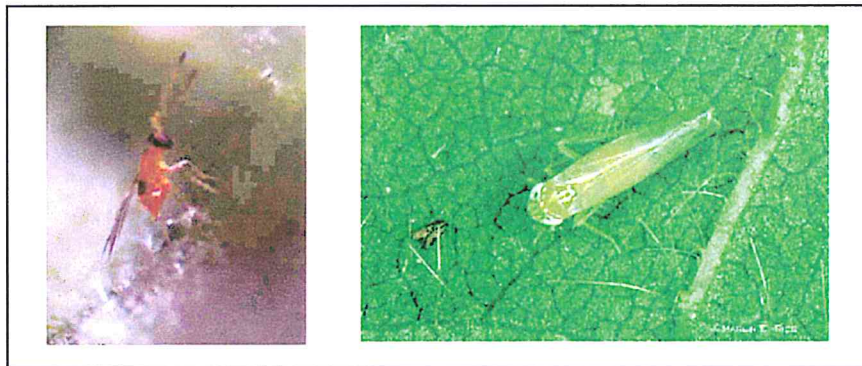


UNIVERSITY OF HANNOVER
FACULTY OF HORTICULTURE
INSTITUTE OF PLANT DISEASES AND PLANT PROTECTION

**LIFE TABLE STUDIES AND SEARCHING BEHAVIOUR OF *ANAGRUS*
ATOMUS L. (HYMENOPTERA: MYMARIDAE), AN EGG PARASITOID OF
EMPOASCA DECIPIENS PAOLI (HOMOPTERA: CICADELLIDAE)**



By
AGBOKA Komi
(Ingénieur Agronome)

**Thesis submitted in partial fulfilment of the requirements for the award of
Master of Science (M.Sc.) in Horticulture**

September 2002

Declaration

I, Komi, Agboka, hereby declare, that the work presented in this thesis is my own and has not been submitted for a degree in any other University.



K. AGBOKA

I certify that, this thesis has been supervised by:

PD Dr. Christian BORGEMEISTER

Prof. Dr. Hans-Michael POEHLING

Dr. Henning VON ALTEN

Abstract

The green leafhopper *Empoasca decipiens* Paoli (Homoptera: Cicadellidae) has recently become a serious new pest of vegetables and ornamentals grown under field conditions and in greenhouses in many European countries. The egg parasitoid *Anagrus atomus* L. (Hymenoptera: Mymaridae) is a natural enemy of *E. decipiens*. The objective of this study was to assess the potential of *A. atomus* for control of *E. decipiens*. Therefore the development, survivorship and reproduction of *A. atomus* were evaluated at three different constant temperatures (i.e., 16, 20 and 24 °C). Moreover the response of *A. atomus* to different densities of leafhopper eggs and its ability to attack different host eggs of different age were investigated. Additionally the searching behaviour of *A. atomus* on four different host plants of *E. decipiens* and the effect of parasitoid densities on the rate of parasitism were examined. The developmental of *A. atomus* periods ranged from 33.6 at 16 °C to 16.3 days at 24 °C. Based on a linear regression between the ranges of the temperatures tested, the lower threshold for development of *A. atomus* was estimated at 8.58 °C. The parasitoid required 251.26 degree-days to complete its development from egg to adult. Based on its thermal requirements *A. atomus* can develop faster and build up faster populations compared to *E. decipiens*. However the fecundity of *A. atomus* is comparatively low. With 30.13 the highest average number of offspring was observed at 24 °C. The combined differences in egg-adult development, adult survivorship and the fecundity produced strong differences in life table parameters of *A. atomus*, especially in term of the intrinsic rate of increase (r_m). Host eggs were parasitized throughout their development but the rate of parasitized eggs was reduced in host eggs older than six days. The maximum rate of parasitism recorded was 62.5%. The number of eggs parasitized by *A. atomus* was positively density dependant. Yet the mean rate of parasitism decreased with increasing host densities. In Petri dish experiments, using leaves of four different host plants of *E. decipiens*, i.e., broad beans (*Vicia faba* L.), sweet pepper

(*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.) and French beans (*Phaseolus vulgaris* L.), the parasitoids could recognize the plants previously exposed to leafhoppers and spent nearly all of the observation time searching for the hosts with many attempts to oviposit on previously exposed to non-exposed plants. The number of parasitized eggs increased with the number of parasitoids released per plant, but the per capita attack decreased with increasing numbers of parasitoids. Likewise, the searching efficiency of *A. atomus* decreased with an increase in parasitoid densities. These results are discussed with regard to the potential use of *A. atomus* biological control of *E. decipiens*.

Acknowledgements

My study and research was supported by a grant of German Academic Exchange Service (DAAD).

I am grateful to Dr. F. Schulthess for his useful comments on earlier versions of this document.

Many thanks to Dr. M.J.W. Copland and D.M. Choudhury at Wye College (UK) for their collaborative work during the early stage of this research project, especially for their valuable help in techniques how to rear and handle *A. atomus*. I am also grateful to Ms. K. Raupach for her technical assistance in rearing *E. decipiens* and useful discussions and suggestions during the course of my research project.

I would like to thank PD Dr. C. Borgemeister for all he has done, in being both a mentor and for allowing me the freedom to learn through my successes and failures. I especially thank him for his support in every aspect of this work. Many thanks to my other supervisors, Prof Dr. H-M Poehling, and Dr. H. von Alten who followed my progress through valuable discussions, comments and suggestions.

My sincere gratitude to my family, for never giving up on me and encouraging me to pursue my dreams.

I am solely responsible for the views expressed in this thesis and hope that the assembled information provides a useful tool for students and scientists working on the biological control of leafhoppers.

Table of Contents

1	GENERAL INTRODUCTION	1
1.1	<i>Empoasca decipiens</i> and related species	1
1.2	Distribution and host plants of <i>E. decipiens</i>	1
1.3	Feeding habits and damage	2
1.4	Biology	3
1.5	Pest management strategies.....	4
1.5.1	Chemical control	5
1.5.2	Cultural control.....	6
1.5.3	Host plant resistance.....	6
1.5.4	Natural enemies – Biological control.....	7
1.6	Statement of the research problem	11
1.7	Objectives	12
1.8	Research Hypothesis	13
2	LIFE-TABLE STUDY OF ANAGRUS ATOMUS, AN EGG PARASITOID OF THE GREEN LEAFHOPPER <i>EMPOASCA DECIPIENS</i>	14
2.1	Abstract	14
2.2	Introduction	14
2.3	Materials and methods.....	16
2.3.1	Insects rearing.....	16
2.3.2	Effect of temperature on life table parameters of <i>A. atomus</i>	17
2.3.3	Within-plant distribution of <i>E. decipiens</i> eggs and parasitism by <i>A. atomus</i>	18
2.3.4	Effect of host eggs age on the parasitism by <i>A. atomus</i>	19
2.3.5	Statistical analysis	19

2.4	Results	20
2.4.1	Egg-adult development.....	20
2.4.2	Adult longevity and reproductive capacity	21
2.4.3	Life table parameters	22
2.4.4	Within-plant distribution of <i>E. decipiens</i> eggs and parasitism by <i>A. atomus</i>	24
2.4.5	Effect of the host egg age on the parasitism by <i>A. atomus</i>	26
2.5	Discussion	26
3	SEARCHING AND OVIPOSITION BEHAVIOUR OF ANAGRUS ATOMUS ON FOUR HOST PLANTS OF ITS GREEN LEAFHOPPER <i>EMPOASCA DECIPIENS</i>	30
3.1	Abstract	30
3.2	Introduction	30
3.3	Materials and Methods	33
3.3.1	Insect colonies	33
3.3.2	Searching behaviour of <i>A. atomus</i> on four host plants of <i>E. decipiens</i>	33
3.3.3	Effect of <i>A. atomus</i> densities on parasitism and on searching efficiency	35
3.4	Results	36
3.4.1	General searching behaviour	36
3.4.2	Interaction between host plant species and prior exposure to leafhoppers	36
3.4.3	Effect of plant species and prior exposure on the searching behaviour of <i>A. atomus</i>	37
3.4.4	Effects of <i>A. atomus</i> densities on parasitism and on their searching efficiency	42
3.5	Discussion	44
4	GENERAL DISCUSSION	47
5	REFERENCES	53

