitoid on *L. sativae* on tomato in mid to late season. The wasp *C. parksi* was thus more abundant on this host or in this habitat than on *Liriomyza* spp. in celery. Experiments in greenhouses using *C. parksi* as biological control agent yielded promising results (Woets and Van der Linden 1986).

Parasitoids of L. bryoniae

The following palaearctic parasitoid species of the families Braconidae and Eulophidae are mentioned by Spencer (1973, p. 212): *Dacnusa hospita* (Förster), *Dacnusa maculipes* Thomson, *Dacnusa sibirica* Telenga, *Chorebus daimenes* (Nixon), *Opius pallipes* Wesmael, *Chrysocharis pubicornis* (Zetterstedt), *Hemiptarsenus zilahisebessi* Erdös and *Pediobius acantha* (Walker). *Aphidius ervi* Haliday, also mentioned by Spencer (1973) is presumably not a parasitoid of *L. bryoniae* but of certain aphid species (Mackauer and Stary 1967). Besides *O. pallipes* and *D. sibirica, D. isaea* and *Halticoptera crius* (Walker) occur as parasitoid of *L. bryoniae. Chrysocharis parksi* has been introduced in European greenhouses. The braconid *Dacnusa areolaris* (Nees), the eulophid *Pnigalio soemius* (Walker) and the pteromalid *Cyrtogaster vulgaris* Walker, which parasitizes the pupae of *L. bryoniae,* were reported in Sweden (B. Nedstam pers. comm.). The following parasitoids from greenhouses are thus known: *Diglyphus isaea* (Walker), *Chrysocharis parksi* (Crawford), *Dacnusa areolaris* (Nees), *Pnigalio soemius* (Walker), *Cyrtogaster vulgaris* Walker and *Halticoptera crius* (Walker).

The most important taxonomic literature on palaearctic parasitoids for the Braconidae with a key to subfamilies is given by Van Achterberg (1976), for the Alysiinae by Griffiths (1968, 1984) and Shenefelt (1974), for the Opiinae by Fisher (1973), for immature stages by Capek (1973), for Eulophidae by Boucek and Askew (1968) and for Pteromalidae by Graham (1969) and Askew (1972). Results of biological studies of some parasitoid species are summarized below.

The parasitoid Opius pallipes Wesmael. Opius pallipes Wesmael (1835) belongs to the subfamily of Opiinae of the Braconidae, which live as endoparasitoids of dipteran larvae and pupate within the puparium of the host. *Opius pallipes* is morphologically very similar to *Opius dissitus* Muesebeck (Wharton 1984). As hosts are reported *C. syngenesiae* on chrysanthemum, *A. spiraeae, L. bryoniae* and *L. strigata* (Spencer 1973, Cornelius and Godfray 1984).

Opius pallipes is a solitary endoparasitoid of *L. bryoniae.* It oviposits in all larval instars. The adult parasitoid emerges from the puparium of the host by making a hole with its mandibles. The sexes can be separated by the

visible protruding ovipositor of the females. Rearings can be hampered by a sex ratio shifting from 50% to 90% or more males. At 22°C the total development takes on average 18.3 days (SD = 1.4, N = 30; Table 1-5). The average longevity is 8.7 days (SD = 5.4, N = 6) in which an average of 89.2 eggs (SD = 57.7, N = 6; Table 1-6) are laid (Hendrikse 1983).

The searching behavior of *O. pallipes* is described by Hendrikse and Zucchi (1979). The female hovers around the leaves. After landing on a leaf, she starts scanning the leaf surface with her antennae and tips it rhythmically with her ovipositor. If the serpentine mine is encountered, the wasp will follow it. Again the antennae and ovipositor are used for scanning the mine and locating the larva. When a host is found, she inserts her ovipositor into it. She may reject it the larva or will lay an egg in it. Older larvae are found faster than younger ones. Host feeding is never observed. *Opius pallipes* is able to distinguish plants infested with *L. bryoniae* from non-infested plants. This parasitoid accepts all larval stages of *L. bryoniae* for oviposition and can discriminate between parasitized and unparasitized hosts (Hendrikse et al. 1980).

The parasitoid Dacnusa sibirica Telenga. Dacnusa sibirica Telenga (1934) belongs to the subfamily of the Alysiinae of the Braconidae. Almost all species are solitary endoparasitoids of dipteran larvae. *Dacnusa sibirica* has a palaearctic distribution, viz. Europe and Siberia. This parasitoid has been introduced into Cleveland (Ohio, USA) (W.J. Ravensberg pers. comm.). As hosts are reported *P. asteris, P. autumnalis, P. plantaginis, P. ranunculi* and *L. bryoniae, C. syngenesiae* on chrysanthemum and *L. trifolii* on tomato (Griffith 1966, 1968, Cornelius and Godfray 1984).

Dacnusa sibirica is a solitary endoparasitoid. It can be sexed by the color of the pterostigma on the wing. The pterostigma is black for the male, whereas in the female it is pale grey. Copulation takes only one to two minutes. The searching behavior of *D. sibirica* is similar to that of *O. pallipes,* although the frequency of touching the leaf and the mine with its ovipositor is higher than in *O. pallipes.* The female parasitizes all larval instars. The adult emerges from the host puparium. At 22°C mean total development took 15.7 days (SD = 1.5; N = 30). The average longevity was 6.1 days (SD = 2.6; N = 7) and mean fecundity was 71.7 eggs (SD = 48.1; N = 7) (Hendrikse 1983).

Older larvae are found faster than younger ones by the wasp. Host feeding is never observed. *Dacnusa sibirica* does not prefer larvae of a specific age for oviposition. It can distinguish unparasitized from parasitized larvae. *Dacnusa sibirica* can distinguish a previously visited leaf from a leaf that was not visited before by conspecifics (Hendrikse et al. 1980). It has been suggested that in addition to marking the host, a marking pheromone is applied to the leaf. A marking pheromone is found for *Dacnusa* sp., a parasitoid of *P. ranunculi* by Sugimoto (1984). Petitt (1984) suggested that *O. dissitus* marks the visited leaf, mainly the mine and larva.

The parasitoid Diglyphus isaea (Walker). The genus *Diglyphus* of the Eulophidae belongs to the subfamily Eulophinae. This subfamily, which includes the genera *Hemiptarsenus* and *Pnigalio,* comprises larval ectoparasitoids only. *Diglyphus* spp. are primary parasitoids. *Diglyphus isaea* (Walker 1838) is holarctic. Its taxonomy is discussed by Gordh and Hendrickson (1979). Hybridization tests between *D. isaea* and *D. intermedius* showed that reproductive isolation between them was not complete. *Diglyphus isaea* is an abundant species in Europe, North Africa and Japan and has been reared from a wide variety of leafminers. It is generally associated with hosts on herbaceous plants and scarce on trees. Eighteen hosts species in five genera of Agromyzidae and the lepidopteran *Lyonetia clerckella* are recorded (Boucek and Askew 1968). New host data are *Liriomyza crucifericola, Cerodontae lateralis, Napomyza carotae* on chicory, carrots and chamomile, *Agromyza albipennis, Agromyza oryzae, Phytomyza horticola, P. ranunculi, L. bryoniae* and *L. trifolii* (Spencer 1973, Van 't Sant et al. 1975, Kamijo 1978, Hendrikse et al. 1980).

Diglyphus isaea is a facultative gregarious parasitoid of leafmining insects. After paralyzing the host, the female usually lays one egg, in exception 2-5 eggs, near or on the host. The eggs are cylindrical and slightly curved. Their size is 0.3 mm by 0.1 mm. The young parasitoid larvae are colorless but become green as they mature. Three larval stages can be distinguished (Ibrahim and Madge 1979). The leafminer becomes flaccid and brown after a few days of paralyzation. The last larval instar displays a characteristic behavior before pupation. It consolidates its pupal chamber with meconial pillars, presumably serving as structural support when the leaf dries out. The parasitoid larva pupates in the leaf and the nymph has a length of ca. 1.5 mm. It is greenish until sclerotization occurs and its eyes are red. The wasp emerges through a round hole, which it cuts through the epidermis of the mine. The wasps can be sexed by their hind tibia. The female has one large black mark on the hind tibia, whereas the male has two small black marks (Askew 1968). The sex ratio is highly variable. The adult females cause mortality in leafminer populations, in addition to parasitization, through host-feeding activities. Host feeding in the laboratory accounted for almost half of the number of larvae killed (Ibrahim and Madge 1979). *Diglyphus isaea* prefers to oviposit the third larval instar of *Liriomyza trifoliearum* Spencer, and second and third larval instars of *C. syngenesiae* (Hendrickson 1975, Ibrahim and Madge 1979). Sometimes parasitoid larvae are cannibalistic and kill conspecific eggs and larvae.

Inoculative releases in 1975-1976 were done in Hawaii against *Liriomyza* spp. and in the North-eastern USA and Canada against *A. frontella* (Nakao and Funasaki 1979, Hendrickson and Barth 1979, Guppy et al. 1984). Establishment of *D. isaea* in north america is now certain. Natural control of agromyzid leafminers in greenhouses in Europe usually occurs during summer. As it overwinters outdoors, *D. isaea* enters the greenhouse in spring. Control of the leafminer population may occur within a few

generations (Scopes 1972, Woets and Van der Linden 1983, Wardlow 1984a, Nucifora and Calabretta 1985). *Chrysocharis pentheus* (Walker), which also acts as a hyperparasitoid, is possibly an important mortality factor of *D. isaea* populations in Japan (Takada and Kamijo 1979).

Predators of Liriomyza spp.

Hendel (1938) observed that some leafminers are eaten by predatory insects, such as ants, true bugs and lacewings. The agromyzid adults may be killed by other dipteran species like *Drapetis subaenescens* (Collin), *Tachydromia annuluta* Fallen (?) (Empididae) and *Coenosia attenuata* (Zetterstedt) (?), which all belong to the Muscidae (Freidberg and Gijswijt 1983). A *Draperis* sp. of the Empididae was described as a predator of agromyzids by Vercambre and Thiery (1983). Prieto and Chaco de Ulloa (1982) observed ants of the family Ponerinae attacking the larvae, and spiders of the Oxyopidae and flies of the Dolichopodidae attacking the adults of *L. trifolii.* The tomato bug *Cyrtopeltis modestus* (Distant), which is a facultative predator of leafminers and is actually considered a pest organism, feeds primarily on tomato stems (Parrella and Bethke 1983). Only the older stages of this predator use leafminers as a food source. Another possibility for leafminer control is the predaceous nematode, *Neoplectana carpocapsae* (Weiser). This nematode may be effective against the stages of *Liriomyza* occurring in the soil (Parrella et al. 1982). Birds, e.g. titmice, sometimes prey on agromyzid larvae and pupae.

Methods for rearing leafminers and their parasitoids

Several laboratory rearing methods of leafminers and parasitoids are described (Freeman and Guyton 1957, Griffith 1962, Hendrickson and Barth 1977, Webb and Smith 1970a, Ketzler and Price 1982). Methods for mass production of both host and endoparasitoids and of ectoparasitoids have also been described (Hendrickson 1975, Hendrikse 1980). The effect of density of *L. trifolii* larvae on the production of flies has been examined (Parrella 1983). Depletion of leaf resources adversely affected larval survival and pupal size. Pupal size was found to be an indicator of longevity and oviposition of adult flies reared on chrysanthemums. Intraspecific competition in *A. frontella* on alfalfa has been investigated extensively (Quiring and McNeil 1984, 1985).

Factors influencing the population development of leafminers and their parasitoids

In spring the predominantly warm and sheltered greenhouse environment and the abundant high-quality food supply can lead to a rapid numerical increase in leafminers, especially in the absence of natural enemies. But the greenhouse environment can also be favorable for a fast development of parasitoid populations. The host and parasitoid populations may proceed at a predictable speed because of the ample food supply, the low variability in abiotic conditions, and the absence of hyperparasitoids and unwanted predators. Nevertheless, there are still many unknown factors, which may influence the host-parasitoid relationship and thus parasitism and the success of a biological control program. The size and growth of both the host and parasitoid populations are determined by (1) abiotic factors, such as temperature, humidity and light, (2) biotic factors, such as host plant quality, and (3) biological characteristics of host and parasitoids. The influence of various factors on flies and parasitoids is summarized below.

Temperature

Temperature affects growth of the host and parasitoid populations. Population growth rate is determined by developmental time, mortality of immature stages, and longevity and fecundity of the adult. Table 1-2 gives some data for *L. bryoniae.* The The total developmental time of *L. trifolii* at different constant temperatures is shown in Table 1-3. The developmental time decreases as temperatures increase from 11.5 °C to 30 °C. Above 30 °C developmental time is near the upper threshold and an air temperature of 35 °C is almost the upper lethal limit. Reductions in population levels of *L. huidobrensis*, which is mentioned as *L. langei* but see Spencer (1973, p. 216), occurred when the maximum daily temperature rose to 40.5 °C (Lange et al. 1957). The theoretical temperature threshold for development is different for each species and instar, and apparently also depend on the host-plant species involved. The real temperature thresholds are probably lower because the theoretical ones are extrapolated and it is commonly found that linear extrapolations lead to values that are too high. The calculated developmental thresholds of *L. trifolii* determined from regression equations for developmental rate are 7.5 °C on bean, 9 °C for pupal stage, 10.1 °C for egg-larva, 10.8 °C for pupal stage on chrysanthemum, 7.8 °C for larval stage on tomato and 12.9 °C for egg stage, 8.4 °C for larval stage and 10.3 °C for pupal stage on celery (Parrella et al. 1981b, Vercambre and Thiery 1983, Leibee 1984, Miller and Isger 1985, Schuster and Patel 1985). The data should be treated with care because of different experimental set-up, host plant species and cultivars, and origin of *L. trifolii.*

Table 1-2. (A). Mean developmental times (days) of *L. bryoniae* and **(B)** mean longevities (days) and fecundities[a] (eggs per female) of *L. bryoniae* at various constant temperatures. The host plant used in all experiments was tomato, *Lycopersicon esculentum* Mill., cv. Moneydor, except for Nedstam (1985), where cv. was Ida.

	Temperature (°C)										
	12	15	18	20	21	22	24	25	27	30	Ref.[b]
A.	-	40	-	24	-	-	-	17	-	-	1
	-	-	-	-	-	19.6	-	-	-	-	2
	77.8	41.4	32.4	-	23.7	-	19.0	-	15.1	14.2	3
	-	40.6	-	26.5	-	-	-	17.1	-	-	4
B.	-	-	-	-	-	8.7 (67)	-	-	-	-	2
	-	13.6 (92)	-	9.0 (144)	-	-	-	6.6 (163)	-	-	4

[a] in parentheses.
[b] References: 1 Van der Linden 1983, 2 Hendrikse et al. 1980, 3 Nedstam 1985, 4 Minkenberg and Helderman 1990.

The optimum temperature for development defined as the temperature with the lowest mortality is near 25°C. On chrysanthemum 68%, 80%, 92.5%, 75.5% and 0% emergence from pupae occurred at 15.6°C, 21.2°C, 26.7°C, 32.2°C and 37.8°C, respectively (Parrella et al. 1981b). On the same host-plant species 33%, 56%, 61%, 75% and 74% pupal emergence was found at 16°C, 28°C, 20°C, 26°C and 30°C respectively (Miller and Isger 1985). On celery at 15°C, 20°C, 25°C, 30°C and 35°C 80%, 83%, 87%, 83% and 9%, respectively, of the pupae survived (Leibee 1984). The mortality of immature stages of *L. trifolii* rises sharply at temperatures above 30°C and at low temperatures. Some data on the developmental time of *L. trifolii* at fluctuating temperatures are collected (Fagoonee and Toory 1984, Miller and Isger 1985). However, more research is necessary for evaluating the effects of alternating temperatures on the development and the mortality of immature stages of *L. trifolii*.

Legumes are more suitable host plants than chrysanthemums, considering developmental time and immature mortality (Charlton and Allen 1981). This seems to be in contrast with the number of suitable genera in Leguminoseae and Compositae (see section Host Plants). The total survival

Table 1-3. Mean developmental times (days) of *L. trifolii* at various constant temperatures.

Host plant	Temperature (°C)											Ref.[e]
	11.5	13.8	15[c]	16	18	20	23.8	25[d]	30	32.5	35	
Phaseolus vulgaris	>116	64.7	51.2	-	-	20.3	16.1	15.8	12.5	12.2	-	1
P. vulgaris (bean)	-	-	61	-	-	23	-	17	15	-	+[a]	2
C. morifolium	-	-	-	-	29.0	-	-	-	-	-	-	3
C. morifolium cv. Show Off[b]	-	-	-	-	-	24.1	-	16.7	13.8	14.3	-	1
C. morifolium cv. Fandago	-	-	-	50.8	39.2	29.6	-	18.6	14.4	-	-	4
L. esculentum cv. Moneydor	-	-	44.0	-	-	24.6	-	16.6	-	-	-	5
A. graveolens cv. Florida 2-14	-	-	64.0	-	-	29.8	-	18.7	15.9	-	14.0	6

[a] 100% mortality of immatures.
[b] This cultivar is considered susceptible.
[c] 15°C includes 14.8°C.
[d] 25°C includes 26°C.
[e] References: 1 Charlton and Allen 1981, 2 Vercambre and Thiery 1983, 3 Prieto and Chacó de Ulloa 1982, 4 Miller and Isger 1985, 5 Minkenberg 1988b, 6 Leibee 1984.

from larvae and pupae at 23.8°C on blackeyed pea, pinkbean, chrysanthemum cultivars 'Show Off' and 'Yellow Knight' was 73%, 73%, 47% and 1%, respectively. Resistance against *L. trifolii* can be quite variable in chrysanthemums. Webb and Smith (1969) examined the effect of temperature on larval developmental time and mortality of *L. trifolii*, which was cited as *L. munda* but see Spencer (1973, p. 203). Mortality in tomato and chrysanthemum but not in lima bean increased significantly with decreasing temperatures. Mortality was highest in the first larval instar, intermediate in the second and lowest in the third larval instar. On chrysanthemum was a correlation between the longer larval developmental time and the higher larval mortality in those cultivars considered resistant. Such a correlation was not found for tomato cultivars.

Cold storage can be an important phytosanitary measure. In cold storage at 0°C, newly laid eggs of *L. trifolii* survived three weeks but all stages of larvae were killed after one to two weeks in the chrysanthemum cuttings at 0°C (Webb and Smith 1970b). Chrysanthemum cuttings in quarantine can be freed of leafminers this way after incubation of the eggs combined with

Table 1-4. Mean longevities (days) and mean fecundities[a] (viable eggs) of *L. trifolii* at various constant temperatures.

	Temperature (°C)										
Host plant	15	15.6	20	21.1	25	26.7	30	32.2	35	37.8	Ref.[c]
C. morifolium, cv. White Hurricane[b]	-	16.7 (42)	-	14.6 (234)	-	12.8 (279)	-	12.3 (189)	-	3.1 (1)	1
C. morifolium, cv. White Hurricane[b]	-	-	-	-	-	13.7 (298)	-	-	-	-	2
A. graveolens L. cv. Florida 2-14[b]	27.7 (24)	-	28.3 (182)	-	16.8 (288)	-	14.6 (406)	-	13.0 (240)	-	3
A. graveolens, cv. Tall Utah 5270-R[b]	-	-	-	-	-	12.1 (212)	-	-	-	-	2
L. esculentum cv. Dwarf Patio[b]	-	-	-	-	-	10.0 (40)	-	-	-	-	2
L. esculentum cv. Moneydor	6.5 (5)	-	14.4 (79)	-	5.6 (59)	-	-	-	-	-	4

[a] In parentheses.
[b] The flies were provided with honey.
[c] References: 1 Parrella 1984, 2 Parrella et al. 1983b, 3 Leibee 1984, 4 Minkenberg 1988b.

a chemical fumigation treatment (Mortimer and Powell 1984). Storage at 1.1°C for at least 16 days is also effective in controlling *L. trifolii* on celery, if pupae are absent (Leibee 1985).

The longevity and fecundity of *L. trifolii* decrease above 35°C. The upper oviposition threshold is near 40°C (Table 1-4). The maximum feeding rate occurred at 32.2°C and the highest oviposition rates were found at 26.7°C on chrysanthemum. The calculated threshold for oviposition determined from regression equations of oviposition rate on temperature was 12.2°C. Experimentally, the temperature threshold for oviposition was established at 10.0°C. Degree-days were calculated using this base temperature. There was a strong relationship between the cumulative percent oviposition and the calculated cumulative degree-days (°D). On the basis of these data it is possible to predict how much a female will oviposit over time. Ninety percent of all oviposition on chrysanthemum occurred within 550°D of adult life (Parrella 1984). The maximum population growth of *L. trifolii* on chrysanthemum and celery can be expected between 25°C and 30°C. When

Table 1-5. Mean developmental times (days) of parasitoids of *Liriomyza* spp. at various constant temperatures.

Parasitoid	Temperature (°C)												Ref.[b]
	12	15[a]	18	20	21[a]	22	23	24	25[a]	27[a]	30	35[a]	
Opius pallipes	-	-	-	-	-	18.3	-	-	-	-	-	-	1
Dacnusa sibirica	-	-	-	-	-	15.7	-	-	-	-	-	-	1
Dacnusa sibirica	54	32.1	26.6	-	18.8	-	-	15.4	-	13.4	12.8	-	2
Diglyphus isaea	-	-	-	14.5	-	-	-	-	-	-	-	-	3
Diglyphus isaea (♀)	-	25.5	-	-	-	-	-	-	9.8	-	-	-	4
Diglyphus isaea (♀)	-	26.0	-	16.6	-	-	-	-	10.5	-	-	-	5
Cyrtogaster vulgaris	82	39.6	30.7	-	22.7	-	-	18.6	-	14.7	13.8	-	2
Opius dimidiatus	-	-	-	-	-	-	20.4	-	-	-	-	-	6
Pnigalio flavipes	-	-	-	-	-	-	16.0	-	-	-	-	-	6
Diglyphus begini	-	-	-	-	-	-	-	-	11	-	-	-	7
D. begini	-	-	-	-	-	-	-	-	10.4	-	-	-	8
D. begini	-	-	-	-	-	-	14.3	-	-	-	-	-	6
D. intermedius	-	-	-	-	-	-	-	-	11	-	-	-	9
D. intermedius	-	22.8	-	-	12.7	-	-	-	-	8.9	-	10.0	10
Chrysocharis viridis	-	-	-	-	-	22.4	-	-	-	-	-	-	11
C. parksi (♀)	-	-	-	-	22.7	-	-	-	-	14.5	-	14.4	6
Halticoptera patellana	-	-	-	-	-	24.6	-	-	-	-	-	-	6
Hemiptarsenus semialbiclava	-	-	-	16.5	-	-	-	-	11.5	-	8.5	6.5	12

[a] The temperatures 15, 21, 25, 27 and 35 °C include 15.5, 21.1, 25.5, 26.7 and 32.2 °C, respectively.

[b] Host, host plant and reference: 1 *L. bryoniae*, tomato (Hendrikse et al. 1980), 2 *L. bryoniae*, tomato (Nedstam 1985), 3 *C. syngenesiae*, chrysanthemum (Ibrahim and Madge 1979), 4 *L. bryoniae*, tomato (Minkenberg 1989), 5 *L. trifolii*, tomato (Minkenberg 1989), 6 *L. sativae*, tomato (McClanahan 1983), 7 *P. atricornis*, *Sonchus oleraceus* (Doutt 1957), 8 *L. trifolii*, chrysanthemum (Allen and Charlton 1981), 9 *L. trifoliearum*, artificial mine (Hendrickson and Barth 1978), 10 *L. sativae*, artificial mine (Patel and Schuster 1983), 11 *L. trifolii*, chrysanthemum (Christie and Parrella 1987), 12 *L. trifolii*, bean (Vercambre and Thiery 1983), respectively.

Table 1-6. Mean longevities (days) and fecundities[a] (eggs) of parasitoids of *Liriomyza* spp. at various temperatures.

	Temperature (°C)							
Parasitoid	15	20	21.1	22	25[c]	26.7	host/host plant	Ref.[d]
Opius pallipes	-	-	-	8.7 (89)	-	-	*L. bryoniae*, tomato	1
Dacnusa sibirica	-	-	-	6.1 (72)	-	-	*L. bryoniae*, tomato	1
Dacnusa sibirica	20.2 (225)	6.0 (94)	-	-	7.4 (48)	-	*L. bryoniae*, tomato	2
C. parksi[b]	-	-	11.4 (58)	-	-	14.7 (135)	*L. trifolii*, chrysanthemum	3
Diglyphus isaea	23 (293)	32 (286)	-	-	10 (209)	-	*L. bryoniae*, tomato	4
D. begini	-	-	-	-	17.0 (268)	-	*L. trifolii*, chrysanthemum	5
D. intermedius[b]	-	-	-	-	24 (40)	-	*L. trifolearum*, bean	6

[a] In parentheses.
[b] Parasitoids provided with honey.
[c] 25° also includes 25.5°C.
[d] References: 1 Hendrikse 1983, 2 Minkenberg 1990, 3 Christie and Parrella 1987, 4 Minkenberg 1989, 5 Allen and Charlton 1981, 6 Hendrickson and Barth 1978.

the average temperature is about 15°C there will be almost no increase in number. However, in the used set-up the longevity and fecundity of *Liriomyza* spp. were strongly influenced by the availability of honey to the adult female (Freeman and Guyton 1957). *Liriomyza trifolii* females on blackeyed pea provided with honey lived more than three times longer and produced a threefold number of eggs. The longevity and fecundity at 23.8°C without and with honey were 7.2 d and 117 eggs and 22.7 d and 439 eggs, respectively (Charlton and Allen 1981). It is likely that some honeydew produced by whiteflies or aphids and nectar will be present in the field but it is not known whether the agromyzid flies utilize these food sources.

More research needs to be done to characterize the microclimatic conditions, in which the immatures and adults of *L. trifolii* exist in order to make better predictions of the population growth (Leibee 1984). As these predictions will usually be based on experiments done at constant temperatures in climate rooms, the effects of fluctuation of the temperature and differences in host-plant quality on the biology of *L. trifolii* must also be determined.

Humidity

The significance of humidity is quite different for the various stages of *L. trifolii*. Except for extreme dryness or extreme moisture influencing the condition of the plant, the eggs, larvae and female adults are insensitive to relative humidity (RH). The larger number of feeding punctures made by the adult female at high temperatures is probably partly due to the necessity for taking up more water than at low temperature when ambient RH is relatively higher. The influence of humidity and free moisture on the pupal stages of *L. trifolii* was studied at a range of constant temperatures by Charlton and Allen (1981). There was an increasing pupal emergence when the air over the pupae became more moist. A RH of 11%, 15%, 32%, 51%, 62%, 76%, 94% and 100% yielded emergences of 6%, 22%, 40%, 64%, 65%, 65%, 72%, 88%, respectively. In sand when no water was added, the emergence was still 49% and in peat it was as high as 79%. When newly formed pupae were submerged in water for 4 hours (h), 24 h or 75 h, 96%, 50% and 0% survived, respectively. Although the pupal stage is very sensitive to drought, humidity seems to play a minor role on the population growth of *Liriomyza* spp. under greenhouse conditions, where RH usually varies between 40% and 60%. The influence of the relative humidity on the population growth of parasitoids has not yet been examined.

Light

Little research on the effect of light intensity and duration on the development of the leafminers and parasitoids and on their behaviour has been done. Adult *Liriomyza* spp. show a positive phototactic response. Therefore, a slowly rotating cage was proposed to eliminate any directional bias due to light or other environmental factors during oviposition experiments (Smith et al. 1970). In darkness *L. trifolii* females do not oviposit (Light:Dark was 16:8 h, Minkenberg unpublished data). In greenhouses, higher densities of mines are usually observed along paths, borders and at the south side, which could be an influence of light. Further research on the effect of light on the dispersal of agromyzid flies is necessary for understanding the distribution patterns of leafminers. Some authors assumed that the effectiveness of the parasitoid *D. isaea*, which was introduced against *C. syngenesiae* in greenhouse chrysanthemum in England, was impaired by the low light intensity or short photoperiod in spring, autumn and winter (Scopes and Biggerstaft 1973). The effect of the diminished radiation at that time of the year could be a relatively lower body temperature of parasitoids, leading to reduced activity and hence, parasitization.

Host-plant suitability and acceptability

The host plant can be of great influence on the population growth of leafmining flies and parasitoids and their interactions. The suitability of host plants for phytophagous insects can be determined by comparing the growth, survival, oviposition or feeding on various host plants. Data presented in Tables 1-3 and 1-4 clearly demonstrate the large variability in the life-history variables of *L. trifolii* on different host-plant species or cultivars.

Liriomyza trifolii, which is introduced into Europe on ornamentals, occurred in large numbers in vegetables only a few years afterwards. The imported individuals fed and laid eggs, and their offspring survived on these plants. Evidently, a genetic basis for this step was present. The following questions have been raised: (1) Are there 'host races' of *L. trifolii*, in other words are there genetically different forms? (2) Do the female flies have a preference for the host plant on which they bred and is this preference genetic or is it caused by preconditioning of adults or larvae? (3) Will offspring which developed on the host plant that was preferred by their parents have a higher fitness than offspring which developed on other host-plant species? (for a review of this subject, see Futuyma and Peterson 1985).

A sympatric host-associated variation in host preference was demonstrated in the polyphagous species *Liriomyza brassicae* (Riley) by Tavormina (1982). He found that flies produced a significantly greater proportion of their offspring on the host plant from which they were collected as larvae. A laboratory strain showed even a greater tendency to lay their eggs on the host plant on which they were reared than wild flies from the same host-plant species. According to Tavormina (1982): "This fact demonstrates there is selection for an increased tendency to produce mines on the host plant an individual develops on and these results are consistent with the hypothesis that selection accounts for the divergence in mine-production behavior observed in the wild population and that further divergence in the wild population is being inhibited by gene flow". He further concluded that conditioning was not the only factor responsible for the differences in mine production behavior, but that there was a genetic basis as well. There was no evidence that larval mortality was lower on the parental host plant and some evidence that larval growth is slightly faster on parental host plants. For another polyphagous leafminer, *L. sativae*, it was found that samples from closely adjacent fields of pea and tomato differed in host-plant preference phenotypically, and genetically in pupal weight (Via 1984a,b). There was no significant genetic correlation of developmental time across host plants. There was a genetic correlation between ovipositional preference and developmental time on the two host plants but the 'populations' differed very little in average responses to the two plant species indicating that population divergence has not occurred in this system. The absence of 'host races' in this species may be due to frequent migration among crops, given the close spatial proximity of the test fields and yearly crop rotation. In

choice experiments with the oligophagous leafminer *Phytomyza matricariae* Hendel it was found that maximum numbers of feeding punctures occurred on the plant on which flies were bred, but the number of eggs laid were not significantly higher than on some other plant species (Seghal 1971). The existence of host races or sibling species in *L. trifolii* could have important consequences for the development of control programs.

For adult flies of *L. trifolii,* chrysanthemum and celery were more favorable hosts than tomato (Parrella et al. 1983b). Significantly fewer punctures and eggs were found on tomato and females lived for a shorter time. In the field, *L. trifolii* was more abundant on celery in adjacent plantings of tomato and celery (Zehnder and Trumble 1984). A comparison of oviposition and development on tomato and three weed species showed that *L. trifolii* laid significantly more eggs on tomato and nightshade, whereas the percentage emergence and pupal weight on the different host plants did not significantly differ. The larval developmental time was significantly shorter on nightshade (Zoebish et al. 1984). These ovipositional and feeding preferences of the females correspond with some biological characteristics of their offspring on the different host-plant species, e.g. larval mortality was significantly higher on tomato (Parrella et al. 1983b). However, the developmental time on tomato was not different from that on chrysanthemum, whereas on celery it was longer than on tomato and chrysanthemum (Table 1-3).

Thirty weed species are listed as host plants for *L. trifolii* with notes on their level of infestation and abundance (Genung and Janes 1975). The relative susceptibility of different varieties of chrysanthemum and of tomato for *Liriomyza* spp can vary remarkably (Kelsheimer 1963, Wolfenbarger 1966, Webb and Smith 1969, Webb et al. 1971, Schuster and Harbaugh 1979a,b, Schuster et al. 1979, 1981, Alverson and Gorsuch 1982, Oetting 1982, Broadbent and Blom 1984). No significant differences in leafminer tolerance among cantaloupe cultivars were found (Chandler and Thomas 1983). A comprehensive review on resistance of chrysanthemum and tomato to *Liriomyza* spp. including a discussion of the possibilities for breeding programs has been given by Schuster et al. (1981).

The ratio of feeding puncture and eggs could be used as an indicator of intraspecific host-plant preference for agromyzids, assuming that on a more nutritious host plant a female fly needs less feeding to produce an equal number of eggs (Hussey and Gurney 1962). Comparing the relative susceptibility of several chrysanthemum varieties for *C. syngenesiae,* they found a positive relation between the feeding puncture/egg ratio and survival of immatures. However, the feeding puncture/egg ratio seemed unsatisfactory as an index for host plant preference to compare different host-plant species or cultivars (Ibrahim and Madge 1977).

Increased plant nitrogen may either cause an increase or decrease in insect development, fecundity and numbers (Scriber 1984). On lettuce supplied with increasing concentrations of nitrogen number of eggs laid increased in

C. syngenesiae (Hussey and Gurney 1962). Increased fertilization which resulted in a heavier attack than before by *Liriomyza* sp. was previously shown (Woltz and Kelsheimer 1958). A lower larval mortality of *L. sativae* occurred in chrysanthemum with a higher level of fertilization (Poe et al. 1976). A linear relationship between fertilizer rate and the density of *L. trifolii* on chrysanthemum was established (Price and Harbaugh 1981, Harbaugh et al. 1983). These authors stressed that a surplus of nitrogen contributes to the problem of leafminers as a pest.

Physical barriers on the leaf surface of the plant can be of great influence on insects. The hooked trichomes on pink bean causes premature death of leafmining flies (Charlton and Allen 1981). Higher density of hairs on plants negatively influences oviposition by agromyzid flies (Lin and Mitchell 1981, MacLean and Byers 1983). Further investigations on the influence of host plants on fly and parasitoid performance and preference are necessary.

Development of a biological control program

After listing parasitoid species of *Liriomyza* spp. and summarizing information from the literature, an evaluation of these species for biological control of *Liriomyza* spp. in the desired crops and under the required climatic conditions should be made.

In greenhouse research the following procedure is recommended (Van Lenteren 1980): (1) literature research on pest and natural enemies, (2) collection of natural enemies, (3) laboratory experiments to study the influence of temperature on biological parameters and to examine behavioural characteristics, (4) trials in experimental greenhouses, (5) trials in commercial greenhouses, (6) development of mass-rearings for parasitoids, (7) development of an introduction method for parasitoids. Steps 4 to 7 are not always performed in this sequence and may be carried out at the same time. This approach has led to the development and application of biological control against several important greenhouse pests in vegetables (Van Lenteren 1986a). The usual method for evaluating parasitoids for biological control is still highly empirical: the trial-and-error method. This method takes about 3-5 years. However, it is difficult to find growers, who will give permission for trials in their greenhouses.

Only a few parasitoid species can usually be tested and the chosen species is not per se the most effective. In the Netherlands a biological control method against leafminers in greenhouse tomatoes has been developed and only four parasitoid species are evaluated with dozens of leafminer parasitoids known. It has been demonstrated that two species are effective, i.e. prevent a pest crossing the economic injury level during the entire growing season. The need for an efficient evaluation method prior to introduction is stressed by most biological control workers and ecologists.

The aim of our research project is to evaluate the capacity of some parasitoid species to control both leafminers, *L. trifolii* and *L. bryoniae*. A general goal is to develop proper evaluation techniques for screening the control potential of parasitoid species prior to their use in practical situations. A compilation of the literature describing the characteristics of an effective natural enemy has been made (Van Lenteren 1980). The choice of criterions is determined by the release program used. We want to control *Liriomyza* spp. in greenhouse tomatoes by seasonal inoculative release of parasitoids. The characteristics of an effective natural enemy which we consider useful as criterions for the selection procedure of a parasitoid, are (1) successful development on host, (2) no negative effects, (3) synchrony between host and parasitoid generations, (4) reproductive capacity, (5) searching efficiency. Several of these criteria are absolute, e.g. successful development on host. The reproductive capacity and searching efficiency are vaguely defined. It is necessary to quantify these criterions more precisely for a good comparison of parasitoids.

An index for the reproductive capacity is the intrinsic rate of increase (r_m). The r_m values vary with temperature and depend on the developmental rate, mortality, longevity, sex ratio and fecundity. These life-history variables can be estimated under laboratory conditions. If host feeding occurs, the host kill rate should be measured, i.e. the combined value of r_m and the additional mortality caused by host feeding. The r_m values may be used to compare parasitoid species mutually and to compare parasitoids with their hosts. A prerequisite in seasonal inoculative release systems is that an effective parasitoid species has r_m values equal to or larger than these of its host (Van Lenteren 1986a). The realized rates of increase of parasitoids are lower in the greenhouse situation than the r_m values, because in greenhouses host densities should remain low. But estimating r_m values is useful for comparing potential population development.

The searching efficiency criterion is only loosely defined and contains aspects like functional and numerical response, aggregation, mutual interference, intrinsic searching capacity, handling time, dispersal and spatial heterogeneity. This criterion can be defined as that part from the reproductive capacity that is realized under the given circumstances. The searching behaviour of a parasitoid, involving the time spent in different phases of host location and the stimuli used, can be investigated in laboratory. But experiments in greenhouses may still be necessary, e.g. to estimate dispersal.

To verify the validity of the examined criterions, the combination of values for different parasitoids should be related to their control capacities in commercial greenhouses. Effective as well as ineffective parasitoid species should be subjected to critical studies in order to establish a more reliable selection procedure of parasitoids for biological control. In the near future we hope to present a reliable selection procedure for natural enemies in seasonal inoculative release systems, based on our studies with parasitoids of leafminers.

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2

Life history of the agromyzid fly *Liriomyza trifolii* on tomato at different temperatures[1]

Abstract

The effects of three constant (15°C, 20°C and 25°C) and one alternating (16-22°C, mean 19.5°C) temperatures on development, mortality, feeding, fecundity and longevity of *Liriomyza trifolii* (Burgess) on tomato plants cv. 'Moneydor' were examined in the laboratory. Developmental rates and thresholds for each instar were estimated by means of linear regression. Further, data on the biology of *L. trifolii* are given and discussed.

The intrinsic rate of increase, r_m, was -0.0023 and 0.1254 viable eggs per female per day at 15°C and 25°C, respectively, and net reproduction varied from one viable female egg per female at 15°C to 26 eggs per female at 20°C. Generation time varied from 48 days at 15°C to 24 days at 25°C. Ninety percent oviposition occurred within the first 115 degree-days of adult life at both 20°C and 25°C. Fecundity and longevity were highly correlated with the number of feeding punctures. No correlation was found between life-history variables and pupal length. The data indicate that tomato is a suitable host plant allowing populations of *L. trifolii* to increase if temperatures are above 16°C.

Introduction

Liriomyza trifolii is an agromyzid fly, which occurs in many agricultural production areas of the world (Minkenberg 1988a). The larvae mine leaves and seriously damage ornamentals and vegetables. Several detailed studies have been conducted on its biology and life history (for reviews, see Minkenberg and Van Lenteren 1986, Parrella 1987).

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Temperature is a factor causing large differences in the development of *L. trifolii* and its reproduction (Charlton and Allen 1981, Leibee 1984, Miller and Isger 1985, Vercambre and Thiery 1983, Parrella 1984). The host plant is a second factor with a considerable impact on the performance of *L. trifolii*, as demonstrated by the feeding and reproduction of females on selected plant species (Parrella et al. 1983b). Other distinct characteristics of the host plant influencing performance of *L. trifolii* are its cultivar (De Jong and Van de Vrie 1987, Schuster et al. 1981) and its growing conditions, e.g. the amount of fertilizers applied (Bethke et al. 1987).

Natural control of this leafminer pest by parasitoids occurring in the field can be very effective (see references in Minkenberg and Van Lenteren 1986). Biological control of this leafminer by parasitoids using inoculative releases (Lai and Funasaki 1985, Johnson 1987) or using seasonal inoculative releases (Minkenberg and Van Lenteren 1987) has given economic control in some vegetable crops. The biological control of the leafminer in flower crops using inundative introductions of parasitoids has been successful (Parrella et al. 1987a). To evaluate the effectiveness of parasitoids for biological control in a pre-introduction study, data on the development and reproduction of *L. trifolii* and its parasitoids are necessary (Minkenberg and Van Lenteren 1987).

The objectives of the research reported here are: (a) to estimate developmental rate and mortality for each instar, adult feeding, fecundity and longevity at three constant temperatures, 15 °C, 20 °C and 25 °C, and at one alternating temperature, 16-22 °C, on tomato; (b) to determine theoretical threshold temperatures for development and oviposition and (c) to estimate r_m values of *L. trifolii* at different temperatures.

Materials and methods

Insect rearing. A hundred larvae of *L. trifolii* were collected from tomato plants grown in commercial glasshouses in the Netherlands in the areas 'Westland' and 'Kring' to start a colony at the end of 1983. The colony was held on *Lycopersicon esculentum* cv. 'Moneydor'. This cultivar, which is no longer grown by commercial growers, was selected as model plant for laboratory studies (Van Lenteren et al. 1976). Plants were grown in pots (10 x 10 cm) on a standard soil mix (Naturado) in a small glasshouse at 25 ± 5°C, RH 70 ± 10%, using techniques similar to those reported by Hendrikse (1980). Artificial light was used to keep the photoperiod at 16 h. Pesticides were not applied. Occasionally, *Thrips tabaci* (Lind.) were controlled by frequent releases of predatory mites, *Amblyseius cucumeris* (Oud.).

Development/mortality. Developmental time and percentage mortality were measured by regular observations of a cohort of individuals from oviposition until emergence. Twelve 'standard' plants, cultured at 20 ± 5 °C, circa

five weeks old and each with eight fully expanded leaves, were exposed to a hundred flies for two hours (0900-1100). At the end of the exposure period, the plants were checked for the presence of flies and then transferred to controlled environment rooms operating at the constant temperatures of 15°C, 20°C and 25°C, which is the temperature range in heated commercial glasshouses in winter and spring, and under the alternating temperature regime of 16-22°C (mean 19.5°C) representing a winter and early spring temperature regime. The alternating temperature changed linearly during 0100-0300 h and 1500-1700 h and was a constant 22°C during 0300-1500 h and a constant 16°C during 1700-0100 h simulating a glasshouse temperature regime. The measured temperatures varied ± 0.5°C. RH was 60-70 ± 10% and photoperiod was L16:D8. The photophase occurred between 0100 (i.e., 'lights on') and 1700 h, provided by 16 High Pressure Lamps (Philips) with a light intensity between 30-80 W/m^2. Eggs, which became clearly visible after a few days due to an increase in size as previously observed by Tilden (1950) and Dimetry (1971) for *Liriomyza* spp., were located using transmitted light at 25 or 50 times magnification and every four hours beginning at 0100 h the developmental stage of each individual was determined. Densities were kept low with a maximum of six larvae per leaflet to avoid negative effects of intraspecific competition on their performance (Parrella 1983). To distinguish the three larval stages occurring within leaves (e.g., Tauber and Tauber 1968), some morphological characteristics, viz. length of mouth hooks, body and mine, were measured in a preliminary experiment. Emerged adults were sexed.

The beginning of pre-adult development was fixed at 1000 h, i.e. at the mid point of the oviposition period. Developmental times were corrected later by minus two hours, which is half the interval of four hours between inspections. The period during which the larva was outside the leaf before pupation was included in the developmental time of the third instar. Developmental times for each instar were based only upon individuals that reached adulthood.

To examine the relation between the fitness of an individual and its size, pupae were measured to provide an index for adult size. Pupal lengths (± 0.01 mm) and weights (± 0.001 mg) were compared as a indicator of size. To select one of these, the relation between these two variables was first examined in a second preliminary experiment at 25 ± 0.5°C, RH 70 ± 10% and L16:D8.

Statistics. A cohort, i.e. all individuals per temperature treatment, was considered to be an experimental unit. Effect of temperature on developmental rate, i.e. 1/developmental time, was tested by linear regression (SAS 1985). Only means of developmental rates at constant temperatures were regressed on temperature and theoretical thresholds for development were calculated for each pre-adult instar using the X intercept method. To evaluate linearity, t-test's on the differences in developmental rate between 20°C and the mean at 15°C and 25°C were performed to check deviation

of the means at 20°C from the midpoint between 15°C and 25°C. The observed value of developmental rate at alternating temperature was tested for its deviation from the expected value of the regression line at 19.5°C for developmental rates based upon constant temperatures (Snedecor and Cochran 1980, p. 164). Correlations between leaf age varying from one to circa five weeks and development, mortality and pupal length were tested using the Spearman rank correlation test. With respect to the correlation between leaf age and mortality, data of the four temperature treatments were analyzed together. The correlation between developmental time and pupal length was analyzed per treatment and per sex, to determine if pupal length might differ between sexes.

Feeding/fecundity/longevity. Feeding and fecundity were estimated by counting daily numbers of feeding punctures and first instars, i.e. viable eggs, produced by individual females. Pupae were randomly removed from the colony, measured and placed singly in small glass vials. Females were released into cylindrical cages, which had a diameter of 35 cm and a height of 65 cm and were made of transparent plastic with openings covered with gauze for air circulation, in the morning of emergence, i.e. Day 1, in a controlled environment room. A standard plant and 2 males, which were replaced if necessary, were added. Oatman and Michelbacher (1958) showed that replacement of males might be necessary for maximum egg production in *Liriomyza sativae* Blanchard, which is mentioned as *Liriomyza pictella* (Thomson) but see Spencer (1973, p. 202). Because sugar sources were not likely to be present under commercial glasshouse conditions, they were not provided to the flies. Three constant temperatures, 15 °C, 20°C and 25°C, and one alternating temperature, 16-22°C, with a mean of 19.5°C were used (RH 70 ± 10%, L16:D8, photophase 0100-1700 h, light intensity 30-80 W/m^2). Plants were changed daily at 1700 h, i.e. 'lights off', and the feeding punctures and viable eggs were counted on each plant.

In order to determine the best moment for changing plants, a third preliminary experiment was carried out to examine when flies were laying eggs or were active during 24 h (at 25°C, N = 10 females, 2 females/cage, males added).

Females which died before 1700 h on the first day, were excluded from results. Females not producing fertile offspring due to e.g., mating problems (Oatman and Michelbacher, 1958), were also excluded from results (ratios of no. non-productive females and total no. females at 15°C, 20°C, 25°C and 19.5°C were 13:31, 1:30, 2:30 and 0:30, respectively). When first instar larvae or feeding punctures were not found on a plant, the female was considered to have died the day before.

Statistics. The effect of temperature on the variables was tested by linear regression. Correlation between pupal length and the variables was tested using Spearman rank correlation test. Correlation as well as partial correlation between feeding, fecundity and longevity were estimated

(Snedecor and Cochran 1980, p. 361). The theoretical temperature threshold for oviposition was estimated using the X intercept method after analysis by means of linear regression based upon mean oviposition rate at constant 15°C, 20°C and 25°C, using life span without post-oviposition period. Data were checked for normality prior to analysis (SAS 1985). Cumulative % oviposition per day was related to cumulative degree-days for all temperatures. To compare with the data of Parrella (1984), degree-days were based upon an experimentally established temperature-threshold for oviposition, 10°C (Parrella 1984).

Reproductive capacity. The intrinsic rate of increase (r_m) at four temperatures was calculated from the variables estimated in aforementioned experiments assuming a sex ratio of 1:1. The Lotka equation (1925) was used to calculate r_m values by iteration. Net reproduction, R_0, and generation time were estimated (Krebs 1982, p. 161, 164).

Results and discussion

Preliminary experiments

Comparison of the morphological variables length of mouth hook, body and leaf mine of the three larval instars of *L. trifolii* revealed that length of mouth hook was a reliable character to distinguish the larval instars. No overlap in size of the mouth hooks occurred between the instars and it could be used most easily (Table 2-1). This characteristic was, therefore, used to distinguish between the three larval instars. The use of it to discriminate *L. sativae* larval instars had been advocated previously (Oatman and Michelbacher 1958). Mines produced by *L. trifolii* larvae on tomatoes

Table 2-1. Mean lengths (mm) of mouth hooks, bodies and mines of the three larval instars of *L. trifolii* (range between parentheses).

Larval instar	first	N	second	N	third	N
Mouth hook	0.10 (0.08-0.11)	15	0.17 (0.15-0.18)	15	0.25 (0.22-0.31)	22
Body	0.39 (0.33-0.53)	8	1.00 (0.55-1.21)	15	1.99 (1.26-2.62)	22
Mine	6.0 (1.3-14.5)	14	23.5 (15.7-35.2)	15	75.6 (33.2-152.7)	22

Table 2-2. Developmental times (day) of *L. trifolii* at three constant and one alternating temperatures (mean ± SE, range).

Stage	Temperature (°C)			
	15	20	25	19.5 (16-22)
Egg	6.6 ± 0.06 (6.2 - 7.0)	3.1 ± 0.02 (2.9 - 3.4)	2.7 ± 0.02 (2.7 - 3.0)	3.8 ± 0.01 (3.5 - 4.5)
First larval	3.3 ± 0.18 (2.5 - 4.5)	2.8 ± 0.08 (0.5 - 3.7)	1.4 ± 0.05 (1.2 - 2.0)	3.4 ± 0.04 (2.5 - 4.3)
Second larval	3.7 ± 0.28 (2.7 - 5.0)	2.1 ± 0.06 (1.7 - 3.2)	1.4 ± 0.08 (1.0 - 2.2)	2.2 ± 0.03 (1.3 - 2.7)
Third larval	3.7 ± 0.14 (3.0 - 4.8)	2.3 ± 0.10 (0.5 - 3.5)	1.8 ± 0.15 (0.8 - 3.5)	2.4 ± 0.05 (1.7 - 5.2)
Pupal	26.8 ± 0.26 (25.8 - 28.5)	15.0 ± 0.05[a] (13.7 - 17.0)	9.3 ± 0.10 (8.8 - 10.5)	16.8 ± 0.06 (15.8 - 19.2)
Total	44.0 ± 0.64 (41.0 - 47.0)	24.6 ± 0.09[a] (23.7 - 27.0)	16.6 ± 0.33 (15.0 - 20.9)	28.5 ± 0.12 (26.9 - 33.9)
N	11	34 a86	17	98

were considerably longer than those on beans (Fagoonee and Toory 1984). These authors showed that mine width was also a distinct characteristic.

Pupal weight and length were significantly correlated (N = 160, r = 0.88, P < 0.05) and both gave an equally good indication of adult size. Pupal length was preferred because of its convenience. Length of pupae was found to stabilize after 3 days in an experiment, in which pupae were measured daily (N = 71, mean reduction in length of pupae as their age increased from 0 to 9 days was 0.02 mm with a maximum value observed of 0.10 mm at 25°C, RH 70%). Shrinking of agromyzid pupae was earlier found by Drolet and McNeil (1984).

No. of eggs and feeding punctures produced by *L. trifolii* during the scotophase, 1700-0100 h, and the photophase, 0100-0900 h and 0900-1700 h, were 0, 8.0 ± 2.9; 10.2 ± 4.1, 283.2 ± 39.4 and 5.4 ± 0.7, 295.2 ± 69.8, respectively (means ± SE). The flies did not lay eggs during the scotophase. Also on chrysanthemum and bean, little activity of flies was observed during night hours (Parrella 1984, Vercambre 1980). Oviposition commenced at the beginning of the photophase (Fagoonee and Toory 1984). 'Lights off' was therefore determined as the optimal time for changing plants because the

Table 2-3. Developmental rates (1/day) for pre-adult instars (N = 3) and oviposition rates (1/day) for adult females (N = 75) of *L. trifolii* regressed on constant temperatures and estimated temperature thresholds for development and oviposition.

	Rate regressed on temperature (°C)	Estimated threshold temperature (°C)
Development		
Egg	Y = 0.02146 X - 0.1475 (r = 0.94, P = 0.22)	6.9
First instar	Y = 0.04357 X - 0.3824 (r = 0.94, P = 0.22)	8.8
Second instar	Y = 0.04548 X - 0.4043 (r > 0.99, P = 0.02)	8.9
Third instar	Y = 0.03294 X - 0.2018 (r = 0.98, P = 0.12)	6.1
Pupa	Y = 0.00705 X - 0.0703 (r > 0.99, P = 0.06)	10.0
Total	Y = 0.00378 X - 0.0343 (r > 0.99, P = 0.02)	9.1
Oviposition		
	Y = 0.79025 X - 9.9587 (r = 0.60, P < 0.0001)	12.6

The X intercept method was used to calculate thresholds.

flies could 'settle down' during night. The number of first instar larvae counted per plant was considered to equal the total number of viable eggs laid by a female during the day preceding plant transfer.

Effect of temperature on development

The duration of development was inversely related to temperature between 15°C and 25°C (Table 2-2). Observed means for second and third instar larvae and total developmental rate at 20°C did not deviate from the calculated values at 20°C (Fig. 2-1). The relationships of developmental rates with temperature for the second instar larva and in total were approximately linear within this temperature range (Table 2-3). Because the minimum temperature under glasshouse regimes is at 15°C and the temperatures considered are in the linear region, the linear approximation for predicting developmental rates is acceptable. The rate of development through the larval stage, which was expressed by Y = 0.0131 X - 0.1069 (r = 0.99, P = 0.003) with a threshold temperature of 8.2°C, compared closely to the developmental rate and threshold found on tomato cv. 'Walter' Y = 0.0118 X - 0.0926 and 7.8°C, respectively (Schuster and Patel, 1985). The theoretical temperature-threshold for total development was 9.0°C. The threshold temperatures for eggs, 6.9°C, and third instars, 6.1°C, were found to be

lower than for other instars, whose thresholds varied between 8.8°C and 10.0°C.

Larval periods spent outside the leaf were relatively short and temperature dependent. The highest mean was found at 15°C with a maximum of 8 h: 3.3 ± 0.7 h (mean ± SE), whereas at 25°C the mean period outside the mine was less than 2 h. These periods on tomatoes were shorter than those on celery (Leibee 1984). Temperature dependency in 'prepupal' developmental times in *Liriomyza* spp. had previously been found (Dimetry 1971, Leibee 1984). The rate of total development (1/day) was significantly less at the alternating temperature regime of 16-22°C (mean = 19.5°C), i.e. 0.035, than the rate, i.e. 0.039, predicted from the regression of developmental rate on temperature using constant temperatures ($z = 34$, $P < 0.05$).

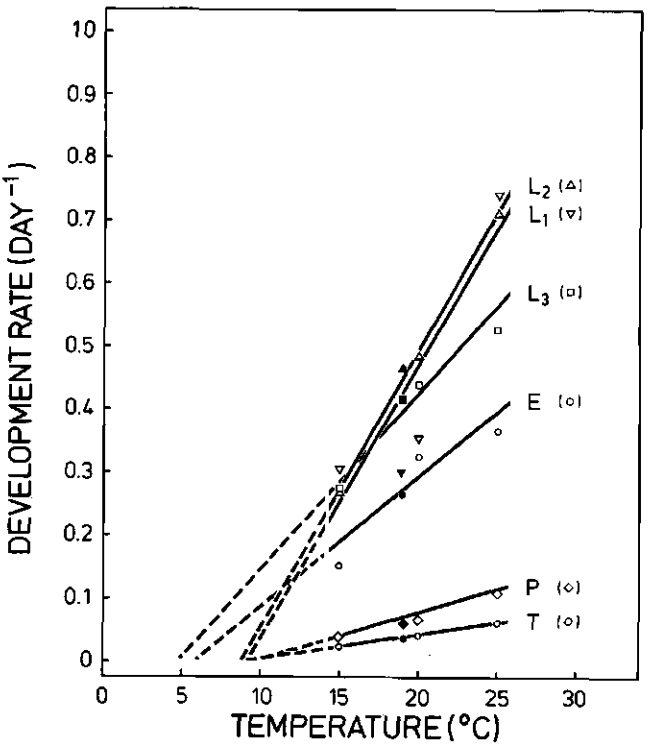

Fig. 2-1. Effect of temperature on developmental rates of *L. trifolii* for immature stages (E = egg, L1 = first instar larva, L2 = second instar larva, L3 = third instar larva, P = pupa and T = total) at 3 constant (open symbols) and an alternating temperatures (filled symbols); one-tailed t-tests, P = 0.05 (for explanation, see text): E, z = -25.66, sign.; L1, z = 2.67, sign.; L2, z = 0.60, n.s.; L3, z = -1.02, n.s.; P, z = 9.37, sign.; T, z = 1.74, n.s.). Regression equations based upon means at constant temperatures (see table 2-3).

The egg stage was proportionally 12% to 16%, the larval stage 24% to 29% and the pupal stage was 55% to 61% of the total time spent as an immature on tomato. Proportionally longer egg and larval stages and shorter pupal stages have been found on bean, chrysanthemum and celery (Charlton and Allen 1981, Leibee 1984). *L. trifolii* larvae were seen feeding both night and day except during molting. This has also been shown for the agromyzid *Phytomyza lanati* Spencer (Tauber and Tauber 1968).

The duration of male and female development was significantly different only at 20°C when males emerged on average 10 h earlier than females (Wilcoxon two-sample test, $Z = -2.4$, $P = 0.02$). Male pupae were significantly shorter than female pupae. At 20°C mean male length ($N = 40$) was 1.53 ± 0.08 mm while females ($N = 46$) were 1.71 ± 0.12 mm long (mean ± s.d.; t-test, $t = 8.09$, $P < 0.0001$). At 19.5°C males ($N = 45$) and females ($N = 53$) were 1.58 ± 0.09 mm and 1.73 ± 0.10 mm long, respectively ($t = 7.36$, $P < 0.0001$). Adult females emerging from larger pupae were also larger than males in *L. sativae* (Oatman and Michelbacher 1958). No correlation was found between the developmental time of an individual and its pupal length.

No diurnal trend was found for the time of egg hatch nor for molting of first and second larval instars. Most third instars left their mines within 4 to 8 h after lights on. At 15°C, 20°C, 25°C and 19.5°C, 91%, 94%, 80% and 79% of the larvae, respectively, dropped from the plants during that interval. Few larvae left their mines during the second half of the photophase. At 15°C, 20°C, 25°C and 19.5°C, 0%, 0%, 0% and 4% of those observed, respectively, dropped from the plants. Most adults emerged within 4 to 8 h after lights on, viz. at 15°C, 20°C, 25°C and 19.5°C, 36%, 94%, 91% and 87% of the flies, respectively, emerged during this period. Only a few adults emerged earlier, shortly before the light would be switched on, or later, during the day. These phenomena of dropping of the majority of larvae from plants and emerging of most adults in *Liriomyza* during early daylight hours have previously been shown (Charlton and Allen 1981, Oatman and Michelbacher 1958).

Effect of temperature on mortality

Mortality showed no consistent relationship with temperature (Table 2-4). Egg mortality varied from 12% to 23%. Mortality was highest in the first, intermediate in the second and least in the third larval instar. Highest total mortality (73%) occurred at 15°C, whereas most immatures reached adulthood near 20°C. At the alternating temperature mortality was relatively low and 64% of the eggs survived to adulthood.

Table 2-4. Percentage mortality of *L. trifolii* stages at different temperatures.

Stage	Temperature (°C)			
	15	20	25	19.5 (16-22)
Egg	23	20	21	12
First larval	45	22	18	5
Second larval	29	9	19	2
Third larval	0	1	9	1
Pupal	8	7	15	20
Total	73	48	60	36
N eggs	40	140	42	152

Within-instar mortality of individuals entering the instar is given for immatures and total mortality is from egg to adult.

Effect of leaf age on development and mortality

No eggs were laid on the lowest leaf of the plants. Only larval and total developmental time in the 20°C experiment were significantly correlated with leaf age (Spearman rank correlation test, N = 34: r_s = -0.44, P = 0.009 and r_s = -0.36, P = 0.039, respectively). Larvae developed faster in the lower leaves of the plant than in the top leaves in that experiment. Pupal developmental time and pupal length did not correlate with leaf age.

With the data of the four temperature treatments added together, percentage mortality from the oldest leaf (no. 7) to the youngest leaves (no. 1) plus two non-expanded leaves was 27%, 39%, 46%, 64%, 66%, 74%, 76%, 100% and 100%, respectively. Mortality and leaf age were significantly correlated (N = 27: r_s = -0.49, P = 0.009). Eggs laid within leaves near the top developed slower and were more likely to die during development than individuals in a lower region of the plant where the leaves were older.

Developmental rates and percentage survival of immatures might thus be underestimated, if relatively small plants, within which the distribution of eggs does not correspond to plants under 'natural' conditions, are used in life history studies as previously stressed by Parrella (1987, p. 211). Because the larvae usually restrict their feeding activity to one leaflet, the egg-laying adult female determines where on a plant her offspring will feed. *L. trifolii* deposits her eggs in the middle stratum of tomato plants with a maximal number on the 7th leaf from the top, independent of plant age, and more than 60% of the larvae occurs on leaves 7 to 10 from the top (Schuster and Beck 1981). In contrast, oviposition by another tomato leafminer, *Liriomyza bryoniae* (Kalt.), has been showed to peak on the 15th

Table 2-5. Mean feeding (no. punctures), fecundity (viable eggs), longevity (day), feeding rates (no. punctures/day), oviposition rates (viable eggs/day), ratios of eggs and feeding punctures and pre- and post-oviposition periods (day) of *L. trifolii* at different temperature [mean ± SE](range).

	Temperature (°C)			
	15	20	25	19.5 (16-22)
Feeding	339 ± 38 c (108 - 656)	1406 ± 128 a (252 - 2989)	914 ± 112 b (191 - 2819)	782 ± 88 b (117 - 2044)
Fecundity	5 ± 1 c (1 - 20)	79 ± 10 a (5 - 208)	59 ± 11 ab (5 - 260)	34 ± 5 bc (2 - 99)
Longevity	6.5 ± 0.5 b (2 - 11)	14.4 ± 1.4 a (4 - 29)	5.6 ± 0.5 b (2 - 14)	7.4 ± 0.7 b (2 - 18)
Feeding rate	53 ± 5 c (22 - 109)	103 ± 5 b (42 - 153)	153 ± 11 a (64 - 299)	107 ± 8 b (39 - 227)
Oviposition rate	0.8 ± 0.2 c (0.1 - 3.7)	5.9 ± 0.6 b (0.8 - 13.1)	9.1 ± 1.1 a (0.8 - 24.9)	4.5 ± 0.5 b (0.3 - 11.4)
Eggs/feeding punctures	0.02 ± 0.00 b (0.002-0.048)	0.06 ± 0.01 a (0.020-0.118)	0.06 ± 0.01 a (0.004-0.132)	0.04 ± 0.01 b (0.005-0.098)
Pre-oviposition	2.4 ± 0.4 (0 - 6)	1.8 ± 0.3 (0 - 7)	1.2 ± 0.1 (1 - 2)	1.3 ± 0.3 (0 - 8)
Post-oviposition	1.6 ± 0.3 (0 - 4)	3.1 ± 0.6 (0 - 14)	0.2 ± 0.1 (0 - 2)	1.1 ± 0.3 (0 - 5)
N	18	29	28	30

Tukey's studentized range test, P = 0.05; means of variable with the same letter are not significantly different.

leaf from the top near half-swollen fruit (Ledieu and Helyer 1985, Westerman and Minkenberg 1986). I speculate that *L. trifolii* may oviposit preferentially on mature leaves, because its offspring will develop faster and have a higher chance of survival.

Effect of temperature on feeding, fecundity and longevity

Feeding activity of female flies on tomato leaves was affected by temperature. Most feeding, i.e. punctures/female, occurred at 20°C and with the least at 15°C (Table 2-5). However, feeding rate, i.e. averaged punctures/female/day, was greatest at 25°C. Feeding activity (no. feeding punctures/female or punctures/female/day) was generally higher on tomato than on chrysanthemum as found by Parrella (1984). Feeding rate at a constant 20°C did not differ from the alternating temperature regime. The feeding activity of a female was age dependent and increased sharply during the first days of her life to a peak at Day 2 or 4, after which feeding rate declined with age (Fig. 2-2).

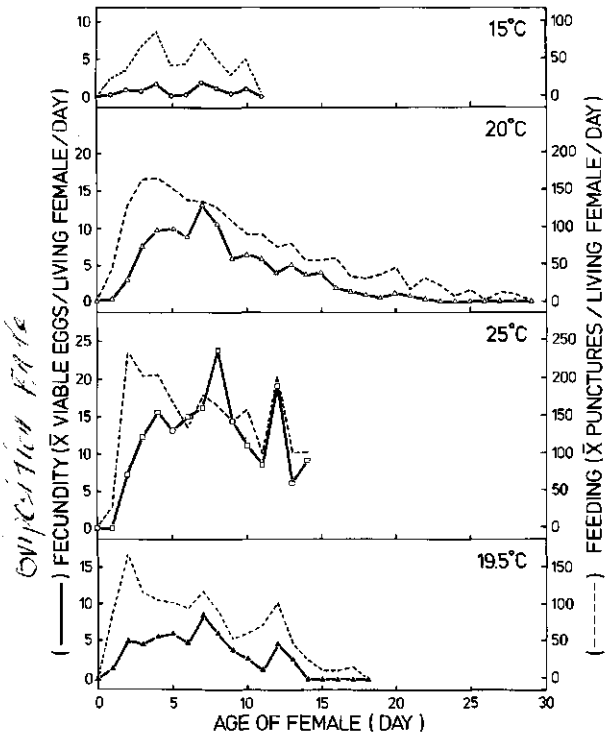

Fig. 2-2. Gross reproduction and feeding of *L. trifolii* at three constant and one alternating temperatures.

Mean fecundity was 79 eggs at 20°C and only five at 15°C (Table 2-5). The estimated fecundity of *L. trifolii* at different temperatures on chrysanthemum and on celery is many times higher than on tomato (Leibee 1984, Parrella 1984). However, comparison of adult performance on the different host-plant species is complicated by the honey provided as an extra food resource to the flies in those studies. Direct access to honey greatly enhances fecundity and longevity of *L. trifolii* flies (Charlton and Allen 1981, Vercambre 1980, Zoebisch and Schuster 1987). Mean oviposition rate varied from nine eggs per day at 25°C to six at 20°C and one at 15°C. Oviposition rate showed a slow increase with a peak at Day 7 or 8, after which a slow decrease followed, with the exception of the 15°C treatment where egg production was constantly low (Fig. 2-2). The typical triangular form of the reproduction curve has also been found in other studies (Leibee 1984, Parrella 1984, Parrella et al. 1983b).

The peak in oviposition was preceded by a peak of feeding. Both activities had a similar pattern throughout female's life at 20°C and 25°C. Similar tendencies in feeding and oviposition in *L. trifolii* has previously been shown (Parrella et al. 1983b, Parrella 1984). The egg laying period was preceded by a preoviposition period, which varied from 1.2 to 2.4 days dependent upon temperature, and was followed by a postoviposition period of 0.2 to 3.1 days (Table 2-5). Temperature dependency in the preoviposition period of the agromyzid fly *Liriomyza congesta* Becker, which is mentioned as L. trifolii but see Spencer (1973, p.95), was demonstrated by Dimetry (1971).

Feeding and fecundity had a correlation coefficient of 0.82, feeding and longevity of 0.75 and fecundity and longevity of 0.54 (Spearman rank correlation test, N = 104, $P < 0.0002$ for all). Partial correlation between feeding and fecundity was 0.65 and between feeding and longevity 0.69. Females without food lived only two to three days (Charlton and Allen 1981, Zoebish and Schuster 1987). From the data presented here, I conclude that feeding on plant sap is of importance both for the production of eggs and for the prolongation of life span.

The vertical distribution of feeding punctures was similar to the distribution of viable eggs. The highest number of feeding punctures was found on the 4th leaf, the maximum egg number on the 5th leaf from the top; percentages feeding punctures and viable eggs were on leaf no. 8 and 7: 2 and 5%, on leaf no. 6: 7 and 18%, on leaf no. 5: 24 and 28%, on leaf no. 4: 32 and 27%, on leaf no. 3: 19 and 14%, on leaf no. 2: 9 and 4%, on leaf no. 1: 3 and 2% and on non-expanded leaves: 3 and 2%, respectively (from the 19.5°C experiment, N = 30 females, total no. feeding punctures = 23158 and viable eggs = 1008). Feeding, fecundity and longevity were greatest at 20°C. Adult mortality was highest at 25°C and lowest at 20°C (Fig. 2-3). The ratios of eggs and feeding punctures at 20°C and 25°C did not differ and were significantly higher than those at 15 °C and at the alternating temperature (Table 2-5). The ratio at 25°C is markedly lower than the ratios found on other host-plant species at 26.7°C (Parrella 1984, Parrella et al. 1983b).

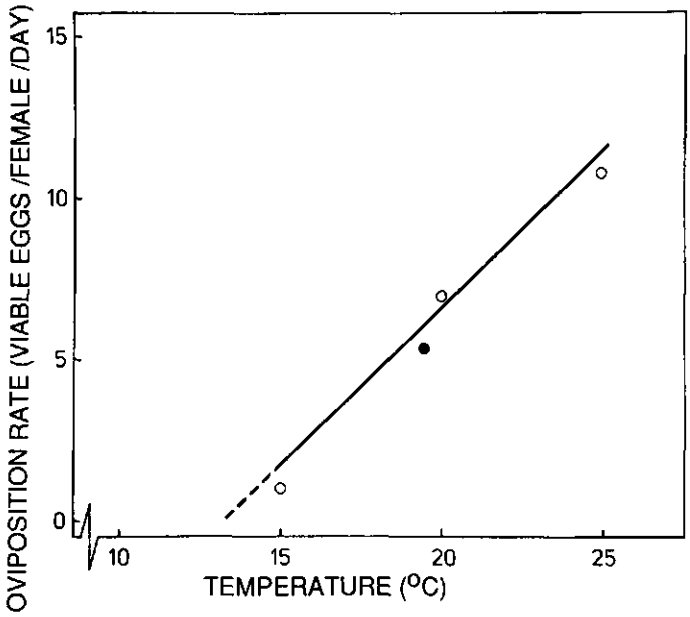

Fig. 2-3. Percent survival of *L. trifolii* at different temperatures.

The oviposition rate varied linearly with temperature (Fig. 2-4). The theoretical temperature threshold for oviposition was 12.6°C (Table 2-3), which closely agrees with the theoretical threshold of 12.2°C found on chrysanthemum (Parrella 1984). The value for 19.5°C, i.e. 5.5 eggs/female/day, predicted from the regression of oviposition rate on temperature using constant temperatures did not deviate significantly from the observed mean fecundity at 19.5°C. Means at 15°C, 20°C, 25°C and 19.5°C were 1.1 a, 6.9 bc, 9.3 c and 5.1 b, respectively (Tukey 's studentized range test, P = 0.05; means followed by the same letter are not sign. different).

No correlation was found between female pupal length and feeding, oviposition during her life or her longevity. In addition, there was no correlation between female pupal length and feeding rate, oviposition rate or ratio of eggs and feeding punctures. In contrast, pupal size was found to be a positive indicator of both oviposition and longevity for *L. trifolii* on chrysanthemum (Parrella 1983).

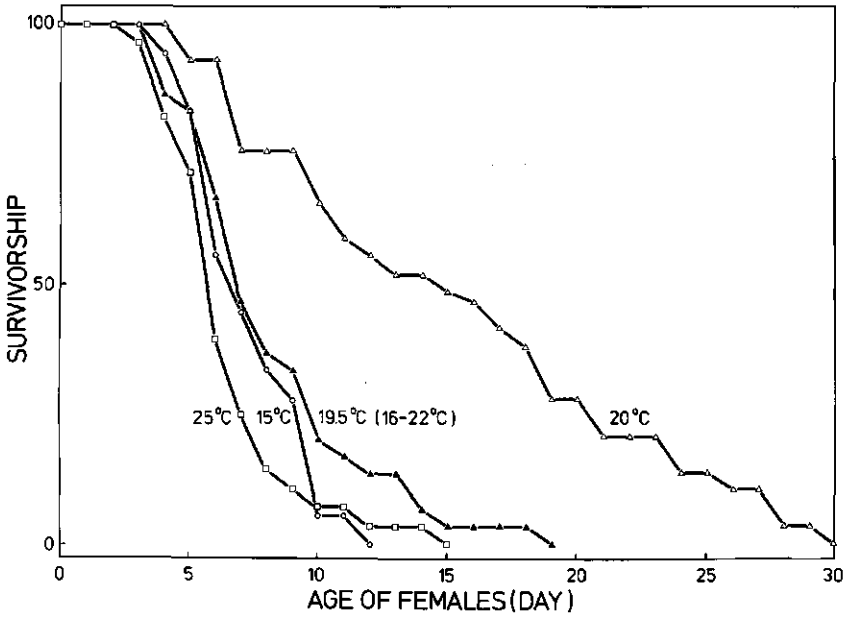

Fig. 2-4. Mean oviposition rates of *L. trifolii* at three constant (open symbols) and one alternating (filled symbol) temperatures. Regression equation is based upon data at the constant temperatures (see table 2-3).

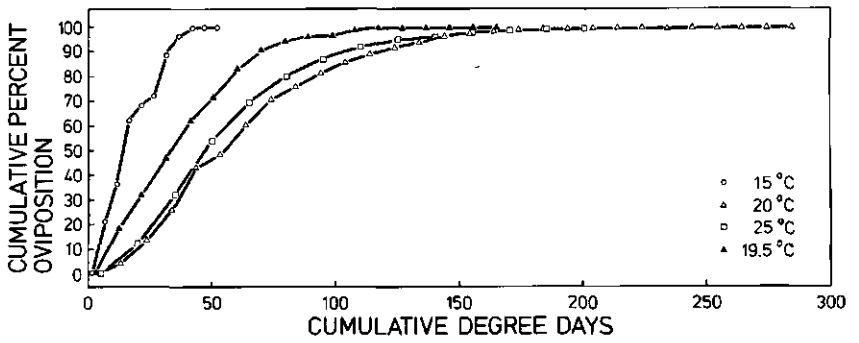

Fig. 2-5. Cumulative percent oviposition per day by *L. trifolii* versus cumulative degree-days at different temperatures. A threshold temperature of 10.0°C was used to calculate degree-days.

Cumulative percent oviposition per cumulative degree-day was similar at 20°C and 25°C (Fig. 2-5). Ninety % of all oviposition occurred within the first 115 DD of adult life, which is considerably less than the 550 DD reported for chrysanthemum (Parrella 1984). The relationship between cumulative % oviposition and cumulative degree-days differed at 15°C but fecundity was only five eggs per female at this temperature and at the alternating temperature (16-22°C), suggesting a strong negative effect of low temperature on fecundity and longevity (see also Fig. 2-3; Parrella 1984).

Temperature effects in general

The optimum temperature for population growth is near 25°C as shown by the intrinsic rate of increase (Table 2-6). The generation time decreases with temperature from 48 days at 15°C to 24 days at 25°C. At 20°C a population may multiply more than 25 times per generation. The r_m values of *L. trifolii* on celery with honey as an extra food resource at 15°C, 20°C and 25°C are -0.033, 0.077 and 0.149 viable female eggs/female/day, respectively (estimated from the data of Leibee [1984]). The r_m value at 15°C is negative and similar to the estimate on tomato, which implies that a *L. trifolii* population at a constant 15°C will slowly decrease in number. The r_m values at 20°C are also similar, whereas the value on celery at 25°C is higher than on tomato without honey, indicating a better performance of *L. trifolii* on celery than tomato at 25°C.

To predict population dynamics of *L. trifolii* under 'field' conditions at fluctuating temperatures, interpolation from data measured in the laboratory at constant temperatures is only possible when the life-history variables react instantaneously to temperature. Near the lower and upper thresholds deviations are to be expected. The upper threshold is above 35°C and 40°C is lethal to larvae (Vercambre and Thiery 1983, Leibee 1984, Parrella 1984, Bodri and Oetting 1985). The lower threshold for both development and oviposition is near 10°C. An experimentally determined threshold for

Table 2-6. Intrinsic rate of increase, r_m, (viable female eggs/female/day), net reproduction, R_0, (viable female eggs/female) and generation time, T, (day) of *L. trifolii* at different temperatures.

Temp. (°C)	r_m	R_0	T
15	-0.0023	1.2	48
20	0.1024	25.5	32
25	0.1254	14.9	24
19.5 (16-22)	0.0744	12.3	34

development is probably lower than 9-10 °C (Charlton and Allen 1981, Miller and Isger 1985). Thus, this threshold for development is a few degrees below that for oviposition, which is 10 °C (Parrella 1984). A close agreement between developmental times observed and estimated at constant 16 °C and 10-22 °C cycling has been found only at those temperatures (Miller and Isger 1985). Deviations between 15 °C and 25 °C are not expected for the insect's development.

Host-plant suitability

The performance of *L. trifolii* has been quantified by measuring development and mortality of different instars, weight and length of pupae, and fecundity and longevity of adults. Ideally, all life-history variables should be estimated under specific, well-defined conditions and the corresponding intrinsic rate of increase (r_m) calculated, before general conclusions are drawn about host-plant suitability. For instance, the r_m values of *Agromyza frontella* (Rondani) on four alfalfa cultivars are very similar indicating little effect of cultivar on the population dynamics of *A. frontella,* although significant differences in several life-history variables on these cultivars have been found (Drolet and McNeil 1984). For *Liriomyza* spp., however, such a comparison of the performance on different host plant species or cultivars is complicated by the differences in experimental design used by various researchers (e.g., colony, feeding regime, etc.). Moreover, there are few data on developmental time (age at first reproduction), which is the most important life-history variable affecting the intrinsic rate of increase in fast growing populations (Caswell and Hasting 1980, Lewontin 1965). An accurate estimation of life-history variables, particularly developmental time, is only possible with precise measurements in a controlled environment. This study stresses that, in order to examine host-plant suitability, developmental times should be estimated in addition to other life history variables.

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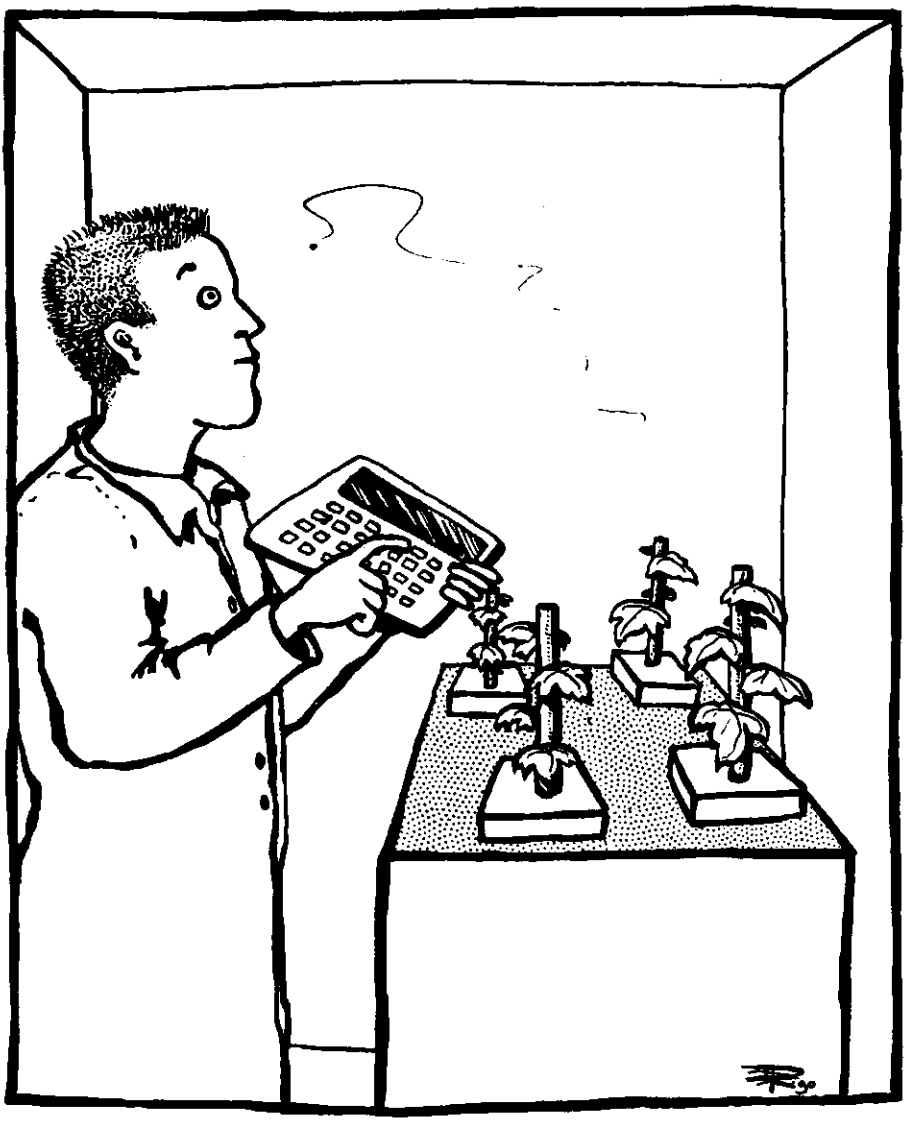
Behavioral research of parasitoids



"flying, ...landing,.....searching.....,handling host.....,
.....seeking eye-contact....."

3

Effects of temperature on the life history of the agromyzid fly *Liriomyza bryoniae* on tomato[1]

Abstract

Effects of three constant (15°C, 20°C and 25°C) and one alternating (16-22°C, mean 19.5°C) temperatures on development, mortality, fecundity and longevity of *Liriomyza bryoniae* (Kaltenbach) on tomato plants cultivar Moneydor were examined in the laboratory. Developmental rates for each pre-adult instar were estimated. Lower thresholds for development and for oviposition of *L. bryoniae* were at least 8°C and approximately 11°C, respectively. Optimum temperature for development and reproduction was 25°C, at least within the range examined. Egg-to-adult developmental rate under alternating temperatures did not differ significantly from that at 20°C, suggesting that developmental rate responds rapidly to a change in temperature. However, fecundity and oviposition rate with the alternating temperature regime were significantly lower than at 20°C, indicating a slower response of reproduction to changes in temperature. At all temperatures examined, more than 85% of oviposition occurred within 100 degree-days of eclosion. Pupal length was positively correlated with temperature but not with developmental time, fecundity, oviposition rate or longevity. Intrinsic rate of increase (r_m) varied from 0.0457 viable female eggs per female per day at 15°C to 0.1841 eggs per female per day at 25°C, net reproduction from nine viable female eggs per female at 15°C to 54 eggs per female at 25°C, and generation time from 49 days at 15°C to 22 days at 25°C. Comparison with previous studies indicates that tomato is a more suitable host plant for *L. bryoniae* than for *Liriomyza trifolii* (Burgess) within the range of 15°C to 25°C.

[1] Published as: O.P.J.M. Minkenberg and C.A.J. Helderman 1990. Effects of temperature on the life history of *Liriomyza bryoniae* (Diptera: Agromyzidae) on tomato. *Journal of Economic Entomology* **83**: 117-125.