List of tables

Table 1.1 Duration of the different developmental stages and the longevity of adult of <i>E. decipiens</i> (in days) at different temperatures (Raupach et al., 2002; Habib et al., 1972)	4
Table 2.1 Egg-adult development time of <i>A. atomus</i> at three constant temperatures	21
Table 2.2 Egg-adult survival, adult longevity, oviposition time, total offspring and sex-ratio (means \pm s.e) of <i>A. atomus</i> at three constant temperatures	22
Table 2.3 Effect of temperature on life table parameters of <i>A. atomus</i>	23
Table 3.1 Probability levels from two-way Analysis of Variance for plant species, infestation status and their interactions	37
Table 3.2 Probe parameters of <i>A. atomus</i> on four different host plants previously exposed to <i>E. decipiens</i>	38

List of figures

Figure 2.1 <i>Anagrus atomus</i> survival and reproduction at A (16°C), B (20°C) and C (24°C).....	23
Figure 2.2 Distribution of unparasitized leafhopper eggs (A) and parasitized eggs (B) in <i>V. faba</i> . Different letters above bars indicate significant differences between means (ANOVA, $P < 0.05$).....	24
Figure 2.3 Relation between host density and number of parasitized eggs (A: $y = 7.05 \cdot \ln(x - 16.84)$, $r^2 = 0.55$, $P = 0.0036$) and rate of parasitism (B: $y = 64.85 - 0.35x$; $r^2 = 0.52$, $P = 0.0053$).....	25
Figure 2.4 Host age and parasitism by <i>A. atomus</i> . Different letters above bars indicate significant differences between means (ANOVA, $P < 0.05$).....	26
Figure 3.1 Proportion of the total observation time that <i>A. atomus</i> females spent on four host plants of <i>E. decipiens</i> with or without prior exposure to leafhoppers.....	39
Figure 3.2 Proportion of resting of <i>A. atomus</i> females on different host plant species with or without prior exposure to leafhoppers.....	40
Figure 3.3 Effects of different <i>A. atomus</i> release densities on the rate of parasitism of <i>E. decipiens</i> eggs in <i>V. faba</i> plants. Bars followed by the same letter are not significantly different (ANOVA, $P < 0.05$)	41
Figure 3.4 (I) Relationship between log transformed searching efficiency (A) and parasitoid density (D). (II) Number of parasitized eggs per female <i>A. atomus</i> as affected by different parasitoid densities	43

1 GENERAL INTRODUCTION

1.1 *Empoasca decipiens* and related species

Empoasca decipiens is a small cicada that was first described by Paoli (1930) in Italy. The adult, entirely green, is 3-4 mm long (Schmidt and Rupp, 1997; Ossiannilsson, 1981). Leafhoppers belonging to the *Empoasca* genus are very similar in appearance. A precise identification to the species level is rather complex, and requires preparations of the male genitalia (DeLong, 1971).

Species of the genus *Empoasca* are important pests of cultivated plants. In the first half of the 20th century *E. fabae* Harris was considered as the most important pest of potatoes in North America (Fenton and Hadzell, 1920; Müller, 1956). The potato leafhopper *E. fabae* is the most common insect pest found on field beans in Ontario (Whitfield and Ellis, 1976) and can be responsible for heavy yield losses if not controlled (Gonzales and Wyman, 1991). It is also a key pest of alfalfa in the northeastern and north central United States and southern provinces of Canada (Lamp, 1990). A closely related leafhopper species, *E. kraemeri* Ross and Moore is considered as an important pest on beans in Latin America (Graham, 1978; van Schoonhoven et al., 1985). Yield losses of up to 79% have been reported for heavily infested bean fields in Columbia (Kornegay and Cardona, 1990). The polyphagous leafhopper *E. vitis* Goethe is a common pest in vineyards in central Europe (Schruft, 1987; Vidano et al., 1987a). The dwarf leafhopper *E. decipiens* is a widespread pest attacking field and protected cultivated crops.

1.2 Distribution and host plants of *E. decipiens*

Empoasca decipiens is polyphagous insect and has been recorded on a wide variety of crops and non-cultivated plants. Apart from *Hauptidia maroccana* Melichar (Hom.: Cicadellidae), an important pest of tomatoes in the UK (Jacobson et al., 1996) and France (Maisonneuve et al.,

1995), *E. decipiens* is the most damaging leafhopper species in European greenhouses (Schmidt and Rupp, 1997; Helyer and Talbaghi, 1994; Loginova, 1992). Recently heavy infestations by and yield losses caused by *E. decipiens* have been recorded in cucumbers in southern Germany (Schmidt and Rupp, 1997).

Empoasca decipiens is widely distributed in central and southern Europe, North Africa, the Middle East and Central Asia (Ossiannilsson, 1981). In Egypt, *E. decipiens* attacks cotton, beans, potatoes and vegetables such as tomatoes, cucumbers, cabbage, sugar beets, sweet pepper and carrots (Habib et al., 1972; El-Dessouki and Hosny, 1969). In Turkey, *E. decipiens* was recorded on sesame (Kersting et al., 1997), on cucumbers and squash (Akkaya et al., 1999), whereas according to older reports in central Europe *E. decipiens* occurs primary on potatoes and sugar beets (Günthart, 1971) and in vineyards (Vidano et al., 1987a). In Poland, the leafhopper was recorded on annual ornamental plants cultivated for seeds (Soika and Labanowsky, 1996).

1.3 Feeding habits and damage

The damage on host plants is caused by the nymphs and adults. Feeding of nymphs and adults cause chlorotic and necrotic lesions as well as leaf deformation. At higher leafhopper densities, the leaves suffer from drought stress and can partially or completely dry out. However, the leafhoppers may also attack the fruits, and the puncturing of the fruit tissue can result in a serious reduction in the quality of the harvested crop.

Leafhoppers are sucking insects, removing plant sap directly from parenchyma cells or from the vascular system in the leaflet, petioles and stems. It has been shown by histological studies on sections of stylets within the plant that *E. decipiens* could feed from the stem parenchyma but also pierce the phloem if this is reachable (Koblet-Günthardt, 1975) and Schmidt and Rupp

(1997) described it as a phloem feeder. Punctured tissue is torn, and distorted and fast necrotic reactions around the feeding site can be observed. During feeding the leafhoppers inject saliva, which may be involved in the strong tissue reactions. While the principal mechanism of feeding is not disputed there are at least two explanations concerning the specific injury of plant tissue caused by this type of feeding: Johnson (1934) and Smith and Poos (1931) asserted that the typical symptoms of so-called “hopperburn” (i.e., a complex of symptoms produced by feeding of the leafhopper adults and nymphs) are caused by interference with the translocation of food materials and water due to the physical plugging of the xylem and the destruction of the phloem cells. Other investigators (e. g., Medler, 1941; Carter, 1939; Granovsky, 1930; Fenton and Hartzell 1923; Beyer, 1922) support the theory that a salivary toxin is injected into the plant during or preceding insect feeding. Symptoms sometimes appear very similar to symptoms caused by diseases or nutrient imbalance but according to Schmidt and Rupp (1997) no evidence of involvement of phytoplasmas, pathogens commonly transmitted by leafhoppers, was found in infested plants by *E. decipiens*.

1.4 Biology

Adult females start to lay eggs after a pre-oviposition period, which may vary with the climatic conditions from one to several days (Habib et al., 1972). In several *Empoasca* spp., the oviposition period lasts from 1.5 to 10 days (DeLong, 1938; Harries and Douglass, 1948; Nielson and Toles, 1968), but in hibernating species this may be several months.