Introduction

The polyphagous fly *Liriomyza bryoniae* is a major economic pest of vegetables such as tomato and cucurbits throughout Europe, northern Africa, Israel and Japan (for reviews, see Spencer 1973, Minkenberg and Van Lenteren 1986). Trading of plant material may facilitate further dispersal of *L. bryoniae*, just as has occurred with the closely related leafminer *Liriomyza trifolii*. The pest insect *Liriomyza trifolii* has become cosmopolitan and occurs regularly on vegetables, particularly in the Mediterranean area (Minkenberg 1988a). The injury threshold for leafminers on glasshouse tomatoes is relatively high, i. e. about 10 mines/plant (Ledieu and Helyer 1985). In glasshouse crops and outdoor crops, leafminers can be controlled naturally by overwintering or immigrating parasites, or biologically by means of seasonal, inoculative releases of commercially available parasitic wasps, e.g. *Dacnusa sibirica* Telenga and *Diglyphus isaea* (Walker) (Lyon 1986, Minkenberg and Van Lenteren 1986, Nedstam et al. 1987 [refs herein], Wardlow 1985, Woets and Van der Linden 1982).

The biology of *Liriomyza* spp. has recently been reviewed (Parrella 1987). The host plant and its growing conditions (e.g., Minkenberg and Fredrix 1989) and temperature have a considerable impact on the life history of agromyzid flies (Minkenberg and Van Lenteren 1986, Parrella 1987; for a review on temperature effects, see Laudien 1973). Few studies have addressed effects of temperature on the development and reproduction of *L. bryoniae*. Development and reproduction for this agromyzid fly at one temperature on tomato was examined by Hendrikse et al. (1980), development at different temperatures was described by Nedstam (1985), and development and reproduction at different temperatures on melon was measured by Saito (1988).

Life-history data of *L. bryoniae*, like those of *L. trifolii* (Minkenberg 1988b), may be useful to predict the effectiveness of parasitoids prior to their introduction into glasshouses and to construct a deterministic, population dynamical model of host and parasitoid populations in the context of our pre- and postintroduction evaluation projects (Van Lenteren 1986, Minkenberg and Van Lenteren 1987).

In this study, we (a) estimated development and mortality for each immature instar, fecundity and longevity of adults at three constant temperatures, 15 °C, 20 °C and 25 °C, and at one alternating temperature, 16-22 °C; (b) determined lower thermal thresholds for development and oviposition; (c) estimated r_m values of *L. bryoniae* at the different temperatures.

Materials and methods

L. bryoniae colony. Two hundred larvae of *L. bryoniae* were collected from tomato plants grown in commercial glasshouses in the Netherlands (Westland and Kring area) to start a colony at the end of 1983. The colony was maintained on *Lycopersicon esculentum Miller* cv. Moneydor grown in pots (10 by 10 cm) on a standard soil mix (Naturado) at 20 ± 5°C and RH 70 ± 10%. Artificial light was used to maintain a photoperiod of 16 h (L16:D8). Pesticides were not applied. The occasionally occurring *Thrips tabaci* (Lind.) was controlled by predatory mites, *Amblyseius cucumeris* (Oud.).

Rearing unit. After they were exposed to flies, plants were placed on a galvanized building-netting (mesh width 7.5 by 7.5 cm) atop a standard aluminum glasshouse bench (1 by 1.5 m) (Fig. 3-1). Mature larvae that crawled from the plants were guided by a slanting plane (Hostalite) into a gutter where they pupated. The inside of the gutter was daubed with Fluon

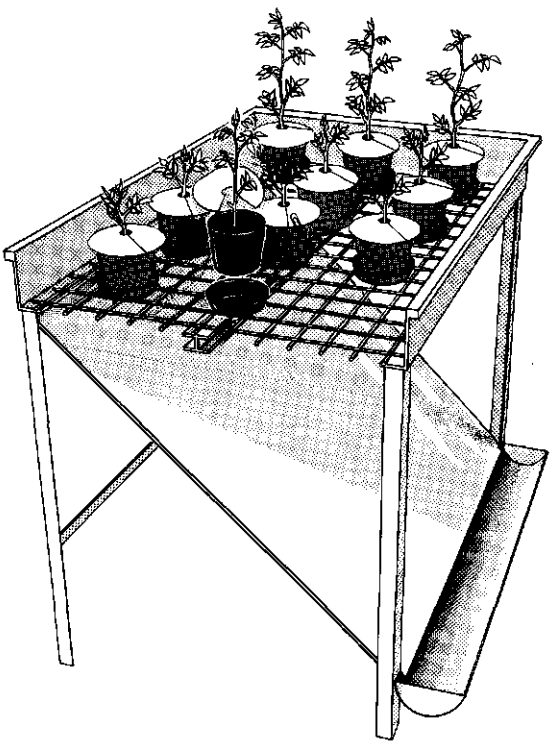


Fig. 3-1. Rearing unit for insects that normally pupate in the soil (e.g., *L. bryoniae;* for more information, see Materials and Methods).

is 19.5°C) differed significantly from that predicted (i.e., at 19.5°C) from the linear equations based upon developmental rates at constant temperatures. We also compared our regression lines with those of Nedstam (1985) using analysis of covariance and models for homogeneity of slopes (SAS 1985, p.436). The relationship between developmental time and pupal length per treatment and per sex was analyzed by a Tukey studentized range test and a Wilcoxon two-sample test, respectively.

Leaf nitrogen. Two plants per treatment of the development had mortality experiment were used to analyze the total nitrogen content in the leaves. Leaves were oven dried and percentage of nitrogen of dry weight was determined (see Minkenberg and Fredrix 1989 for details).

Observed lower thresholds for development. Threshold temperatures were determined by observation of eggs, first instars, and pupae maintained in growth cabinets at 6°C and 8°C (mean temperatures ± 0.5°C, RH > 50% and L16:D8).

Fecundity and longevity of adult females. Fecundity was estimated by daily counts of first instars produced by individual females. Pupae were randomly chosen from the colony, their length was measured (for the 20 and 25°C treatment), and they were placed singly in small glass vials. On the morning of emergence (Day 1), females were released into the cylindrical cages in the controlled environment room. Each cage contained a plant with three expanded leaves (total area 500-600 cm^2) and two males, which were replaced if necessary. Sugar sources were not provided to the flies. Plants were changed daily between 1600 h and 1700 h (the onset of scotophase). Viable eggs were counted from Day 2. Females which died on the first day before 1700 h, and those that laid only unfertile eggs were excluded from analysis.

The effect of temperature on fecundity, longevity and oviposition rate was determined by analysis of variance. Means were compared by Tukey's studentized range test. Correlations between pupal length and the life history variables were determined by the Spearman rank correlation test. The theoretical temperature threshold for oviposition was estimated using the X intercept of linear regression of oviposition rate (excluding the postoviposition period) against temperature (15°C, 20°C and 25°C). Cumulative % oviposition per day was depicted vs. cumulative degree-days for all examined temperatures. For comparison with reproduction of *L. trifolii* on tomato and chrysanthemum (Minkenberg 1988b, Parrella 1984), degree-days were based upon an oviposition threshold temperature of 10.0°C. The intrinsic rate of increase (r_m) at four temperatures was calculated assuming a sex ratio of 1:1 by the Lotka (1925) equation. Voucher specimens were deposited in the insect collection of the Agricultural University of Wageningen.

Results and discussion

Pre-adult development and mortality

Developmental time for *L. bryoniae* was inversely related to temperature for all instars (Table 3-1). Mean total developmental time varied from 41 days at 15°C to 17 days at 25°C. Developmental rates for each immature instar and in total were not linearly related to temperature (Table 3-2). Regression lines are shown in Fig. 3-3. The lower threshold temperature differed among developmental stages. Neither eggs nor pupae developed at 6°C, whereas larval development required 60 d. At 8°C, eggs did not hatch, whereas larval and pupal development each required ca. 70 d. The estimated lower threshold temperature for egg development was 5.8°C and for larval and pupal development, 8.7 and 8.2°C, respectively. Thus, observed temperature thresholds for larvae and pupae were lower than the estimated threshold and for eggs higher than the estimated thresholds. Overall, the lower threshold for development of *L. bryoniae* was apparently higher than 8°C. The upper threshold temperature for development of *L. bryoniae* on melon is between 30°C and 35°C (Saito 1988).

Table 3-1. Developmental time (day) of *L. bryoniae* at three constant and one alternating temperatures (mean ± SE, range).

Stage	Temperature (°C)			
	15	20	25	19.5 (16-22)
Egg	6.1 ± 0.1 (5.5 - 7.1)	4.2 ± 0.0 (4.0 - 4.7)	3.0 ± 0.0 (2.7 - 3.3)	4.0 ± 0.0 (4.0)
First larval	4.6 ± 0.2 (3.0 - 6.7)	3.3 ± 0.1 (2.3 - 4.0)	1.4 ± 0.0 (1.0 - 2.3)	2.0 ± 0.0 (2.0 - 3.0)
Second larval	3.7 ± 0.2 (2.7 - 5.0)	2.5 ± 0.1 (2.0 - 3.7)	2.0 ± 0.0 (1.3 - 2.4)	3.1 ± 0.1 (2.0 - 5.7)
Third larval	4.0 ± 0.2 (3.0 - 5.7)	2.7 ± 0.1 (2.0 - 3.7)	1.6 ± 0.0 (0.3 - 2.7)	3.0 ± 0.1 (1.7 - 4.7)
Pupal	22.2 ± 0.2 (20.7 - 23.0)	13.9 ± 0.1 (13.0 - 15.0)	9.2 ± 0.1 (8.0 - 11.0)	14.4 ± 0.1 (13.0 - 16.0)
Total	40.6 ± 0.2 (38.8 - 42.1)	26.5 ± 0.1 (25.0 - 28.0)	17.1 ± 0.1 (16.0 - 19.0)	26.5 ± 0.1 (25.0 - 31.0)
N	17	42	72	75

Table 3-2. Developmental rates (1/day) for pre-adult instars[a], oviposition rates (1/day) for adult females[b] of *L. bryoniae* regressed on constant temperatures and estimated temperature thresholds for development and oviposition.

	Rate regressed on temperature (°C)	Estimated threshold temperature (°C)[c]
Development		
Egg:	Y = 0.01742 X - 0.1008 (r = 0.996, P = 0.05)	5.8
First instar:	Y = 0.05139 X - 0.6021 (r = 0.931, P = 0.24)	11.7
Second instar:	Y = 0.02201 X - 0.0466 (r = 0.997, P = 0.05	2.1
Third instar:	Y = 0.04662 X - 0.4743 (r = 0.967, P = 0.16)	10.2
Pupa:	Y = 0.00643 X - 0.0530 (r = 0.996, P = 0.06)	8.2
Total:	Y = 0.00338 X - 0.0273 (r = 0.991, P = 0.08)	8.1
Total larva:	Y = 0.01190 X - 0.1039 (r = 0.975, P = 0.14)	8.7
Oviposition		
	Y = 1.70432 X - 18.604 (r = 0.58, P = 0.0001)	10.9

[a] Mean developmental rates, Y (1/d), regressed on temperatures, X; only means from the observations at constant temperatures are used in this analysis (N = 3).

[b] Oviposition rate, Y (viable eggs/day), regressed on temperature, X; only observations at the constant temperatures were used in this analysis (N = 102).

[c] The X-intercept method was used to calculate thresholds.

Developmental times observed in this study are similar to those reported for *L. bryoniae* on tomato cv. Ida (Nedstam 1985). The regression of developmental rates vs. temperature within the range 12-30°C for eggs, larvae, pupae and in total based upon her data are Y = 0.0142 X - 0.0913 (r = 0.991), Y = 0.0157 X - 0.1558 (r = 0.979), Y = 0.0059 X - 0.0444 (r = 0.993) and Y = 0.0033 X - 0.0268 (r = 0.996), respectively (P < 0.0001 for all). Estimated thresholds are 6.4°C, 9.9°C, 7.5°C and 8.1°C, respectively. The temperature threshold estimated by the X intercept method for complete preadult development is only a vague indicator for the observed threshold for development. Furthermore, the highest threshold temperature of the different immature stages should actually be used to estimate the threshold for development (i.e., 9.9°C in stead of 8.1°C). These regressions do not differ significantly from ours (P > 0.05), implying that development of *L. bryoniae* immatures from glasshouse populations from Sweden and the Netherlands is similarly affected by temperature. Development of *L. bryoniae* from Japanese glasshouses on melon, shows similar patterns: total development Y = 0.00347 X - 0.0256, threshold 7.4°C, (15-25°C, Saito 1988).

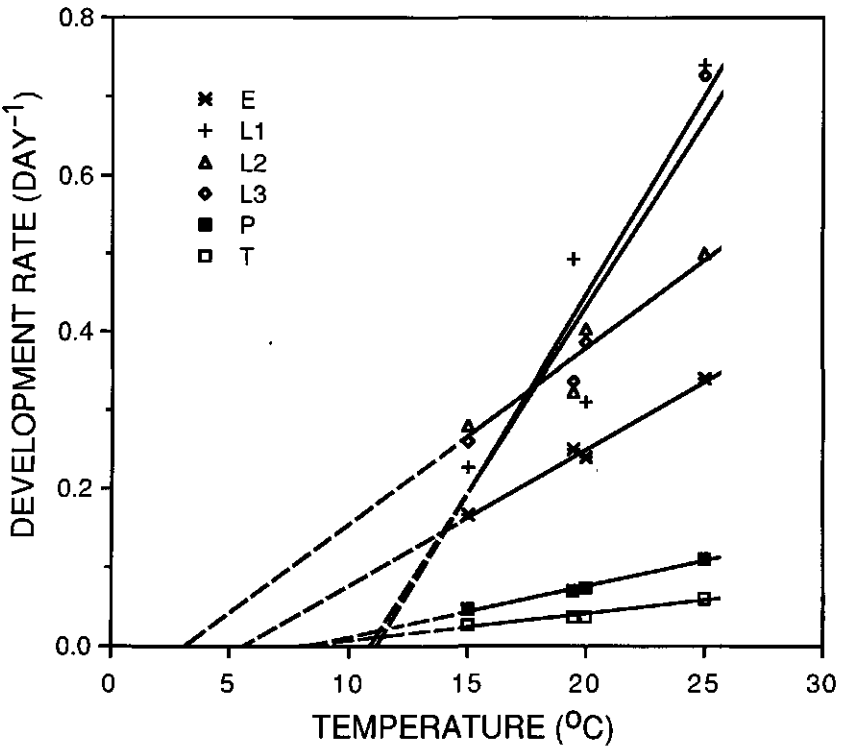


Fig. 3-3. Effect of temperature on developmental rates of *L. bryoniae* for immature stages (E = egg, L1 = first instar larva, L2 = second instar larva, L3 = third instar larva, P = pupa and T = total) at three constant (15 °C, 20 °C and 25 °C) and one alternating temperatures (16-22 °C, mean = 19.5 °C). Regression lines were based only upon means at constant temperatures (see Table 3-2).

The total developmental rate, 0.0378/day, under the alternating temperature regime was not significantly different from the predicted rate, 0.0386/day ($z = 1.65$, $p > 0.05$). The similarity in developmental rate strongly suggests a rapid response of development to changing temperatures. Of the total developmental time, the egg stage accounted for 15-18%, the larval stage 29-31% and the pupal stage 52-55%. Relative to *L. trifolii* (Minkenberg 1988b), *L. bryoniae* requires more time for larval development but less time for pupal development. Within the larval stage, however, the respective developmental times were highly variable for both leafminer species.

Mean developmental time of males was 2-6 h shorter than that of females, although the difference was significant only in two cases (total

larval developmental time at 20°C: Z = -2.4, P = 0.049; total developmental time at 25°C: Z = -1.97, P = 0.049). The short larval developmental time of males accounted for most of this difference. The result is consistent with the general observation that in insects, males develop slightly faster than females (Ratte 1985). Male pupae were significantly shorter than female pupae (Table 3-3). Similar differences between the sexes in development and pupal length have previously been shown for *L. trifolii* on tomato (Minkenberg 1988b).

Consistent correlations between developmental time and pupal length were not found (e.g., for females: larval developmental time and pupal length at 20°C and 19.5°C, r_s = +0.50, P = 0.02 and r_s = -0.48, P = 0.006, respectively). The cause for these results might have been food quality. Pupal length (for both sexes) was directly correlated with temperature within the examined range (Table 3-3). This is in contrast to the general rule that, except at low temperatures, size is inversely related to temperature (Hinton 1981, p.31). Positive correlations between pupal length and temperature determined in our study may indicate that temperature affected food quality, i.e. tomato plants kept at high temperature provide a better food. Temperature treatment influenced leaf nitrogen content of the plants in the controlled environment rooms. The percentage leaf nitrogen content of plants in the environment rooms at 20°C and at 19.5°C, the alternating regime, was 4.3% (after 14 days) and 2.2% (after 13 days), respectively. Plants in the glasshouse contained 7.8% leaf nitrogen. Plants at 25°C after the larvae dropped from the leaves contained 6.0% leaf nitrogen (after 9 days). Because leaf nitrogen content and the performance of *L. trifolii* correlates (Minkenberg and Fredrix 1989), it will probably be impossible to determine temperature effects on leafminers without considering an indirect effect of the host plant.

Total mortality was approximately 35% at 20°C, 25°C and 19.5°C, whereas at 15°C it was 80% (Table 3-4). Thus, our results do not agree with those of Nedstam (1985), who found that pupal mortality was only 43% at 12°C and 8% at 24°C. The relatively high mortality of pupae in our study may have been due to the low humidity (ca. 70%) of the chambers; pupal emergence and ambient humidity are positively correlated in *L. trifolii* (Charlton and Allen 1981). In conclusion, the optimum temperature for development (i.e., with the highest developmental rate and the lowest mortality) was 25°C, at least within the temperature range examined.

Table 3-3. Effect of temperature treatment and sex on pupal length (mm) of *L. bryoniae* (mean ± SD).

Temperature	Female	N	Male	N	Wilcoxon
20 °C	2.02 ± 0.11 b	22	1.86 ± 0.08 b	20	Z = 5.51, P > 0.0001
25 °C	2.12 ± 0.12 a	31	1.91 ± 0.11 a	24	Z = 7.08, P > 0.0001
19.5 °C	1.96 ± 0.13 c	31	1.82 ± 0.12 c	44	Z = 4.84, P > 0.0001

Means within a column with the same letter are not significantly different (P > 0.05); Tukey's studentized range test. Analysis of variance showed a significant effect of temperature treatment (F = 31.83, df = 188, P < 0.05) and sex (F = 124.97, df = 188, P < 0.05) on pupal length. Means between sexes within a row were tested by the Wilcoxon two sample test.

Table 3-4. Percentage of mortality of *L. bryoniae* preadult stages at different temperatures.

Stage	Temperature (°C)			
	15	20	25	19.5 (16-22)
First larval	61	3	3	6
Second larval	20	3	13	6
Third larval	4	3	5	4
Pupal	37	29	18	23
Total	80	35	34	38
N first instars	89	65	110	122

Within-instar mortality of individuals entering the instar is given for each and total mortality is from first larval instar to adult.

Fecundity and longevity of adult females

Mean fecundity varied from 92 eggs/female at 15°C to 163 eggs/female at 25°C (Table 3-5). The largest number of eggs, 662, was produced by a *L. bryoniae* female at 25°C. The optimum temperature for adults defined as the temperature with the highest fecundity, was 25°C. Oviposition rate was age dependent and it peaked at 4 or 5 d and declined thereafter (Fig. 3-4). Longevity was inversely correlated with temperature (Fig. 3-5). Females lived on average for ca. 1 week at 25°C and almost 2 weeks at 15°C. Longevity and fecundity were positively correlated within a given temperature (r_s = 0.64, 0.78, 0.50 and 0.77, P = 0.0001 for all, at 15°C, 20°C, 25°C and 19.5°C, respectively). Preoviposition period of females was temperature dependent as has been demonstrated for other *Liriomyza* spp. (Dimetry 1971, Minkenberg 1988b). The postoviposition period was very short at all temperatures examined. Fecundity of *L. bryoniae* on tomato was slightly higher than on melon (Saito 1988).

Table 3-5. Mean fecundity (viable eggs), longevity (day), oviposition rates (viable eggs/day), pre-and postoviposition periods (day) of *L. bryoniae* females at different temperatures (mean ± SE, range).

	Temperature (°C)			
	15	20	25	19.5 (16-22)
Fecundity	92 ± 12 ab (21 - 283)	144 ± 22 bc (17 - 637)	163 ± 24 cd (5 - 662)	65 ± 12 a (1 - 305)
Longevity	13.6 ± 1.1 a (5 - 27)	9.0 ± 0.9 b (3 - 24)	6.6 ± 0.5 b (2 - 15)	6.9 ± 0.7 b (2 - 18)
Oviposition rate	6.7 ± 0.7 a (2.3 - 14.9)	15.2 ± 1.3 b (3.3 - 35.9)	23.3 ± 2.3 c (0.8 - 50.9)	7.7 ± 0.9 a (0.3 - 20.3)
Pre-oviposition	2.5 ± 0.2 (1 - 6)	1.6 ± 0.2 (1 - 6)	1.1 ± 0.0 (1 - 2)	1.8 ± 0.2 (1 - 6)
Post-oviposition	0.6 ± 0.3 (0 - 8)	0.2 ± 0.1 (0 - 3)	0.6 ± 0.3 (0 - 11)	0.4 ± 0.2 (0 - 7)
N	30	32	40	34

Means of variable with the same letter are not significantly different (Tukey's studentized range test, P > 0.05).

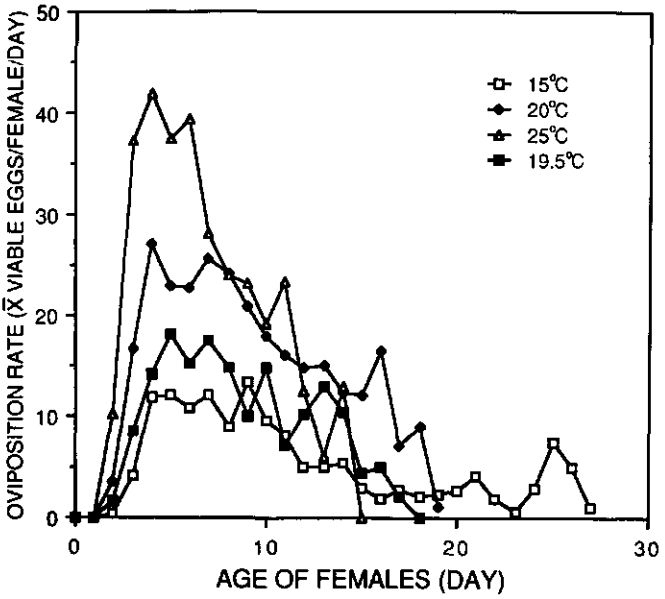


Fig. 3-4. Gross reproduction of *L. bryoniae* at three constant and one alternating temperatures.

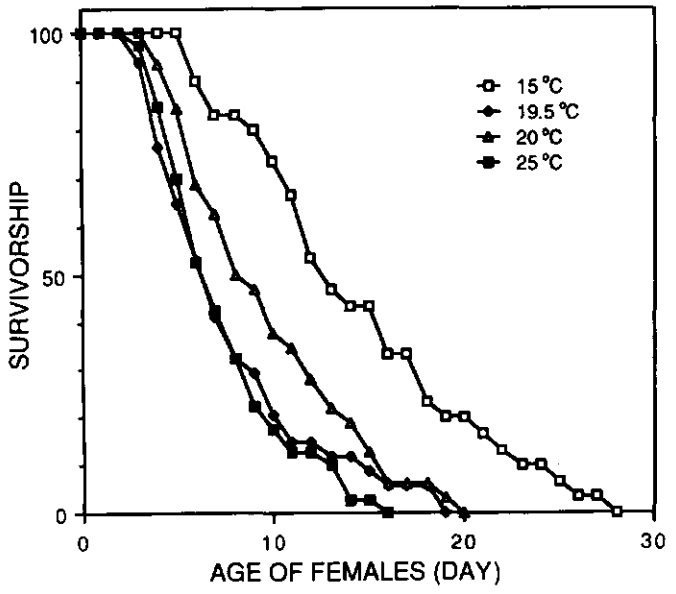


Fig. 3-5. Percentage of survival of *L. bryoniae* at different temperatures.

Fecundity and oviposition rate at the alternating temperature regime (mean 19.5°C) were significantly lower than at a constant 20°C (Table 3-5). Rate of oviposition for *L. bryoniae* thus responded relatively slowly to changes in temperatures.

Although reproduction for many insects is a function of size (Hinton 1981, Ratte 1985), no significant correlation was found between pupal length and fecundity, longevity or oviposition rate at either 20°C or 25°C. Similarly, no significant correlation was found between pupal length and total fecundity after 2, 3 and 5 days of female life at either temperature. Thus, within the examined temperature range, pupal length is not useful as an indicator of fitness.

The ovipositional threshold temperature was estimated to be 10.9°C (Fig. 3-6). To permit comparison with results of other studies, cumulative % oviposition/day vs. cumulative degree-days was depicted assuming a threshold of 10.0°C (Fig. 3-7). More than 85% of all oviposition occurred within 100 DD for all temperatures. Similar patterns have been shown previously for *L. trifolii* on tomato (Minkenberg 1988b), though total fecundity is higher for *L. bryoniae* .

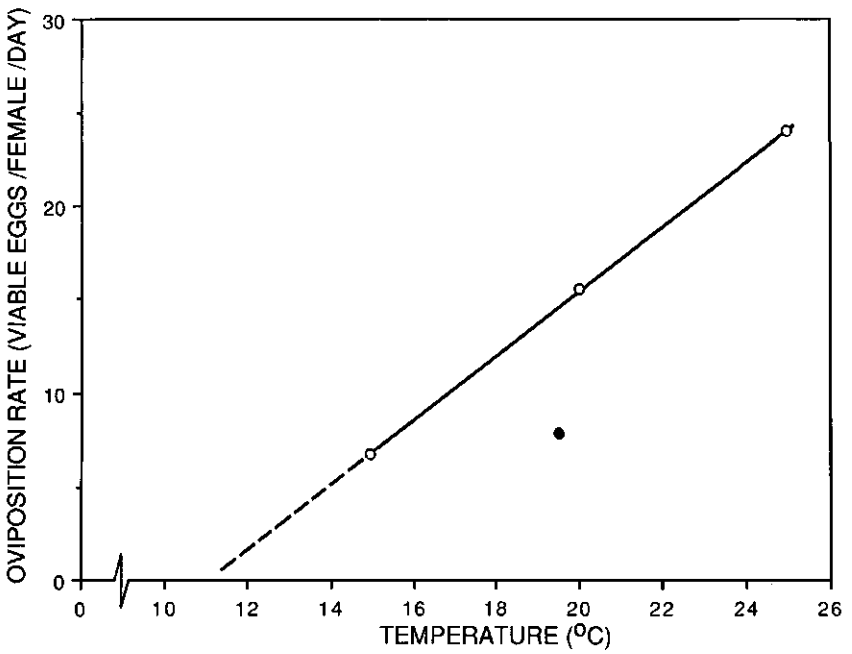


Fig. 3-6. Oviposition rate of *L. bryoniae* females at constant temperatures (open circles) and at an alternating temperature (filled circle). Regression line is based only upon data at constant temperatures (see Table 3-2).

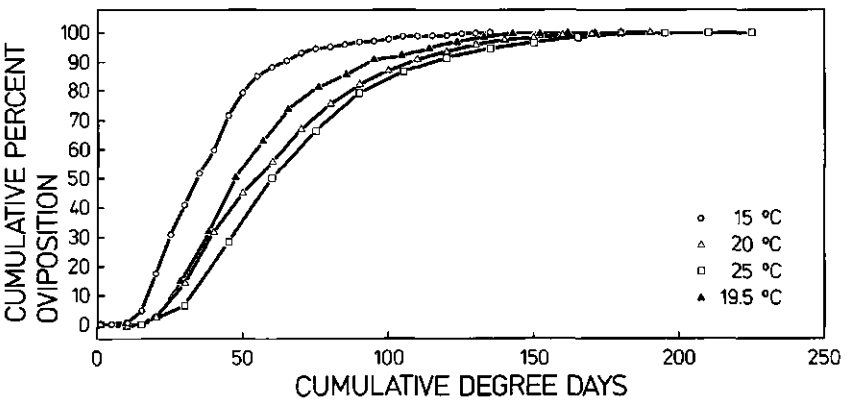


Fig. 3-7. Cumulative percentage of oviposition per day by *L. bryoniae* versus degree-days at different temperatures. A threshold temperature of 10.0 °C was used to calculate degree-days.

General temperature effects

The intrinsic rate of increase indicated that the optimum temperature for population growth between 15 °C and 25 °C is 25 °C and that population density might increase 54 times per generation at this temperature (Table 3-6).

The dramatic negative effect of the alternating temperature on *L. bryoniae* females was apparent in the r_m value (0.09) and the net reproduction (19), which were markedly lower than those at 20 °C (0.12 and 46, respectively), despite similar generation times. Since feeding and egg-laying

Table 3-6. Intrinsic rate of increase, r_m, (viable female eggs/female/day), net reproduction, R_o, (viable female eggs/female) and generation time, T, (day) of *L. bryoniae* at different temperatures.

Temperature (°C)	r_m	R_o	T
15	0.0457	9.3	49
20	0.1159	45.9	34
25	0.1841	53.6	22
19.5 (16-22)	0.0910	19.1	35

take place almost exclusively during the day, we speculate that a physiological process related to the digestion of food or the production of eggs (e.g., ripening) occurs primarily at night and is therefore slowed by the alternating temperature regime. For *L. trifolii* also, reproduction under an alternating temperature regime is less than that at a comparable constant temperature and thus does not react rapidly to changes in temperature (Minkenberg 1988b). The negative effect of fluctuating temperature on the performance of these agromyzid flies on tomato suggests that predictions based upon constant temperatures may overestimate reproduction in the glasshouse.

Comparison of performance with L. trifolii on tomato

Compared to *L. bryoniae, L. trifolii* exhibits higher temperature thresholds for both development (9.1°C vs. 8.1°C) and oviposition (12.6°C vs. 10.9°C). At intermediate temperatures (20-25°C), *L. trifolii* develops more rapidly than *L. bryoniae* but has higher immature mortality and lower fecundity (Minkenberg 1988b). As a result population growth rate of *L. bryoniae* exceeds that of *L. trifolii* on tomato. This observation is consistent with their importance as a pest in western and northern European glasshouse tomatoes. However, studies with *L. trifolii* on other host plants (Leibee 1984, Parrella 1984) and its importance as a pest on tomatoes in Mediterranean countries indicate that the optimum temperature for *L. trifolii* population growth is probably between 25°C and 30°C. Thus, at high temperatures, *L. trifolii* may perform better than *L. bryoniae* on tomato crops.

Acknowledgments

We thank Laurens van Miltenburg for participating in the experiments, David Karowe, Joop van Lenteren, Rudy Rabbinge and Mitch Trimble for critically reading the manuscript and improving the English, M. van Montfort for statistical advice, Wies de Goffau (Plant Protection Service, Wageningen) for identification of the species, Henk Snellen for rearing the flies and Frederik von Planta and Piet Kostense for the drawings.

4

Reproduction of *Dacnusa sibirica*, an endoparasitoid of the tomato leafminer *Liriomyza bryoniae*, at constant temperatures[1]

Abstract

The parasitoid *Dacnusa sibirica* Telenga is currently used for seasonal inoculative biological control of the leafminers *Liriomyza bryoniae* (Kaltenbach) and *Liriomyza trifolii* (Burgess) on glasshouse vegetables in northwest Europe. The braconid is effective from the beginning of the season until July, when average temperatures vary between 15 °C and 20 °C. To estimate the potential reproduction (R_0) of the parasitoid, the effect of temperature (15 °C, 20 °C and 25 °C) on its fecundity, longevity and oviposition rate was examined in the laboratory. Fecundity and longevity decreased with increasing temperatures, but did not differ significantly between 20 °C and 25 °C. Oviposition rate was highest at 20 °C, apparently the optimum temperature for oviposition. The R_0 of *D. sibirica* decreased with increasing temperatures, suggesting that *D. sibirica* may be less effective at high temperatures. The use of potential reproduction, R_0 or r_m, to evaluate parasitoid effectiveness is discussed.

Introduction

The polyphagous agromyzid flies *Liriomyza bryoniae* (Kaltenbach) and *L. trifolii* (Burgess) occur on several economically important vegetable crops and ornamentals (Spencer, 1973). Most of the damage is caused by the larvae, which mine the leaves and reduce the yield or the aesthetic value of the plants. The rate of population growth is high. Chemical control of the flies is difficult, partly because the larvae develop inside the leaf (for reviews on their biology, natural enemies and distribution, see Minkenberg 1988a, Minkenberg and Van Lenteren 1986, Parrella 1987).

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[1] Published as: O.P.J.M. Minkenberg 1990. Reproduction of *Dacnusa sibirica* (Hymenoptera: Braconidae), an endoparasitoid of the leafminer *Liriomyza bryoniae* (Diptera: Agromyzidae) on tomatoes, at constant temperatures. *Environmental Entomology* 19: 625-629.

Parasitoids naturally present on tomatoes can at times effectively control leafminers (Woets and Van der Linden 1982, Wardlow 1985, Minkenberg and Van Lenteren 1986). This situation has led to the development of biological control of leafminers by releases of the native *D. sibirica* (Hymenoptera: Braconidae) (Hendrikse et al. 1980, Minkenberg and Van Lenteren 1986). Seasonal inoculative releases are applied at the beginning of the growing season and the parasitoids are introduced during a short space of time, preferably a few times during one host generation. This method is usually applied against multivoltine pests during one growing season. Contrary to inundative biological control, the number of natural enemies released is relatively small, which makes it more attractive for growers from a financial standpoint. Parasitoids are now commercially available and have been used on glasshouse vegetables under integrated control in northwest Europe since 1984 (Hussey and Scopes 1985, Minkenberg 1988c, Van Lenteren and Woets 1988). This technique has been applied over an estimated area of 100 ha in 1987 (Nedstam et al. 1987). Also biological control has been initiated against leafminers in ornamentals and plants grown for seed production (Heinz et al. 1988, Jones et al. 1986, Parrella et al. 1987b, Wardlow 1985). Very low pest densities can be achieved by weekly releases of natural enemies during the entire season, i.e. inundative biological control. However, phytosanitary restrictions on the export of plant material, e.g. on the presence of natural enemies, might hinder the development of integrated control programs in ornamentals in Europe (Van Lenteren 1987).

The palaearctic species *D. sibirica* is a solitary endoparasitoid. All larval instars of *L. bryoniae* and *L. trifolii* are accepted for oviposition by *D. sibirica* females, although long mines with old larvae are found more often than young ones and are preferred for oviposition (Hendrikse et al. 1980). The adults emerged from fly puparia, usually in the soil. This parasitoid was chosen for evaluation because: (1) it oviposits into both *Liriomyza* spp. and successfully develops on them; and (2) it is well adapted to the glasshouse ecosystem, e.g. it can overwinter there. Nevertheless, additional data are needed prior to determine whether *D. sibirica* is the most suitable choice as a biological control agent of leafminers.

In biological control where inoculative or seasonal inoculative natural enemy releases are made, it is generally assumed that, to be effective, a parasitoid should have a high reproductive capacity. From December until July, *D. sibirica* is considered to be an effective biological control agent of leafminers on tomato in northwest Europe. It is therefore hypothesized that the potential reproduction of *D. sibirica* is high at the temperatures commonly occurring in glasshouses at that time.

The main objective of this research was to determine the reproductive capacity of *D. sibirica* at three constant temperatures (15°C, 20°C and 25°C) that corresponded to the mean glasshouse temperatures from December to July.

Materials and methods

Rearing. The agromyzid *L. bryoniae* was reared in a glasshouse at ca. 20 °C ± 5 °C at 80% ± 10% RH on tomato cv. Moneydor (for details, see Minkenberg and Helderman 1990). The braconids, *D. sibirica* (circa 100 individuals), were collected in commercial tomato glasshouses in the Netherlands and reared at ca. 22 °C at 50% RH in a controlled environment room. Tomato plants infested with *L. bryoniae* larvae were exposed for 2 to 3 days to *D. sibirica* put in cages (60 by 60 by 60 cm) for parasitization.

Fecundity and longevity. Fly pupae were chosen randomly from the parasitoid colony. On the morning of emergence, i.e. Day 1, single female parasitoids were put into cylindrical cages (described by Minkenberg and Helderman 1990) in controlled environment rooms at 15 °C, 20 °C or 25 °C. A tomato plant, 50 cm high and infested by 25 to 30 second or third instars of *L. bryoniae,* was introduced into each cage together with 2 or 3 *D. sibirica* males. The parasitoids were not provided with food. Temperatures were accurate to ± 0.5 °C and RH was 65-80% ± 10%. The photoperiod was 16L:8D with light intensity equivalent to 30-80 W/m^2 from 0100 h to 1700 h. Plants infested with larvae were replaced daily between 1600 h and 1700 h before the onset of the scotophase.

The fecundity of individual parasitoid females was estimated daily by counting the number of eggs and first instars. To do so, all leafminers from the infested plants had to be dissected under a microscope after the exposure; this was done after a few days. Because parasitoid eggs increase dramatically in size during the first days, they are readily visible. Female parasitoids that died before 1700 h on Day 1 or did not lay any eggs, were excluded from analysis.

Statistical analysis. Analysis of variance and Tukey's studentized range test were used to compare the effect of temperature on fecundity, longevity and oviposition period (i.e., longevity without postoviposition period) of *D. sibirica.* I used the Spearman rank test to analyze correlations between these life history variables. Cumulative percent oviposition per day was related to cumulative degree-days for all three temperatures using a threshold temperature for oviposition of 10.0 °C (Minkenberg 1988b).

Values of R_0 and r_m for *L. bryoniae* (Minkenberg and Helderman 1990) and *L. trifolii* (Minkenberg 1988b) previously have been estimated at the three temperatures. To estimate R_0 and r_m under similar conditions for *D. sibirica,* the developmental times and mortality of immatures were based on data from Nedstam (1985). Development at 15 °C took 32 days (value observed by Nedstam) and at 20 °C and 25 °C, 21 days and 15 days, respectively (estimated from developmental rate (1/day), i.e. the reciprocal of developmental time, regressed on temperatures between 12 °C and 27 °C: $Y = 0.0038\ X - 0.0272$, $r = 0.996$, $P < 0.0001$. Larval-pupal mortality at 15 °C was 19%

(observed value) and at 20°C and 25°C estimated to be 15% and 12%, respectively. Values for r_m at each temperature were calculated with the Lotka (1925) equation assuming a 1:1 sex ratio.

Results and discussion

Effect of temperature

Fecundity and oviposition period of *D. sibirica* decreased with increasing temperature (Table 4-1). Oviposition rate was highest at 20°C, but not significantly different from that at 15°C. The optimal temperature for oviposition defined as the temperature at which the oviposition rate is highest, was 20°C. Fecundity of *D. sibirica* at 25°C was substantially lower than at 20°C, indicating that 25°C was above the optimum and close to its upper threshold temperature for oviposition (Fig. 4-1).

Table 4-1. Fecundity, oviposition period, longevity and oviposition rate of *D. sibirica* female parasitoids at three constant temperatures (mean ± S.E., range between brackets).

	Temperature (°C)		
Variable	15	20	25
Fecundity (eggs)	225 ± 52 a (31 - 499)	94 ± 24 b (10 - 318)	48 ± 7 b (14 - 92)
Oviposition period (days)	18.4 ± 3.7 a (4 - 36)	6.0 ± 0.7 b (3 - 10)	5.6 ± 0.4 b (4 - 8)
Longevity (days)	20.2 ± 3.6 a (8 - 38)	-*	7.4 ± 1.0 b (4 - 15)
Oviposition rate (eggs/day)	11.4 ± 0.7 ab (7.8 - 13.9)	14.2 ± 2.2 a (2.5 - 31.8)	8.6 ± 1.0 b (1.3 - 13.1)
N	9	12	12

Tukey's studentized range test, $\alpha = 0.05$; means of a life-history variable followed by a different letter across rows differ significantly. * No data available.

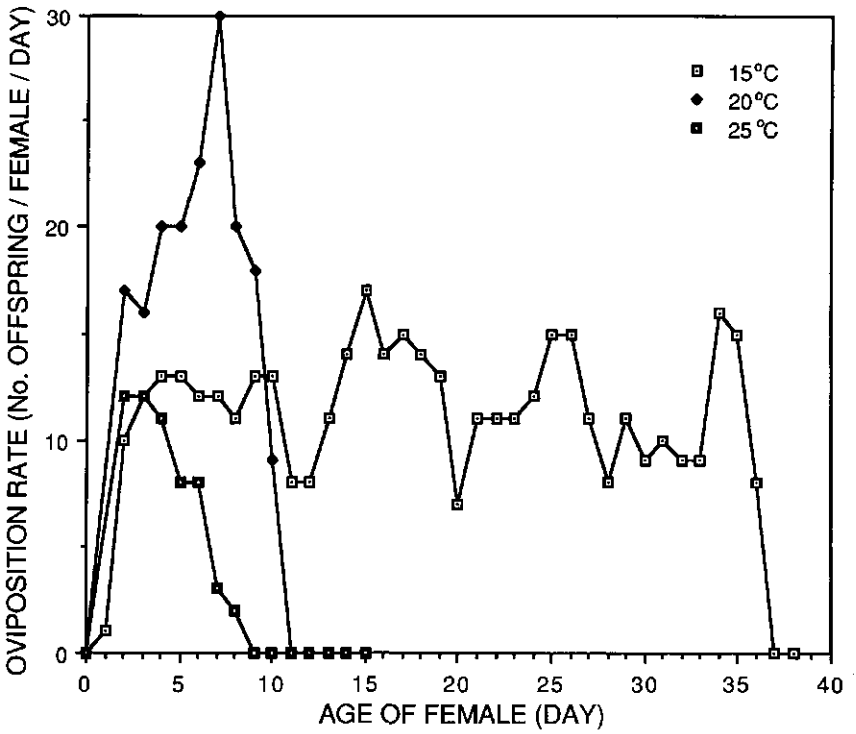

Fig. 4-1. Gross reproduction of *D. sibirica* at three constant temperatures.

The mean oviposition rate of ca. 10 eggs per day at 15°C was relatively constant during most of the female's life span, whereas it peaked at 20°C and 25°C. The fecundity was highly correlated with the oviposition period at 15°C and 20°C (r_s = 0.97, P < 0.0001 and r_s = 0.93, P < 0.0001, respectively). Survival was highest at 15°C, which was much greater than at 20°C and 25°C (Fig. 4-2). Survivorship at the latter two temperatures were similar. Mean fecundity at 22°C for *D. sibirica* estimated by Hendrikse et al. (1980), i.e. 56 eggs, is consistent with our results.

Preoviposition periods were not found for *D. sibirica*, which is a synovigenic species. Furthermore, the female did not sting hosts to feed, although occasionally individuals were seen feeding on the exudate from an oviposition opening.

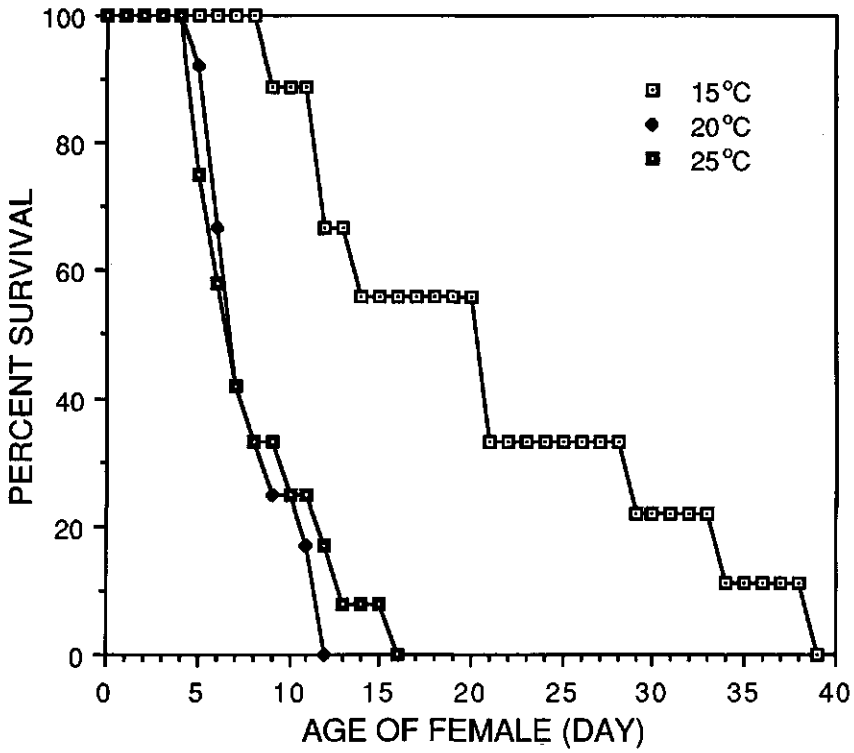

Fig. 4-2. Survival of *D. sibirica* at three constant temperatures.

The graph of cumulative percent oviposition vs. cumulative degree-days (Fig. 4-3) shows the oviposition rate at 20 °C and 25 °C deviating from the curve at 15 °C. This indicates that reproduction does not respond rapidly to changes in temperature. At the higher temperatures, 90% of the eggs were laid after 75 degree-days, whereas at 15 °C after 125 degree-days. Temperature apparently affected the performance of *D. sibirica* in a non linear way, which implies that predictions can not be made on basis of heat units, at least within the range 15-25 °C.

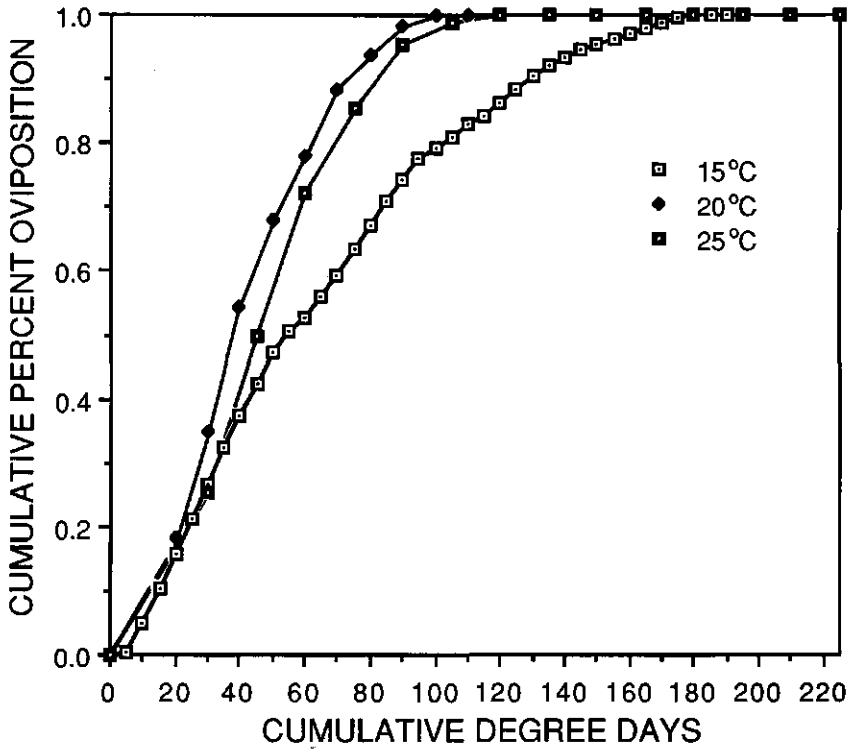

Fig. 4-3. Cumulative percent oviposition per day by *D. sibirica* vs. cumulative degree-days at three constant temperatures. A lower threshold of 10.0 °C was used to calculate degree-days.

The generation time was 45 days at 15°C, 26 days at 20°C and shortest at 25°C, 19 days. Net reproduction, R_0, decreased from 91 at 15°C to 21 at 25°C (Table 4-2), but this decrease at 25°C was compensated for by a large decrease in developmental time, that resulted in a higher r_m at 25°C than at either 20°C or 15°C. However, it is not known if there are any differences in the viability of parasitoid offspring developing at different temperatures.

Table 4-2. Net reproduction rate (female eggs/female/generation), R_0, and intrinsic rate of increase (female eggs/female/day), r_m, of the parasitoid *D. sibirica* on *L. bryoniae* at different temperatures.

	Temperature (°C)		
	15	20	25
R_0	91	40	21
r_m	0.107	0.145	0.163
T	45	26	19

Values partly based on developmental times and pre-adult mortality data from Nedstam (1985).

Evaluation of D. sibirica for biological control

Life-history data suggest that biological control will be more successful for *L. trifolii* than for *L. bryoniae,* because tomato is a less suitable host plant for *L. trifolii* (Minkenberg and Helderman 1990). It is known from experience that *D. sibirica,* if appropriately introduced, suppresses *Liriomyza* populations on tomato in commercial glasshouses in the Netherlands from December until July. After mid-May another indigenous parasitoid, *Diglyphus isaea* (Walker), usually invades glasshouses and plays a part in the control of leafminer populations. A similar cycle, namely initial parasitization by *D. sibirica* and later by *D. isaea* immigrating into the glasshouse, has been observed on *Liriomyza* leafminers in English and Scandinavian tomato glasshouses. A similar pattern has been observed in biological control of the leafminer *Chromatomyia syngenesiae* (Hardy) on chrysanthemum (Wardlow 1985). The invader seems to be an essential component of this biological control method.

To compare potential reproduction of the parasitoid, reproductive potential was expressed as net reproduction (R_0) rather than the intrinsic rate of increase (r_m). The use of the life table variable r_m is inappropriate because, by following Lotka (1925), a stable age distribution in the population is assumed. In Dutch glasshouses, the pest population is usually composed of a single developmental stage in the beginning, e.g. *L. bryoniae* adults simultaneously emerged after diapause (Minkenberg and Van Schelt unpubl.), and subsequently the pest population increases in numbers. Throughout the entire glasshouse, leafminer generations do not overlap for at least three generations. There are about 8 to 10 leafminer generations on tomato per growing season, i.e. per year. To predict population development in such situations, it is assumed better to compare R_0 of the parasitoid at different temperatures or with that of other parasitoids rather than their respective r_m's. In other areas, e.g. south Europe, where an approximate stable age distribution might be achieved after a few months, r_m seems to be more appropriate to compare population development of parasitoids. However, data on the age distribution of the insects in the glasshouse or in the field are lacking.

The R_0 and r_m values are estimated under optimal conditions in the laboratory and may represent a maximal value for the conditions specified. I do not assume that reproduction estimated approximates values occurring in the glasshouse and they are unlikely to have a single precise value because other factors than temperature apparently play a significant role in the glasshouse (Westerman and Minkenberg 1986). But these life history data indicate the natural limits and therefore, they might be useful for evaluation of parasitoids.

Development of *D. sibirica* is well synchronized with that of its hosts, allowing a comparison on the basis of R_0. Because its potential reproduction is lower as average temperatures rise above 20 °C, it is expected that *D. sibirica* might be less effective at high temperatures, assuming that other operating factors stay equal. One such important factor is host density, making that probably only a part of the reproduction is realized, particularly at the low host densities in commercial glasshouses. In summary, the results suggest that *D. sibirica* becomes less effective as a biological control agent of leafminers, *L. bryoniae* in particular, at high average temperatures.

In seasonal inoculative biological control the parasitoids should control a pest over numbers of generations. The reproductive capacity of a parasitoid is of importance for the success of a biological control method, but in addition to, for instance, the density of the pest insect relative to that of the parasitoid at the moment of parasitoid introduction. Therefore, parasitoids cannot be simply discarded because of their low reproductive capacity, relative to that of their host. Conversely, a relatively high reproductive capacity of a parasitoid does not necessarily confer effectiveness, because other factors might constrain success.

Reproduction realized by parasitoids is expected to be influenced by, for example, the way of searching for pest insects. Knowledge of the specific mechanisms used in host location and selection may allow accurate forecasting of searching capacity. Examination of searching at low host densities is necessary for a thorough evaluation of its ability as a biological control agent.

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5

Temperature effects on the life history of the eulophid wasp *Diglyphus isaea*, an ectoparasitoid of *Liriomyza* leafminers[1]

Abstract

The effect of constant and alternating temperatures on the life history of *Diglyphus isaea*, as an ectoparasitoid of *Liriomyza* leafminers on tomato plants (cv. Moneydor), was examined in the laboratory.

Parasitoid development and size were significantly affected by temperature. Males showed a shorter developmental time and pupal size than female parasitoids. Both developmental time and pupal size in *D. isaea* differed according to the host species. Pupal size showed no consistent relationship with temperature. The lower thermal threshold for development and oviposition was determined. Fecundity with *L. bryoniae* as host did not differ significantly between temperature regimes. Furthermore, larger females did not produce more offspring. Development and reproduction under the alternating temperature regime did not differ from those at the comparable constant temperature, suggesting a rapid response to changes in temperature.

The intrinsic rate of increase (r_m) and net reproduction (R_0) of *D. isaea* nearly doubled from 15°C to 25°C and 20°C, respectively, and generation time at 25°C was less than half that at 15°C. Because the parasitoid's population growth is higher than that of these pest insects at all temperatures, *D. isaea* is a promising candidate for seasonal inoculative biological control of *Liriomyza* on tomato crops in Scandinavian and western European glasshouses. Possible constraints to its effectiveness are discussed.

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Introduction

The originally palaearctic species *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) is now a generally occurring, primary larval ectoparasitoid of a number of leafmining diptera on herbaceous plants in Europe, northern Africa and Japan. Through recent inoculative introductions into the USA (including Hawaii), Canada and New Zealand, this species has become cosmopolitan (Hara, 1986, Cameron et al. 1987). The biology of *D. isaea* and related parasitoid species has been reviewed by Minkenberg and Van Lenteren (1986). Female *D. isaea* accepts only late larval instars of *Liriomyza* for oviposition but feeds on all larval instars whereby, without ovipositing, she stings these hosts many times to feed upon the exudate (Minkenberg and Van Lenteren 1987).

Diglyphus isaea has become important in several southern European countries, in both glasshouses and open fields as a biological control agent of agromyzid leafminers in vegetable crops (Pena 1983, Lyon 1986, Nedstam et al. 1987, Van Lenteren and Woets 1988). In Scandinavia and western Europe, *D. isaea* is only predominant during summer, causing substantial mortality of leafminers in glasshouse crops (Hendrikse et al. 1980, Woets and Van der Linden 1983, Wardlow 1984, 1985). Experimental use of *D. isaea* in integrated control programmes on chrysanthemum and tomato indicate that it is an ineffective control agent in winter and spring, suggesting that low temperature may limit the development and reproduction of this parasitoid. However, there are too few data to critically evaluate this hypothesis.

The main objective of the research reported here was to determine the reproductive capacity of *D. isaea*, measured as the intrinsic rate of increase, r_m, at three constant temperatures that correspond to typical glasshouse temperatures from winter to early summer (15°C to 25°C). In addition, r_m was estimated under an alternating temperature regime in order to determine how development and reproduction of *D. isaea* respond to fluctuations in temperature. Intrinsic rates of increase of its hosts *Liriomyza trifolii* (Burgess) and *Liriomyza bryoniae* (Kalt.) have previously been estimated under similar conditions (Minkenberg 1988b, Minkenberg and Helderman 1990). If the reproductive capacity of *D. isaea* exceed those of its hosts at comparable temperatures, *D. isaea* may be considered a promising candidate for biological control of these leafminers (Van Lenteren 1986a).

Materials and methods

Glasshouse colonies. L. bryoniae and *L. trifolii* were reared at ca. 20°C and ca. 25°C, respectively, at 70% ± 10% RH, on tomato cv. Moneydor (for details, see Minkenberg 1988b, Minkenberg and Helderman 1990). *Diglyphus*

isaea was cultured at ca. 25°C at 80% RH in an experimental glasshouse with *L. bryoniae* as host.

Temperature regimes. All experiments were carried out in controlled environment rooms at 15°C, 20°C and 25°C, and at the alternating temperature of 18-22°C, mean 20.3°C. The alternating temperature increased linearly from 0100 to 0300 h, decreased from 1500 to 1700 h, was fixed at 22°C from 0300 to 1500 h and at 18°C from 1700 to 0100 h. Temperatures were accurate to ± 0.5°C, RH was 70% ± 10%. The photoperiod was 16:8 (light: dark) with the photophase from 0100 h to 1700 h (light intensity 30-80 W/m^2).

Development/mortality. Eight tomato plants cv. Moneydor, about 4 weeks old with six leaves, each infested with 20 to 40 third larval instars of the host, *L. trifolii* or *L. bryoniae,* were exposed to about 100 parasitoids from 0900 to 1100 h. The plants then were transferred to the environment rooms. At 16.00 h development time and percent mortality of immatures were measured by examining all individuals every eight hours.

Developmental time was measured as the time from hatching to pupation. Pupal length (± 0.01 mm) was measured as a possible index for fitness. The onset of development was assumed to be the time of plant transfer, i.e. 1100 h. The pre-pupal phase, i.e. after construction of a pupal chamber with meconial pillars, was included in the pupal developmental time. Mean developmental times for each instar were calculated only for those individuals that reached adulthood.

Host feeding/fecundity/longevity. Parasitoid pupae were randomly chosen from the glasshouse colony. After measuring their length, they were placed singly in small glass vials. On the morning of emergence, i.e. Day 1, the females were released into cylindrical cages (described by Minkenberg and Helderman 1990) in the controlled environment rooms. Each cage contained a tomato plant infested with 30-40 third instar *L. bryoniae* and 2-3 *D. isaea* males. The parasitoids were not provided with sugar. Plants were changed daily between 1600 h and 1700 h, before the onset of the scotophase.

A few days after exposure when healthy leafminers (recognizable by their violently moving mouth hooks) left the plants, all mines within the leaves of exposed plants were dissected under a microscope using transmitted light, in order to estimate host feeding and fecundity of the parasitoid females. Host feeding by individual females was estimated by daily counts of leafminers, which once fed upon remained as empty sacks with up to a dozen black spots, probably caused by stings of parasitoid females (Fig. 5-1). However, it was not always possible to determine the cause of death (natural or by host feeding); recently punctured leafminers did not show any melanization, whereas old dead leafminers were almost decayed. But the stung leafminers were recognizable by the additional amount of frass.

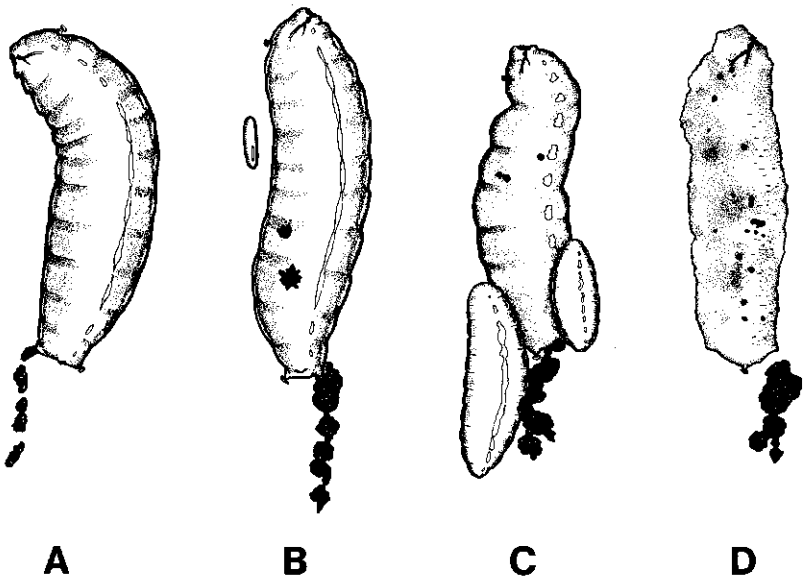

Fig. 5-1. Third instar leafminers *(Liriomyza),* ca. 1.8 mm, under different conditions: A) healthy leafminer; B) turgid paralysed leafminer with a *D. isaea* egg close by; C) leafminer stung only a few times for paralyzation with *D. isaea* larvae eating on it and D) dead leafminer after host feeding with many black spots characteristic for stings.

To estimate fecundity, turgid paralyzed leafminers with parasitoid eggs or larvae were counted (parasitized hosts, whose development was apparently stopped, were usually stung only once or a few times). Parasitoid females which died before 1700 h on the first day or did not lay any eggs or laid only infertile eggs, were excluded from analysis.

Observed lower thresholds for development and oviposition. The lower thresholds for development were determined by daily observing the third larval instars and pupae maintained in growth cabinets, with one plant per cabinet at a constant temperature. Development was followed at 13 °C and successively lower temperatures at 1 °C intervals until development ceased.

The temperature threshold for oviposition was determined by a similar procedure. Ten adult females were kept in a cage containing an infested tomato plant for several weeks in a growth cabinet. The exposed plant was then incubated at 20 °C and checked for parasitoid offspring.

Statistical analysis. The effect of temperature on development was determined by linear regression analysis (General Linear Models [SAS, 1985]). A t-test was used to determine whether observed mean developmental rate (1/day), i.e. 1/developmental time, under the alternating temperature regime, mean 20.3°C, differed significantly from that predicted for the same temperature (Sokal and Rohlf 1981), the predicted value being calculated from the linear regression equation based upon developmental rates at constant temperatures. Differences in developmental time and pupal length between sexes were analyzed using the Wilcoxon 2-sample test and the Students t-test, respectively. Correlations between developmental time and pupal length were analyzed by the Spearman rank correlation test. The effect of temperature on developmental time, host feeding, fecundity, longevity (without postoviposition period), host feeding rate, oviposition rate and preoviposition period was determined by analysis of variance, followed by a Tukey's studentized range test. Using a threshold temperature for oviposition of 10.0°C, we compared the relationship between cumulative percent oviposition per day and cumulative degree-days for *D. isaea, L. trifolii* and *L. bryoniae.* The intrinsic rate of increase (r_m) at each temperature was calculated by the Lotka equation (1925). It should be noted, however, that while we assumed a 1:1 sex ratio in this equation, *Diglyphus* spp. occasionally exhibit sex ratios that are extremely male biased (e.g., Hendrickson and Barth 1978; Coote and Ellis 1986). This is apparently related to the relatively small size of these agromyzid hosts (Heinz and Parrella 1990b).

Results and discussion

Effect of temperature and host species on D. isaea development.

Temperature and sex strongly affected the developmental time of *D. isaea* (Table 5-1A). Egg-to-adult development on *L. trifolii* was about 2.5 times as fast at 25°C than at 15°C. Mean developmental times for *D. isaea* pupae were usually slightly shorter than its egg-larval developmental time, as reported previously for *D. intermedius* (Patel and Schuster 1983).

Males developed faster than females at 20°C and 20.3°C (Wilcoxon 2-sample test: $Z = 4.35$ and $Z = 3.97$, respectively, $P < 0.0001$ for both). Therefore, results on development were split with respect to the sexes. This phenomenon has also been found in *D. isaea* on the chrysanthemum leafminer *Chromatomyia syngenesiae* (Hardy) (Cheah 1987). Male pupae were significantly shorter than female pupae, only at 20°C and 25°C (t test: $t = 4.11$ and $t = 3.69$, respectively, $P < 0.0005$ for both).

Although, for insects in general, size is inversely related to temperature within the normal temperature range (Hinton 1981), like demonstrated for the parasitoids *Cotesia glomeratus* (L.), *Cotesia rebecula* (L.) and *Pteromalus*

Table 5-1A. Mean developmental times (days) and pupal lengths (mm) reached by *D. isaea* females and males at three constant and one alternating temperatures [mean ± SE] (range) on *L. trifolii*.

Stage	Temperature (°C)			
Female	15	20	25	20.3 (18-22)
Egg-larval	13.3 ± 0.3 (10.8 - 16.2)	8.8 ± 0.1 (7.8 - 10.2)	5.0 ± 0.1 (4.1 - 7.3)	8.2 ± 0.1 (7.2 - 9.2)
Pupal	12.8 ± 0.3 (10.7 - 14.7)	7.9 ± 0.1 (7.3 - 8.7)	5.5 ± 0.1 (4.0 - 6.0)	7.4 ± 0.1 (6.7 - 8.3)
Total	26.0 ± 0.3 (23.5 - 29.2)	16.6 ± 0.1 (15.8 - 17.8)	10.5 ± 0.1 (8.8 - 11.7)	15.7 ± 0.2 (14.8 - 16.8)
Pupal length	1.51 ± 0.05 (1.01 - 1.86)	1.47 ± 0.03 (1.17 - 1.70)	1.55 ± 0.03 (1.25 - 1.92)	1.55 ± 0.04 (1.23 - 1.81)
N	21	22	36	18
Male				
Egg-larval	14.5 ± 0.8 (11.5 - 18.2)	8.4 ± 0.1 (7.8 - 9.2)	5.4 ± 0.2 (4.1 - 7.3)	7.9 ± 0.1 (7.2 - 8.5)
Pupal	12.3 ± 0.3 (11.0 - 13.3)	7.4 ± 0.1 (6.3 - 8.0)	5.1 ± 0.1 (4.3 - 6.0)	6.9 ± 0.1 (6.3 - 7.7)
Total	26.8 ± 0.9 (22.5 - 30.9)	15.8 ± 0.1 (14.8 - 16.8)	10.3 ± 0.2 (8.4 - 12.3)	14.7 ± 0.1 (14.1 - 15.8)
Pupal length	1.42 ± 0.08 (1.09 - 1.69)	1.29 ± 0.03 (0.97 - 1.67)	1.41 ± 0.03 (1.15 - 1.80)	1.47 ± 0.04 (1.23 - 1.78)
N	8	32	32	20

puparum (L.) (Nealis et al. 1984), the pupal length of *D. isaea* showed no linear relationship with temperature (Table 5-1A; e.g., linear regression analysis for females, r = 0.10, P > 0.36). Furthermore, pupal length was not significantly correlated with egg-larval, pupal or total developmental time. These results do not agree with the general observation that developmental time and size of individuals raised under the same conditions are correlated in insects (Gordon 1984, Ratte 1985).

On *L. bryoniae,* developmental time and pupal size were also significantly affected by temperature and sex (Table 5-1B). *Diglyphus isaea* significantly faster developed on this host than on *L. trifolii* at 15°C and 25°C (Z = -2.24, P < 0.03 and Z = -3.08, P < 0.002, respectively). At 25°C, *D. isaea* pupae from *L. bryoniae* were significantly larger than those from *L. trifolii* (females, t = -2.17, P = 0.04). Thus, *L. bryoniae* seems to be the more suitable host. The developmental times reported here on both *L. bryoniae* and *L. trifolii* are much faster than on the leafminer *C. syngenesiae* (Ibrahim and Madge 1979, Cheah 1987). Since *L. bryoniae* is smaller than *C. syngenesiae,* differences in developmental time of *D. isaea* are probably due to differences in host 'quality' rather than host size. The differences in size on the different hosts is in contrast with the general pattern (Ratte 1985) where size of the adult is independent of host quality (if enough food is available).

Equations of the rate of development against temperature were calculated, assuming that the mean developmental rates, i.e. the reciprocals of developmental times, were linearly related to temperature between 15°C and 25°C. (Table 5-2). The slope of the regression of this parasitoid was relatively steep compared with that of its hosts (Minkenberg 1988b, Minkenberg and Helderman 1990), implying that the developmental rate of the parasitoid stages within the mine was strongly raised by a slight increase in ambient temperature. The nearctic species *D. intermedius* on the vegetable leafminer *Liriomyza sativae* Blanchard develops faster than *D. isaea* on *L. trifolii,* but its developmental rates are similar to *D. isaea* on *L. bryoniae* (from the 15.5°C to 26.7°C data of Patel and Schuster [1983]: Y = 0.0061 X - 0.0507, r = 0.999, and predicted developmental times at 15°C, 20°C and 25°C are 24.4 days, 14.0 days and 9.8 days, respectively).

The estimated lower threshold temperatures for development were approximately 9.5°C for eggs and 8°C for pupae (Table 5-2). Since in these experiments, all larvae and some pupae of *D. isaea* developed to the next instar at 6°C, the actual thresholds are probably ca. 3°C lower than those estimated. For poikilothermic animals, the threshold estimated by the X intercept method is usually higher than the observed threshold, because the curve of the developmental rate versus temperature has a sigmoid shape (Laudien 1973). This study also indicates that the X intercept method is too inaccurate and therefore not useful to estimate lower threshold temperatures.

Table 5-1B. Mean developmental time (days) and pupal lengths (mm) reached by *D. isaea* females and males at two constant temperatures [mean ± SE] (range) on *L. bryoniae*.

	Temperature (°C)				
Female	15	25	Male	15	25
Egg-larval	13.7 ± 0.2 (13.2 - 14.2)	4.7 ± 0.2 (4.1 - 6.1)		13.0 ± 0.8 (11.9 - 14.5)	4.8 ± 0.1 (4.5 - 5.5)
Pupal	11.8 ± 0.2 (11.3 - 12.7)	5.2 ± 0.1 (4.7 - 6.0)		10.4 ± 0.2 (10.0 - 10.7)	5.0 ± 0.1 (4.7 - 5.3)
Total	25.5 ± 0.3 (24.5 - 26.2)	9.8 ± 0.2 (9.1 - 11.5)		23.4 ± 0.9 (22.5 - 25.2)	9.7 ± 0.2 (8.5 - 10.5)
Pupal length	1.58 ± 0.06 (1.44 - 1.73)	1.67 ± 0.05 (1.15 - 1.86)		1.37 ± 0.08 (1.25 - 1.54)	1.53 ± 0.09 (1.09 - 1.92)
N	5	15		3	9

Table 5-2. Equations of developmental rate (1/day), Y, for *D. isaea* females and males on *L. trifolii* (A) or *L. bryoniae* (B) on temperature (°C), X, and estimated threshold temperatures (°C) for development

Female	Regression equations	Estimated threshold temperature
A)Egg-larva	Y = 0.0125 X - 0.1196 (r = 0.98, P = 0.14)	9.6
Pupa	Y = 0.0106 X - 0.0813 (r = 0.999, P = 0.03)	7.7
Total	Y = 0.0057 X - 0.0502 (r = 0.99, P = 0.09)	8.7
B)Total	Y = 0.0063 X - 0.0550 (r = 1)	8.8
Male		
A)Egg-larva	Y = 0.0120 X - 0.1122 (r = 0.99, P = 0.06)	9.4
Pupa	Y = 0.0115 X - 0.0926 (r = 0.999, P = 0.03)	8.0
Total	Y = 0.0060 X - 0.0538 (r = 0.997, P = 0.05)	9.0
B)Total	Y = 0.0060 X - 0.0478 (r = 1)	7.9

Regression were done using means of each temperature treatment (N = 3 for *L. trifolii* and N = 2 for *L. bryoniae*). Only observations from constant temperatures were used. The X intercept method used to calculate thresholds.

At the alternating temperature, the observed rates for the total development of females, 0.0638 (1/day) and males, 0.0680 (1/day) did not differ significantly from those predicted from the regression equations at 20.3°C (females and males: 0.0655 (1/day) and 0.0680 (1/day), respectively; females: $z = 0.87$, $P > 0.05$; males: means are the same). This shows that the development of *D. isaea* responds rapidly to temperature changes within the range 18-22°C, which agrees with the general rule (Ratte 1985, p. 55), that temperature changes around the inflexion point of the curve of developmental rate versus temperature cause neither a considerable retardation nor an acceleration in development. The relationship between the developmental rate of *D. isaea* on *C. syngenesiae* and temperature was also found to be linear between 19°C and 25°C (Cheah 1987). However, the mean developmental rate at 16°C diverged from the regression line, suggesting that the lower food quality of this host raises the lower thermal threshold (female, 12.8°C; male, 12.9°C) in comparison with *Liriomyza* spp. (see Table 5-2). At present, one can only speculate about causes for the differential performance of *D. isaea* with regard to host species.

D isaea adults nearly all emerged in the light, primarily in the morning; 83% emerged by 08.00 h, i.e. within 7 h after the beginning of the photophase, 14% between 0800-1600 h and 3% during 1600-2400 h. However, we found no diurnal trend in larval molting. In conclusion, *D. isaea* on tomato is best reared on a temperature of 25°C with *L. bryoniae* as host.

Effect of temperature on mortality

Mortality was significantly higher at 15°C than at either 20°C or 25°C (Table 5-3). Mortality at the fluctuating temperature did not differ from that at the constant temperature of 20°C. Most deaths occurred during the egg-larval stage. These observations agree with the conclusion of Ratte (1985) that mortality versus temperature curves of insects are generally U-shaped.

Effect of temperature on host feeding, fecundity and longevity

There was significantly more host-feeding activity by adult females at 15°C than at the other temperatures (Table 5-4), but no significant differences in fecundity between the different temperatures. There are only a few examples of insect species with a fecundity that is not significantly affected by temperature within such a temperature range (Hinton 1981).

The percentage of attacked leafminers used for host feeding varied from 15% to 40%. Similar results are found for *D. isaea* on the chrysanthemum leafminer *C. syngenesiae,* where host feeding accounts for almost a third of larvae attacked (Ibrahim and Madge 1979). In contrast, *Diglyphus pusztensis* (Erdös and Novicky) kills far more hosts (*Phytomyza ranunculi* Schrank) for

Table 5-3. Percentage of mortality of *D. isaea* preadult instars on *L. trifolii* at different temperatures

Stage	Temperature (°C)			
	15	20	25	20.3
Egg-larval	52	15	23	7
Pupal	3	4	0	7
Total	54 a	18 b	23 b	14 b
No. eggs	63	66	88	44

Means followed by a different letter differ significantly; G-test ($P < 0.05$) on numbers of fully developed versus dead immatures.

feeding than for oviposition at low temperatures, 13-15°C (Sugimoto et al. 1982). The ectoparasitoid of leafminers *Chrysocharis pentheus* (Walker) uses ca. 50% of the hosts killed for feeding (Sugimoto and Ishii 1979), whereas the endoparasitoid *Chrysocharis parksi* Crawford feeds on only ca. 25% of the leafminers attacked (Christie and Parrella 1987). Host feeding substantially contributed to host mortality in all these parasitoid species.

The oviposition rate of *D. isaea* was significantly affected by temperature. It was highest at 25°C with 19 eggs per female per day compared to between nine and 13 eggs per female per day at the other temperatures. In general, the oviposition rate is maximal at temperatures approaching the upper threshold for oviposition, falling off steeply at higher and more gradually at lower temperatures (Bursell 1974). However, the oviposition rate at 15°C did not conform to this rule, making it impossible to use the X intercept method for estimating the lower threshold for oviposition. Furthermore, although the total egg production in insects reaches its maximum at a temperature slightly lower than the optimum temperature for the oviposition rate (Ratte 1985), this was not found for *D. isaea* between 15°C and 25°Ca.

D. isaea is a synovigenic species and showed a preoviposition period which varied from 1 to 3 days. The preoviposition period showed no consistent relationship with temperature. In contrast, the preoviposition period of *D. pusztensis* was clearly inversely related with temperature within this range (Sugimoto et al. 1982).

Both host feeding and oviposition rates were dependent on the age of the female (Fig. 5-2). Oviposition rate reaches its maximum on the 14th day after emergence (31 eggs/female) at 15°C, on the 9th day (21 eggs/female) at 20°C, on the 8th day (20 eggs/female) at 20.3°C and on the 10th day (35 eggs/female) at 25°C.

Table 5-4. Mean total host feeding (no. of leafminers), fecundity (no. of eggs), longevity (days), host feeding rates (no. of leafminers/day), oviposition rates (no. of eggs/day) and preoviposition periods (days) of *D. isaea* at different temperatures (mean ± SE; range)

	Temperature (°C)			
	15[a]	20	25	20.3 (18-22)
Host feeding[b]	[1]92 ± 26 a (84 - 269)	70 ± 4 b (56 - 90)	73 ± 24 b (9 - 197)	48 ± 6 b (19 - 92)
Fecundity	293 ± 37 a (66 - 446)	286 ± 20 a (196 - 411)	209 ± 47 a (64 - 441)	263 ± 30 a (74 - 406)
Longevity	23 ± 1 a (8 - 30)	32 ± 2 b (24 - 45)	10 ± 2 c (5 - 21)	21 ± 2 a (10 - 28)
Host feeding rate	8.1 ± 0.6 a (4.4 - 10.1)	2.2 ± 0.1 b (1.3 - 2.8)	5.8 ± 1.1 c (1.8 - 10.9)	2.2 ± 0.2 b (1.4 - 16.6)
Oviposition rate	12.7 ± 1.0 a (6.0 - 19.4)	9.0 ± 0.4 a (6.2 - 10.8)	18.9 ± 1.4 b (12.8 - 26.3)	12.6 ± 1.0 a (4.7 - 16.6)
Pre-oviposition period	1.4 ± 0.2 a (1 - 2)	2.2 ± 0.2 b (1 - 3)	1.1 ± 0.1 a (1 - 2)	2.0 ± 0.2 b (1 - 3)
N	11	10	9	12

Means with a different letter significantly differ; Tukey's studentized range test, $P < 0.05$. The 15°C experiment was ended after 26 days for 7 females.

Survival of females was lowest at 25°C (Fig. 5-3). Their longevity at 20°C was significantly higher than that under the alternating regime, but feeding, fecundity and oviposition rates were not (Table 5-4), suggesting a rapid response of the parasitoid's reproduction to temperature changes (cf. Table 5-5).

A lower threshold for oviposition at ca. 8°C was observed; at 5°C the parasitoids stayed motionless at the bottom of the cage. At 8°C, some females were observed on the plant and 12 days after their release two parasitoid larvae were found. Parasitoids could be kept alive for months at these low temperatures.

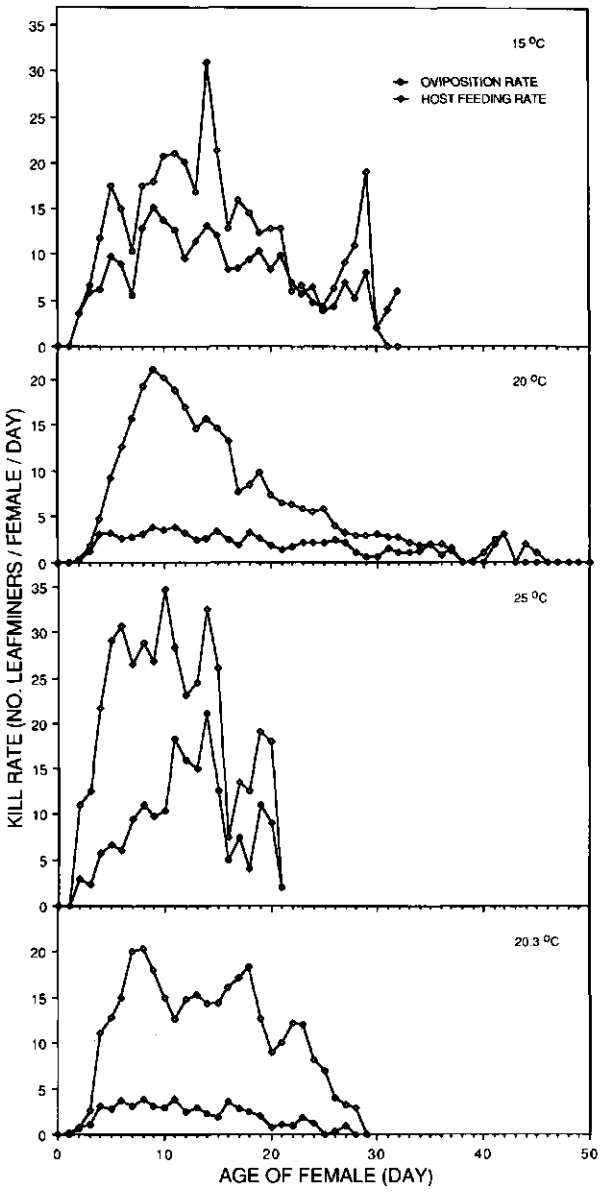

Fig. 5-2. Gross host feeding and reproduction of *D. isaea* at three constant and one alternating temperature, 18-22 °C (Note: the no. of leafminers killed by oviposition might be lower than presented here, because *D. isaea* is facultatively gregarious and may lay more than one egg per host).

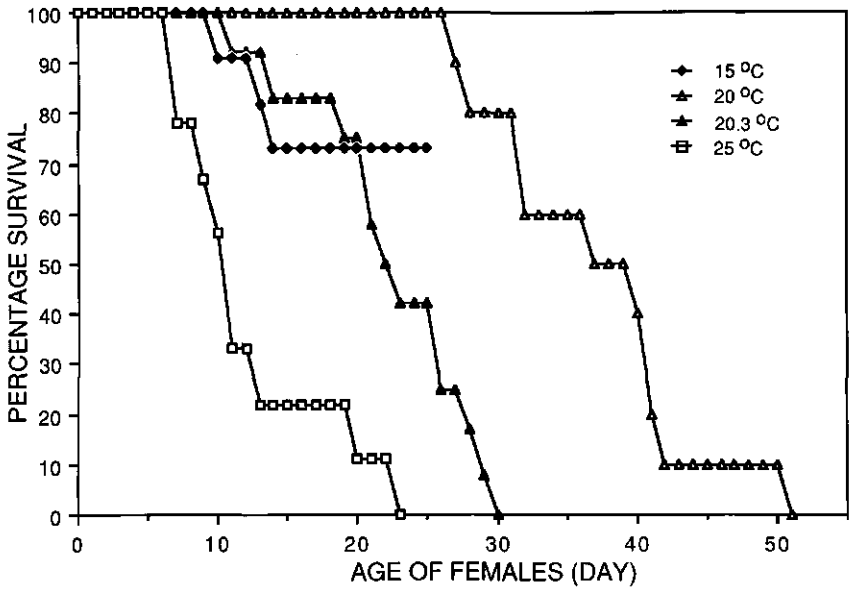

Fig. 5-3. Survival of *D. isaea* females at different temperatures (alternating temperature: mean = 20.3 °C, range 18-22 °C; the 15 °C experiment was ended after 26 days for 7 females).

Table 5-5. Intrinsic rate of increase, r_m, (female eggs/female/day), net reproduction, R_0, (female eggs/female) and generation time, T, (days) of *D. isaea* on *L. bryoniae* at different temperatures (sex ratio of 1:1 assumed).