The eggs of *E. decipiens* are laid within the plants tissues (beneath the leaf surface, in the petiole and in the stem) and are visually difficult to detect. They are cylindrical, slightly curved, broader and more bluntly rounded at the posterior end (Habib et al., 1972). The incubation period of the egg is negatively correlated with temperature (table 1.1). *Empoasca decipiens* passes through five

nymphal instars. The temperature also affects nymph development. At 27°C total nymph development (all stages) takes 11.8 days (Habib et al., 1972). No nymphal development was observed at 37 and 40°C (Raupach et al., 2002). In addition to temperatures host plants can have a profound effect on the duration of the nymphal stages, the number of offspring and the longevity of the adults. Broad beans (*Vicia faba* L.) have been shown to be the most suitable host plant for the development of the nymphs (Raupach et al., 2002; Habib et al., 1972). The longest nymphal development was observed on aubergine and tomato (Raupach et al., 2002).

Table 1.1 Duration of the different developmental stages and the longevity of adult of *E. decipiens* (in days) at different temperatures (Raupach et al., 2002; Habib et al., 1972)

Temperatures (°C)	egg stage	total nymph stage	adult longevity
15	28.29	36.87	*
20	14.88	18.80	*
22	11	19.5	38.5
24	11.11	14.85	*
27	7.2	11.8	31.9
29	5	9.2	31.5
35	8.19	10.84	*

* Values not determined. Data at 22, 27, and 29 °C are from Habib et al. (1972).

1.5 Pest management strategies

The control strategies of leafhoppers include the use of chemicals, cultural measures and resistant varieties. In addition, leafhoppers have many natural enemies, including lady beetles, lacewings, spiders and egg parasitoids. They are also subject to diseases and parasites that can help to keep their numbers down. For forecasting populations can be monitored with yellow sticky traps placed close to the foliage; low populations can be managed using such traps.

1.5.1 Chemical control

A broad range of synthetic organic insecticides as well as botanicals like pyrethrins and even soaps can provide effective leafhopper control (Missouri Botanical Garden: IPM information, 1999). Old studies (i.e., Zimmerman, 1948) reported good control of leafhoppers by sulfur dust or a pyrethrum-sulfur dust and satisfactory control by pyrethrum-talc dust. Combinations with chlorpyrifos and with profenofos increased the effectiveness against *E. decipiens* (El-Nawawy et al., 1983). But such treatments negatively affect natural enemies like *Scymnus interruptus* (Goeze) (Col.: Coccinellidae), *Paederus alfieri* Koch (Col.: Staphylinidae) and *Chrysoperla carnea* (Steph.) (Neu.: Chrysopidae) thereby increasing the development of secondary pests outbreaks.

The insect growth regulator Buprofezin, which is also commonly used for control of the rice leafhopper *Nephotettix virescens* (Distant) (Hem.: Cicadellidae), has shown strong effects against the nymphal stages of *E. decipiens* (100% of nymphs are killed) in laboratory, but little activity against adults and eggs was recorded (Helyer and Talbaghi, 1994). Yet, no effects of Buprofezin on various biological control agents, commonly used in the greenhouse environment, were observed, indicating the potential use of Buprofezin in IPM programs against *E. decipiens*.

Because of possible negative effects on the environment and human health, and the increasing demand of consumers for non-chemically produced vegetables, farmers are encouraged to adopt a sustainable approach to managing pests, using methods that minimize environmental, health, and economic risks.

1.5.2 *Cultural control*

Cultural control measures may provide some relief from leafhopper damage. It includes early harvest (early cutting in the case of alfalfa), intra-field trap cropping, and removal of crop residuals. One management strategy could be the use of alternative cropping techniques such as intercropping. Lamp (1991) found that an alfalfa-oat intercrop reduced the densities of potato leafhopper adults and nymphs by as much as 82.6 and 89.5 per square meter, respectively, when compared with alfalfa monocultures. But intercropping also reduced alfalfa biomass and maturity. Further research by Roda et al. (1997) showed that potato leafhopper emigration was greater from alfalfa grass intercrops than from alfalfa monoculture and the number of ovipositions was reduced. Though the intercropping did not suppress the pest population below the current economic thresholds (2 leafhoppers per sweep when alfalfa is <25 cm tall (Rice, 1996)) these findings on *E. fabae* could be an important component for an integrated control strategy against *E. decipiens* but only with little relevance for greenhouse conditions.

1.5.3 *Host plant resistance*

Host plant resistance has been developed as a major factor in the control of certain species of leafhoppers; especially those attacking alfalfa, beans, cotton, potato and sugar beet have been studied in detail. The resistance of the potato variety Sequoia to *E. fabae* has provided nearly as much protection from leafhopper damage as has the use of insecticides (DeLong, 1971). Evidence has been presented that solanaceous alkaloidal and glycosides, and aglycons like solanadine and demissidine may be effective feeding deterrents and responsible for the avoidance of *E. fabae* to certain varieties of potatoes (Dahlman and Hibbs, 1967). Breeding programs for potatoes seek to develop genotypes that are resistant to both hopperburn and stunting (NAAIC, 1998). One resistance factor could be the presence of glandular trichomes on the plant surface and/or some

co-varying traits carried through generations (Elden and McCaslin, 1997). New alfalfa cultivars have been developed by seed companies with glandular trichomes as traits that confer resistance to potato leafhoppers (Elden and McCaslin, 1997). Despite the importance of host plant resistance in control strategies against *Empoasca* spp., no resistant variety has so far been developed against *E. decipiens*.

1.5.4 Natural enemies – Biological control

Predators and adult leafhopper parasitoids

In the literature several species of predatory bugs of the genus *Orius* (Het.: Anthocoridae) are frequently cited as predators of leafhoppers (Müller, 1956), in particular *O. minutus* (L.) and *O. insidiosus* Say (Vietmeier et al., 1996). However, attempts to use *Orius* spp. for biological control of *E. decipiens* were not successful, possibly because the adults and nymphs are too fast and can easily escape and could not be captured successfully in high numbers by these predators (Helyer and Talbaghi, 1994). On the other hand the predatory bug *Melacocoris chlorizans* Panzer (Het.: Miridae) is described as an important predator of the first and second instars of *E. vitis* (Vidano et al., 1987a). According to Müller (1956) also the lacewing *Chrysoperla carnae* Stephens (Neu.: Chrysopidae) is a natural enemy of nymphs of *Empoasca* spp. In cage trials, *E. decipiens* nymphs were preyed by *C. carnae* larvae (Schmidt and Rupp, 1997). However, the pubescent nature of many cucumber varieties may limit the possible use of *C. carnae* as a biological control agent of *E. decipiens* since pubescence of plant surface can impede the movement of the predators, often affecting searching time and weakening the predator response (Price et al., 1980). Moreover, in preliminary choice experiments *C. carnae* larvae preferred aphids to *E. decipiens* nymphs as prey (Raupach, 1999) and very often leafhopper occur together with aphids on host plants. Predatory flies of the genus *Coenosia* spp. (Dipt.: Muscidae) are the only natural enemies described so far

of adult leafhoppers (Kühne, 1998). At present, three *Coenosia* spp. are tested for possible use as biological control agents in greenhouses (Bünger et al., 2002). However, in first experimental releases under practical greenhouse conditions *Coenosia* spp. could not prevent high infestation levels of *E. decipiens* (Schmidt and Rupp, 1997). Certain mite species are also occasionally cited as important predators of *E. fabae* (Müller, 1956). For instance *Anytis* spp. (Acari: Anytidae) are considered to be important natural enemies of *E. vitis* (Vidano et al., 1987b). Finally high rates of parasitism of up to 90% by *Charletonia* spp. (Acari: Erythaedae) were recorded on *E. vitis* and *E. decipiens* (Maixner et al., 1998).

Many species of Dryinidae have also been reported as parasites of adult leafhoppers. Drynids, probably *Lonchodryinus ruficornis* Dalman (Hym.: Dryinidae) was detected in *E. decipiens* in potato and grapevine (*Vitis vinifera* L.) fields (Demichelis and Manino, 1995).

Egg parasitoids

Several mymarid species in the genus *Anagrus* (Hym.: Mymaridae) are commonly known to parasitize the eggs of leafhoppers (Williams and Martinson, 2000; Triapitsyn, 1998; Cronin and Strong, 1990).

Anagrus atomus is a minute parasitic wasp that attacks eggs of the leafhoppers. The adult is brown. Haliday (1833) gives the following description of *A. atomus*: “The *Anagrus atomus* female is approximately 0.6 mm long; the fore wing is also approximately 0.6 mm long so that the width of the insect with the wings extended is a little more than its total length; the antennae are rather shorter than the body and the basis of the antennae are widely separated. In the female, the antennae have nine segments the scape is about twice as long as the pedicel and the first funicular segment is very short. The remaining segments tend to increase in size towards the

apex, but the last segment is much larger than the others and forms a club. The most striking external difference between the females and males is the form of the antennae. In the male the antennae have thirteen segments, the scape is slightly longer than the pedicel and the terminal segments of the antennae are similar to each other except the last, which is pointed at the tip". Adult females can reproduce parthenogenetically, although males are also found. According to Vidano et al. (1987b), *A. atomus* is polyvoltine. Parasitized eggs can be easily identified because of their conspicuous red colour (Cooper, 1993). The day length does not seem to influence the development of the parasitoid, whereas the temperature is the crucial factor for the development of *A. atomus*. Under favourable conditions *A. atomus* can reproduce continuously throughout the whole year; there is no diapauses (Cooper, 1993). In Great Britain, *A. atomus* was identified as a parasitoid of *H. maroccana* and is now already commercially available (Wardlow and Tobin, 1990) to control generally leafhoppers and particularly *H. maroccana* and *E. decipiens* on tomatoes and ornamentals such as *Primula* (Primulaceae), *Ipomoea* (Convolvulaceae) and *Streptocarpus* (Gesneriaceae) species.

The parasitoid is polyphagous and attacks many Cicadellidae. *Anagrus atomus* was detected in field studies in Turkey on *Arboridia* (= *Erythroneura*) *adanae* Dlab. (Hom.: Cicadellidae). There it used *Edwardsiana rosae* L. (Hom.: Cicadellidae) on *Rosa* sp. and *Rubus* sp. (Rosaceae) as over-wintering refuges (Yigit and Erkilic, 1992). It is also reported on *Lindbergina aurovittata* Douglas (Hom.: Cicadellidae) with an average parasitism of 16 % (Bosco and Arzone, 1991). Lists of natural enemies of the maize pest *Zyginidia pullula* Boheman (Hom.: Cicadellidae) and other Cicadellidae infesting blackberry and other plants in Italy include the egg parasitoid *A. atomus* as well (Vidano and Arzone, 1988). For *E. vitis*, *A. atomus* is described as the most important mortality factor, which can eliminate up to 90 % of the leafhopper eggs (Cerutti et al.,

1990) compared to other egg mortality factors. Wardlow and Tobin (1990) reported high rates of parasitism (approximately 70-80%) of *H. maroccana* eggs from July onwards, i.e., during a rather advanced time in the vegetation period of the tomatoes, indicating again a strong temperature dependence of the efficiency of *A. atomus*. Vidano et al. (1987b) found almost 50% of *E. vitis* eggs parasitized by *A. atomus* in Italian vineyards, indicating the high potential of the parasitoid for control of leafhoppers. However, little is known about the economic efficacy of *A. atomus* for control of leafhoppers. According to Cooper (1993) in 40% of the cases, releases of *A. atomus* resulted in sufficient control of *H. maroccana* in tomatoes in Great Britain. Due to the short life span of the adult parasitoids, Cooper (1993) recommends, however, weekly releases of *A. atomus*, at least during a period of four weeks. The parasitoid can be also used to combat *E. decipiens* outbreaks (Schmidt and Rupp, 1997), but first releases of *A. atomus* on the island Reichenau in southern Germany did not lead to a sufficient control of *E. decipiens* (Rupp, 1999). The reasons for this failure in biological control are yet poorly understood. However, a final evaluation of the efficiency of *A. atomus* as a natural enemy of *E. decipiens* is at present not possible since crucial data on the biology, ecology and behaviour of the parasitoid are still lacking. Particularly the host identification behaviour, the temperature dependence and the influence of the various host plants of *E. decipiens* on the regulatory potential of *A. atomus* needs to be gathered. In two recent studies in California, the influence of different host plants of the sugar beet leafhopper of *Circulifer tenellus* (Baker) (Hom.: Cicadellidae) on the rate of parasitism by and on different behavioural parameters of the closely related egg parasitoid *A. nigriventris* Girault were observed (Al-Wahaibi and Walker, 2000a&b). In sugar beet fields the rate of parasitism of *C. tenellus* eggs by *A. nigriventris* can reach very high levels, sometimes exceeding 90% (Flock et al. 1962; Meyerdirk and Hessein, 1985; Meyerdirk and Moratorio, 1987). However, augmentative releases of *A. nigriventris* in the winter-spring breeding habitats of *C.*

tenellus did not result in considerable reductions in the infestation pattern of leafhoppers on the summer hosts (Al-Wahaibi and Walker 2000a). In subsequent laboratory studies, Al-Wahaibi and Walker (2000a&b) could show that individual host plant species of *C. tenellus* had a very pronounced and sometimes negative effect on the performance, i.e., rate of parasitism of *A. nigriventris*. Likewise Lovinger et al. (2000) observed a negative influence of pubescent lucerne varieties on the searching behaviour of *A. nigriventris*. Moreover, in olfactometer experiments with *A. nigriventris*, Honda and Walker (1996) observed a clear preference for volatiles of sugar beets compared to *Salsola tragus* (L.) (Chenopodiaceae), an alternative host plant of *C. tenellus*. Additionally, the host plant of the leafhopper on which both host and parasitoid were reared can significantly affect the oviposition behaviour of *A. nigriventris* (Honda and Walker, 1996). These results indicate a possibility to manipulate the oviposition preference of *A. atomus* by a careful selection of the host plants.

1.6 Statement of the research problem

The leafhopper *E. decipiens* has become a serious pest of vegetable and ornamental crops in European greenhouses. High infestation levels can lead to significant economic losses, both in terms of quantity and quality of the crops (Lamp et al., 1991; Cuperus et al., 1983).

Theoretically the leafhopper can be controlled through application of synthetic insecticides, though this threatens to disrupt the already existing and very efficiently used biological control programs against other important greenhouse pests like white flies, leafminers and aphids. Moreover, chemical control of leafhoppers is difficult due to the lack (i.e., lack of registration) of appropriate insecticides and yet unclear relationships between infestations and economic losses caused by many *Empoasca* spp. (Maixner et al., 1998). At present no well developed biological control strategies for *E. decipiens* are available. Rational pest management strategies to maintain

E. decipiens populations at acceptable levels should be based on a sound knowledge of the population dynamics of the pest. Moreover, the important biotic regulation factors need to be identified; particularly egg parasitoids should be stressed, as the pest is here killed in the egg stage and hence further damage by the pests can thus be prevented.

Previous findings indicate that the egg parasitoid *A. atomus* is a key mortality factor of *E. decipiens* (Schmidt and Rupp, 1997). In a model, Cerutti et al. (1991) showed that without the impact of *A. atomus*, simulated densities of the closely related leafhopper species *E. vitis*, a serious pest in European vineyards, exceeded the economic damage threshold, indicating that the egg parasitoid is able to maintain *E. vitis* population densities on a level that does not negatively affect the yield. However, at present detailed knowledge on the biology, ecology and behaviour of the egg parasitoid is still lacking. Hence these gaps in our understanding of the *A. atomus* biology need to be filled before a successful biological control program for *E. decipiens* can be developed. For a comprehensive judgement of the potential efficiency of *A. atomus* as a natural enemy of *Empoasca* spp. in general and of *E. decipiens* in particular crucial data on the host identification behaviour, the influence of temperature and the various host plants of *E. decipiens* on the biotic potential of the parasitoids is still lacking. These information, coupled with a thorough understanding of the foraging and host discrimination behaviour of *A. atomus*, and detailed data on the fecundity of the parasitoids, will provide the basis for the development of a biological control program against *E. decipiens*.