Temperature (°C)	r_m	R_0	T
15	0.114	68	39
20	0.175	118	30
25	0.273	81	18
20.3 (18-22)	0.190	113	27

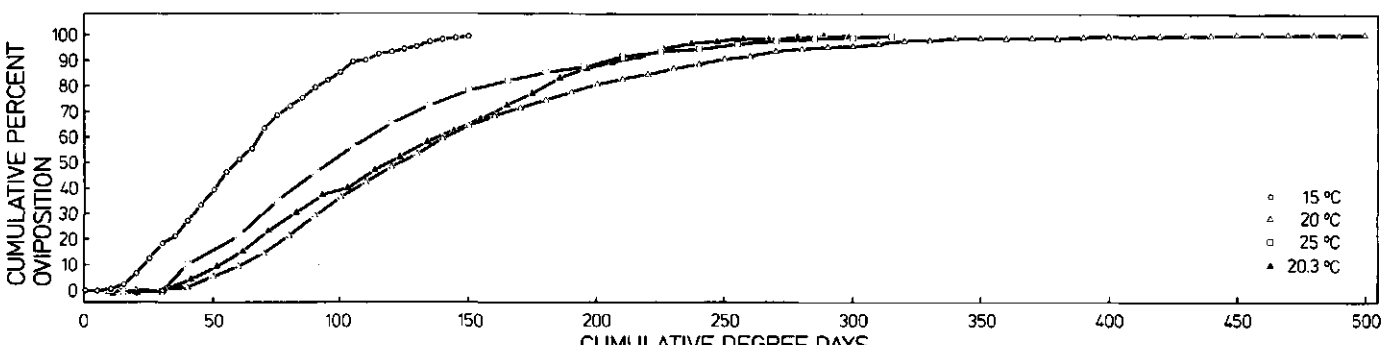

Fig. 5-4. Cumulative percent oviposition per day by *D. isaea* versus cumulative degree-days at different temperatures. A threshold temperature of 10.0 °C was used to calculate degree-days (alternating temperature: mean = 20.3 °C, range 18-22 °C).

Cumulative percent oviposition versus cumulative degree-days showed that 80% of the oviposition by *D. isaea* occurred after 200 degree-days within the range 20-25°C (Fig. 5-4), whereas the flies *L. trifolii* and *L. bryoniae* laid more than 80% of their eggs after only 100 degree-days (Minkenberg 1988b, Minkenberg and Helderman 1990).

Correlations between life-history variables

At 15°C, 25°C and the alternating temperatures, mean host feeding and fecundity were significantly and positively correlated (r_s = 0.63, P = 0.04, r_s = 0.90, P = 0.001 and r_s = 0.70, P = 0.01, respectively), and also for all but four of 41 individual females, fecundity was also significantly positively correlated with host feeding at all temperatures. *Diglyphus isaea* differs in this respect from the endoparasitoid *C. pentheus* where fecundity was found not to be correlated with host feeding. Though such a relationship may have been obscured because this parasitoid also feeds on previously parasitized leafminers. *Chrysocharis pentheus* uses ca. 64% of the attacked hosts (*P. ranunculi)* exclusively for host feeding (Sugimoto and Ishii 1979), a much higher percentage than that by *D. isaea.*

Fecundity was highly correlated with longevity at 15°C, 20°C, 25°C and 20.3°C (r_s = 0.78, P = 0.005, r_s = 0.63, P = 0.05, r_s = 0.97, P = 0.0001 and r_s = 0.77, P = 0.003, respectively), as reported for *C. pentheus* (Sugimoto and Ishii 1979), suggesting for *D. isaea* that host feeding is essential for increasing egg production and prolonging its life span.

Neither host feeding, fecundity nor longevity of *D. isaea* females was correlated with their original pupal length. Furthermore, assuming that pupal length reflects adult size, fitness of adult *D. isaea* females, defined by the number of offspring, seems to be independent of their size. In general, fecundity and size are positively correlated (Hinton 1981, Ratte 1985), like found in the parasitoids *C. glomeratus, C. rebecula* and *P. puparum* (Nealis et al. 1984). Apparently, host feeding by adult *D. isaea,* which is essential for egg production, influences fecundity far more than the size of female (for examples on the effects of food components on fecundity, see Engelmann 1984). Differences in rearing conditions such as temperature regime and host quality, may also affect the viability of the adult (Laudien 1973, p. 371-372).

Temperature effects on population growth

From the above results, the population growth of *D. isaea* is expected to be highest at 25°C, with an intrinsic rate of increase of 0.273 female eggs per females per day, and lowest at 15°C, with 0.114 female eggs per female per day (Table 5-5). The net reproduction indicates that under optimum conditions a *D. isaea* population may multiply 68 to 118 times every generation depending on the temperature, the estimated generation time varying from 4.4 weeks at 15°C to 2.5 weeks at 25°C.

Since temperature is a key factor in the population dynamics of insects, this sort of data is necessary to predict the reproductive potential of *D. isaea* populations in glasshouses. *D. isaea* populations in glasshouses are not exposed to temperatures lower than 15°C or higher than 25°C during winter and spring, the rapid response of development and reproduction to alternating temperatures found in this laboratory study is also likely be found in these glasshouse populations. However, these results may not apply in the field, if the temperature range is outside that investigated here, and moreover, natural longevity is probably shorter than that in the laboratory. Nonetheless, the results are an estimate of potential population growth to which field data can be compared.

In a temperate climate, the lower thermal thresholds for development of parasitoids in nature are in general higher than that of their hosts. The reason is supposedly that only those parasitoid individuals have been selected due to their simultaneous presence with host instars (Campbell et al. 1974, Campbell and Mackauer 1975, Sugimoto et al. 1982, Nealis et al. 1984). In contrast, the lower thermal threshold for development of the parasitoid *D. isaea* was similar to that of its host *L. bryoniae* and much lower than that of *L. trifolii*. This observation may be explained by the current artificial agroecosystem, viz. the practice of heated glasshouses under integrated control. The polyphagous parasitoid *D. isaea* only temporarily inhabits glasshouse crops, since it cannot hibernate in situ. Considering the lower thermal thresholds, the palaearctic species *L. bryoniae* found on British glasshouse tomatoes since 1926 (Speyer and Parr 1948), is better adapted to the glasshouse temperature regime and the tomato plant than the originally subtropical *L. trifolii* found only since 1980 in European glasshouse vegetables (Minkenberg 1988b, Minkenberg and Helderman 1990). Further comparison is limited by lack of information on the overwintering instar, place of overwintering and the part played by diapause.

Is host feeding a positive characteristic for an effective parasitoid?

Host feeding provides a protein diet for some parasitoid species; this is necessary for continued egg production (Bartlett 1964, Leius 1967). Other parasitoids, for which host feeding is unnecessary, can use every host they discover for reproduction; no time is lost in host feeding.

In seasonal inoculative biological control, the numbers of parasitoid offspring produced from hosts during the season is even more important than the numbers of pest insects killed just after the release of parasitoids. When evaluating a parasitoid as a biological control agent, it is therefore important that 'host-feeders' and 'non host-feeders' should be first compared in respect to their reproductive capacities rather than upon the, sometimes impressive, host-feeding habit of the 'host-feeder'. Furthermore, to quantify effectiveness in respect to host feeding, behavioral observations at the individual level followed by simulations with parasitoid-host models are necessary.

Is D. isaea a suitable candidate for biological control?

The parasitoid *D. isaea* developed faster, showed a lower mortality in its immature stages, the adults lived longer and produced more eggs than either of its hosts *L. trifolii* and *L. bryoniae* between temperatures of 15°C and 25°C. Hence, the population growth rates of this parasitoid were greater than those of the flies, particularly at the higher temperatures (Table 5-6). This study shows that the reproduction of *D. isaea* is not limited by the temperature regimes specific for western European glasshouses. Thus, basing our recommendations on its relative reproductive capacity, *D. isaea* is considered a suitable candidate for the biological control of leafminers on tomato.

Table 5-6. Intrinsic rates of increase, r_m, (female eggs/female/day) of *L. trifolii, L. bryoniae* and *D. isaea* on *L. bryoniae* at different temperatures (sex ratios of 1:1 assumed).

Insect	Temperature (°C)		
	15	20	25
L. trifolii	-0.002	0.102	0.125
L. bryoniae	0.046	0.116	0.184
D. isaea	0.114	0.175	0.273

Whether these results can be used to predict the effectiveness of *D. isaea* as a biological control agent depends upon the similarity between the experimental conditions where the parasitoid was offered a surplus of hosts within a limited space during the adult stage, and the conditions in a glasshouse. In Dutch glasshouses for several month after start of cultivation of the tomato plants in mid December *L. bryoniae* shows discrete generations. This means that adult parasitoids have to bridge a period of about one week before late leafminer instars suitable for oviposition appear. There is a similar situation in tomato fields on the west coast of Florida, where in spring no overlap occurs during two to three generations of *Liriomyza* leafminers; adults of *Diglyphus* parasitoids emerging from one leafminer generation apparently do not survive long enough to be able to oviposit on the next leafminer generation (Patel and Schuster 1983). The non-ovipositing females of some parasitoid species live longer, probably due to egg-resorption (Quednau 1967, Sugimoto et al. 1983a, Melton and Browing 1986). Further, there is always some variation in host appearance. But the suitable instar is only present for a short period of about one to four days, depending on temperature. However, a proportion of the host population will probably escape in time parasitoid attack due to generation asynchrony between the parasitoid and *Liriomyza* populations (e.g., Westerman and Minkenberg 1986). The asynchrony between parasitoid and leafminer generations can thus not be obviated by weekly introductions. Introductions repeated every leafminer generation are not commercially feasible yet.

Furthermore, because the densities of leafminers in glasshouses are usually far below 30 hosts per plant, the parasitoid will probably be unable to find sufficient hosts during her life span to realize her total potential reproduction. Thus, potential reproduction is likely to be limited by searching efficiency.

With the development of an evaluation procedure for the suitability of parasites for the biological control of pests using seasonal inoculative introduction (Van Lenteren 1986a), five criteria for selection have been proposed. Three of them, reproductive capacity, generation synchrony and searching efficiency, have been previously discussed by Minkenberg and Van Lenteren (1987). The present paper shows that the reproductive capacity of *D. isaea* is higher than that of the pest insects at all examined temperatures. But, it is only likely to realize a part of its reproductive capacity in glasshouses. Generation synchrony and searching efficiency must be further examined to gain insight into the effectiveness of *D. isaea* as an agent for seasonal inoculative biological control in western European glasshouses.

Acknowledgments

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6

Preference and performance of the herbivorous fly *Liriomyza trifolii* on tomato plants differing in leaf nitrogen[1]

Abstract

Host-plant selection by *Liriomyza trifolii* (Burgess) was examined on tomato plants, *Lycopersicon esculentum* Miller, containing 4.2, 4.5, 5.1, or 5.6% leaf nitrogen. In no-choice tests, the acceptability of plants for feeding and oviposition was not substantially influenced by nitrogen level. In choice tests, flies that had been exposed previously to plants of high nitrogen preferred to feed and oviposit on high nitrogen plants, whereas flies previously exposed to plants with the least nitrogen showed no preference. Performance variables such as egg-larval developmental rate and survivorship, and pupal size were increased with increasing nitrogen levels; this relationship suggests a functional explanation for the preference shown by *L. trifolii* flies exposed previously to plants with high nitrogen.

Introduction

The herbivorous fly *Liriomyza trifolii* (Burgess) is one of the few polyphagous agromyzid species (Spencer 1973). The flies occur on several economically important vegetable crops and ornamentals in many areas of the world (Minkenberg 1988a). Their reproductive capacity is great, they can defoliate plants of many species, and they are resistant to several groups of insecticides (for reviews, see Minkenberg and van Lenteren 1986, Parrella 1987). Damage is caused mainly by the larvae, which mine leaves and reduce yield or the aesthetic value of plants.

An intriguing characteristic of the *L. trifolii* female is that she makes hundreds of punctures with her ovipositor for feeding on plant sap. This feeding greatly enhances her reproductive capacity (Minkenberg 1988b).

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While feeding, a female also may assess the nutritional value of the leaf, which may influence the decision whether to continue feeding and ovipositing or leave (Bethke and Parrella 1985). Because the larvae usually restrict their feeding to one leaf, the ovipositing female also "determines" where, and thus what food, her offspring will eat. Females both feed and oviposit primarily on leaves of the middle region of tomato plants (Schuster and Beck 1981).

A general characteristic of cultivating plants is the provision of a surplus of nitrogen fertilizers, which may facilitate establishment of *L. trifolii* on new crops and accelerate its population growth (Harbaugh et al. 1983, Bethke et al. 1987). Although many studies have addressed life-history variables of *L. trifolii* on different host plants, only one study has considered the variability in host-plant quality within or between fields due, for example, to climatic differences or cultural practices (Knodel-Montz et al. 1985). In agricultural habitats, an individual fly normally feeds and oviposits on plants of the same species. Insects do not generally accept all individuals of those plants with equal probability (for a review on intraspecific host-plant discrimination, see Rausher 1983). The ability of an ovipositing female to detect the physiological condition of a plant may strongly influence her fitness. To explain the spatial patterns and growth of populations, it is necessary to elucidate the behavioral mechanisms, which lead to the aggregated distributions of insects observed in the field (Stimac 1982, Papaj and Rausher 1983). *L. trifolii* leafminers show a clumped distribution in the field and in greenhouses (Jones and Parrella 1986, Lynch and Johnson 1987, Schuster and Beck 1981).

But why are some plants and some leaves within plants consumed and others not? Insects in general tend to prefer plants of a specific physiological state or age (Kogan 1986), perhaps to maximize their nutritional intake. Often, plants are selected according to leaf nitrogen content (McNeill and Southwood 1978, Mattson 1980, Scriber 1984). In this case, a plant of high quality is defined as a plant containing much nitrogen. The variation in nitrogen level between plants may explain the distribution and growth of leafminer populations to some extent (e.g., Hanna et al. 1987). The specific questions addressed in this study are: Is feeding or oviposition directly affected by the complex of plant variables characteristic of a certain nitrogen level (no-choice tests)? Do flies prefer (in sense of Singer 1986) plants of a certain nitrogen level, for feeding and oviposition (choice tests)? Does nitrogen level affect performance variables, such as development, mortality, and size, of females' offspring?

Materials and methods

Tomato plants, *Lycopersicon esculentum* Miller cv 'Moneydor', were sown in perlite and regularly moistened with a fertilizer containing 11.9 mille equivalent, me (mmol), nitrate per litre in an experimental glasshouse at 23 ± 3°C, RH 80 ± 20%, and 17L:7D. One month later, plants were transplanted to rockwool cubes, 10 by 10 by 10 cm, on racks and placed in blocks of four, in which each plant was randomly assigned a different treatment. Plants within a block received a different fertilizer containing 3.00, 5.00, 8.24 or 11.9 me nitrate. The amount of the solution was adapted to plant size. Plants were watered daily; 30% excess of the usual amount of solution was applied to avoid accumulation of salts in the substrate. The pH varied from 5.6 to 6.4 and the electric conductivity from 0.94 to 1.30 mmho. After seven weeks on rockwool, the unsprayed plants had 11-12 leaves and were used for experiments. At that time, mean ± SE leaf areas of the plants of the four levels, N1 to N4, (two plants per treatment) was 583 ± 31, 939 ± 47, 1289 ± 69, 1763 ± 94 cm^2, respectively. The higher fertilizer levels produced greener and larger plants with larger leaves and higher leaf nitrogen content. To analyze leaf nitrogen content, five leaves of the middle region, where adult feeding and larval mining were likely to occur (Minkenberg 1988b), were removed (two plants per treatment). Total nitrogen was determined by the Kjeldahl method (McKenzie and Wallace 1954). The leaf nitrogen content for the plants of levels N1 to N4 was 3.4, 3.9, 4.6 and 4.9% total nitrogen of dry weight, respectively, which brackets a range found in commercial glasshouses (young and old tomato plants is 4.6 and 5.3%, respectively; spring 1987).

The rearing of flies was described by Minkenberg (1988b). Female flies of two to three days old were used. Younger flies lay few eggs because of a preoviposition period. Flies had to be exposed to plants immediately after emergence to prevent initial high mortality. To examine the effect of this exposure before experiments, flies were divided into two groups by releasing them either on plants of the lowest or highest nitrogen level on the first or second day (0900-1800 h) after emergence in cages (60 by 60 by 60 cm). At least 20 males were added to each cage. After exposure, females were kept separately for 15 h in small glass vials without food. This deprivation period was long compared with the normal eight h scotophase; during this period flies produce no eggs and only a few feeding punctures (Minkenberg 1988b).

In no-choice tests, eight blocks of four plants each were randomly placed in cages. Females previously exposed to the lowest nitrogen level (15 per cage) and to the highest nitrogen level (20 per cage) were released for 6 h (0900-1500 h). One experiment per treatment (four plants per cage) was conducted. Feeding punctures and eggs were counted with transmitted light under a microscope 3-5 days after the experiment and were combined for each treatment.

Table 6-1. Number of feeding punctures and eggs per *L. trifolii* female on plants with different nitrogen content in a no-choice test; females previously exposed to plants with 4.2% or 5.6% nitrogen (% dry weight total nitrogen).

% Nitrogen level	Previously exposed to nitrogen level 4.2% feeding punctures	4.2% eggs	5.6% feeding punctures	5.6% eggs
4.2	28	3	41	2
4.5	23	3	39	1
5.1	43	3	61	2
5.6	25	4	30	2
Mean ± SE	30 ± 5	3 ± 0	43 ± 7	2 ± 0

Number equals total number for one trial divided by number of released flies. Number of released flies per treatment for exposure to 4.2% is 15 and to 5.6% is 20. Statistical analysis was impossible because each treatment was conducted only once.

Table 6-2. Number of feeding punctures and eggs (mean ± SE) made by *L. trifolii* females in a choice test[a] with four plants of different leaf nitrogen content; females previously exposed to plants with 4.2% or with 5.6% nitrogen.

% Nitrogen level	Previously exposed to nitrogen level 4.2% feeding punctures	4.2% eggs	5.6% feeding punctures	5.6% eggs
4.2	175 ± 75 a	6 ± 2 a	28 ± 8 a	2 ± 1 a
4.5	58 ± 14 a	5 ± 1 a	83 ± 19 ab	3 ± 1 ab
5.1	134 ± 60 a	7 ± 2 a	130 ± 27 b	5 ± 1 ab
5.6	171 ± 62 a	8 ± 2 a	148 ± 30 b	8 ± 2 b

Tukey's studentized range test, $\alpha = 0.05$; means followed by the same letter are not significantly different. Analysis of variance for the number of feeding punctures for exposure 4.2% and 5.6% is $F = 0.91$ (df = 19, $P = 0.46$) and $F = 8.17$ (df = 19, $P = 0.002$), respectively, and for the number of eggs for exposure 4.2% and 5.6% is $F = 0.50$ (df = 19, $P = 0.69$) and $F = 4.47$ (df = 19, $P = 0.02$), respectively. Number of released females per experiment is 20; 5 trials per treatment were conducted. Data are corrected for differences in leaf area between plants of different nitrogen content; square root transformed data are used for analysis.

In choice tests, blocks of plants were placed in cages and flies (20 per cage) were released for 6 h (0900-1500 h). For each treatment, five experiments were done, in which positions of the differently treated plants were changed. The total leaf area of plants differed among nitrogen levels. The numbers of feeding punctures and eggs per plant were then corrected for differences in leaf area. It was assumed that a plant with twice the leaf area was twice as likely to be encountered by a fly, if searching is random.

To promote normality, a square root transformation was carried out on data corrected for leaf area. Plants with a different nitrogen level were considered to differ qualitatively in the behavioral experiments, because many important characteristics of plants (e.g., leaf color, amino acid composition of nitrogen content, and secondary plant substances) might have differed among treatments. Data were therefore analyzed by means of analysis of variance rather than regression analysis.

To estimate egg-larval developmental time and mortality, gauze bags were put over mined leaves and checked daily for pupae at ca. 1700 h. The lengths of pupae were measured on the first or third day after they dropped from the leaf. Afterwards, pupae were oven-dried at 70°C for a week and weighed. The effect of leaf nitrogen on the performance of the females' offspring was examined by analysis of variance and comparison of means tests. Voucher specimens have been deposited in the insect collection of the Agricultural University of Wageningen.

Results

In a no-choice situation, tomato plants of the four nitrogen levels were accepted by *L. trifolii* for feeding and oviposition. Furthermore, whether exposed previously to plants with the lowest or the highest levels of nitrogen, females did not strongly discriminate among plants in subsequent exposures; rather, they made approximately equal numbers of feeding punctures and laid similar numbers of eggs in all plants (Table 6-1). Thus plants of every level were acceptable for feeding and oviposition (compare differences in response in Table 6-2).

In the choice tests, flies previously exposed to plants with 4.2% nitrogen produced the same number of feeding punctures and eggs on each type of plant (Table 6-2). However, flies previously exposed to plants with 5.6% nitrogen were more selective, and fed and oviposited significantly more on plants with higher nitrogen level (Table 6-2).

The performance of offspring of the *L. trifolii* females was affected by host-plant nitrogen (Tables 6-3 and 6-4). Egg-larval developmental time decreased significantly with increasing nitrogen level of the plants. Egg-larval mortality was higher on plants with a low nitrogen content than on plants with a high nitrogen content. Pupae were significantly longer and heavier on plants with a high nitrogen content. An increase of 0.6% in leaf nitrogen content within the examined range resulted in significantly larger pupae.

Table 6-3. Mean (± SE) egg-larval developmental time (day) and percentage mortality of *L. trifolii* on tomato plants of different leaf nitrogen content.

% Nitrogen level	Developmental time[a]	N	Mortality[b]	N[c]
4.2	13.2 ± 0.2 a	61	52 a	117
4.5	12.9 ± 0.1 a	80	33 b	112
5.1	12.3 ± 0.1 b	151	29 b	182
5.6	12.2 ± 0.1 b	185	30 b	249

[a] Tukey's studentized range test, $\alpha = 0.05$; means followed by the same letter are not significantly different. Analysis of variance for the developmental time is $F = 30.75$ (df = 476, $P < 0.0001$).

[b] Dead eggs and larvae divided by initial number of eggs. Chi-square test ($\alpha = 0.05$) on numbers observed; percentage of mortality followed by a different letter indicate a significant difference.

[c] Initial number of eggs, laid by females used in behavioral experiments.

Table 6-4. Mean lengths (mm) and dry weights (mg) of *L. trifolii* pupae developed on plants of different leaf nitrogen content; offspring produced by females, which were previously exposed to plants with 4.2% or 5.6% nitrogen.

%	4.2% nitrogen[a]				5.6% nitrogen[b]			
Nitrogen level	Length[c]	N	Weight[c]	N	Length[c]	N	Weight[c]	N
4.2	1.50 a	39	0.158 a	39	1.49 ab	17	0.165 a	17
4.5	1.54 a	58	0.175 a	54	1.53 ab	17	0.173 a	17
5.1	1.61 b	81	0.195 b	78	1.56 bc	49	0.200 b	48
5.6	1.63 b	95	0.205 b	95	1.60 c	80	0.222 c	79

[a] Measured on the 3rd day after pupation.

[b] Measured on the 1st day after pupation.

[c] Pairwise t-test, $\alpha = 0.05$; means in a column followed by the same letter are not significantly different.

Discussion

Among herbivorous insects, feeding and oviposition preferences within and between plants can usually be explained, at least in part, by differences in expected reproductive success (Miller and Strickler 1984). In this study, pupae from larvae fed on plants with a high nitrogen level are larger and heavier than those from plants with a low nitrogen level, and it is probable that these size differences constitute real differences in fitness. Parrella (1983) found *L. trifolii* flies from large pupae on chrysanthemum to have a higher fecundity and longevity than flies from smaller pupae. However, pupal size of *L. trifolii* on tomato does not appear to be correlated with pre-adult developmental rate, mortality of the instars, adult fecundity, longevity or oviposition rate (Minkenberg 1988b). This study did not address the influence of plant nitrogen level on fecundity of *L. trifolii*. Still, the high developmental rate and low mortality on plants with a high nitrogen level suggest that fitness of *L. trifolii* increases with increasing nitrogen level, at least within the range of 4.2% to 5.6%.

At present, no definitive explanation can be made for the differential preference and performance of *L. trifolii* with regard to plant quality.

Our results are consistent with the hypothesis that a preference is induced as the result of an experience with high-nitrogen plants. Inexperienced flies may have a low acceptance threshold (see Singer 1982, Papaj and Rausher 1983) and therefore show no preference, and that this threshold is not altered by exposure to plants containing 4.2% nitrogen. In contrast, flies exposed to plants containing 5.6% nitrogen may have a high acceptance threshold and therefore avoid using less suitable plants. The threshold may be set by gut satiation, assimilation of particular nutrients or egg production, after having encountered a host plant (for a review on induction of preference, see Papaj and Prokopy 1989).

Another, more complex, mechanism of threshold may involve associative learning. For instance, the chemical constitution of the imbibed plant sap may be associated with gut satiation, nutrient assimilation or egg production. Sensory perception of amino acids has been demonstrated in several lepidopterans (e.g., van Loon 1988, p. 13). However, the use of such a cue would probably lead to a more rapid rejection of subthreshold hosts, and therefore result in a stronger preference than the one we observed.

We further speculate that, when high nitrogen plants are abundant, *L. trifolii* use plants that maintain or raise the acceptance threshold and disperse relatively quickly from plants that have a lower nitrogen level than the current threshold. With increasing deprivation, subthreshold hosts become more acceptable. This strategy is adaptive because host acceptability is correlated with host suitability. Our data indicate, at the population level, that the preference shown by *L. trifolii* adults that had been previously exposed to plants of high nitrogen is related to success in development, growth rate and survival of immatures.

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7

Influence of leaf nitrogen content of tomato plants on performance and intraplant preference of a leafmining fly[1]

Abstract

In a study on intraspecific host plant acceptability, *Liriomyza trifolii* females that had previously been exposed to plants of high nitrogen content, showed a feeding and oviposition preference for plants of high nitrogen (Minkenberg and Fredrix 1989). Females showed a preference to feed and oviposit on the high middle leaves within plants. It was hypothesized that the preference between plants was related to a better performance of females and offspring on high nitrogen plants compared to low nitrogen plants. Different nitrogen dosages were applied to tomato plants, resulting in plants containing 3.4, 3.9, 4.6 or 4.9% leaf nitrogen. *Liriomyza trifolii* females responded to increased leaf nitrogen with significantly increased feeding and fecundity, longer oviposition periods, and higher feeding and oviposition rates. Their offspring on the same plants showed reduced developmental time, lower mortality and increased pupal size. Consequently, intrinsic rate of increase was positively linearly related to leaf nitrogen. Size of *L. trifolii* females appeared to be independent of fecundity, longevity and developmental time. Pupal length of males increased with increasing developmental time.

These results indicate that *L. trifolii* is well adapted in its intraspecific host plant selection, because the ability to distinguish between plants with differences in leaf nitrogen content will directly lead to an increase in its fecundity, longevity and overall fitness. The implications of leaf nitrogen as a significant factor in the behavior and population dynamics of *L. trifolii* are discussed.

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Introduction

In herbivorous insects, ovipositing females that can discriminate plant characteristics associated with performance, e.g. survival of their offspring, usually have a selective advantage over females that are not able to do so. Therefore, the female's host plant preference is expected to be correlated with juvenile performance as a result of natural selection. However, many studies could not confirm such a relationship between preference and performance (Wiklund 1975, Holdren and Ehrlich 1982, Rausher 1983, Singer 1983, Whitham 1983, Mackay 1985). To date, only seven studies support the predicted relationship (Rausher and Papaj 1983, Williams 1983, Leather 1985, Myers 1985, Via 1986, Taylor and Forno 1987, Singer et al. 1988). Reasons for this inconsistency in the relationship between preference and performance in the field reviewed by Papaj and Rausher (1987) and Thompson (1988) include: (1) inappropriate measurements of females' preference and performance of their offspring (2) evolutionary time lag between the observed preference and performance; (3) patch dynamics which may cause a rather weak relationship between preference and performance; (4) specific performance traits related to preference; and (5) enemy free space as an important factor in performance. An additional cause is recently given by Ng (1988), who found in a single population of *Euphydryas editha* butterflies that some individuals show an intraspecific host plant preference, whereas others are generalists and equally accept all plants encountered. Moreover, the preference shown by the specialists correlated with the survival of their offspring on the accepted and non-accepted plants; suitability of plants did not differ among the offspring of the generalists.

Herbivorous insects often remain at low densities because of limited access to high quality food (White 1978, Crawley 1989, for an exception see Stiling et al. 1982). Leaf nitrogen content is generally an indicator of food quality (Jones 1976, McNeill and Southwood 1978, Scriber and Slansky 1981) and sensory perception of amino acids has been demonstrated for some insects (Van Loon and Van Eeuwijk 1989). In addition, leaf water content is important (Stanton 1980 in Stanton 1982) but is usually positively correlated with nitrogen content (Mattson 1980). Nitrogen content within plants may vary greatly (Denno 1983, Schultz 1983). Although increased nitrogen levels do not always improve juvenile performance (Scriber 1984), leaf nitrogen is a plant characteristic that usually dramatically affects performance (Scriber & Slansky 1981, Strong et al. 1984).

The polyphagous leafmining fly *Liriomyza trifolii* is an economically important pest in ornamentals and vegetables (Minkenberg and Van Lenteren 1986, Parrella 1987). Given its recent introduction on chrysanthemums into Europe, its present abundance on many plant species and the frequent migration between crops, it is very unlikely that there are host races adapted to particular host plants in Europe. Adult fecundity and longevity on tomato is lower than that of the congeneric, native leafminer

species on tomato, *Liriomyza bryoniae* (Minkenberg 1988b, Minkenberg and Helderman 1990). Actually, tomato is a suboptimal host for *L. trifolii* adults compared to other plant species (Parrella et al. 1983b). *Liriomyza trifolii* females prefer plants with a high nitrogen content over plants with a low nitrogen content, if they have previously been exposed to high nitrogen plants (Minkenberg and Fredrix 1989). Females determine what food offspring will eat because larvae usually restrict their feeding to the leaf from which they hatch. Agromyzid females puncture and feed on host plants (Spencer 1973, Bethke and Parrella 1985). After alighting females will probe the plant by feeding and then leave or continue feeding. Some feeding punctures are also used for deposition of solitary eggs. Distribution patterns of eggs within plants are equal to those of feeding punctures (Minkenberg 1988b). Thus, females seem to feed on similar plants and plant parts as do their offspring. The within-plant preference was further examined in this study.

Furthermore, developmental rate and survival of offspring increase with increasing nitrogen levels, suggesting a functional explanation for the between-plants preference shown by the adults (Minkenberg and Fredrix 1989). Since there might be a direct link between plant nitrogen content and adult performance, we addressed the question whether female fecundity and longevity increase with increasing nitrogen levels. To express the effect of nitrogen on fitness of females by intrinsic rate of population increase (r_m), immature developmental time and survival on plants of different nitrogen levels were examined as well. Finally, pupal length of all individuals was measured to examine the relationship between fitness traits and size.

Materials and methods

Variation in leaf nitrogen. Tomato plants in most western European glasshouses are grown on rockwool and individually watered and fertilized by drip-system irrigation to minimize water stress and nitrogen deficiency. To estimate the between-plant variation in leaf nitrogen of plants grown commercially, leaves were collected from the high middle region of plants where mining occurred; collections were made monthly from two commercial glasshouses during the season of 1988, and nitrogen content of leaves was determined. All determinations in this study were Kjeldahl nitrogen (total nitrogen, % dry weight).

Plant and insect rearing. Tomato plants, *Lycopersicon esculentum* cultivar 'Moneydor', were sown in perlite and regularly watered with a fertilizer containing 11.9 mM nitrate in an experimental glasshouse at 23 °C (range 19-35 °C), 75% RH (range 60-100%) with a photoperiod of 19:5 h (L:D) from May until August 1985. Seedlings were transplanted to rockwool cubes (320 cm^3) and placed in blocks of four. Each plant within a block was randomly

assigned to a different treatment and received a fertilizer containing 3.00, 5.00, 8.24 or 11.9 mM nitrate (N_1 to N_4, respectively). Fly rearing is described in Minkenberg and Helderman (1990).

Intraplant preference. To estimate within-plant variation in leaf nitrogen, 28 plants (seven plants per treatment) of about 12 weeks in age were used. Leaves were divided into five classes according to their age. The age classes were determined according to position of leaves on plants.

Distributions of feeding punctures and eggs of individual females (N = 7) during their life span were examined on the highest nitrogen plants (11.9 me NO_3^-) at alternating temperatures (16-22 °C, mean is 19.5 °C; experimental procedures as described below). Observed versus expected distribution within the plant was analyzed by a Chi-square test (Sokal and Rohlf 1981). Numbers expected were corrected for leaf biomass (dry weight), because larger leaves have a higher chance of visit, if females randomly select leaves. Dry weight for the bottom, low, middle, high and top leaf classes was 3.2, 8.6, 16.7, 9.6 and 4.3 g, respectively.

Feeding/fecundity/longevity. Eight weeks old plants with 7-10 fully expanded leaves were used for these experiments. Every week two plants per treatment were used for measuring leaf surface and nitrogen content. The higher fertilizer levels produced larger plants and higher leaf nitrogen content. The mean leaf area (± SE) of the plants of the levels N1 to N4 was 583 ± 31, 939 ± 47, 1289 ± 69 and 1763 ± 94 cm^2, respectively, and their leaf nitrogen content 3.4, 3.9, 4.6 and 4.9%, respectively.

Feeding and fecundity were estimated by counting feeding punctures and first instars (i.e., viable eggs) produced by individual females daily. Pupae collected from the rearing were measured (± 0.01 mm) to examine the relationship between performance variables of an individual and its size. They were placed singly in small glass vials. Newly emerged females randomly assigned to plants of the four regimes (N = 25, 24, 24 and 17 for treatment N_1 to N_4, respectively) were released into cylindrical cages (Minkenberg and Helderman 1990) containing a treated plant in a controlled environment room at 25.4 ± 0.5 °C at RH 70 ± 10% and a photoperiod of 16:8 h (L:D) with the photophase between 0100 and 1700 h. Plants were changed daily between 1630 and 1700 h, from Day 2 onwards.

Females which died before 17.00 h on the first day, or did not produce offspring were excluded from the analysis. Effects of treatment on the variables feeding, fecundity, oviposition period (i.e., longevity minus postoviposition period), feeding rate, oviposition rate, ratio of egg and feeding punctures, and preoviposition period of the flies reared in the colony were determined by analysis of variance and Tukey studentized range test (SAS Institute 1985). Correlations between the variables and pupal length were analyzed by the Spearman rank correlation test (Sokal and Rohlf 1981). The effect of female size on these variables and on offspring pupal lengths and developmental time was determined by linear regression analysis. Concerning

size, randomization of females between treatments was checked by linear regression and a difference in female size between treatments was not found ($F = 2.38$, $P = 0.07$, $df = 89$).

Development/mortality. Developmental time and percentage mortality were measured at 25 °C for individuals produced by the females of the 'feeding/fecundity/longevity' experiment. Offspring were observed daily, after 13.00 h, from egg hatch until adult emergence in a controlled environment room operating at the same regime as the previous experiment. Pupae were collected in net bags and their pupal length (± 0.01 mm) was measured. The period during which the larva was outside the leaf before pupation was included in the time of egg-larval development. Developmental times were based only on individuals that reached adulthood.

Effects of treatment on developmental time were analyzed by linear regression and Tukey studentized range test. Mortality for different treatments were compared using G tests. Since pupal length and developmental time in *L. trifolii* differ between sexes (Minkenberg 1988b), correlations between these variables within treatments were analyzed separately for the sexes. Intrinsic rates of increase (r_m) were calculated by the Lotka (1925) equation on basis of a 1:1 sex ratio (Minkenberg and Van Lenteren 1986, this study).

Results

Variation in leaf nitrogen of tomato plants

Differences between plants in commercial glasshouses were considerable (range 1.6%). Total leaf nitrogen found in leaves of the high middle region of tomato plants where most mining occurred was, on average, 4.5% and varied between 3.8 and 5.4% during the season. With respect to variation with plant age or season, no trend was found in the nitrogen content of the leaves from the commercially grown glasshouse tomatoes, contrary to the generalization that nitrogen content of whole plants declines with age or time (Scriber and Slansky 1981). In field tomatoes, nitrogen content decreases during the season from an average of 4% to about 2%, both because the nitrogen level within leaves decreases and because, as the season progresses, there is a lower initial nitrogen level of young leaves. The leaf nitrogen content of field tomatoes is between 1.5% for a very old leaf and 7% for the youngest leaf at the start of a growing season (L.T. Wilson pers. comm.).

Table 7-1. Nitrogen content (% dry weight) of tomato leaves in five age classes (determined by position on the plant) taken from four plants of increasing nitrogen levels (single samples from seven plants; only the highest nitrogen plants were further used for the within-plant preference experiments, see Table 7-2)

	Nitrogen level of plant			
Class (leaf no.)	2.6%	3.2%	4.2%	4.5%
Top (10,11,12)	-	3.3	4.8	4.9
High middle (8,9)	2.8	4.0	4.8	4.8
Middle (6,7)	2.9	3.4	4.5	4.5
Low middle (4,5)	2.7	3.0	3.9	4.4
Bottom (1,2,3)	2.1	2.6	3.1	3.8
Range	0.7	1.4	1.7	1.1
NO_3^- doses (mM)	3.00	5.00	8.24	11.90
Dry weight (g)	2.39	3.93	4.95	6.04

Intraplant preference

Nitrogen levels within tomato plants differed largely with a maximal range of 1.7% (Table 7-1). Females, which were individually kept during their entire life span on daily renewed plants, fed and oviposited primarily in the middle and high middle leaves of the experimental tomato plants (Table 7-2). Numbers of feeding punctures and eggs were significantly higher in the high middle leaves than in other areas. The vertical distribution of feeding punctures was similar to that of eggs, suggesting that adult and larval food does not differ substantially.

Performance

Feeding, fecundity, oviposition period, feeding rate and oviposition rate of adult females increased with increasing nitrogen level (Table 7-3). Feeding on plants of the highest nitrogen level was nearly four times as great as feeding in the lowest level, while fecundity was three times as great. Females on the plants of the highest nitrogen level lived twice as long as females on the lowest nitrogen plants. The preoviposition period of the females was not significantly affected by plant nitrogen. The ratio between eggs and feeding punctures, which might be regarded as conversion factor of food into eggs assuming that other food sources, e.g. honeydew, were absent, was negatively correlated with nitrogen content.

Table 7-2. Preference for feeding and oviposition of *L. trifolii* females within the highest nitrogen plants (4.5% dry weight) on leaves of different ages (determined by position on plant). Chi-square tests at P = 0.01 were performed for equal distributions of feeding punctures and eggs among leaf classes; numbers expected were corrected for leaf biomass between the classes before analysis; s.h. and s.l. mean significantly higher and lower, respectively, than expected for that class and n.s. indicates not significantly different from expected

Class (leaf no.)	% feeding	test	% eggs	test
Top (10,11,12)	8	s.l.	11	n.s.
High mid (8,9)	36	s.h.	36	s.h.
Middle (6,7)	39	n.s.	37	n.s.
Low mid (4,5)	16	s.l.	12	s.l.
Bottom (1,2,3)	1	s.l.	4	s.l.
N	4128		219	

Reproduction increased with increasing leaf nitrogen levels of the plants (Fig. 7-1). Feeding, fecundity and oviposition period were highly correlated within the four treatments and overall (Spearman: r_s is between 0.55 and 0.85, $P < 0.01$ within treatments and $P < 0.0001$ overall, $N = 90$). Fecundity between females differed greatly within all treatments. Some individuals laid only a few eggs, whereas others produced hundreds. Offspring of some females did not reach adulthood at all (for treatments N_1 to N_4 for 4, 3, 3 and 2 females, respectively). Similar numbers of females got only daughters or sons. Preoviposition period of females being, as previously shown, independent of plant nitrogen was negatively correlated with their fecundity (Spearman: $r_s = -0.21$, $P < 0.02$, $N = 90$), whereas it was independent of feeding ($P > 0.73$) and oviposition period ($P > 0.56$) over all treatments.

Leaf nitrogen content significantly affected *L. trifolii* immature growth, survival and size (Table 7-4). Mean developmental time decreased with increasing leaf nitrogen content. Both egg-larval and pupal development differed significantly between plants with different nitrogen levels. Both developmental time and pupal length were significantly different for the sexes, which has been previously found in *Liriomyza* spp. (Via 1986, Minkenberg 1988b, Minkenberg and Helderman 1990). Pupal length increased with increasing nitrogen content of leaves. Mortality of immatures decreased with increasing nitrogen content and was significantly different at all four nitrogen levels (Table 7-4). Sex ratio of offspring was 644 females - to 605 males, which was not significantly different from a 1:1 ratio (G test, $G = 0.64$, $P = 0.42$).