1.7 Objectives

The main objective of this study was to develop a suitable biocontrol program for *E. decipiens* by using the egg parasitoid *A. atomus*. Initially various life table parameters of *A. atomus* were recorded and a suitable methodology to assess the impact of the egg parasitoid on the population

dynamics of *E. decipiens* were developed and subsequently implemented. Moreover, searching and oviposition behaviour on different host plants were studied.

1.8 Research Hypothesis

(i) Temperature is a crucial factor affecting the development, the fecundity and longevity of *A. atomus*. Temperature plays a critical role in determining the rate of development, survival, and reproduction of insect species. Understanding the relationships between temperature and various life history processes is important in developing biological control programmes. In addition, understanding the effects of temperature on life history parameters such as the intrinsic rate of increase can be useful for examining the potential effectiveness of a species as a biological control agent, estimating its potential for population growth, and determining the response of a species to temperatures (Messenger, 1964).

(ii) Host density and host age can influence the parasitism of *A. atomus*. Foraging parasitoids are routinely confronted with host populations that are spatially fragmented among different parts of the plant, and different clumps of the host plant. Parasitoids should forage longer and parasitize proportionally more hosts in patches of high host density (Hassell and May, 1974; Cook and Hubbard, 1977).

(iii) The efficacy of the egg parasitoids *A. atomus* is related to its ability to locate the infested plant and subsequently parasitize the host. Foraging parasitoids are generally assumed to employ indirect means to distinguish features of patches and alter their foraging effort accordingly. In searching behaviour, the response of *A. atomus* towards different host plants exposed or non-exposed to *E. decipiens* was evaluated. Effects of *A. atomus* densities on the parasitism and on the searching efficiency were additionally examined.

2 LIFE-TABLE STUDY OF *ANAGRUS ATOMUS*, AN EGG PARASITOID OF THE GREEN LEAFHOPPER *EMPOASCA DECIPIENS*

2.1 Abstract

The objective of our study was to assess the potential of the egg parasitoid *Anagrus atomus* L. (Hymenoptera: Mymaridae) for control of the greenhouse leafhopper *Empoasca decipiens* Paoli (Homoptera: Cicadellidae). The egg-adult development time, survivorship and reproduction of *A. atomus* were evaluated at three constant temperatures (16, 20 and 24°C). Developmental time ranged from 33.6 at 16°C to 16.3 days at 24°C. Based on a linear regression of development rate on temperature the lower threshold was estimated at 8.58°C. *Anagrus atomus* required 251.2 degree-days to complete its development from egg to adult. The egg-adult survival and the sex ratio were not influenced by the temperatures tested. The intrinsic rate of increase (r_m) varied significantly between all three temperatures. The potential of *A. atomus* to attack different host ages was additionally investigated. Host eggs were parasitized throughout their development but rate of parasitism was reduced in host eggs older than six days. The number of eggs parasitized was positively density dependant but the rate of parasitism decreased with increase of host density. A maximum rate of parasitism of 62.5% was recorded. The potential impact of the egg parasitoid on the population dynamics of *E. decipiens* is discussed.

2.2 Introduction

In recent years leafhoppers have become serious pests of vegetables and ornamentals in European greenhouses. Apart from *Hauptidia maroccana* Melichar (Hom.: Cicadellidae), an important pest of tomatoes in the UK (Jacobson et al., 1996) and France (Maisonneuve et al., 1995), the most damaging leafhopper species in European greenhouses is *Empoasca decipiens* (Schmidt and Rupp, 1997; Helyer and Talbaghi, 1994; Loginova, 1992). *Empoasca decipiens* is widely

distributed in central and southern Europe, North Africa, the Middle East and Central Asia (Ossiannilsson, 1981). Recent reports on outbreaks from the Netherlands, Great Britain (Helyer and Talbaghi, 1994), Bulgaria (Loginova, 1992), Switzerland (Anonym, 1998aandb) and Germany (Schmidt and Rupp, 1997) led to an increased interest in developing alternative control strategies for *E. decipiens*. Particularly serious outbreaks of *E. decipiens* were recorded on Reichenau island in southern Germany, where the leafhopper threatened commercial cucumber production in greenhouses (Schmidt and Rupp, 1997).

Empoasca decipiens can be controlled through application of synthetic insecticides, though this threatens to disrupt the already existing and very efficiently used biological control programs against other important greenhouse pests like white flies, leafminers and aphids. Research on the natural enemy complex of *Empoasca* spp. indicated that because of the high mobility of leafhopper adults and nymphs, predators are not very likely to efficiently control the pests (Helyer and Talbaghi, 1994). Presently, the most promising candidate for augmentative biological control in greenhouses is the egg parasitoid *A. atomus* (Schmidt and Rupp, 1997; Cerutti et al., 1991). In several surveys, *A. atomus* proved to be the most common egg parasitoid associated with *E. decipiens* on vegetables (Rupp, 1999; Schmidt and Rupp, 1997). Although mymarid egg parasitoids are important natural enemies of many agricultural pests, they have only occasionally been used in biological control (Cronin and Strong, 1990a; Meyerdirk and Moratorio, 1987). However, the high control potential of mymarids merits further research on their biology, ecology and their behaviour, prerequisites for their effective use in biological control programs. In this study we intended to provide such basic information on *A. atomus*, particularly with regard to its life statistics at different temperature regimes and its potential to regulate populations of *E. decipiens*. These data, coupled with already existing information on the population dynamics

of *E. decipiens* (Raupach et al., 2002; Habib et al., 1972), could provide the basis for the development of a successful biological control strategy against this leafhopper species.

2.3 Materials and methods

2.3.1 Insects rearing

A laboratory culture of *E. decipiens*, originally obtained from the Federal Biological Center for Agriculture and Forestry (BBA) in Braunschweig, Germany, has been established at the Institute of Plant Diseases and Plant Protection (IPP), Hannover University, Germany. The insects are reared continuously on broad beans plants (*Vicia faba* L. [Fabaceae]) at 24°C at a photoperiod of L16:D8, following the protocol developed by Raupach et al. (2002). *Anagrus atomus* was obtained from English Woodlands Biocontrol, the commercial supplier of *A. atomus* in the UK. The parasitoid is commercially distributed as pupae in leaf pieces of primrose (*Primula vulgaris* Huds. [Primulaceae]). Mass rearing of *A. atomus* at IPP followed the protocol developed by Triapitsyn and Moratorio (1998) for the closely related species *A. nigriventris* Girault with certain modifications. Potted broad bean plants bearing leafhopper eggs (0-3 days) were collected from the adult *E. decipiens* cage and then placed in an insectary room (24°C ± 1, 65-70% relative humidity [r.h.], 16:8 h light: darkness [L:D.]). Each plant was then covered with a cylindrical Plexiglas cage (32 cm tall and 13.5 cm diameter) with two screened windows in the sidewall and a nylon mesh covering one top. Three to five female *A. atomus* were released in each cage for oviposition. After 12 days, the plants were gently shaken to discard leafhopper nymphs that had emerged from unparasitized eggs. Thereafter, stems and leaves were cut in small pieces (approximately 1-2 cm) containing individual parasitized eggs, as indicated by their red colour (Cooper, 1993), and placed into 1.5 ml Eppendorf tubes with a piece of wet filter paper. The tubes were kept in a sandwich plastic box in the incubator (24°C ± 1, 65-70% r.h., 16:8 h (L:D)

and were daily checked for emergence of parasitoids. Condensed water was removed daily since *A. atomus* does not tolerate high relative humidity.

2.3.2 Effect of temperature on life table parameters of *A. atomus*

Developmental time from egg to adult was studied under three constant temperature regimes, i.e. 16, 20 and 24°C. The regimes were chosen because they represent temperatures frequently encountered in European greenhouses. Mated female *A. atomus*, <24h-old, were individually released in Plexiglas cylinders containing a broad bean plant, with only one seedling per pot, that harboured *E. decipiens* eggs; the host eggs were obtained by exposing broad beans to adult leafhoppers for 72h (for details see previous section). Parasitoids were allowed to oviposit for 24h. Thereafter, the development of the parasitoid progeny was followed in the three respective temperature-controlled chambers (i.e. 16, 20 and 24 ± 1°C, 65-75 r.h., 16:8 h L:D). Each temperature treatment was repeated seven times with individual females. When the parasitized eggs started to turn red, stems and leaves were cut into individual pieces, harbouring a single parasitized egg, and each piece was then individually kept in an 1.5 ml Eppendorf tube until emergence of the adult parasitoids. For each temperature regime tested, egg-adult development time were computed as

$$D = \frac{\sum nix_i}{\sum n_i},$$

where n_i is the number of adults emerging and x_i the time required for each individual to complete development, and mortality as total number of emerged parasitoids/total number of host eggs that had been parasitized.

To study the effect of temperature on the fecundity and longevity of female wasps, 15 F1 females from each temperature treatment were selected at emergence and were then mated separately by placing individual pairs in 1.5 ml Eppendorf tubes. Thereafter, each female was placed in a separate cylinder, containing a broad bean plant harbouring *E. decipiens* eggs (for details see previous section), and was kept at the same temperature regime as that of its pre-adult development. Every 24h until death the female parasitoids were provided with a newly infested plant, harbouring *E. decipiens* eggs, and each day the number of females alive was recorded. The removed plants were kept until parasitized eggs turned red. Plant parts harbouring parasitized eggs were then cut in small pieces as described for the previous experiment and kept in 1.5 ml Eppendorf tubes until the emergence of the F2. Emerging adults were counted and sexed, and the fecundity, longevity of the tested females and the sex ratio of the F2 were calculated for each temperature regime tested.

2.3.3 *Within-plant distribution of E. decipiens* eggs and parasitism by *A. atomus*

The objective of the experiment was to assess the impact of *A. atomus* as a mortality factor on its leafhopper host. Thus the parasitism was calculated and distribution of *E. decipiens* eggs and parasitized eggs was determined within the broad bean plant. Mated female parasitoids, <24h-old, were individually released in a cylinder containing a broad bean plant harbouring *E. decipiens* eggs. After 12 days of incubation period, the number of leafhopper nymphs, parasitized eggs (as indicated by their red colour) and unparasitized still in the plant (visible by their eye-spotted stage) were recorded and the percent parasitism was calculated using the formula of Van Driesche (1983): $\% PA = 100 * PE / (N + PE + U)$ where: PE = number of parasitized eggs, N = number of leafhopper nymphs, U = number of unparasitized eggs.

The within-plant distribution of parasitized eggs in the host plant was quantified by counting the parasitized eggs in the stems of the plant, the petioles and in the stems of the leaf. This data was then related to the oviposition pattern of *E. decipiens* in three plants where no parasitoids were released. These plants were first introduced into the *E. decipiens* oviposition cage for 48 h. After eight days of incubation at 24°C, the leaf limbs, petioles of these plants were cut and put on agar (8g/l), the rest of the plant (stems) covered with cylindrical cage previously described. The number of hatching nymphs from each part of the plant was determined and their proportion in relation to the total number of eggs in each plant was then calculated.

2.3.4 Effect of host eggs age on the parasitism by *A. atomus*

Vicia faba plants, two weeks old and in the three-four leaves stage were introduced into oviposition cages and exposed to adult leafhoppers for 48 h. Thereafter, the plants were removed, placed in empty cages and were incubated at 24°C for zero, two, four, six and eight days to generate one-two, three-four, five-six, seven-eight and nine-ten days-old leafhopper eggs, respectively. For each age group, three potted-plants were covered with a cylinder and one mated <24h-old female was released per cylinder for one day. After 12 days of incubation, the number of emerged leafhopper nymphs as well as non-parasitized and parasitized eggs counted. The experiment was conducted at 24°C, 65-70% r.h. and 16:8 h L:D:, and each age group was repeated three times.

2.3.5 Statistical analysis

Differences in developmental time, survivorship and reproduction at different constant temperatures, host age and rate of parasitism were analysed by analysis of variance (ANOVA) and means were separated using Duncan multiple range test (GLM procedure, SAS institute, 1992). A linear regression was computed to estimate the lower developmental threshold and the

thermal constant by using the developmental rate [R (T)] as dependant variable and constant temperature (T) as independent variable (Campbell et al., 1974): $R(T) = a + b \cdot T$. The lower developmental threshold was determined as the intercept of the linear equation and the degree-day requirements for immature development, as the value of the inverse of the linear equation slope. Life statistics were calculated according to Hulting et al. (1990), using the jackknife program. Differences in r_m values among populations were calculated following the protocol of Dixon (1987) and compared with Newman-Keuls sequential tests (Sokal and Rohlf, 1995) based on jackknife estimates of variance for r_m values (Meyer et al., 1986).

2.4 Results

2.4.1 Egg-adult development

The parasitoids completed development at all three temperatures tested (Table 2.1). Adult emergence began after 29, 20 and 15 days at 16, 20 and 24°C, respectively. Both males and females developed at the same rate for each of the three tested temperature regimes (two way ANOVA, $df = 1$; $F = 1.57$; $p = 0.21$). The proportion of males emerging each day was low, leading to a female-biased sex ratio at each temperature tested.

The development rate [Rate (T)] of the parasitoids increased linearly with temperature (T) [Rate (T) = $-0.03433 + 0.00398T$; $r^2 = 0.954$; $p < 0.0001$]. The lower developmental threshold for *A. atomus*, at which no development occurred, was estimated at 8.28°C. Based on this development threshold, *A. atomus* required 251.26 Day-Degrees (DD) to develop from egg to adult.

Table 2.1 Egg-adult development time of *A. atomus* at three constant temperatures

Temperatures (°C)	Mean development time (in days \pm s.d.)					
	$\text{\textcircled{M}}$		$\text{\textcircled{F}}$		$\text{\textcircled{M}}\text{\textcircled{F}}$	
16	33.16	\pm 2.81a	33.86	\pm 1.80a	33.60	\pm 2.06a
20	22.35	\pm 0.75b	22.75	\pm 1.27b	22.64	\pm 1.16b
24	16.26	\pm 1.37c	16.32	\pm 0.83c	16.30	\pm 0.98c

Means in the same column followed by the same letter are not significantly different at $P < 0.05$.

2.4.2 Adult longevity and reproductive capacity

Temperature had a significant effect on the adult longevity (i.e. survival), but not on the sex ratio and the survival rate in immature *A. atomus* (Table 2.2, Figure 2.1).

At emergence *A. atomus* females started laying eggs without any pre-oviposition period. Oviposition was greatest during the first two days. Temperature had a significant influence on the oviposition period and the total number of offspring per female (Table 2.2).

Females *A. atomus* lived a maximum of 22, 12 and 9 days at 16, 20 and 24°C, respectively (Figure 2.1 A-C). For all three temperatures tested, maximum daily progeny decreased with increasing age of the females (Figure 2.1 A-C).

Table 2.2 Egg-adult survival, adult longevity, oviposition time, total offspring and sex ratio (means \pm s.e) of *A. atomus* at three constant temperatures

	Temperatures ($^{\circ}$ C)					
	16		20		24	
Survival (%)	79.2	\pm 6.7a	77.7	\pm 5.07a	83.6	\pm 4.47a
Adult longevity	15.1	\pm 0.92a	8.3	\pm 0.59b	5.9	\pm 0.35c
Oviposition period	4.4	\pm 0.24a	4.3	\pm 0.35a	3.3	\pm 0.59b
Total offspring	19.7	\pm 0.94b	21.3	\pm 1.07b	30.1	\pm 1.36a
Sex ratio	72.7	\pm 0.02a	73.7	\pm 0.62a	71.1	\pm 0.06a

Means in the same line with different letters are significantly different at $P < 0.05$

2.4.3 Life table parameters

Computed life table parameters for three temperature regimes are presented in Table 2.3. The net reproduction R_0 decreased from 21.45 at 24 $^{\circ}$ C to 14.31 at 16 $^{\circ}$ C. However, the mean generation time and the doubling time (DT) decreased with increasing temperature. The intrinsic rate of increase (r_m) and the finite rate of increase (λ) were significantly higher at 24, than at 20 and 16 $^{\circ}$ C. The combined differences in egg-adult development, adult survivorship and fecundity produced strong differences in life table parameters of *A. atomus*, especially in r_m .