Table 7-3. Mean feeding (no. of feeding punctures), fecundity (viable eggs), oviposition periods (days), feeding rates (no. punctures/day), oviposition rates (viable eggs/day), ratios of eggs and feeding punctures and preoviposition periods (days) of *L. trifolii* on tomato plants of different leaf nitrogen content (mean ± SE; range). Means followed by a different letter differ significantly at $P < 0.05$ by Tukey's studentized range test; analysis of variance, df = 89 for all variables. Data were transformed by square root before analysis (* means $P < 0.05$, ** $P < 0.001$, and *** $P < 0.0001$)

	Nitrogen level				
	3.4%	3.9%	4.6%	4.9%	F value
Feeding	327 ± 39 a (73-836)	547 ± 67 a (94-1463)	964 ± 121 b (158-2336)	1300 ± 170 b (367-2659)	18.98***
Fecundity	16 ± 3 a (2-63)	20 ± 3 a (1-55)	32 ± 9 ab (1-181)	50 ± 8 b (1-112)	5.56*
Oviposition period	2.5 ± 0.1 a (2-4)	3.2 ± 0.2 ab (1-5)	4.0 ± 0.5 b (2-11)	4.3 ± 0.5 b (1-7)	7.12**
Feeding rate	125 ± 12 a (37-279)	166 ± 14 a (60-293)	249 ± 30 b (79-832)	315 ± 26 b (150-541)	18.6***
Oviposition rate	6.1 ± 0.9 a (1.0-21.0)	5.8 ± 0.6 a (0.5-11.0)	6.5 ± 1.1 a (0.5-19.4)	10.4 ± 1.2 b (1.0-19.3)	3.81*
Eggs/feeding punctures	0.06 ± 0.01a (0.01-0.18)	0.04 ± 0.00ab (0.01-0.08)	0.03 ± 0.01b (0.00-0.10)	0.04 ± 0.01ab (0.00-0.11)	4.06*
Pre-oviposition	1.0 ± 0.0 a (1-2)	1.1 ± 0.1 a (1-2)	1.2 ± 0.1 a (1-3)	1.2 ± 0.1 a (1-2)	0.78
N	25	24	24	17	

Table 7-4. Effects of leaf nitrogen on mean ± s.e egg-larval, pupal and total developmental times (days), pupal lengths (mm) and percentage mortality of *L. trifolii* on tomato plants. Linear regression on females (f) and males (m): all the dependent variables were significantly affected by nitrogen content, $P < 0.0001$, and sex, $P < 0.012$. Mortality was analyzed by a G test ($P < 0.05$); means followed by a different letter differ significantly; n of eggs indicates the initial number of viable eggs

Developmental stage		Nitrogen level 3.4%	3.9%	4.6%	4.9%	F (df)
Egg-larval	f	8.2 ± 0.1	8.3 ± 0.1	8.0 ± 0.1	7.7 ± 0.1	28.15
	m	8.3 ± 0.1	8.0 ± 0.1	7.8 ± 0.1	7.6 ± 0.1	(1241)
Pupal	f	9.5 ± 0.1	9.4 ± 0.1	9.2 ± 0.1	9.3 ± 0.0	15.69
	m	9.3 ± 0.1	9.2 ± 0.1	9.1 ± 0.0	9.1 ± 0.0	(1241)
Total	f	17.7 ± 0.1	17.6 ± 0.1	17.2 ± 0.1	17.0 ± 0.1	47.77
	m	17.6 ± 0.1	17.2 ± 0.1	17.0 ± 0.1	16.7 ± 0.1	(1241)
Pupal length	f	1.60 ± 0.02	1.64 ± 0.01	1.70 ± 0.01	1.71 ± 0.01	195.56
	m	1.51 ± 0.02	1.53 ± 0.01	1.56 ± 0.01	1.56 ± 0.01	(1240)
N	f	75	123	188	250	
	m	59	104	206	237	
Mortality		36.6 a	22.5 b	14.2 c	9.8 d	
N eggs		396	475	773	847	

Fig. 7-1. Net reproduction (viable female eggs/female) of *L. trifolii* on plants of four different nitrogen levels.

Table 7-5. Effects of leaf nitrogen on intrinsic rate of increase, r_m, (viable female eggs/female/day), net reproduction, R_0, (viable female eggs/female) and generation time, T, (day) of *L. trifolii* on tomato

Nitrogen level	r_m	R_0	T
3.4%	0.084	5.0	19.3
3.9%	0.104	7.7	19.4
4.6%	0.127	13.8	20.9
4.9%	0.159	22.8	19.7

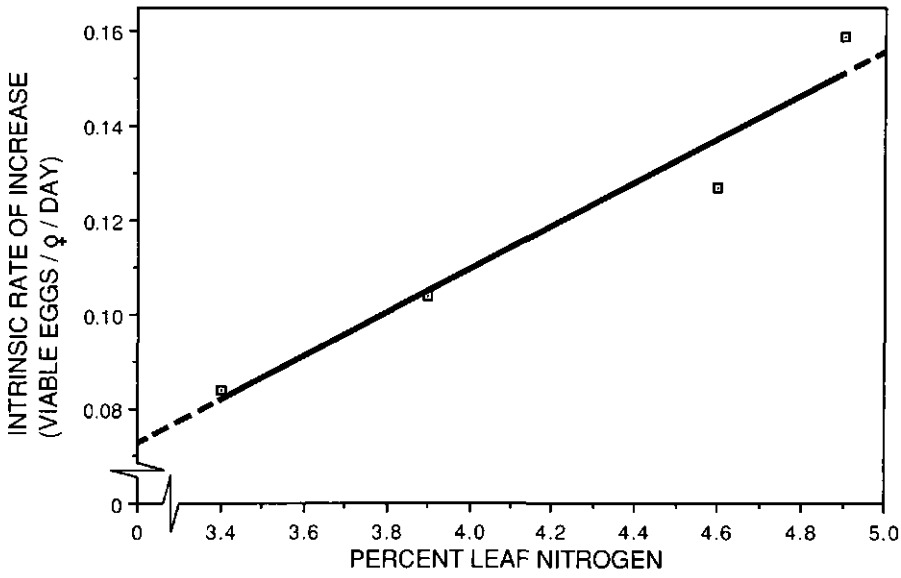

Fig. 7-2. Intrinsic rate of increase (viable female eggs/female/day) of *L. trifolii* on plants of four different nitrogen levels. Linear regression of r_m, Y, against nitrogen level, X: Y= 0.0462 X - 0.0754 (r = 0.97, df = 3, P = 0.028).

Thus, all life history variables of L. trifolii, with the exception of sex ratio and preoviposition period, were affected by increasing leaf nitrogen content of tomato plants to benefit its intrinsic rate of increase, at least within the range 3.4% to 4.9% at 25°C (Table 7-5). As a consequence, net reproduction rate, R_0, of L. trifolii was significantly related to nitrogen levels (Table 7-5) and r_m was positively, and linearly, correlated with leaf nitrogen level (Fig. 7-2).

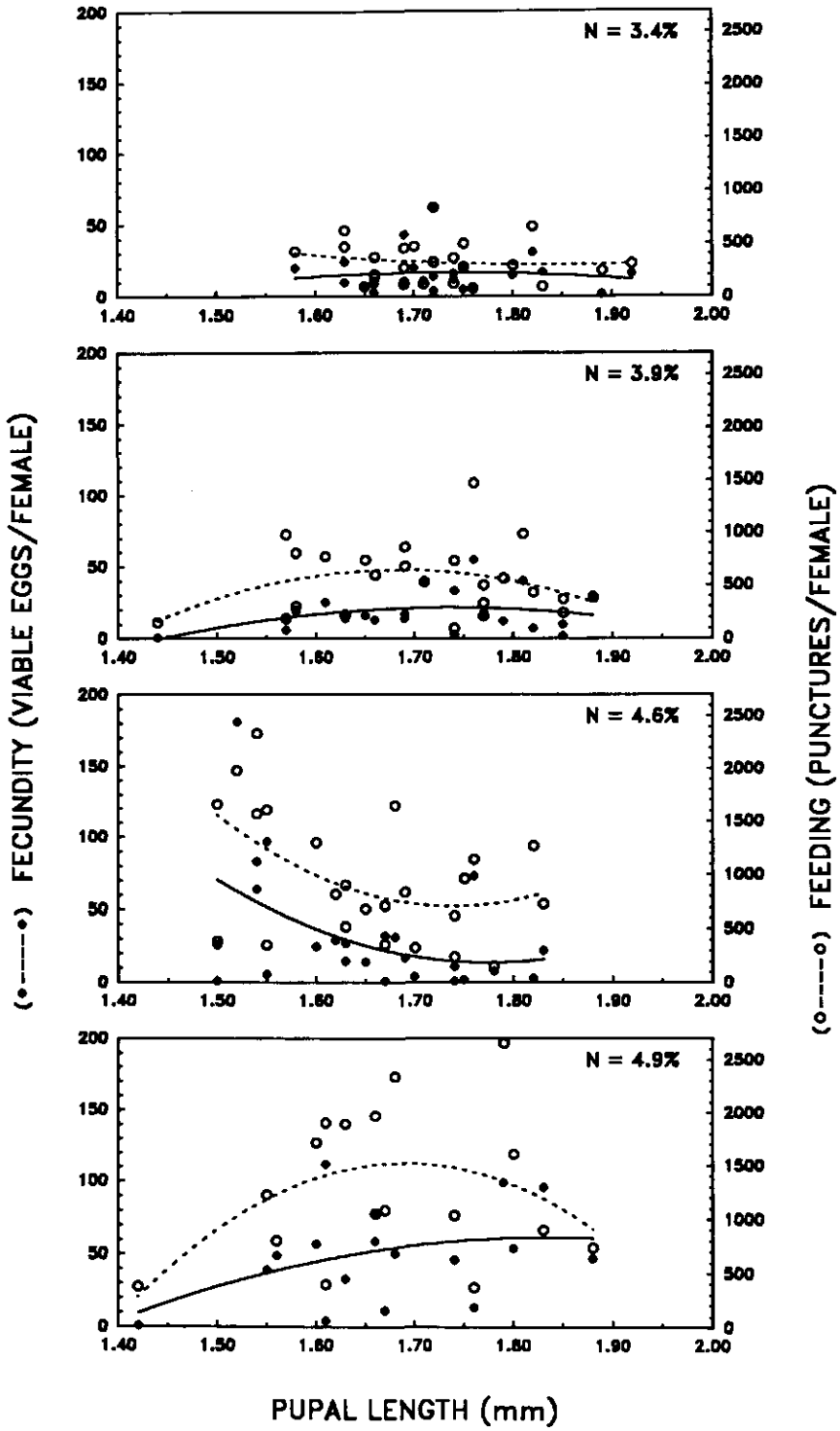
N = 3.4%
N = 3.9%
N = 4.6%
N = 4.9%
(•——•) FECUNDITY (VIABLE EGGS/FEMALE)
(o——o) FEEDING (PUNCTURES/FEMALE)
PUPAL LENGTH (mm)
0
50
100
150
200
500
1000
1500
2000
2500
1.40
1.50
1.60
1.70
1.80
1.90
2.00

Table 7-6. Linear regression of developmental time of offspring on their size split for the two sexes within treatments and for all data pooled (for the latter analysis, nitrogen level was included). Mother of offspring was included as an independent classification variable. The model applied was: pupal length of offspring = M (mother) * DT (developmental time transformed by log)

	Daughters					Sons				
%N	F	df	P total	P M	P DT	F	df	P tot	P M	P DT
3.4	3.08	74	0.0007	0.017	0.0002	1.52	58	0.13	0.20	0.052
3.9	2.01	122	0.012	0.015	0.07	1.97	103	0.017	0.11	0.077
4.6	2.71	186	0.0003	0.0002	0.69	2.42	205	0.002	0.002	0.63
4.9	5.63	249	0.0001	0.0001	0.20	4.19	236	0.0001	0.0001	0.037
Total	3.73	634	0.0001	0.0001	0.71	2.67	605	0.0001	0.0001	0.005

Body size and life-history traits

Females' size had no effect on their performance (Fig. 7-3), with the exception of the 4.6% nitrogen treatment, remarkably, where size was significantly inversely related to fecundity (linear regression: Y (fecundity) = -13 X (size) + 27, $F = 4.84$, $P < 0.039$, $df = 23$). In general, feeding and fecundity appeared to be independent of body size, at least within the range of pupal length examined.

The achieved pupal length by females was found to be unrelated to developmental time over all treatments (Table 7-6), indicating that small females had not developed faster than large ones, although within treatments size of females on plants of the lowest nitrogen level significantly decreased with developmental time. In contrast, male size was found to be positively correlated with developmental time over all treatments. Female size was significantly affected by her mother within all treatments (Table 7-6). This was only the case in two treatments for the males.

Fig. 7-3. Effect of size of females on their fecundity and feeding within the four leaf nitrogen treatments. Polynomial regression lines are drawn to show trends.

Discussion

Preference

The agromyzid *L. trifolii* prefers to feed and oviposit on leaves of a specific age. This is consistent with field observations in that most mining occurs in leaves of a certain age within the plant (Minkenberg et al. unpubl.). Nitrogen content decreases with the age of the leaves (Raupp and Denno 1983). Since nitrogen content is highest in the top leaves and, among different plants, females prefer plants of the highest nitrogen content, we speculate that additional proximate factors are involved in the within-plant preference. One of the possible factors operating are phenols, the effects of which increase with the protein content of the food (Kennedy 1986). However, quality of the nitrogen, viz. levels of specific amino acids or the 'structural configuration' of proteins reducing its digestibility might be a factor as well (Duffey and Bloem 1986). Furthermore, increased nitrogen levels are likely to affect differently the abundance and density of trichomes on tomato leaves, which might act as a physical barrier and a sticky trap, and in addition show insecticidal effects (Lin et al 1987). The preference for feeding and oviposition on the higher middle region leaves is probably adaptive, because there is some evidence that developmental rate and survival of immatures is higher on these leaves than on the top leaves (Minkenberg 1988b).

With respect to the between-plant preference, females that have previously been exposed to plants with high nitrogen content prefer to feed and oviposit on plants of a high nitrogen level (Minkenberg and Fredrix 1989). Performance of adult female *L. trifolii* and their offspring are directly affected by leaf nitrogen content. In addition to a higher nitrogen content, increased nitrogen availability to the plant might also lead to a higher production of nitrogenous metabolites that have insecticidal effects, such as alkaloids, by the plant; however, the concentration of such secondary compounds might actually decrease within the leaves with increasing nitrogen content (Gershenzon 1984). The feeding and oviposition preferences of females can thus far be explained only functionally: their reproductive success increases on plants with a high nitrogen level.

Performance

Low nitrogen content may lead to a very low population increase in *L. trifolii*. At 25°C, which is the approximate optimum temperature for population increase in *L. trifolii* (Minkenberg 1988b), population growth is predicted to become zero on tomato plants with 1.6% leaf nitrogen (regression equation in Fig. 7-2). Since our study was done at the approximate optimum temperature, population growth is expected to be lower under a different temperature regime.

This polyphagous species may maximize its nutritional intake by choosing among plants. This is, however, only reflected in its preference and cannot be deducted from its feeding behavior, i.e. the number of feeding punctures. When flies are confined to low nitrogen plants, the low nitrogen content is not compensated for by a high feeding rate. The egg-feeding ratio, which is assumed to increase on a highly nutritious host, has been shown to be an indicator for host plant suitability in no-choice experiments for *Chromatomyia syngenesiae,* an agromyzid fly on lettuce (Hussey and Gurney 1962), and interspecifically for *L. trifolii* (Parrella et al. 1983b). In contrast, we found a decrease in the egg-feeding ratio with increasing leaf nitrogen for *L. trifolii* on tomato. Possibly, females can imbibe more sap per puncture on the low nitrogen plants because of a difference in thickness of the leaves. The use of the ratio as an index for host plant suitability is thus questionable.

The relatively prolonged net reproduction curve for the 4.6% nitrogen plant treatment (Fig. 7-1) was based on two individuals: one female lived for 11 days producing most eggs of all females, viz. 181, and the other lived until day 13 after having laid 83 eggs. Flies of this size seemed to perform very well on those plants (Fig. 7-3), in comparison to the flies of different size and to flies on the other treatment plants.

Body size

A correlation between size and life history variables of *Liriomyza* spp. has not been found thus far on tomato (Via 1984b, Minkenberg 1988b, Minkenberg and Helderman 1990), whereas on other host plant species positive correlations between size and development or reproduction were shown for females (Parrella 1984, Via 1984b). Life history theory predicts that a trait in females is expected to evolve so as to maximize reproduction. But we found that there is apparently no correlation between any of the life history traits and size over a broad range of sizes. This is in contrast with the general supposition that large females have a selective advantage, because they potentially produce more offspring (Roff 1981, Via 1986). The fecundity advantage model mainly applies to animals that are energy limited (Shine 1988), but there are no obvious reasons why the synovigenic *L. trifolii* females would have limited access to food in agricultural habitats. Body size, at least within the natural limits, appears to be loosely connected to fitness, i.e. development and reproduction (see also Leather 1988).

General conclusions

A positive correlation was found between host plant preference and performance in *L. trifolii*. In contrast, many studies on other insects found no such correlation. This might be explained in our system by the direct link between the plant chosen by the female and her reproductive success. In addition to a greater own fecundity on a high nitrogen plant, she provides her offspring with a greater fitness opportunity, because the food selected by the adult female will be similar for the immatures.

We have demonstrated that an increase in leaf nitrogen of nearly 50% (from 3.4% to 4.9%) dramatically enhances population growth of these flies: r_m becomes almost twice as large. Given the impact of variation between plants on intraspecific host plant preference and performance, this study supports the suggestions by Rausher and Papaj (1983), Scriber (1984) and (White 1984) that the conditions under which plants grow might alter both the acceptability of these plants for adult insects and the suitability of these plants for their offspring, and might thus complicate comparison of preference and performance on different host plant species (in sense of the patch dynamics hypothesis postulated by Papaj & Rausher [1987] and Thompson [1988], see also Williams 1983). As concluded by Thompson (1988), the evaluation for both agricultural and natural systems must ultimately be made in the field.

Acknowledgments

Our thanks go to Marion van Rosevelt for participating in some of the experiments and to our colleagues Joop van Lenteren and Joop van Loon (Wageningen Agricultural University), Gregory English-Loeb, Kevin Heinz, David Krainacker, Judy Nelson and Theodore Wilson (University of California, Davis), and Sara Via (Cornell University, Ithaca) for their valuable comments on the manuscript.

8

Role of volatile infochemicals in foraging behavior of the leafminer parasitoid *Dacnusa sibirica*[1]

Abstract

Previous investigations suggested that the leafminer parasitoid *Dacnusa sibirica* Telenga does not use a volatile kairomone in foraging for hosts: parasitoids landed equally frequent on an uninfested tomato plant and on a tomato plant infested with larvae of *Liriomyza bryoniae* (Hendrikse et al. 1980). However, in wind-tunnel observations we found that volatile infochemicals emitted by uninfested and leafminer-infested tomato plants did affect differently the parasitoid's foraging behavior. This was obvious from the proportion of wasps flying upwind, but not from the proportion of wasps landing on the leaves. In addition, latency time, i.e. time prior to take-off, and duration of preflight antennal behavior were influenced by the presence of upwind uninfested or infested tomato leaves; though, these parameters appeared to be affected predominantly by visual stimuli of the leaves. The proportion of upwind flights with wind only depended on whether the wasps had experience with tomato leaves with or without hosts: host-experienced wasps flew predominantly downwind and wasps experienced with only plant material flew predominantly upwind. Our data provide a new view on the foraging behavior of *D. sibirica*.

Introduction

Many species of parasitic wasps are known to respond to volatile kairomones during foraging for hosts (Weseloh 1981, Vinson 1984). Although it is likely that all parasitoids employ such infochemicals (*sensu* Dicke and Sabelis 1988), for several species a response to a volatile kairomone has not been found. The conclusion about the ability of a parasitoid species to perceive hosts by olfaction, however, may depend on the type of bioassay used and the parameters quantified. The parameters measured are usually selected

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(4) four uninfested tomato leaves in a vial *under* the tunnel; air was led through the vial and introduced into the tunnel through a nozzle.
(5) like (4), but leaves heavily infested by all three larval stages of *L. bryoniae*.

In experiment A, wasps (both tomato-experienced and host-experienced) reared on bean plants were used. During the night prior to the experiment the wasps were kept at 15 ± 1 °C in darkness. The wasps of each experience pre-treatment were used in the wind tunnel under treatments (1), (2) and (3). Each wasp was tested only once. All six combinations (2 pre-treatments, 3 experimental treatments) were tested on the same day.

Experiment B was similar to experiment A, but differed in that the wasps had been reared for one generation on *L. bryoniae* in tomato plants. In experiment C wasps reared on *L. bryoniae* in bean plants and given a host experience were observed under treatments (4) and (5). The experiments were run between November 1988 and February 1989. Experimental conditions were 25 ± 1 °C and 60 ± 10% RH.

Statistical analyses. The effects of treatment and stimulus on the parameters latency and proportion of latency spent 'aerially antennating' was analyzed by two-way ANOVA and multiple comparison tests (see legends of figures). To obtain normal distributions the following transformations were used: inverse of latency and arcsin of the proportion of latency spent 'aerially antennating'. The effects of treatment and stimulus on the parameters proportion of wasps flying upwind and proportion of wasps landing on leaves were analyzed by three-factor G-test (Sokal and Rohlf 1981).

Results

Experiment A: Parasitoids reared on hosts in bean plants

Latencies of wasps (Fig. 8-1) were affected by the stimulus offered, but no effect of previous experience was found (Table 8-1a). Latency was shorter when infested leaves were upwind than when uninfested leaves or no leaves at all were upwind. For the percentage latency spent 'aerially antennating' (Fig. 8-2) the effect of stimulus was different for the two experience groups (Table 8-1A): for tomato-experienced wasps this parameter was not affected by stimulus, but host-experienced wasps antennated less frequently when uninfested leaves or no leaves were offered than when infested leaves were upwind.

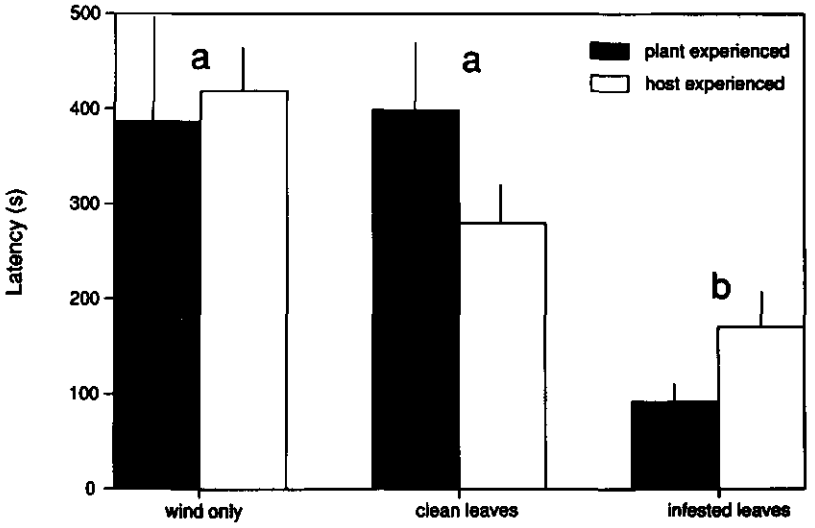

Fig. 8-1. Latency time (mean and SE) of *Dacnusa sibirica* females on a tomato leaflet in a wind tunnel in which uninfested tomato leaves, *L. bryoniae*-infested tomato leaves or no leaves at all (control) were offered upwind. Parasitoids have been reared on *L. bryoniae* in bean plants. Effects of experience are not significant (Table 1A). Effects of stimuli have been tested by ANOVA followed by Tukey's multiple range test (α = 0.05). Significant differences are indicated by different letters (N = 17 for each bar).

The effect of stimulus on proportion of wasps flying upwind (Fig. 8-3) was different for the two experience groups (three-factor G-test, G = 17.0, df = 2, P = 0.006). Host-experienced wasps flew more upwind towards infested leaves than towards uninfested leaves but only a small proportion (12%) flew upwind in an empty tunnel. In contrast, plant-experienced wasps flew predominantly (79%) upwind in an empty tunnel, which does not differ from the response to infested leaves. However, when uninfested tomato leaves were offered, a significantly lower proportion of tomato-experienced wasps flew upwind. No effects of stimulus or experience were seen for the proportion of wasps whose first flight ended on the offered leaves (Table 8-2A). When landing on the leaves was analyzed only for wasps that landed upwind, relative to the release point, the same conclusion was obtained.

These data show that *D. sibirica* behaved differently towards uninfested and infested tomato plants. The only parameter that did not show this is the proportion of wasps whose first flight ends on the leaves. Furthermore, prior experiences significantly affected *D. sibirica*'s behavior in the tunnel. This is obvious from the proportion of latency spent aerially antennating and the proportion of wasps flying upwind.

Table 8-1. Two-way ANOVA for behavioral components of *D. sibirica* females in a wind tunnel. Superscripts indicate significance levels. Data were transformed before analysis.

A: *Wasps reared on hosts in bean plants*

Variable		*F* values		
	Residual mean of squares	Between host-experienced and tomato experienced	Between stimuli	Interaction
Latency	$3.8 * 10^{-4}$	2.37^{ns}	$7.60^{0.0009}$	2.02^{ns}
% of latency spent 'aerially antennating'	$3.9 * 10^{-2}$	$20.58^{<0.0001}$	$6.84^{0.002}$	$4.89^{0.01}$

B: *First generation of wasps reared on hosts in tomato plants.*

Variable		*F* values		
	Residual mean of squares	Between host-experienced and tomato experienced	Between stimuli	Interaction
Latency	$4.9 * 10^{-5}$	0.02^{ns}	0.36^{ns}	2.47^{ns}
% of latency spent 'aerially antennating'	$6.0 * 10^{-2}$	$7.54^{0.007}$	$3.44^{0.04}$	$4.19^{0.02}$

Table 8-2. Landing of *D. sibirica* females on tomato leaves in a wind tunnel.

Experience	Stimulus	Landing on plant?		
		n(yes)[1]	n(no)	proportion yes
A: *Wasps reared on hosts in bean plants*				
Tomato leaf	uninfested	8	12	0.40 a[2]
	infested	9	11	0.45 a
L. bryoniae in tomato leaf	uninfested	4	16	0.20 a
	infested	9	12	0.43 a
B: *Wasps reared on hosts in tomato plants*				
Tomato leaf	uninfested	3	17	0.15 a[2]
	infested	6	14	0.30 a
L. bryoniae in tomato leaf	uninfested	4	11	0.27 a
	infested	6	14	0.30 a

1 N(yes)= number of wasps whose first flight ended on the offered leaves.
N(no) = number of wasps whose first flight did not end on the offered leaves.
2 Percentages of wasps landing on the plant, followed by the same letter are not significantly different (G-test, $\alpha = 0.05$).

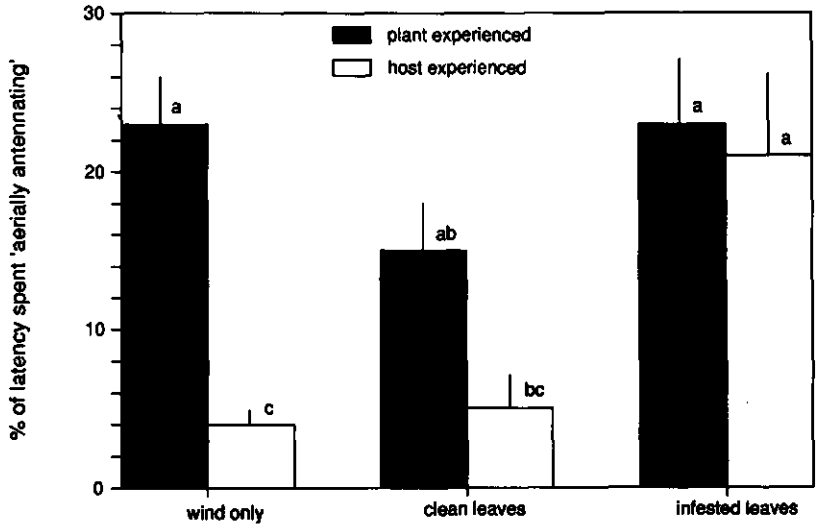

Fig. 8-2. Percentage of latency spent 'aerially antennating' (for description of behavior see Methods and Materials) by *D. sibirica* females on a tomato leaflet in a wind tunnel in which uninfested tomato leaves, *L. bryoniae*-infested tomato leaves or no leaves at all (i.e. control, only wind) were offered upwind. Parasitoids have been reared on *L. bryoniae* in bean plants for many generations. Bars with the same letter are not significantly different (repeated G-test, α = 0.05; N = 17 for each bar).

Experiment B: Parasitoids reared on hosts in tomato plants

Why would tomato-experienced wasps reared on hosts in bean plants have a lower tendency to fly upwind to uninfested tomato leaves than in an empty tunnel as described above (Fig. 8-3)? An explanation may be found in the experiences the wasps had had. They may have been stimulated by bean-related chemicals upon emergence (cf. Corbet 1985, Hérard et al. 1988b). Subsequently, they were kept in a petridish with an uninfested tomato leaflet which naturally did not result in host-encounter. The observed behavior towards uninfested tomato plants (Fig. 8-3) may have been caused by induced aversion towards this novel host plant. To investigate this, we reared wasps from the bean-strain for one generation on tomato plants infested by *L. bryoniae*. These wasps were given the same experiences as the bean-strain wasps and were observed in the wind tunnel.

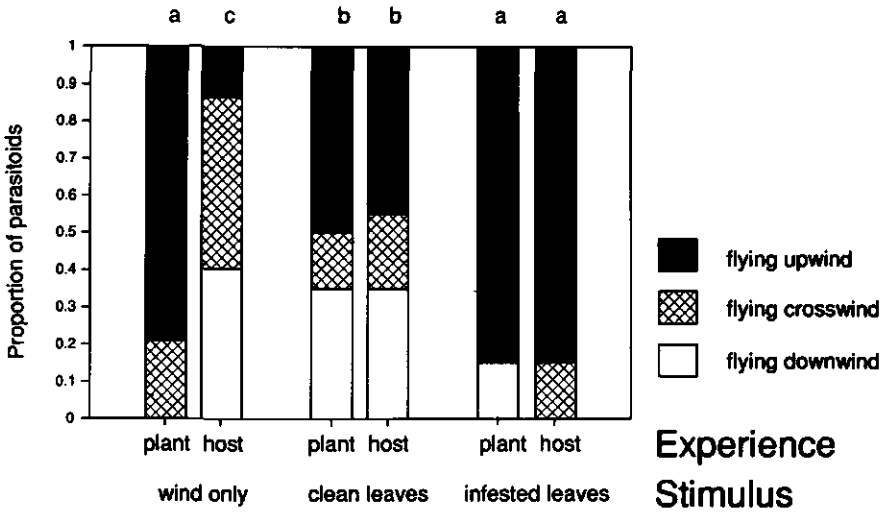

Fig. 8-3. Proportion of *D. sibirica* females flying upwind, downwind or crosswind in a wind tunnel in which uninfested tomato leaves, *L. bryoniae*-infested tomato leaves or no leaves at all were offered upwind. Parasitoids have been reared on *L. bryoniae* in bean plants for many generations. In all experiments 19 to 21 wasps have been used. Different letters above bars indicate that the proportion of wasps flying upwind is different between those bars (G-test on numbers, $\alpha = 0.05$).

The results (Fig. 8-4, 8-5 and 8-6, Tables 8-1B, 8-2B) were similar to those obtained for the bean-strain. The most striking difference lies in the parameter 'proportion of wasps flying upwind': in contrast to bean-reared wasps, the effect of stimulus was not dependent on experience (three-factor G-test, G = 1.25, df = 2, $P >> 0.05$) and the wasps responded similarly to clean leaves and wind only; an increased proportion of wasps flew upwind towards infested leaves (Fig. 8-6). This indicates that rearing history affects the response of *D. sibirica* to uninfested tomato leaves.

Another difference is that latencies (Fig. 8-4), although showing the same trend as observed for the bean strain (Fig. 8-1), are not significantly affected by stimulus or experience (Table 8-1B).

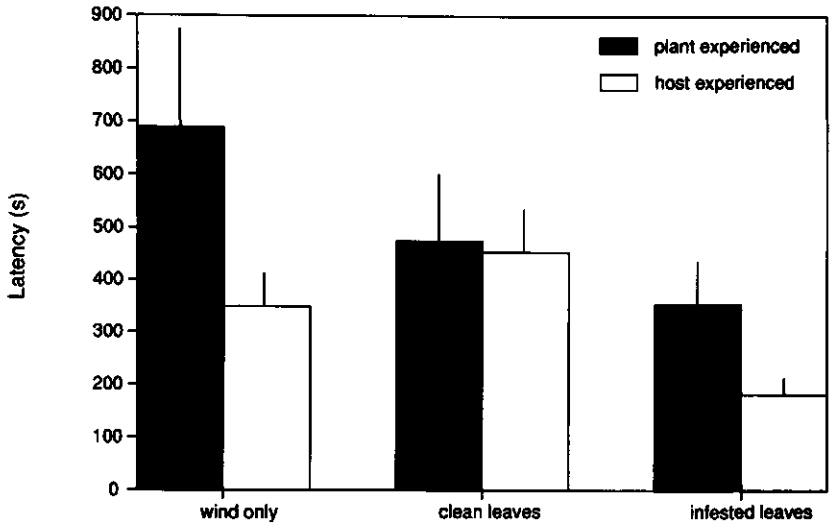

Fig. 8-4. Latency (mean and SE) of *Dacnusa sibirica* females on a tomato leaflet in a wind tunnel in which uninfested tomato leaves, *L. bryoniae*-infested tomato leaves or no leaves at all were offered upwind. First generations of parasitoids reared on *L. bryoniae* in tomato plants. No significant effects of experience or stimulus have been found (N = 15 for each bar; see also Table 8-1B).

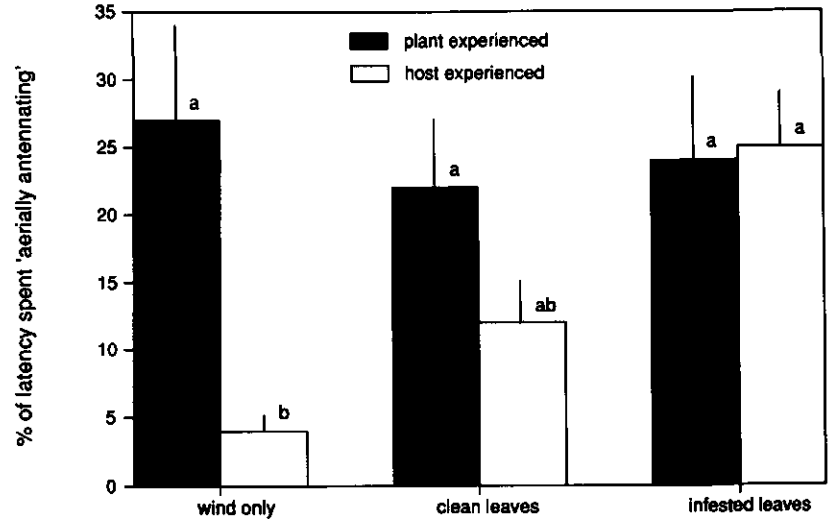

Fig. 8-5. Percentage of latency spent 'aerially antennating' (for description of behavior see Methods and Materials) by *Dacnusa sibirica* females on a tomato leaflet in a wind tunnel in which uninfested tomato leaves, *L. bryoniae*-infested tomato leaves or no leaves at all were offered upwind. First generations of parasitoids reared on *L. bryoniae* in tomato plants. Bars with the same letter are not significantly different (repeated G-test, α = 0.05; N = 15 for each bar).

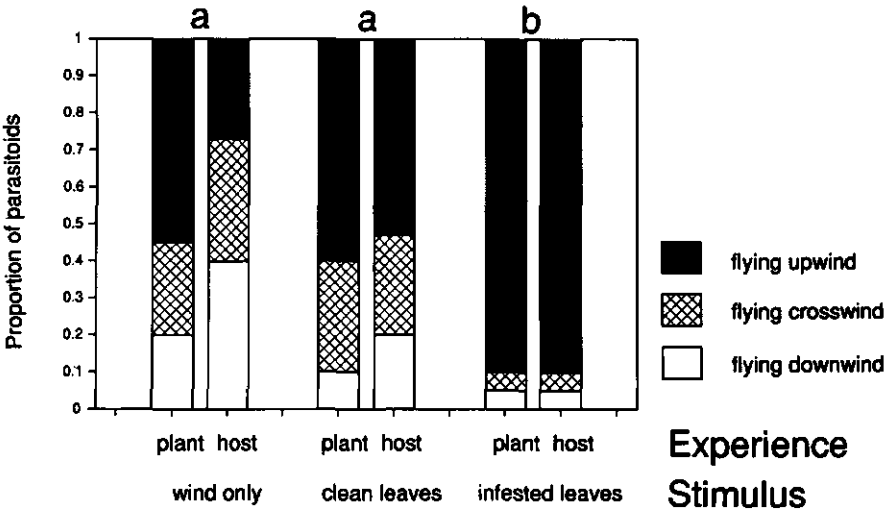

Fig. 8-6. Proportion of *Dacnusa sibirica* females flying upwind or downwind (crosswind flights excluded from analysis) in a wind tunnel in which uninfested tomato leaves, *L. bryoniae*-infested tomato leaves or no leaves at all were offered upwind. First generations of parasitoids reared on *L. bryoniae* in tomato plants. In all experiments 19 to 21 wasps have been used. The effect of the stimulus is not dependent on experience (three-factor G-test, $G = 1.25$, $df = 2$, $P >> 0.05$). Pairs of bars indicated by different letters differ in proportion of wasps flying upwind (G-test on numbers, $\alpha = 0.05$).

Experiment C: Visual plant stimuli absent; host-experienced parasitoids reared on host in bean plants

In the above experiments the leaves were offered in the flight tunnel and therefore these data do not provide conclusive evidence about the complex of the stimuli evoking the behavioral response. To investigate whether olfactory or visual stimuli were involved, we offered odor of uninfested or infested plants through a nozzle to host-experienced wasps of the bean-strain. In this situation without visual stimuli from the leaves, the proportion of wasps flying upwind differs significantly between treatments (Table 8-3). Latencies and percentages of latency spent aerially antennating were not significantly different (Table 8-3). For conclusions about the role of infochemicals on flight behavior the proportion of wasps flying upwind appears to be the most important parameter. For conclusions about the role of visual cues further experiments are needed.

Table 8-3. Behavioral response of D. sibirica in a wind tunnel towards tomato leaves. Wasps reared on hosts in bean plants for many generations. Odor injected into tunnel through a nozzle.

Stimulus n	Latency (s) spent aerially antennating	%Latency	No. wasps flying upwind	downwind	crosswind	Proportion[1] flying upwind
uninfested leaves 18	443 ± 100 a[2]	12 ± 2 a	2	11	5	0.15 a[3]
infested leaves 17	351 ± 53 a	9 ± 0.5 a	9	5	3	0.64 b

[1] Expressed as number flying upwind divided by total number of wasps.
[2] Values in same column, followed by same letter are not significantly different (Mann-Whitney U test, $\alpha = 0.05$).
[3] Values in same column, followed by same letter are not significantly different (G-test done on numbers of wasps, $\alpha = 0.05$).

Correlation between 'aerially antennating' and proportion of wasps flying upwind

The proportion of latency spent aerially antennating was recorded because this behavior of chemoreceptor-carrying extremities appeared to be relevant in perception of volatile infochemicals. All take-offs were preceded by this behavior, but it was also displayed without subsequent take-off. The behavior suggested that the wasps were sampling the air for presence and/or direction of volatile cues. The parameter shows a positive correlation with the proportion of wasps flying upwind (Linear regression analysis $F = 18.6$, $r^2 = 0.61$, $df = 13$, $P < 0.001$), which suggests that it is of significance in interception of information about the surroundings.

Discussion

Distinguishing between uninfested and infested tomato plants: olfactory cues

Our data agree with previous observations of *D. sibirica*: the proportion of first landings on the plant is similar for uninfested and infested plants (cf. Hendrikse et al, 1980). However, we observed that latency on an uninfested tomato leaflet is shorter when infested leaves are offered than when uninfested leaves are offered. Our observations show that the proportion of latency spent aerially antennating and the proportion of wasps flying upwind are affected by the upwind stimulus. The latter parameter is affected by olfactory stimuli (Table 8-3). Thus, present data show that *D. sibirica*

employs a volatile kairomone, emitted from tomato leaves infested by *L. bryoniae,* in long-range host location. However, this kairomone does not result in differences in landing on infested and uninfested leaves in the wind tunnel.

Visual cues in parasitoid flight behavior

In wind tunnel investigations of parasitoid host-location behavior published to date, visual stimulation has not been observed (Drost et al. 1988, Hérard et al. 1988a). Our data suggest that visual stimuli affect preflight behavioral parameters in *D. sibirica.* The visual differences (color) between infested and uninfested tomato leaves were very apparent in the present investigation. However, just as observed by Hendrikse et al. (1980) this clear visual difference did not significantly affect the proportion of flies that landed on the plant. Interestingly, the behavior of another braconid leafminer parasitoid, *D. rufiventris,* on a leaf is affected by visual stimuli (Sugimoto et al. 1988a). For more conclusive evidence about effects of visual cues affecting *D. sibirica*'s foraging behavior wind tunnel investigations are needed with artificial plants that have visual stimuli similar to natural plants, but do not release infochemicals. Some other interesting examples of visual cues affecting parasitoid flight behavior have recently been discovered (Van Alphen and Vet 1986, Van Giessen et al. unpubl., Wäckers et al. unpubl.).

Effect of previous experiences on parasitoid flight behavior

The behavioral response of *D. sibirica* was affected by previous experience, as was evident from proportion of latency spent aerially antennating and proportion of wasps flying upwind. It is especially noteworthy that in the latter case the most obvious difference is seen for the response in an empty tunnel (Fig. 8-3 and 6): tomato-experienced wasps fly upwind and host-experienced wasps fly downwind. This indicates that the difference in experience does not affect the behavior towards uninfested and infested plants, but rather affects their reference behavior: the behavior when no information about plants is available at all. This resembles observations on the predatory mite *Phytoseiulus persimilis* Athias-Henriot in a wind tunnel: starved predators walked upwind in clean air but satiated predators walked downwind (Sabelis and Dicke 1985). Starvation of predators is the result of prey deprivation, whereas tomato-experienced *D. sibirica* had been subjected to host deprivation. In both cases, deprived animals move upwind and endowed animals move downwind. This behavior enables recently endowed animals to return to a prey or host patch after leaving it at the windward side. It will be interesting to see how long the effect of host experience on this behavior remains present.

Host-location behavior of D. sibirica: a new point of view

Current data indicate that perception of host and plant stimuli does not lead to increased landing on plants infested with leafminers in a wind tunnel. Olfactory and presumably also visual stimuli rather affect decisions about departing from uninfested leaves: (1) visit duration on uninfested leaves seems to be affected by visual cues of uninfested and heavily infested plants (in combination with infochemicals ?) and (2) once the parasitoid departs, the direction of take-off is affected by presence/absence of a volatile host kairomone or host-plant odors in combination with previous oviposition experience of the wasp. So far, no cues have been identified which affect landing of the parasitoid: landing seems to occur with equal frequencies on infested and uninfested leaves, despite clear visual differences. Recently, it has been found that *D. sibirica* has an initial landing preference for plants with a high host density over plants with a low host density (Chapter 9).