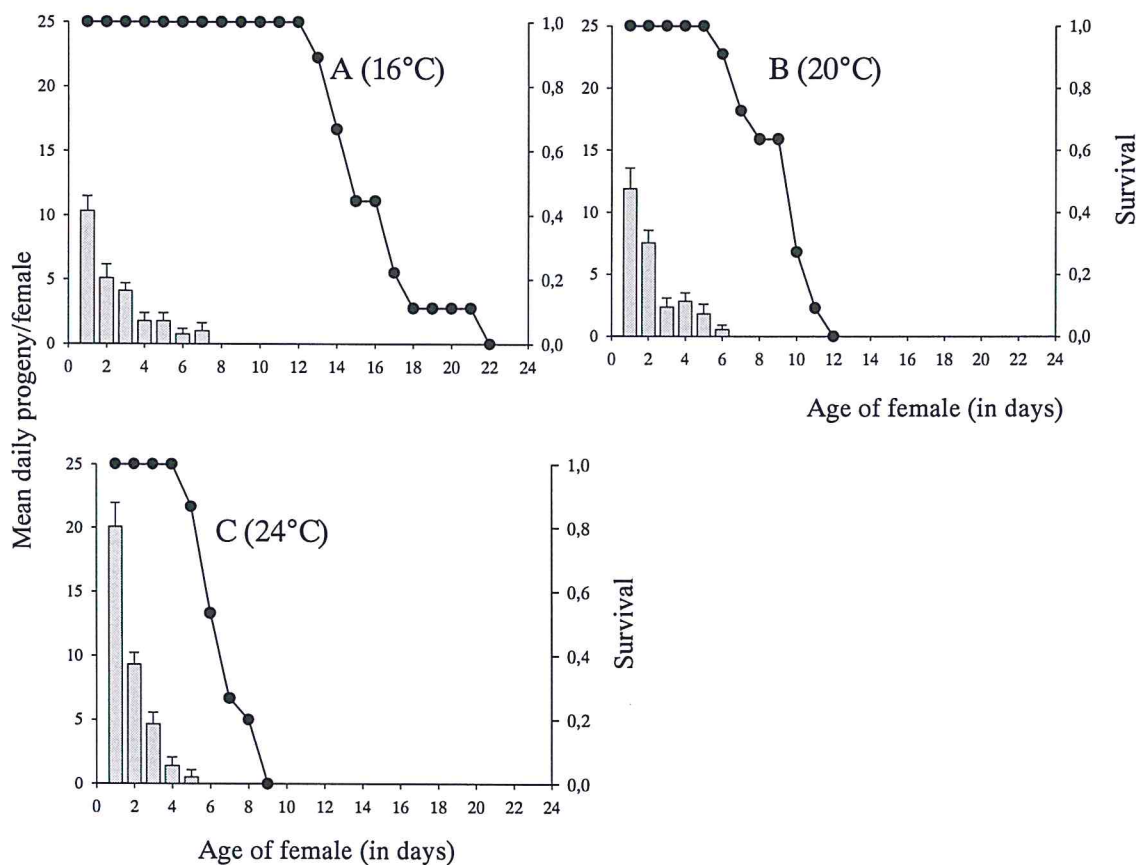


Figure 2.1 *Anagrus atomus* survival and reproduction at A (16°C), B (20°C) and C (24°C)

▬ Daily progeny; —●— Survival

Table 2.3 Effect of temperature on life table parameters of *A. atomus*

Parameters	Temperatures (°C)		
	16	20	24
r_m	$0.086 \pm 0.002c$	$0.128 \pm 0.003b$	$0.191 \pm 0.003a$
R_o	14.3 ± 0.7	15.7 ± 1.0	21.5 ± 1.1
G	30.9	21.6	16.1
DT	8.0	5.4	3.6
λ	1.09	1.14	1.21

r_m = jackknife estimate of the intrinsic rate of increase (per capita rate of population growth),
 R_o = net reproductive rate (female offspring per adult female), G = mean generation time (days),
 DT = doubling time (days) and λ = infinite rate of increase for population.

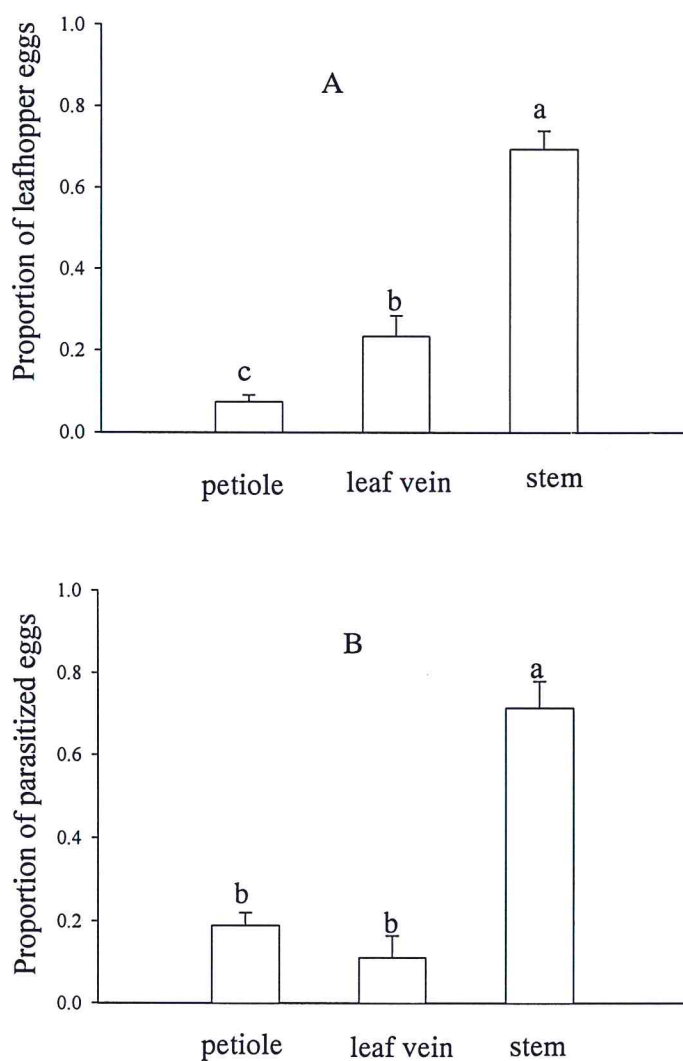


Figure 2.2 Distribution of unparasitized leafhopper eggs (A) and parasitized eggs (B) in *V. faba*. Different letters above bars indicate significant differences between means (ANOVA, $P < 0.05$)

2.4.4 Within-plant distribution of *E. decipiens* eggs and parasitism by *A. atomus*

Mean parasitism of *A. atomus* was 44.7% ranging from 27.2-62.5%. The spatial distribution pattern of parasitized eggs revealed that significantly higher numbers of parasitized eggs were found in the stem than in any other parts of the plant (Figure 2.2). This pattern corresponded with

the spatial distribution of *E. decipiens* eggs in the plants (59% of all leafhopper eggs were found in the stems). Thus, egg laying in *A. atomus* follows the oviposition pattern of *E. decipiens*. Regression analyses revealed a significant negative relationship between host density and level of parasitism (Figure 2.3 A and B).