Effect of rearing history on foraging behavior

Our data indicate that the plant aspect of rearing history of *D. sibirica* affects the wasp's behavior towards uninfested tomato leaves. Wasps reared on hosts in tomato are less affected by a non-rewarding experience on a tomato plant than wasps reared on hosts in bean (Fig. 8-2). Although it concerns a minor effect in this instance, the phenomenon in itself should make one alert. Examples of rearing effects through host or prey on behavior of parasitoids and predators have been recorded previously (Vet 1983, Dicke et al 1986, Noldus 1989b), and recently also effects of the host's diet on behavior of members of the third trophic level have been reported (Hérard et al. 1988b, Drost et al. 1988, Ding et al. 1989, Dicke et al. 1990). This may have implications for mass production of natural enemies for biological control, if the effects are more pronounced than found in this study.

Acknowledgments

We thank Mario Nagtzaam for help with preliminary experiments and Joop van Lenteren and Lucas Noldus for critical review of a previous version of the manuscript.

9

Influence of host density on intra- and interplant foraging behavior by the leafminer parasitoid *Dacnusa sibirica*[1]

Abstract

Behavior of the parasitoid *Dacnusa sibirica* Telenga foraging among four tomato plants with two different densities of the leafminer *Liriomyza bryoniae* (Kaltenbach) was directly observed in the laboratory to examine whether aggregation of searching time would lead to directly density dependent parasitism under these conditions. Time allocation and parasitism were analyzed at a plant and leaf level. Parasitoids showed an initial landing preference for plants with high densities of hosts over plants with fewer hosts. Both visiting time and searching time increased with an increasing number of hosts at the plant and leaf level. Consequently, the proportion of leafminers parasitized was significantly higher on high host density plants in comparison with low density plants, strongly suggesting direct density dependence at the plant level. However, parasitism at the leaf level seems density independent. Oviposition rates based on visiting time or on searching time were significantly higher on high host density plants than on low density plants and also at the leaf level, oviposition rates positively correlated with host density, implying an over all enhanced searching efficiency with increasing numbers of hosts.

Initial GUT, i.e. searching time of visits during which no ovipositions occurred, was significantly higher on leaflets within high host density plants than on leaflets within low host density plants. Apparently, a high number of hosts induced area-restricted search through a prolonged initial GUT, which partly explains the searching time aggregation on leaves within high host density plants and the direct density dependence found at the plant level. The density dependent parasitism occurs at that spatial level probably because these parasitoids are informed about the distribution of their hosts among plants.

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[1] To be published as: O.P.J.M. Minkenberg, M. van der Haagen and L.E.M. Vet. Influence of host density on intra- and interplant foraging behavior by the leafminer parasitoid *Dacnusa sibirica*. Submitted to *Entomologia experimentalis et applicata*.

Introduction

The way in which parasitism is related to host density might form a key component in successful biological control (Walde and Murdoch 1988, Noldus and Van Lenteren 1990). Parasitoids as biological control agents are expected to show non-random search, which will cause aggregation of foraging time, i.e. the total time spent, in areas with high densities of hosts (Beddington et al. 1978, Waage and Hassell 1982). Aggregation of foraging time may lead in theory to directly density dependent, density independent or inversely density dependent parasitism (Royama 1971, Hassell 1982). Foraging time aggregation leading to density independent parasitism has been observed in field studies reported by Waage (1983), Smith and Maelzer (1986) and Hammond et al. (1990). Density independent parasitism in the field has often been reported and seems the commonest form of parasitism (Morrison and Strong 1980, Murdoch et al. 1984, Lessells 1985, Stiling 1987, 1988, Walde and Murdoch 1988, Hirose et al. 1990).

Since some components of foraging behavior e.g., resting, are activities of parasitoids that are unrelated to finding hosts, the relationship between where the parasitoids spent most of their time foraging and the way in which parasitism is distributed, is likely to be concealed. Waage (1983) and Morrison (1986a) speculate that only aggregation of searching time might result in direct density dependence. To date, there is only one field example of searching time aggregation resulting in direct density dependence (Summy et al. 1985). Moreover, searching time aggregation in patches of high host densities does not necessarily lead to density dependence (Morrison 1986a, Rosenheim et al. 1989). Since variability in searching time may obscure an underlying density dependent trend, Morrison (1986b) emphasizes the necessity of measuring aggregation of searching time in individual parasitoids and of determining distribution of parasitism as a result of their behavior. Further, the scale at which time aggregation is related to parasitism may influence the outcome of a study (Heads and Lawton 1983).

Foraging parasitoids that maximize the rate at which they encounter suitable hosts have a selective advantage (for a review of foraging theory, see Stephens and Krebs 1986). When hosts are patchily distributed, encounter rate can be increased by searching in places with a high density of suitable hosts (Cook and Hubbard 1977, Hubbard and Cook 1978). This can be achieved by (1) locating hosts or associated material from a distance (Van Alphen and Vet 1986); hence, parasitoids should prefer to land in places with hosts or the probability of landing should be related to the level of infestation; and by (2) concentrating search within places of high host density, i.e. area-restricted search (Charnov 1976, Kareiva and Odell 1987). Both characteristics, especially when combined in one forager, are expected to lead to searching time aggregation and so, to directly density dependent parasitism, at least at an individual level.

We investigated time allocation from the perspective of foraging theory by assuming that the parasitoid uses "simple rules" to decide how long to

search for hosts in a specific area. A model applied to examine these decision rules is Giving Up Time (GUT). This model assumes that a wasp uses a time threshold and constantly measures time elapsed since certain events such as an oviposition. If the time since her last oviposition exceeds some threshold, then the wasp will leave the specific area. As the threshold is presumed to vary according to the information about hosts present, the model assumes a variable GUT. The initial GUT, i.e. searching time when no hosts are encountered or oviposited in a 'patch' with hosts (Morrison and Lewis 1981), might be affected by stimuli associated with the presence of hosts (Waage 1979). Also, GUT might depend on the size of the area searched (Van Lenteren and Bakker 1978) and might be reset after encounters with hosts that led to ovipositions (Van Lenteren and Bakker 1978, Waage 1979) or after encounters with parasitized hosts (Galis and Van Alphen 1981, Bakker and Van Alphen 1988). A different threshold model has recently been proposed, particularly for leafminer parasitoids (Sugimoto et al. 1987, Sugimoto and Tsujimoto 1988). These parasitoids apparently mark the mines or the leaf area visited with a pheromone. This model assumes that a parasitoid after arrival on a leaf measures the concentration of pheromone deposited and it leaves when the concentration exceeds a certain threshold. This threshold also may be variable.

The braconid *D. sibirica* is synovigenic and attacks sessile larval instars of, e.g. *L. bryoniae*, which is a leafmining fly common on glasshouse tomato (Minkenberg 1990, Minkenberg and Helderman 1990). This parasitoid is presently used as biological control agent for leafminers on vegetables in northwestern European glasshouse (Minkenberg and Van Lenteren 1986). The foraging behavior of this species has previously been described by Hendrikse and Zucchi (1979). Our main objective was to examine the relationship between time aggregation and parasitism in a simple setup. The specific questions addressed were whether (1) the probability of landing was related to host density of plants, (2) individual parasitoids would allocate most of their searching time to areas with high host densities, (3) a possible searching time aggregation would result in direct density dependence, and (4) a variable GUT model would be consistent with the results. The responses of the parasitoid were analyzed at different spatial levels, viz. plant, leaf and leaflet. Foraging behavior of parasitoids had to be observed directly in a patch choice situation with variable durations of observations, depending on when the parasitoid exited (Van Lenteren and Bakker 1978). Certain processes occurring at the population level as in the field, such as mutual interference, egg limitation and others were excluded. Our setup represented a situation between the traditional petri dishes and the field.

Materials and methods

Experimental design. Freshly emerged female parasitoids were removed from rearing (Minkenberg 1990) and kept singly in small glass vials; the next day females were given oviposition experience by exposing them individually to a whole tomato plant, *Lycopersicon esculentum* cv. Moneydor, with ca. ten leaves infested by at least 40 *L. bryoniae* leafminers for ca. eight hours in a transparent, cylindrical cage (Minkenberg and Helderman 1990). After that they got access to non-infested plants only until the experiment 48 hours later to complement their egg load. The foraging behavior of individual, four-day old females of *D. sibirica* was observed in a controlled laboratory environment at 20°C ± 0.5°C with 80% ± 10% RH.

Observations were initiated an hour after the onset of the photoperiod, i.e. 9000 h. Wasps were released from a non-infested leaflet placed in a glass vial, which was in the center of a square table (70 cm) with a tomato plant at each corner (Fig. 9-1). Plants were partly artificial in that they consisted of a plastic stake as stem with five small plastic vials attached to it for holding the tomato leaves. Every leaf was reduced to five leaflets. Artificial stems were used to simplify the experimental environment and to facilitate manipulation of host densities on plants and leaves. However, keeping leafminer densities constant at both the plant and leaf level was not feasible and a certain variation in the densities of hosts offered had to be tolerated. To examine landing preference, two "plant types" were offered: two plants contained a low number of leafminers, 13.0 ± 2.9, and the other two a high number, 40.7 ± 10.3 (Mean ± SD). The position of plants was chosen randomly. Leaves with the highest number of leafminers were placed in the middle (leaf 3), lowest numbers on the top (leaf 1) and at the bottom (leaf 5) and intermediate numbers on leaf 2 and 4, which corresponds to a clumped vertical distribution found in glasshouse tomato (Westerman and Minkenberg 1986). Vertical distribution of mean (± SD) host density on leaf 5 to 1 for low host density plants was 0, 3.5 (± 1.7), 5.4 (± 1.7), 3.8 (± 1.8) and 0 leafminers, respectively (range 0 - 10), and for high host density plants 5.4 (± 3.1), 9.2 (± 3.5), 13.1 (± 3.6), 7.4 (± 4.3) and 4.4 (± 3.6) leafminers, respectively (range 0 - 20). Only late second or third instars were offered to the parasitoids to avoid host size selection.

A square tent of white cloth (2 m height by 1.7 m) covered over the table to ease observations of the black parasitoid. The observer wore a white hat and laboratory coat for camouflage, and a mouth filter for reducing air flow during speech while following the wasp from a distance of 0.5 m within the tent.

Collection of behavioral data. A second person outside the tent recorded the events on a computer. Three classes of events were distinguished: (1) position on plants, (2) position on leaves, and (3) behavior. Plant position was between plant 1 to 4 or off plant e.g., on the table. Leaf position was between leaf 1 to 5, or off leaf e.g., on the stem of a plant. Four behavioral

components were recorded: flying, searching i.e., walking on leaf surface off the mine or standing still with antennae or ovipositor in action, handling i.e., all behaviors on a mine including oviposition, and standing still. Mostly encountering a mine led to oviposition, if the host had not previously been parasitized (92% of the 271 leafminers found contained eggs). Both starting time and ending time for each behavior were registered, which enabled us to calculate visiting time (T) i.e., the total time spent in a specific area, as the sum of searching time (T_s), handling time (T_h) and time standing still (T_r). Observations were ended when wasps flew away from the plants and landed on the tent cloth or when they stayed motionless for at least 20 minutes. After each set of observations leafminers stung by wasps were dissected to determine the presence of eggs. Observations were made of thirteen females, one set of observations per female.

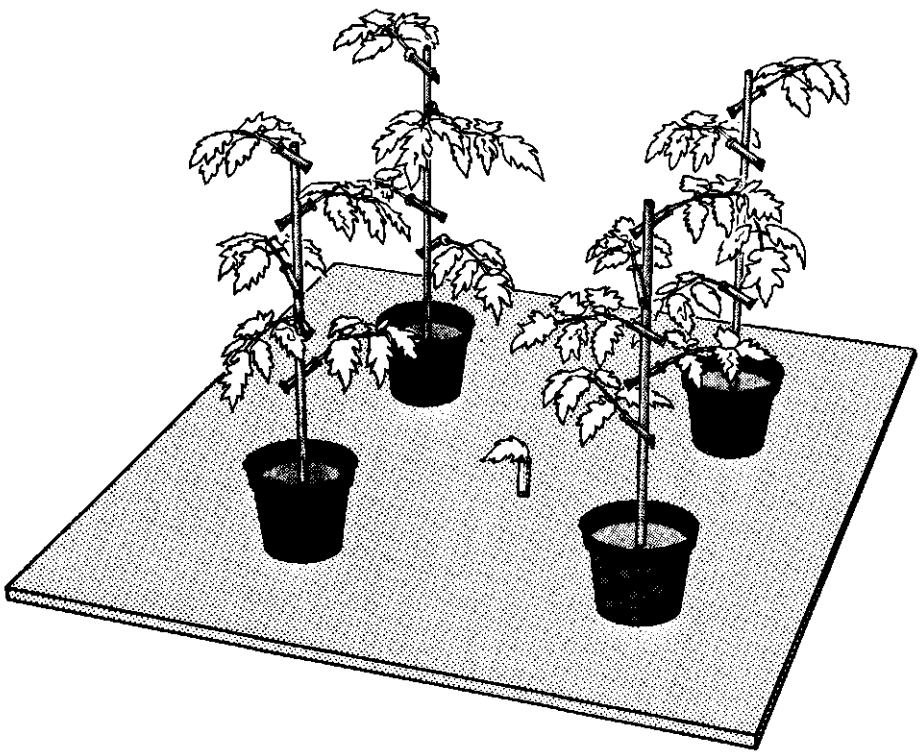

Fig. 9-1. The setup with four plants consisting of an artificial stem with five test tubes each holding a single tomato leaf. Two high and two low host density plants were prepared for every set of observations. Female parasitoids were individually released from a vial with a small tomato leaflet in the center of the arena and her behavior subsequently observed.

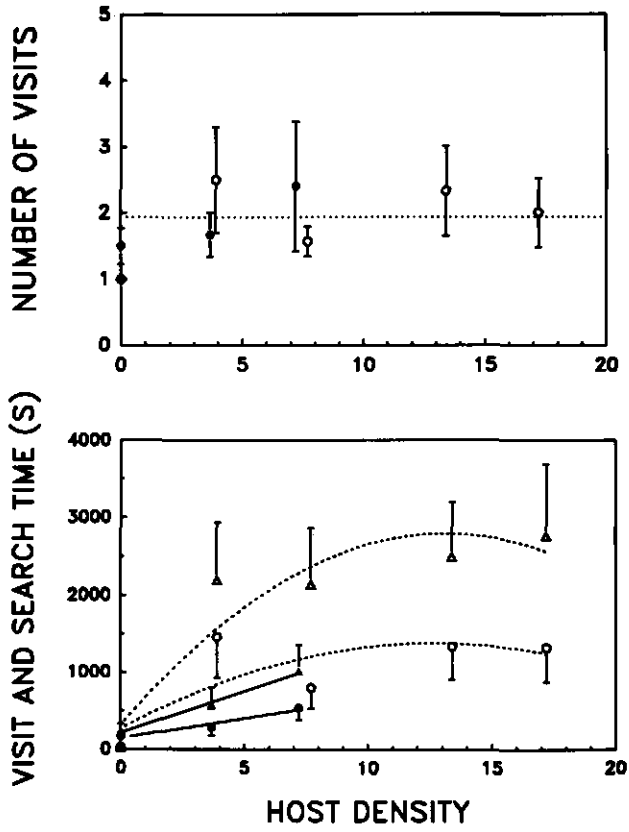

Fig. 9-2. Mean ± SE total numbers of visits per parasitoid to leaves versus host density of leaf (linear regression: P = 0.20, df = 66). Dotted line shows mean number of visits.

Mean ± SE visiting times (triangles) and searching times (circles) at leaves within high host density plants (open symbols) and low host density plants (filled symbols) versus host density of leaf. Linear regression of square root transformed time against host density on leaves within the low density plants gives for T: Y = 2.53 X + 15.66, r2 = 0.39, df = 62, $P < 0.0001$, and for T_s: Y = 1.58 X + 13.1, r^2 = 0.30, df = 61, $P < 0.0001$. Polynomial regression lines for time spent on leaves within high host density plants are given to show non-linear trends.

Visits and parasitism related to host density. The relationship between host density and (1) number of visits, (2) visiting time and searching time, (3) number and proportion of leafminers parasitized and (4) oviposition rate (number of leafminers parasitized divided by either visiting time or searching time) during the observation was analyzed by linear regression (SAS 1985). Before analysis a logit transformation i.e., log ((P + 1)/(N - P + 1)), for the proportion parasitized was conducted (P = number of hosts parasitized, N = number of hosts) and a square root transformation on all other dependent variables to promote normality. In the case of superparasitism, an encountered host was only counted once as an oviposition. All observations were pooled for the analysis. Concordance between females within pooled data was examined using a homogeneity-of-slopes model (SAS 1985). Since data for individual females were unbalanced at leaf level, female was included as a classification variable i.e., the data pooled per wasp, and the type III Sums of Squares were interpreted. All regression analyses were checked for significant interactions between independent variables. Because there was significant heterogeneity between individuals at the leaflet scale, linear regression was only applied at the plant and leaf level. For graphical purpose only, leaves were divided in five classes according to host density, viz. 0, 1-5, 6-10, 11-15 and 16-20 leafminers; the linear regression was done on the original data. GUT on leaflets was transformed by log and was not significantly different from a normal distribution.

Results

Landing preference and number of visits

Ten of the thirteen wasps observed flew initially to a high host density plant (G test: G = 3.974, $P < 0.05$). Thus, females were able to distinguish between high and low host density plants from a distance. Regarding the total number of visits, high host density plants were visited not significantly more often than low host density plants (23 vs. 15 times; G test: $P > 0.05$). Although the wasps could move freely between the plants, most wasps visited only two of them during the period of observation. None of the wasps visited all four plants.

Total number of visits to leaves did not depend on density of hosts on the leaves (Fig. 9-2). The number of leaves visited within high host density plants were higher, however not significantly, than those visited within low host density plants (Table 9-1).

Table 9-1. Influence of plant type (mean ± SE numbers of leafminers/plant) on mean ± SE numbers of visits to plants, numbers of newly visited leaves during first plant visit, numbers of newly visited leaves during all plant visits, and total numbers of leaves visited within a plant; on visiting times (s), searching times (s), numbers of hosts parasitized on plant, proportions parasitism (of the number of leafminers/plant), oviposition rates (number of hosts parasitized/hour) based on visiting time or based on searching time.

	Host density on plant		Test
Response	Low (13.0 ± 0.9)	High (40.7 ± 2.9)	Significance
			Wilcoxon
No. plant visits	1.2 ± 0.1	1.5 ± 0.2	Z = -1.48, P = 0.14
No. leaves newly visited during:			
first plant visit	1.8 ± 0.3	2.4 ± 0.4	Z = -0.67, P = 0.50
all plant visits	2.1 ± 0.4	2.9 ± 0.4	Z = -1.30, P = 0.19
Total no. leaf visits	4.0 ± 1.2	6.0 ± 1.7	Z = -1.00, P = 0.32
			Linear regression (df = 27)
Visiting time	1071 ± 307	5489 ± 1053	F = 22.9, P < 0.0001
Searching time	596 ± 122	2724 ± 578	F = 12.6, P = 0.002
No. hosts parasitized	1.9 ± 1.0	13.5 ± 2.9	F = 26.0, P < 0.0001
Proportion parasitism	0.12 ± 0.06	0.30 ± 0.05	F = 7.0, P = 0.01
Ovip. rate (T)	4.0 ± 2.1	7.9 ± 1.1	F = 10.9, P = 0.003
Ovip. rate (T_s)	7.4 ± 3.8	25.4 ± 8.1	F = 10.3, P = 0.004

Visiting and searching time

Parasitoids allocated most part of visiting time to searching, on average 54.9 ± 2.3% of the time observed. Handling time took 39.5 ± 1.8%, whereas time spent standing still was only 5.4 ± 2.0%. Most *D. sibirica* females left the setup of four plants without depleting their egg load. However, a few wasps stopped searching and stood still for an extended period, probably because eggs were not available for oviposition. Dissection of these wasps indeed showed a few eggs; mean (± SE) numbers of mature eggs in the wasps that left the plants after observation versus those that were inactive for more than 20 minutes, was 28.3 ± 7.8 (N = 6) versus 9.7 ± 3.3 (N = 3).

Wasps spent more time on plants with high leafminer densities (Table 9-1). Of the visiting time allocated, significantly more time was spent searching on high density plants compared to on low density plants.

Table 9-2. Analysis of variance on the influence of plant type, viz. low and high host density, on square root transformed visiting time and searching time on leaves. Model applied was Time = Plant Type * Wasp * Host density. Only leaves of which host density varied between zero and ten were included. The analysis shows that both visit and searching time on leaves with a comparable host density were significantly influenced by the number of hosts present in the plant visited.

Dependent variable: visiting time

Source	*df*	Sum	Mean Squares	*F* value	Significance
Model	13	$7.0 * 10^7$	$5.3 * 10^7$	7.36	$P < 0.0001$
Error	34	$2.5 * 10^7$	$0.7 * 10^7$		
Corrected total	47	$9.5 * 10^7$			
Plant type	1	$4.0 * 10^7$		4.91	$P = 0.008$
Wasp	11	$0.6 * 10^7$		7.83	$P = 0.0002$
Host density	1	$0.8 * 10^7$		11.38	$P = 0.002$

Dependent variable: searching time

Source	*df*	Sum	Mean Squares	*F* value	Significance
Model	13	$1.2 * 10^7$	$1.0 * 10^7$	3.38	$P = 0.002$
Error	34	$1.0 * 10^7$	$0.3 * 10^7$		
Corrected total	47	$2.2 * 10^7$			
Plant type	1	$0.8 * 10^7$		6.04	$P = 0.02$
Wasp	11	$0.2 * 10^7$		2.53	$P = 0.02$
Host density	1	$0.1 * 10^7$		3.77	$P = 0.06$

To examine time allocation at the leaf level, leaves within the range of zero to ten hosts only were first compared between the two types of plants. Linear regression analysis showed a significant effect of plant type on the relation between visiting time or searching time and host density per leaf (Table 9-2). Both visiting time and searching time on leaves with low host densities within high host density plants were significantly longer than those within low density plants. Therefore, responses of parasitoids to leaves had to be analyzed separately for the high and low density plants. Within low density plants, visiting time and searching time were significantly positively correlated with host density, whereas within the high density plants a non-linear relationship was found (Fig. 9-2). Within high density plants, parasitoids spent more time on leaves with hosts than on non-infested leaves. Their time spent on infested leaves did not correlate with the number of hosts available. This might partly result from different responses

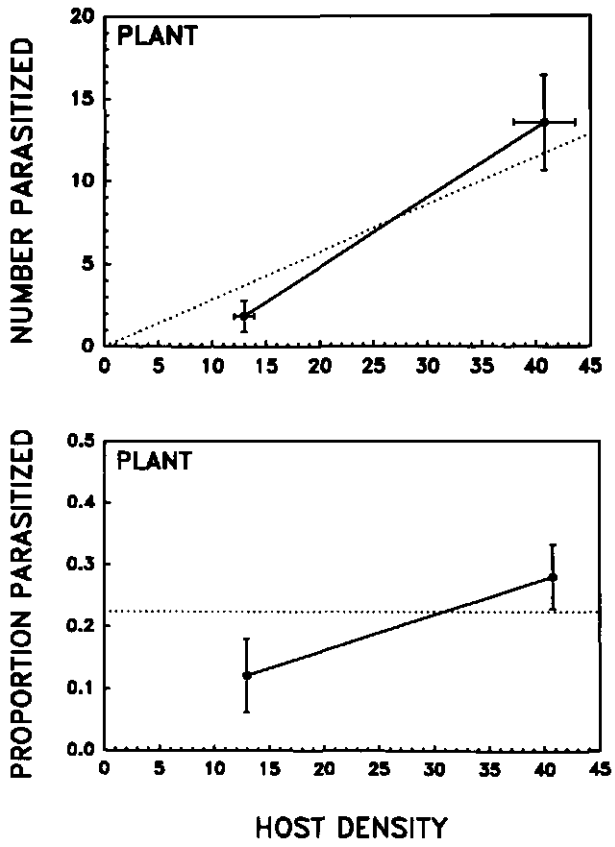

Fig. 9-3. Mean ± SE number of hosts parasitized per female on plants with low and high densities of leafminers (mean ± SE host densities present showed by horizontal error bars). Square root transformed number of hosts parasitized is significantly related with host density: $F = 15$, $df = 27$, $P = 0.0006$. Dotted line represents linear regression of number of hosts parasitized against hosts present if there is density independence.

Mean ± SE proportion of hosts parasitized per parasitoid versus host density on plants with high and low host densities. Linear regression of proportion of parasitized hosts against host indicates a significant relationship (F = 7.0, df = 27, P = 0.01). Dotted line at mean proportion of hosts parasitized on plants represents density independence.

at the ranges in host densities of leaves visited by the wasps: a significant interaction between wasp and host density was found against searching time on leaves within high host density plants (F = 3.4, df = 37, P = 0.007; for Wasp * Host Density, P = 0.006).

Parasitism and host density

Parasitism is directly density dependent when the number of hosts parasitized as a function of numbers available increases at an increasing rate. To demonstrate direct density dependence, numbers parasitized have to be non-linearly related to host density or when there is a significant linearity, the intercept of the linear regression line has to be significantly different from zero. Thus, proportion of hosts parasitized should be significantly correlated with host density i.e., the slope of that linear regression line should be larger than zero. We examined whether these conditions were met at the two spatial levels. At the plant level (Fig. 9-3), the number of leafminers parasitized was positively correlated with increasing host density with an intercept significantly different from zero (T test: T = -5.1, P < 0.0001). In addition, the proportion of leafminers parasitized was significantly positively correlated with host density with a slope significantly different from zero (T = 2.6, P = 0.01), strongly suggesting density dependent parasitism at the plant level.

By contrast, parasitism on leaves was found to be independent of host density. Although it appears from Fig. 9-4 that average parasitism on leaves within low density plants was lower, a significant difference due to plant type, viz. low or high host density, between both number of hosts parasitized and proportion parasitism against host density was not found (analysis of covariance: P = 0.66). Therefore, data for both plant types were pooled, after which a significant, positive relationship was found between number of hosts parasitized and host density per leaf. Furthermore, the proportion parasitism was not significantly related with host density (P = 0.66), suggesting density independent parasitism at the leaf level.

Oviposition rates and host density

Oviposition rates at high host density plants were significantly higher than those at low density plants (Table 9-1). While visiting time and searching time were approximately five times as high on high density plants, oviposition rate based on searching time was more than three time as high on high density plants, whereas oviposition rate based on visiting time was only twice as high, in comparison with oviposition rates on low density plants. At leaves, oviposition rates based on visiting time significantly increased with increasing host density, as did oviposition rates based on searching time (Fig. 9-5).

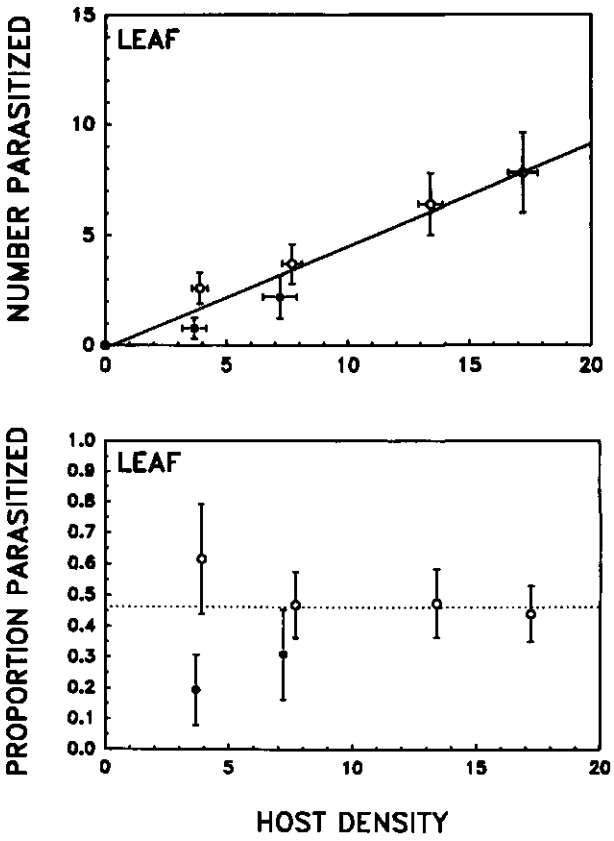

Fig. 9-4. Mean ± SE number of hosts parasitized on leaves within high density plants (open circles) and low density plants (closed circles) versus host density of leaves. Linear regression of number of hosts parasitized against host density suggests density dependence at the leaf level (F = 8.2, df = 66, P < 0.0001). Analysis was done on the original data.

Mean ± SE proportion of hosts parasitized per parasitoid versus host density on leaves within high host density plants (open circles) and low density plants (closed circles). Dotted line shows mean proportion of hosts parasitized on leaves.

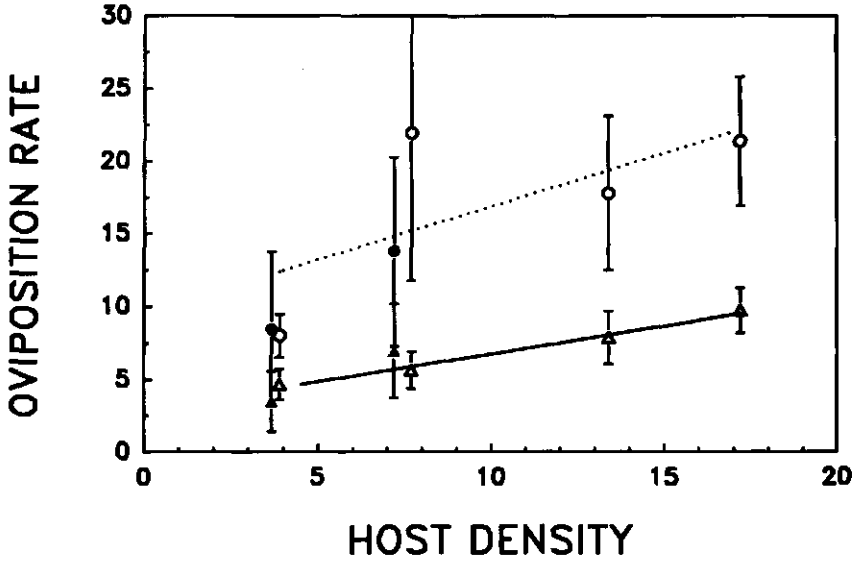

Fig. 9-5. Mean ± SE oviposition rates (leafminers parasitized/hour/parasitoid) based on visiting time (triangles) and searching time (circles) within high density plants (open symbols) and low density plants (closed symbols). Linear regression line of T based oviposition rates (F = 1.7, df = 50, P = 0.009) and of T_s based oviposition rates (F = 1.4, df = 50, P = 0.01) against host density are shown.

Giving Up Time

We defined initial GUT as total searching time of visits during which no mines were encountered and during which no ovipositions occurred, whether or not mines were present. There was no significant difference between initial GUT on leaflets or leaves with or without leafminers (Wilcoxon; leaflet: first visit only, P = 0.31, df = 115; total, P = 0.33, df = 357; leaf: first visit only, P = 0.50, df = 27; total, P = 0.24, df = 68). Therefore, these data were pooled. Initial GUT on leaflets was significantly higher within high than within low host density plants, whereas initial GUT on leaves was similar (Table 9-3). It was concluded that the wasp "sets" her initial GUT at the smallest scale, viz. leaflet, and that the host density of the plant influences the height of the time threshold set. We further examined whether the number of hosts present on a leaflet influenced initial GUT. However, initial GUT on leaflets was not correlated with host density (low host density plants: P = 0.15; high host density plant: P = 0.58). Initial GUT on both plant types was significantly shorter than the respective GUTs (low host density plant: P = 0.04; high host density plants:

Table 9-3. Comparison of the mean ± SE initial GUTs (s) on leaflets and leaves within low and high host density plants. Values were log transformed before analysis.

	Plant			
	Low density	High density	*F* value	Significance
Leaflet	57.3 ± 17.6 (n = 101)	75.6 ± 7.6 (n = 258)	3.3	$P = 0.006$
Leaf	171.1 ± 67.2 (n = 31)	113.4 ± 29.0 (n = 39)	2.1	$P = 0.42$

$P = 0.01$). Searching time after having located a leafminer and oviposited in it was longer than when it would have been without a successful encounter with a leafminer. Further GUT increased with the number of ovipositions within both low and high host density plants (Fig. 9-6). Analysis of covariance showed that there was a significant influence of plant type on the relationships between GUT and number of ovipositions (Table 9-4). Similar correlations were found between GUT and host density, although less significant than with number of ovipositions. Our observations indicate a variable GUT model, whereby searching time on leaflets is influenced by host density of the plant visited and as a consequence, the number of ovipositions.

Table 9-4. Analysis of covariance on the influence of plant type, viz. low and high host density, on the relationship between log transformed GUT and the number of ovipositions (ranging from zero to two only). Model used was LogGUT = Plant Type * Wasp * Oviposition. The analysis shows that the regression lines between logGUT and number of ovipositions for the two plant types have significantly different intercepts.

Dependent variable: logGUT

Source	*df*	Sum	Mean Squares	*F* value	Significance
Model	14	223.2	15.9	11.56	$P < 0.0001$
Error	445	613.7	1.4		
Corrected total	459	836.9			
Plant type	1	11.4		8.3	$P = 0.004$
Wasp	12	65.3		3.9	$P < 0.0001$
Oviposition	1	120.3		87.2	$P < 0.0001$

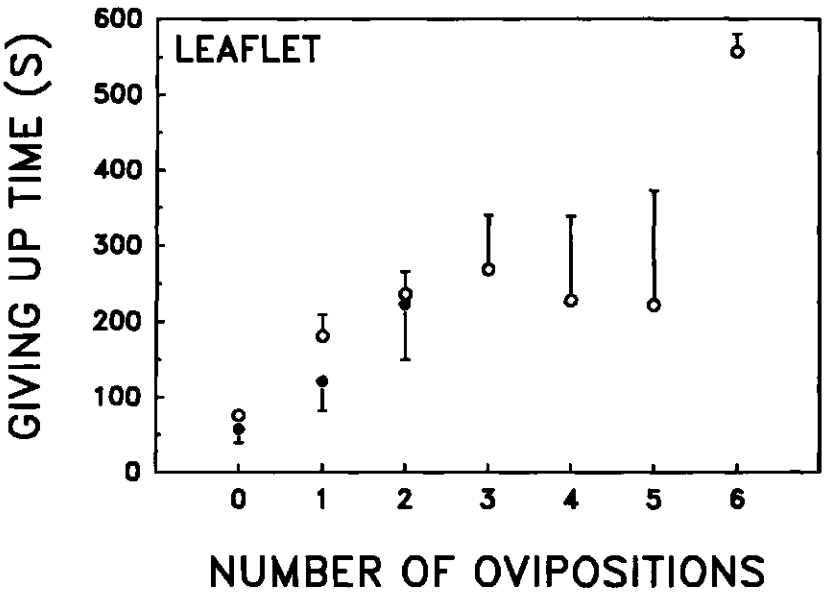

Fig. 9-6. Mean ± SE Giving Up Times (s) versus number of ovipositions on leaflets and initial GUT i.e., time spent searching on leaves without encountering a host, within low host density (closed circles) and high host density (open circles) plants. Log transformed GUT significantly correlated with number of ovipositions for both plant types; low host density plant: $F = 4.9$, $df = 55$, $P = 0.0003$ with for no. of ovipositions $P = 0.008$; high host density plant: $F = 7.8$, $df = 295$, $P < 0.0001$, with for no. of ovipositions $P < 0.0001$.

Discussion

The parasitoids can distinguish from a distance between the two levels of host density as shown by their interplant movement in our setup. By contrast, Hendrikse et al. (1980) found an equal landing response to infested and non-infested plants in *D. sibirica*. It is not mentioned whether these wasps had an oviposition experience before the experiment. Non-experienced wasps may respond differently (Vet and Van Opzeeland 1984). As air movements were limited in our setup, landing behavior is most likely based on perception of diffused volatile kairomones. We have observed wasps to be regularly in an upright position waving their antennae i.e., "aerial antennating," suggesting they might have sampled the environment for volatile kairomones. They show this typical behavior when being at a leaf edge or curve and usually just before taking-off from the leaf. Wind tunnel

experiments have confirmed that *D. sibirica* females do distinguish infested from non-infested plants through volatile chemicals (Chapter 8), although visual cues may be involved as well. By perception of volatile chemicals, *D. sibirica* may get information about the distribution of hosts over neighboring plants.

Prasad et al. (unpubl.) found that *D. sibirica* visits significantly more leaves in plants with leafminers or feeding punctures present than in plants without any hosts or damage. These differences in intraplant movement may be explained by the likely hierarchy in host distribution at different spatial levels. Leaves within high host density plants have a higher probability to contain hosts and in addition have more hosts (> ca. five leafminers in our case) than leaves within low density plants. Once encountering a high density leaf, the probability to find hosts on surrounding leaves is high. More visits to leaves within high host density plants than to leaves within low host density plants enhances the encounter rate with hosts.

The term patch is sometimes difficult to define (Van Alphen and Vet 1986). The strong relationship at the leaf level between number of host parasitized and host density would suggest that a leaf is the spatial level at which *D. sibirica* responds to hosts (Fig. 9-4). If we consider GUT as satisfactorily explaining time allocation, leaflet is actually the level at which *D. sibirica* decides to stay or to leave (Table 9-3). However, host densities of plants influence GUT (Table 9-4) and initial landing preference. In addition, density dependent parasitism could be shown only at the plant level (Fig. 9-3), suggesting a plant or group of plants is the "true patch" defined as the area over which variation in host density most strongly influences parasitoid foraging (Rosenheim et al. 1989). Thus, what is perceived as a patch by *D. sibirica* is hard to specify.

At the leaflet scale, initial GUT appears to be influenced by plant type visited. The parasitoids may perceive hosts from surrounding areas through volatile chemicals. Host density of the leaf might influence initial GUT on leaflets as well, because it cannot be excluded as a factor due to the intrinsic hierarchy of host distribution. Initial GUT may be predetermined by the absolute or relative number of hosts present e.g., within a plant, or initial GUT is changed through experience during visiting a plant. Observations by Prasad et al. (unpubl.) showing that searching time on a "clean" leaf is prolonged by the presence of hosts or other host-derived stimuli on other leaves in the plant, support the first option. A relatively high initial GUT as a mechanism may explain the longer searching on leaflets and consequently on leaves within high host density plants. Searching is prolonged after an oviposition experience as has been found previously for other parasitoids (Van Lenteren and Bakker 1978, Waage 1979). In summary, *D. sibirica* once arrived on a leaflet uses a variable GUT that is influenced by the host density of the plant and that is probably independent from the number of hosts present on the leaf or leaflet visited, to determine its searching time. During searching GUT is reset by every oviposition, perhaps by a constant increment.

The use of a variable GUT to "decide" searching time is probably a response to the clumped host distribution of *L. bryoniae* among and within plants (Westerman and Minkenberg 1986). We did, however, not investigate the influence of contacts with previously parasitized leafminers. Searching time may be reduced by contacting already parasitized hosts (Roitberg and Prokopy 1984, Bakker and Van Alphen 1988). Recent work by Nelson and Roitberg (unpubl.) shows that the GUT of the leafminer parasitoid *Opius dimidiatus* (Ashmead) is slightly increased after contact with parasitized hosts as compared to initial GUT. The presence of empty mines increases initial GUT in *D. sibirica* (Sugimoto et al. unpubl.).

When *D. sibirica* revisits a previously searched mine, its behavior is different from an encounter with a mine housing a non-parasitized host: she usually spends a short time on the mine (Hendrikse et al. 1980). This change in behavior suggests the presence of an external marking pheromone on the mine or on the area near the mine (Sugimoto et al. 1986). Marking the leaf area surrounding the mine may be functional in that leafminers continue to eat and may get disconnected from the external marker due to displacement. The frequent use of the ovipositor by *D. sibirica* during searching on the leaf and the mine is also meant to apply a marking pheromone (Sugimoto et al. unpubl.). An external marker might persist for a short period, viz. several hours (Sugimoto et al. 1986). This is plausible when marking hosts is for the female's own benefit instead of deterring other searching females (Roitberg and Mangel 1989), assuming the probability of encountering previously parasitized hosts decreases over time.

Foraging theory predicts that the most profitable places should be visited first or longest. In the case that parasitoids are not limited by their number of eggs, they are expected to allocate their searching time to maximize their encounter rate with suitable hosts (Charnov and Skinner 1985). In agreement with these predictions, most *D. sibirica* females initially visited a high host density plant and allocated most time to searching of leaves within high host density plants, including those leaves with relatively low densities of hosts. Since oviposition rate on leaves significantly increased with increasing host densities, the number of leafminers parasitized was greatly enhanced as well. Nevertheless, parasitism at the leaf level appeared to be density independent within the high density plants probably due to the allocation of time to leaves irrespective of their host density. At the plant level, however, the preference of landing, the increased searching time at high host density plants compared to low density plants and a higher oviposition rate led to directly density dependent parasitism, at least at the level of the individual.

Acknowledgments

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10

Evaluation of criteria to select parasitoids for seasonal inoculative biological control: a case study[1]

Abstract

To evaluate parasitoids and to develop a preintroduction selection procedure in seasonal inoculative biological control, four criteria are examined: (1) Complete Development and Offspring Quality, (2) Generation Synchrony, (3) Population Growth, and (4) Searching Efficiency. Their concepts are discussed and ways to measure the criteria in the laboratory described. Measurements will produce sets of values for each parasitoid. Empirical evidence and theory show that all criteria should be examined, giving complete sets of values, before selecting parasitoids. Theoretical considerations on the criteria are given, with an emphasis on population growth. Since not one case study on preintroduction selection exists, the criteria are evaluated for two effective parasitoids, *Dacnusa sibirica* and *Diglyphus isaea*, which are presently used for the biological control of agromyzid leafminers on tomatoes in two distinct areas: northwestern and southern Europe, respectively. Population Growth and Generation Synchrony partly explain the difference in effectiveness of these parasitoids in temperate versus warm Europe. However, additional data and experiments, e.g. on their searching efficiency, are necessary.

Introduction

Augmentation of natural enemies provides a diverse way to suppress arthropod pests. For field crops, feasible and reliable biological control methods have been developed (Anonymous 1979, King et al. 1985). Pilot studies were conducted by Huffaker and Kennett (1956) and Oatman et al. (1968) to get effective control of spider mites on strawberries releases of predatory mites. Only until recent augmentative biological control has become implemented on strawberries on a large scale in California

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(Grossman 1989). Various reasons may be adduced for this rather late success, but it demonstrates the potential of the inoculative or inundative release programs in the field.

In greenhouse vegetables particularly, biological control by mostly seasonal inoculative releases and using a variety of predators and parasitoids has been applied successfully over the last two decades (Hussey and Scopes 1985, Van Lenteren and Woets 1988). The greenhouse provides a model ecosystem for studying theoretical concepts in biological control, because it is a relatively closed and homogeneous habitat with a limited number of species present and thereby, climate and plant fertilization is often controlled. Concepts developed for greenhouse ecosystems may further be examined for more complex and variable systems as field crops.

New effective biological control methods have to be developed in the near future to meet the goal of reducing pesticide use in agriculture. Therefore, it is desirable to change from the traditional approach to finding effective natural enemies with a minimum of fundamental research to a thorough preintroduction selection procedure. The selection procedure should enhance the probability of identifying an effective natural enemy compared to randomly testing natural enemies known to attack the pest. One method is using characteristics of effective natural enemies as criteria to select beneficial candidates: the reductionist approach (Waage 1990).

To establish criteria for selecting parasitoids in preintroduction studies, biological characteristics contributing to their effectiveness as control agents, must be identified (Van Lenteren 1980). Selection criteria depend on the type of release strategy: 1) inoculative (classical), 2) seasonal inoculative and 3) inundative introductions (Van Lenteren 1983). Selection criteria have been reviewed by Van Lenteren (1980, 1986a) and are specifically discussed for classical biological control by Huffaker et al. (1977) and for inundative biological control by Hirose (1986), Smith and Hubbes (1987) and Pak (1988). This paper specifically deals with seasonal inoculative biological control using parasitoids.

What is seasonal inoculative biological control?

In a seasonal inoculative release program (Fig. 10-1), parasitoids are introduced during a short period of time to control a pest population over several generations. This method is usually applied against multivoltine pests during one growing season (Van Lenteren 1986a). Compared to classical biological control, the ratio of the number of natural enemies introduced and the number of the insect pest present, i.e. introduction ratio, is relatively large in seasonal inoculative biological control. An important distinction from inundative releases is an initial increase in pest population

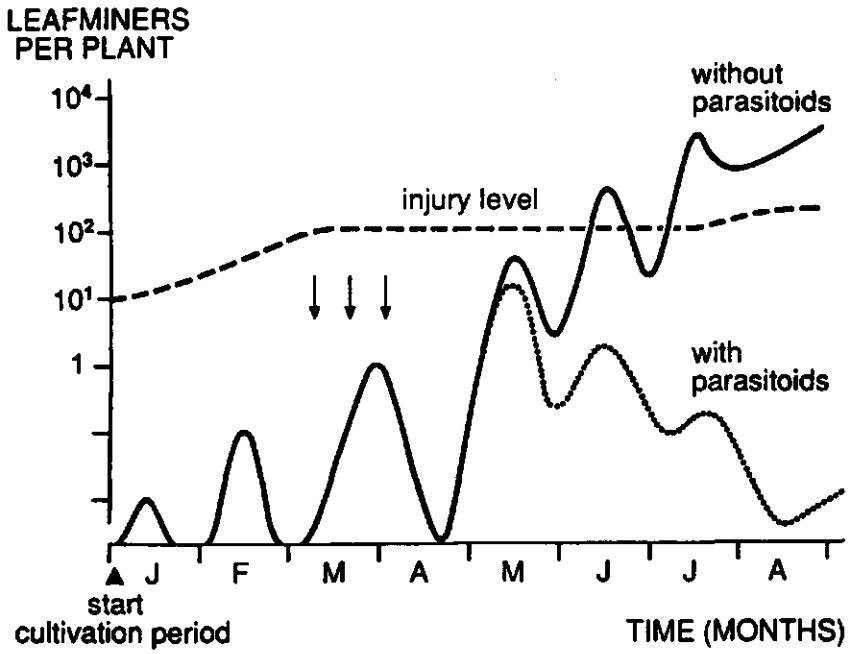


Fig. 10-1. Seasonal inoculative biological control of *Liriomyza* spp., expected leafminer density without control (——) and once biological control (-----) takes effect (hypothetical case). Arrows indicate repeated releases of parasitoids during one leafminer generation.

after the initiation and prior to achieving control when a seasonal inoculative program is used (Sabelis 1981, Eggenkamp et al. 1982a,b, Wyatt 1983, Minkenberg et al. unpubl.). As long as the economic injury level is not exceeded anytime during the growing season, an increase in host density is tolerated and the seasonal inoculative program is considered effective. Correspondingly, an effective natural enemy can be defined as a natural enemy that prevents a pest from exceeding the injury level in commercial greenhouses.

In classical biological control a stable pest-enemy equilibrium has been proposed as a necessary component (Varley et al. 1973) and regulation may occur by density dependent mortality of the pest (Hassell et al. 1976), although field data on successful biological control do not always yield evidence of stability (Murdoch et al. 1985). Apparently, occurrence of stability depends on the specific pest system (Kareiva 1987, Waage and Greathead 1988). For a further review on biological control and stability, I refer to Murdoch (1989). In the greenhouse, long-term stability cannot play a role simply because of the limited production period of ca. 6 to 12 months

(Van Lenteren 1986a). However, densities of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) and its parasitoid *Encarsia formosa* Gahan seem to be quite stable, at least over a few generations in commercial greenhouses (Eggenkamp et al. 1982a,b, Noldus and Van Lenteren 1990).

Non-random search by natural enemies causing their spatial aggregation in patches of high host densities is considered to be a key factor in density-dependent regulation (Beddington et al. 1978, Waage and Hassell 1982, Heads and Lawton 1983). Aggregation may stabilize but under very specific conditions only (Murdoch and Stewart-Oaten 1989). Murdoch et al. (1984) show that effective natural enemies may search randomly, in which case spatial aggregation does not necessarily occur. Other examples of successful classical biological control suggest that density-dependent parasitism seldom occurs (Walde and Murdoch 1988). Presently, it cannot be concluded from theory or practice that direct density-dependent regulation is essential in seasonal inoculative biological control.

Development of a preintroduction selection method: why?

Current selection of natural enemies often proceeds by trial-and-error method: the empirical approach (Ehler 1990a). This usually involves the separate release of different natural enemies into commercial greenhouses and subsequent evaluation of the results by monitoring the pest and natural enemy population. Although the approach has yielded several commercially feasible biological control agents for greenhouse crops, its great disadvantage is that it provides little information on the causes of success or failure of control. Consequently, a comparison of natural enemies to determine which may be the most effective agent is extremely difficult. Furthermore, dependence on this technique makes it difficult to manipulate or improve the use of natural enemies.

The preintroduction selection method outlined here, the analytical approach (cf. predictive *sensu* Ehler 1990a), is conducted in the laboratory for two reasons: (1) *to reduce time and labor* and (2) *to provide a controllable space.* Research in commercial greenhouses is time-consuming and often labor intensive, especially when accurate sampling is necessary. A highly controlled environment is needed whereby single variables relative to the natural enemy's biology can be studied. This approach supposes that the outcome of laboratory studies can indicate effectiveness of parasitoids.

After completion of the laboratory selection procedures, greenhouse tests are still needed to validate the effectiveness of the selected natural enemies. The main aim of the laboratory selection is in the first place to discard those natural enemies which cannot be effective. When initiating the preintroduction selection procedure, all natural enemies known from the literature for a specific pest or collected in the field should be considered. Often a number of candidates can immediately be rejected based on literature information and their biology (see criterion 1). After a first selection

the other candidates should, ideally, further be evaluated with this procedure, although this will rarely be possible.

Development of a preintroduction selection method: how?

On the basis of a literature review of attributes of effective natural enemies, Van Lenteren (1986a) selected six criteria: (1) Development on host, (2) Climatic adaptation, (3) No negative effects, (4) Good culture method, (5) Great reproductive potential and (6) Good density responsiveness. After reconsideration, I put together Van Lenteren's criterion 1 and 3 because they are related to each other. Further, climatic adaptation, criterion 2, is dropped because abiotic factors play a role in the measurement of most attributes of an insect. Thus, attributes are not necessarily constant (Ehler 1990a). Criterion 4, a good culture method, is dropped as well because this is not specifically an attribute of the natural enemy (see also below). Criterion 5 and 6 are maintained and renamed. Finally, I add the criterion generation synchrony, which has not been listed previously but just implicitly acknowledged by Van Lenteren (1986a). Since density dependence is presently not considered to be essential for success, it is not regarded as a selection criterion. All together, I examine four criteria for the preintroduction selection: (1) Complete development and Offspring Quality, (2) Generation Synchrony, (3) Population Growth and (4) Searching Efficiency. These criteria are related to different fields in biology, viz. insect physiology and life history theory (cohort analysis), phenology, population dynamical and behavioral ecology. To verify the validity of the criteria, the set of values assigned to the parasitoids examined should be related to their effectiveness.

In this paper I describe the development of a methodology for selecting parasitoids, which should be applied prior to their mass rearing and releases in the greenhouse. Criteria for the preintroduction selection procedure are discussed and illustrated mainly for two hymenopterous parasitoids, *Dacnusa sibirica* Telenga and *Diglyphus isaea* (Walker), which parasitize two agromyzid leafminers, *Liriomyza bryoniae* (Kalt.) and *L. trifolii* (Burgess) on greenhouse tomatoes.

The agromyzid flies *L. bryoniae* and *L. trifolii* are economically important pests on tomatoes in Europe. *D. sibirica* is currently used for leafminer control on greenhouse vegetables in northwest Europe; *D. isaea* is released in both greenhouse and outdoor vegetables in southern Europe (Minkenberg and Van Lenteren 1986). The commercial use of both *D. sibirica* and *D. isaea* provides empirical evidence for their effectiveness. However, the magnitude of their effect as biological control agents still needs to be shown experimentally.

From the initial studies of a pest problem until the full-scale application of a newly developed biological control method, three phases can be identified and each with its own subjects of research and limitations: (1)

Research; (2) *Development;* and (3) *Implementation.* The *Development* phase involves designing the methods of introduction, mass-rearing and storage, and quality control of natural enemies (Van Lenteren 1986a,b, Dicke et al. 1989, Noldus 1989). *Implementation* is the introduction of the new method in greenhouse practices (Van Lenteren 1987). This paper only deals with the *Research* phase. It should be noted that a parasitoid selected by the preintroduction procedure may be discarded later due to constraints in the next phases (e.g., cost-benefit analysis).

Evaluation framework

Criterion 1: Complete Development and Offspring Quality

Concept. Natural enemies must be able to complete their development on the pest insect successfully in order to control successive generations. In addition to pre-adult survival and adult reproduction, lower and upper (e.g., temperature) thresholds for development and oviposition may be examined. Further, parasitoid adults should maintain quality, e.g. reproductive fitness, over generations of the specific target insect. At the moment of release, the viability of natural enemies should be optimal as a result of good mass rearing techniques (Van Lenteren 1986b). In addition, effective seasonal inoculative programs might require high intraspecific genetic variation in the parasitoids to be released (cf. Roush 1990). The reason is that the conditions in the greenhouse highly differ from those in the mass-rearing (Van Lenteren 1986a), probably urging a strong selection of parasitoids released during their first generation. Only a relatively low number will survive after that first generation in the greenhouse (i.e., a 'population bottle-neck').

Furthermore, parasitoids showing negative effects, such as hyperparasitism, should be eliminated (Caltagirone and Huffaker 1980). The environmental impact of biological control, a possible negative side-effect, is not discussed in here (for a recent review, see Ehler 1990b).

Example. Dacnusa sibirica and *D. isaea* complete development on the leafminers *L. bryoniae* and *L. trifolii,* and were thus suitable for further consideration. Life history studies have shown that survival and reproduction are high on *L. bryoniae* (Minkenberg and Helderman 1990, Minkenberg 1989). In contrast, *Opius pallipes* Wesmael, indigenous to Europe, was discarded for further evaluation because its eggs are encapsulated by the imported leafminer *L. trifolii* (Woets and Van der Linden 1983). *Dacnusa sibirica* and *D. isaea* show no hyperparasitic activity. The Japanese parasitoid *Chrysocharis pentheus* Walker should not be introduced for leafminer control because it is a facultative hyperparasitoid on the key parasitoid *D. isaea* (Takada and Kamijo 1979).

Table 10-1. Waiting time, i.e. average number of days that adult parasitoids must bridge between emergence and appearance of suitable host instars) in the case of discrete host generations (predictions based on life history studies, after Minkenberg 1989, 1990). For *D. isaea* the period without host feeding, which mainly occurs on the early, small larval instars, is given.

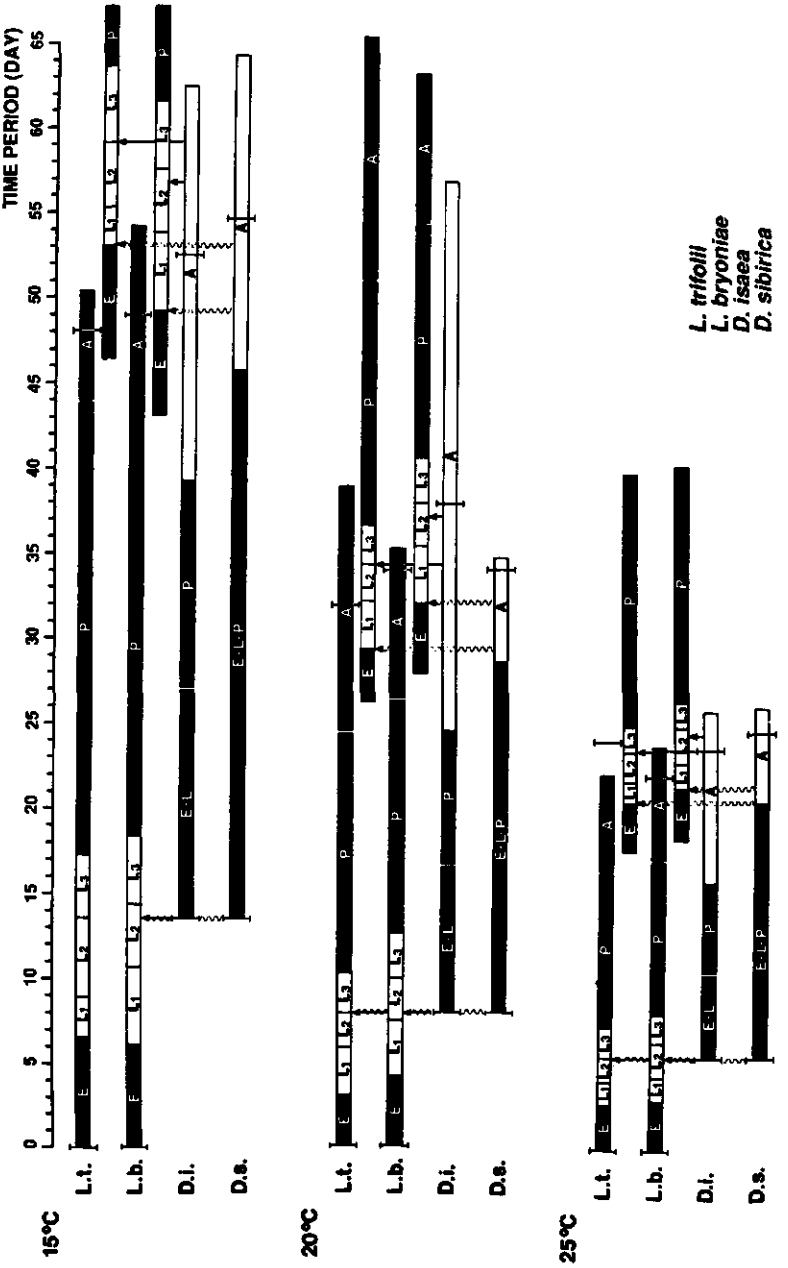
Temperature	Host	*D. sibirica*	*D. isaea*
15 °C	*L. bryoniae*	4	10
	L. trifolii	7	14
20 C	*L. bryoniae*	1	12
	L. trifolii	3	10
25 °C	*L. bryoniae*	1	9
	L. trifolii	0	8

Criterion 2: Generation Synchrony

Concept. Development of parasitoid and host must be synchronous when suitable host instars are present only during a short period per generation and when host generations are not overlapping. A relatively short generation time of the natural enemy cannot be overcome by making two or more smaller releases during one host generation, because a large proportion of its offspring might not survive until the next host generation. The synchrony between parasitoid and host generations is analyzed from life-history studies by estimating the minimum period that a newly emerged parasitoid has to bridge before a suitable host is available, i.e. 'waiting period' (see also example below). The choice of host instars, which can be used for oviposition or feeding by a parasitoid, plays a significant role in the synchronization.

If host generations overlap with suitable hosts constantly present or all host instars are accepted for feeding or oviposition, this criterion is of minor significance.

Example. Dacnusa sibirica develops somewhat faster than its host *L. bryoniae,* resulting in a waiting period for the adult female of one day at 20-25 °C to 3.6 days at 15 °C (Table 10-1). *D. sibirica* parasitizes all larval instars and does not feed on hosts, whereas *D. isaea* successfully parasitizes only late larval instars and feeds on all leafminer instars (Minkenberg et al. unpubl.); for *D. isaea* the period without host feeding is given. With *L. trifolii* as a host, the waiting period is 0.2 to 7.4 days at the same temperatures. The development time of *D. isaea* is much shorter than that of *D. sibirica.* The *D. isaea* females have to wait between ca. 8 days at 25 °C


TIME PERIOD (DAY)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
15°C
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L.t.
L.b.
D.i.
D.s.
E
L1
L2
L3
P
A
E-L
E-L-P
L. trifolii
L. bryoniae
D. isaea
D. sibirica

and ca. 12 days at 15 °C before they can start feeding on the hosts and even longer before encountering a suitable host instar for oviposition (Fig. 10-2). Long waiting periods are likely to reduce reproduction by *D. isaea* in the greenhouse. Leafminer populations in southern Europe comprise several overlapping generations during much of a growing season. This overlapping is generally caused by immigration of flies into the field from weeds and other crops where generation cycles are shifted or show a different periodicity (Chandler and Gilstrap 1987). Waiting by *D. isaea* is less likely to play a role there.

During summer in western Europe, the short generation time of *D. isaea* is a relative advantage when the pest has overlapping generations and there is no waiting time for the adult. Adult survival might then be enhanced implying a greater population growth on successive host generations: a high 'turn over', i.e. a number of generations per host generation (cf. Askew 1971, p. 218, Campbell et al. 1974). This may lead to the relatively high numbers observed during summer.

Criterion 3: Population Growth

Concept. Population growth can be estimated by the intrinsic rate of increase, r, or by net reproduction, R_0. The population characteristic r is defined as the rate of natural increase in a closed population which has been subject to constant age-specific schedules of fertility and mortality for many years and has converged to be a stable population (Pressat 1985). The r values are, by definition, only applicable for the (experimental) conditions under which they have been assessed (Lotka 1925). For example, a change in food quality would require a new series of laboratory experiments. Furthermore, its use is thus only appropriate, by following Lotka (1925), when the population has reached stable age distribution. Assumptions of stable population theory are a closed population, one sex and fixed birth and death rates (Coale 1972, Keyfitz 1985).

Another population statistic is the net reproduction, R_0, which is the mean number of female eggs produced by an average newborn female during her life time. This is a suitable measure of population growth rate per generation, because it also equals the factor by which the population density will be multiplied in the next generation. Its use implies that generations do not overlap. Such a situation occurs in Dutch greenhouses where the population of the leafmining fly *L. bryoniae* is composed of a single develop-

Fig. 10-2. Development of agromyzid flies *L. bryoniae* and *L. trifolii* and their parasitoids *D. sibirica* and *D. isaea* at three constant temperatures. White boxes indicate interacting stages, arrows indicate parasitization and vertical bars (read off against time scale) indicate generation time (E = egg, L_1 = first, L_2 = second, L_3 = third instar, P = pupa and A = adult).

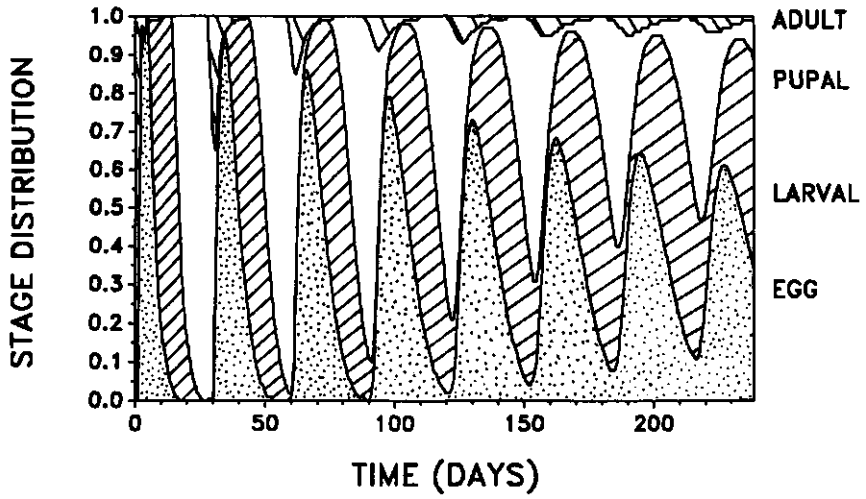

Fig. 10-3. Convergence of an initial adult population of leafmining flies, *L. bryoniae,* consisting out of 190 females with ten females per age class of one day, towards a stable age distribution. The first eight generations are shown. Note that the first generations of leafminers are discrete, hence there are host-free periods for the parasitoids, whereas later generations overlap.

mental stage in the beginning, as adults emerge rather simultaneously after diapause (Minkenberg and Van Schelt, unpubl.). Throughout the entire greenhouse, the generations of larvae do not overlap for at least three generations; there are 8 to 10 generations per growing season, i.e. per year. Population projections for *L. bryoniae* using the Leslie (1945) matrix to examine the convergence of a leafminer population towards a stable age distribution showed that this situation will not be reached within 40 generations (initial cohort is 50 ovipositional females; based on laboratory life history) and that generations do not overlap for 4 generations (Fig. 10-3). The fraction of adults at the stable age distribution is fairly low (Table 10-2), as result of a relatively short female longevity (ca. one week), which might explain the relatively long convergence time of the initial adult population towards a stable age distribution. This implies that r and R_0 of flies will change constantly during the season.

Two basic questions can be distinguished in relation to the use of estimates of population growth: 1) Can we use population growth parameters to compare potential growth of parasitoids with that of the pest insect?

Table 10-2. Stable stage distribution of the leafmining fly *L. bryoniae* compiled by summing proportions in stable age distribution over the appropriate age classes. A subdivision is given for the adult stage only; data from Minkenberg and Helderman (1990).

	Temperature		
Stage	15 °C	20 °C	25 °C
Egg	46.2%	39.0	41.9
Larval	35.5	41.9	40.6
Pupal	16.3	17.0	14.9
Adult	2.0	1.7	2.2
Preovipositional females	0.5	0.3	0.2
Ovipositional females			
first half	1.5	1.4	1.9
second half	0	0	0.1

2) What is the value of comparing potential growth of parasitoids between each other in order to evaluate their effectiveness?

With regard to the first question it has often been stated in the theory on classical biological control (Doutt and DeBach 1964, Huffaker et al. 1974, Rosen 1984) that an effective natural enemy should have a high population growth. It might be valid for two reasons. First, a high reproduction is considered to increase the probability of establishment for a colonizing species, which is an essential trait in classical biological control. Second, the number of natural enemies surviving at the beginning of every growing season is not the decision of the practitioner but depends on the dynamics of the interaction between pest and natural enemy in classical biological control. However, Huffaker and Messenger (1964, p. 107) remark that with regard to reproductive capacity, attempts to ascertain the value of a given natural enemy simply by use of comparisons of its inherent fecundity and generation time with that of the host or prey species has been overstressed. A parasitoid may have a lower reproductive capacity than its host and yet be effective.

An hypothesis was introduced in the theory on seasonal inoculative biological control (Van Lenteren 1986a) that an effective parasitoid has a population growth represented by its intrinsic rate of increase, r_m, that is at least equal to that of its host. When a natural enemy increases faster than the pest, the pest will always be controlled eventually. However, also in the case of a lower population growth rate of the natural enemy compared to

that of the pest, effective control can be achieved. In that case the relative number of natural enemies introduced is crucial, but it can be lower than that of pest insects present to get control (Wyatt 1983, Minkenberg and Damsté 1990).

Use of population growth parameters estimated in the laboratory to predict population growth in the greenhouse for different insects assumes that their population development is equally affected by factors operating in the greenhouse. Population growth of parasitoids in the greenhouse is more likely to deviate from the population parameters estimated than that of the host (see below). The population growth values estimated in the laboratory are unlikely to be useful to compare population growth of parasitoids with that of their host under practical condition for three reasons:

(1) *Linearity.* Stable population theory, or the Lotka model, assumes a linear change in log numbers of a insect over time. Only a constant proportion of the host population can be removed for parasitism, otherwise it will not show constant growth. However, population development of the host is expected to be differently affected by the parasitoid population during the season. The parasitoid may show constant growth only with a superabundance of hosts.

2) *Density-dependent effects.* Hosts usually have easy access to host plants and reproduction is usually only limited at high host density. In contrast, realization of potential reproduction by the parasitoid depends on, among others, their searching for hosts. Parasitoid reproduction is thus likely to be limited at low host densities due to low encounter rates. Reproduction by parasitoids further does not depend on age but mainly on the ability to find hosts; and searching may be individually affected by specific experiences and stimuli, which may alter the ability to find hosts during aging (Van Alphen and Vet 1986, Vet 1988). Another aspect is that the number of flights in the enlarged space increases in search for hosts, which may negatively affect parasitoid longevity and fertility, especially when food sources are absent (Mazanec 1988, cf. Willers et al. 1987).

3) *Introduction Ratio.* Effects of the introduction ratio are not considered. Relative numbers at the beginning will partly determine the outcome of a release (Wyatt 1983, Minkenberg and Damsté 1990).

The difference supposed between parasitoid fecundity and fertility is supported by data, as is the idea of a similar reproduction of the host in the laboratory and the field under comparable circumstances, at least at the beginning of the growing season. The R_0 of *L. bryoniae* is ca. 50 at 20-25 °C (Minkenberg and Helderman 1990), which is consistent with rates found in the glasshouse (Westerman and Minkenberg 1986), suggesting that the rate at which a population of *L. bryoniae* in the absence of natural enemies increases, approximates R_0 estimated in the laboratory. However, a decrease in the net reproduction after a few generations to approximately 10 has been observed (Westerman and Minkenberg 1986, Minkenberg et al. unpubl.). The R_0 estimated in the laboratory of *D. sibirica* is 40 (Minkenberg 1990) and its R_0 found in the glasshouse is ca. 10 (range is 1-16) at

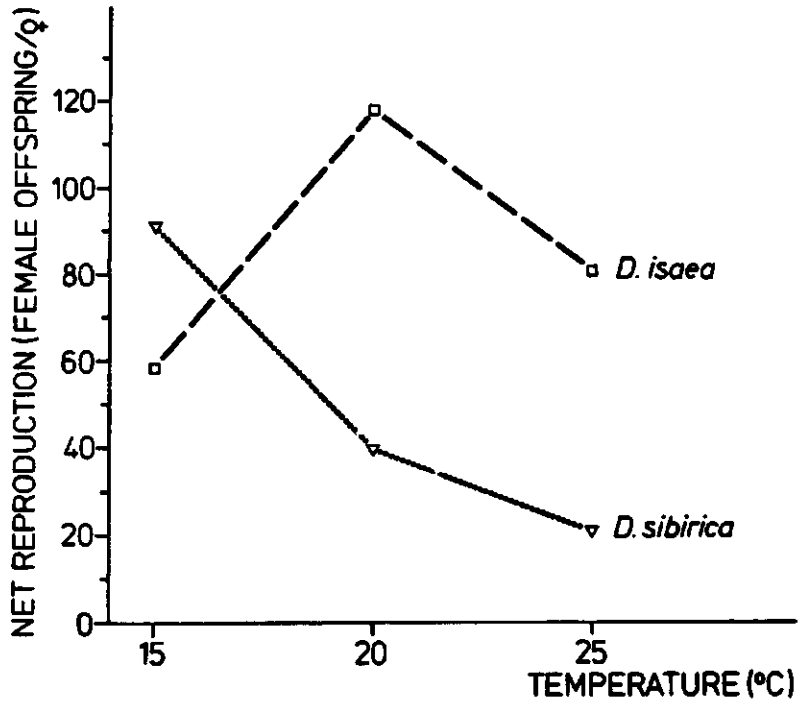

Fig. 10-4. Net reproduction rate, R_0 (female offspring/female/generation) of the parasitoids *D. sibirica* and *D. isaea* at different temperatures (data from Minkenberg 1989, 1990).

host densities of 1 leafminer per 100 plants to 10 leafminers per plant (Minkenberg et al. unpubl.). Thus, R_0 in the glasshouse for *D. sibirica* is only a fraction (ca. 25%) of R_0 estimated in the laboratory.

In seasonal inoculative programs, parasitoids should control a pest over several generations. In order to get effective control, the introduction ratio is of importance in addition to their relative population growth rate. The host population endures extra mortality due to parasitism in addition to its 'natural' mortality in comparison with the parasitoid population (Huffaker and Messenger 1964). Therefore, parasitoids cannot be discarded simply because of their low population growth parameters.

An answer to the second question, what is the value of comparing potential growth of parasitoids between each other to evaluate their effectiveness, is that estimates of population growth rate may be valuable to compare population growth of parasitoids between each other (e.g., in the case where these are the only data available), assuming that population growth of the parasitoids is equally affected in the greenhouse. However, population growth of parasitoids may be differently affected by, among other factors, host density, distribution and spatial heterogeneity in the green-

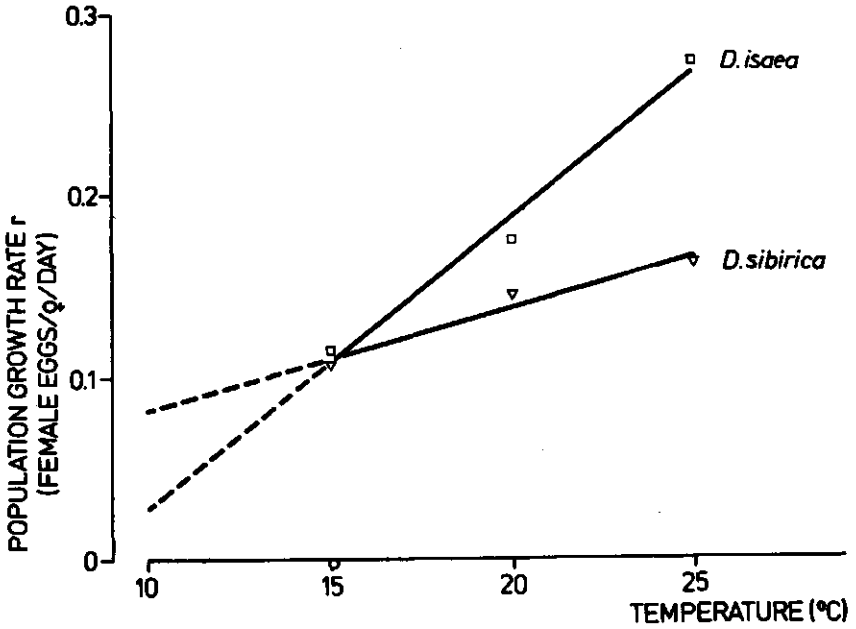

Fig. 10-5. Population growth rate, r (female offspring/female/day), of the parasitoids *D. isaea* and *D. sibirica,* at three constant temperatures; population growth rate (Y) regressed against temperature (X) by linear regression (*D. isaea:* Y = 0.0159 X - 0.1307, r = 0.991, threshold for population development (LT) is 8.2 °C; *D. sibirica:* Y = 0.0056 X - 0.0263, r = 0.979, LT is ca. 5 °C; data from Minkenberg 1989, 1990).

house. Therefore, additional data on the other criteria, e.g. Searching Efficiency, should preferably be collected. Only in the case that the values for the other criteria turn out to be similar, the parasitoids with the highest population growth should be chosen.

A trait typical for parasitoids is their ability to manipulate the sex of their offspring and hence their population growth, which further complicates comparisons. For example, *Diglyphus* spp. vary offspring sex with relative host size, leading to highly male biased sex ratios on the relatively small *Liriomyza* spp. (Heinz and Parrella 1990b). Other natural enemies may use food sources available in the greenhouse to enhance reproduction and to stay alive.

Example. Since generations of leafminers are discrete during the first part of the growing season in northwest Europe, R_0, is a better indicator of population growth than r (knowing that they can reproduce only once per

host generation). The R_0 of the leafminer parasitoids do not show similar trends (Fig. 10-4); at 15°C, *D. sibirica* has a higher R_0 than *D. isaea*, whereas at high temperatures (20-25°C) *D. isaea* has the higher R_0 (Minkenberg 1989, 1990). Thus, *D. sibirica* should be selected for a cold regime. With regard to southern Europe, where leafminer generations overlap, *D. isaea* would be the better candidate, because r is higher than that of *D. sibirica* at all temperatures (Fig. 10-5).

Criterion 4: Searching Efficiency

Concept. Much of the theoretical considerations in classical biological control now concentrates on the dispersion of parasitoids within and between host generations in relation to the distribution of suitable host instars (Morrison and Strong 1980, Hassell and May 1985, Thompson 1986, Strong 1988, Murdoch and Stewart-Oaten 1989). Also in seasonal inoculative biological control it might be important to direct research towards searching behavior of parasitoids in order to get more insight into the underlying mechanisms leading to their effectiveness. Searching by parasitoids amounts to a complex of behavioral components and many factors are involved. Therefore, it will be difficult to make predictions at the population level on the basis of laboratory experiments.

A behavioral bioassay may consist of two parts:

A) *To identify the ability to locate hosts at long distance.* The natural enemy should be able to locate the host at low host density. This can be investigated by e.g., wind tunnel and olfactometer. Janssen et al. (1990) found that of the eleven phytoseiids species tested four were attracted by the odor of cassava leaves infested with the cassava green mite. This simple experiment might increase the chance of finding an effective predator against the cassava green mite.

B) *To measure the searching and parasitization once arrived at a patch.* Estimates on searching can further be derived from examining foraging behavior of parasitoids at different host densities in the laboratory (cf. Huffaker et al. 1977, p. 562). To get 'realistic' results, parasitoids should be directly observed in a patch choice situation with variable observation times (Van Lenteren and Bakker 1978). Searching behavior and parasitism can be measured in different ways; a standard procedure has not been developed yet (Minkenberg and Parrella 1990). A drawback of the laboratory setup is that only relatively high host densities can be offered (a minimum is ca. 1 host per 10 plants). An extensive review on models including searching behavioral parameters and on how they might be used to evaluate predators is given by Kareiva and Odell (1987).

Preintroduction selection

Concept. Some measurements of the first criterion are qualitative such as complete development on the host or not being a primary parasitoid (Table 10-3). When a parasitoid does not meet this criterion, it can be discarded immediately. Another example, for generation synchrony, is when the waiting time for a particular parasitoid is long and there is evidence that this parasitoid cannot survive such a host-free period. The other measurements are quantitative. Estimates may range between zero and hundreds and it is not possible yet to indicate values that will predict effectiveness. Therefore, the entire set of values for the criteria measured should be interpreted for each parasitoid. Parasitoids with the most optimal sets of values will be selected to start releases.

The aim of the preintroduction selection procedure is not to predict which parasitoids will control the pest insect or which parasitoids will be effective. It is probably unlikely that we will ever be able to predict the consequences of introducing a particular natural enemy (Way 1973). However, it might be possible to identify those parasitoids that are likely to fail as biological control agents. In fact, a preintroduction procedure should select, on the basis of the arbitrary criteria, the best candidates for greenhouse trials *by making comparisons between parasitoids.*

Table 10-3. Four criteria and examples on their measurements for the procedure to select parasitoids in seasonal inoculative biological control.

Criterion	Examples on measurements
1. Development and Quality	- Complete development on host - Viability offspring after one generation - Viability offspring over several generations
2. Generation Synchrony	- Waiting time for parasitoids, together with host (stage) selection and convergence to stable age distribution of hosts
3. Population Growth	- Intrinsic rate of increase, r, or net reproduction, R_0
4. Searching Efficiency	- Host (habitat) location - Host location within a patch and parasitism - Functional response

Evaluation of the selection criteria. The outcome of the evaluation of criteria for the leafminer parasitoids does not contradict the empirical evidence: *D. sibirica* is effective in the cooler, temperate region and *D. isaea* in the warmer areas of Europe. In northwestern Europe, the R_0 of *D. sibirica* in combination with the introduction ratios applied is apparently sufficient to control effectively leafminers; its generation time synchronizes well with that of its hosts. Although the population growth of *D. isaea* is high at low temperatures, development of its populations is expected to be severely hindered by the generation asynchrony with leafminer populations in spring. In southern Europe, average temperatures are high and pest generations overlap, conditions that benefit *D. isaea*, given its r values and generation time. The low r at high temperatures of *D. sibirica* may suggest that this biological control agent will be less effective in southern than in western Europe. Regrettably, the effectiveness of *D. sibirica* on tomatoes in southern Europe has not been determined yet. Generation synchrony and relative population growth may indicate why they are effective in the different regions. These criteria seem, therefore, to be important in a preintroduction selection procedure.

The importance of criteria four, searching efficiency, has been shown for some other pest-natural enemy. The effectiveness of *E. formosa* at low temperatures (10-20°C) has been questioned. The population growth, expressed as r, of *E. formosa* is high on tomato, also at low temperatures (Van Lenteren and Hulspas-Jordaan 1983). More important, they are able to search and disperse at low temperatures (Kajita and Van Lenteren 1982, Van der Laan et al. 1982). *Encarsia formosa* is effective at these temperatures as shown in a greenhouse experiment (Hulspas-Jordaan et al. 1987). In addition to abiotic factors, searching behavior can also be influenced by plant architecture. For example, the poor control of greenhouse whitefly on cucumbers is in sharp contrast with the excellent results on tomato (Woets and Van Lenteren 1976). Searching by *E. formosa* on cucumber is impeded by the many large hairs on the leaves (Hulspas-Jordaan and Van Lenteren 1978, Li et al. 1987). Due to a new introduction scheme for cucumbers, whiteflies can now be controlled effectively: high numbers of parasitoids released apparently compensate for the limited searching capabilities (Ravensberg pers. comm.). Another example is the control of the two-spotted spider mite, *Tetranychus urticae* Koch by the predatory mite *Phytoseiulus persimilis* Henriot-Athias, which gives poor results on tomato but excellent ones on cucumber. The population growth rate of *P. persimilis* on both crops is high (Sabelis 1986). When predators move around they are caught by sticky hairs on the tomato stems (Van Haren et al. 1987). Since this predator is very effective on cucumber, it is suggested that the predatory mites are not bothered by cucumber hairs. Some caution in interpreting these results is necessary because these findings give only one possible explanation for the biological control results.

General conclusions

1. Four criteria, viz. (1) *Complete development and Offspring Quality*, (2) *Generation Synchrony*, (3) *Population Growth* and (4) *Searching Efficiency*, appear to be valuable for laboratory selection of parasitoids for seasonal inoculative biological control. With regard to criterion 1, in the case of incomplete development on the host or obligate hyperparasitism, a parasitoid can be discarded immediately.

2. With respect to criterion 2, *Generation Synchrony*, the waiting time, i.e. time between parasitoid emergence and appearance of hosts, seems to be valuable to measure generation synchrony between parasitoid and pest populations. In the case of discrete host generations, a long waiting time might reduce the value of a parasitoid for biological control.

3. The use of criterion 3, *Population Growth*, for comparison of the population growth rate of parasitoids with that of their hosts, is meaningless to predict their effectiveness because of the assumptions of stable population theory, among others a stable age distribution, and neglect of the effects of the relative number of introduced parasitoids. *Population Growth* may be applied to parasitoids for comparison between each other under the assumptions that their respective populations are equally affected in the greenhouse. In the case of discrete host generations, R_0 is a better indicator of population growth than r.

4. Measurement of criteria will produce a set of values for each criterion. Parasitoids with optimal sets of values should be selected. However, interpretation of the outcome of selection procedures needs further verification by greenhouse experiments. Selection should be based on all criteria, because a strength in one criterion may be counteracted by another criterion. Although the evaluation based on these four criteria will lead to a more scientific approach of biological control, just one factor omitted from the procedure, e.g. hairs on plants, may prevent a parasitoid from being effective.

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