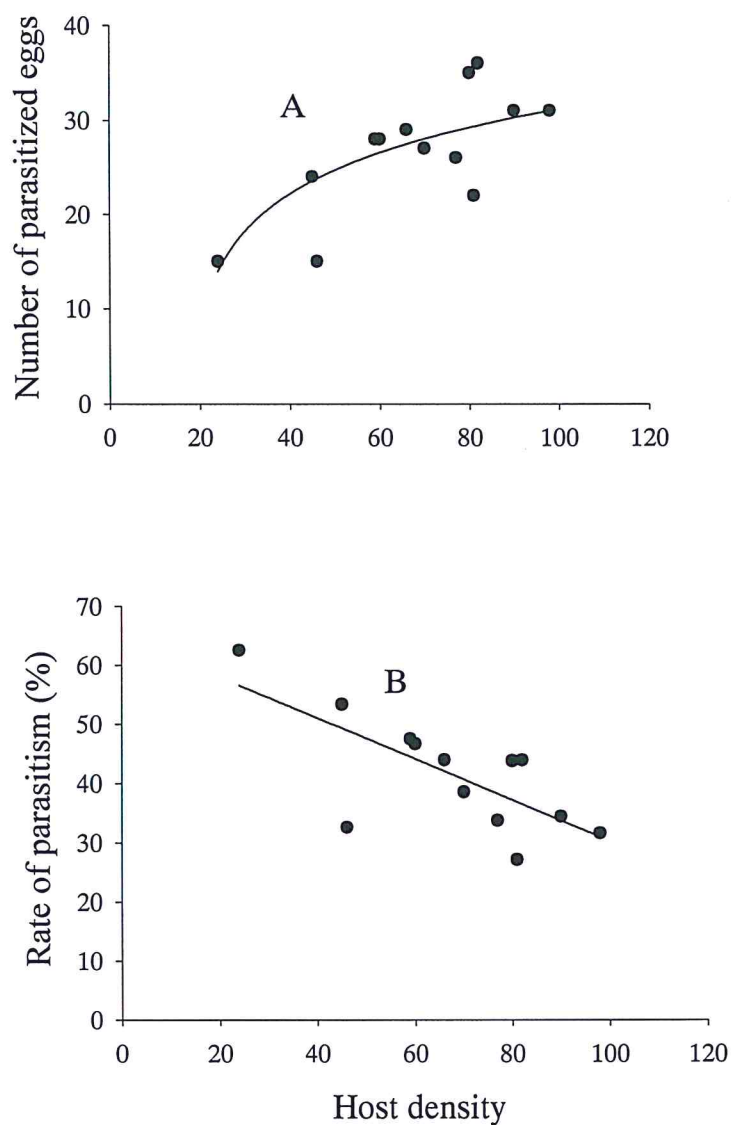


Figure 2.3 Relation between host density and number of parasitized eggs (A: $y = 7.05 \cdot \ln(x - 16.84)$, $r^2 = 0.55$, $P = 0.0036$) and rate of parasitism (B: $y = 64.85 - 0.35x$; $r^2 = 0.52$, $P = 0.0053$)

2.4.5 Effect of the host egg age on the parasitism by *A. atomus*

Anagrus atomus females were capable of successfully parasitizing leafhopper eggs of all age classes tested (Figure 2.4). However, the number of host eggs that were visibly parasitized declined significantly in eggs older than 5-6 days.

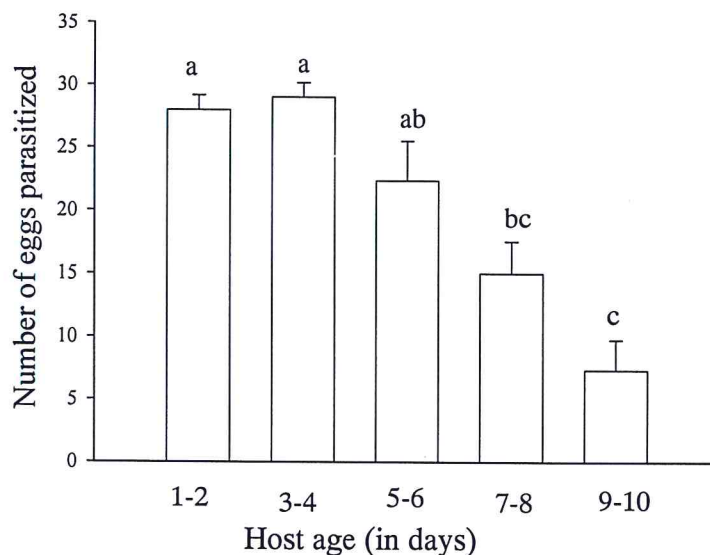


Figure 2.4 Host age and parasitism by *A. atomus*. Different letters above bars indicate significant differences between means (ANOVA, $P < 0.05$)

2.5 Discussion

Temperature is one of the most important factors influencing the development of arthropods. In our study the lower development threshold for *A. atomus* was calculated to be 8.28°C, which is below the range of temperatures used in greenhouse vegetable production systems in Germany (Fricke et al., 1999). Hence the parasitoid is capable to develop during almost the whole production period in greenhouses. Parasitoid development times are variable among the species within the genus *Anagrus*. For example, at 24°C, *A. takeyanus* Gordh required almost two weeks longer to emerge than *A. giraulti* Crawford (Meyerdirk and Moratorio, 1987). The thermal

developmental requirements for *A. atomus* are much lower than the 504 DD for *A. delicatus* Dozier (Cronin and Strong, 1990a) but higher than 244.5 DD evaluated for *A. epos* Girault (Williams, 1984). The differences in the development of these *Anagrus* spp. may be an adaptation to the development of their hosts. The lower development threshold in *A. atomus* is very close to the 8.84°C computed for *E. decipiens* using the data from Raupach et al. (2002) within temperature range of 15-28°C. Moreover, the thermal constant of *A. atomus* was considerably shorter than the 400 DD estimated for *E. decipiens*. Thus, in terms of thermal requirements *A. atomus* is considerably superior to its host.

Female parasitoids laid more eggs during the first days, indicating that egg production in *A. atomus* is proovigenic (Hokyo et al., 1966). Due to the shortest developmental time and highest number of progeny r_m was the highest at 24°C.

Parasitism by *A. atomus* showed a negative host density-dependent relationship. Similar response has been observed in *A. incarnatus* Haliday (Cronin and Strong, 1993). Liljeström and Virla (2001) studying the spatial parasitism of *A. flaveolus* Waterhouse in *Delphacodes kuscheli* Fennah (Hom.: Delphacidae) showed that parasitism was directly density dependent. Likewise, Murphy et al. (1998) observed that the density of *Erythroneura elegantula* Osborn. (Hom.: Cicadellidae) eggs influence the rate of parasitism in *A. epos*. In contrast, Cronin and Strong (1990b, 1993) found that the spatial parasitism by *A. delicatus* and *A. optabilis* Perkins was independent of host density. They showed that the lack of host density response in the *A. delicatus* - *Prokelisia marginata* Van Duzee (Hom.: Delphacidae) system is a consequence of *A. delicatus* dispersion from host clutches after laying only a small fraction of their eggs. In our study, *A. atomus* adults were caged on the same plant; this could give the parasitoid a chance to search and parasitize more eggs on the same plant than if they were free to disperse. Lessells

(1985) demonstrated that parasitoids limited by their egg load produce inverse density dependent parasitism. In fact, the fecundity of *A. atomus* is low, which is a general trait in *Anagrus* spp. (Cronin and Strong, 1990a; Williams, 1984), so that a limited number of eggs were laid even if the host density is increasing, leading to the comparatively low levels of parasitism recorded in this study. Studies of spatial density-dependence relationships are experimental, however, both temporal and environmental factors may obscure the true response of parasitism to host density (Hassell, 1985). In our study a precise assessment of the impact of *A. atomus* and its ability to reduce leafhopper populations was difficult, because in *E. decipiens* it is impossible to determine the exact number of eggs laid in the plant tissue. Moreover, the survival of *A. atomus* from eggs to pupae, the latter indicated by their red colour (Cooper, 1993), and egg mortality in *E. decipiens* cannot be determined.

Higher numbers of *E. decipiens* eggs were parasitized in the stems than in leaves of *V. faba* plants, corresponding to the oviposition pattern of *E. decipiens*. This could mean that parasitoids search longer for hosts in parts of the plant where host oviposition is more likely to occur or could indicate a positively density-dependant response of *A. atomus*. This oviposition pattern of *A. atomus* in broad beans could also indicate that the structure of the plant could provide a barrier to detect of *E. decipiens* eggs since the eggs can be inserted in different positions and depth in the plant tissue. Raatikainen (1967) found that in *Javesella pellucida* Fab. (Hom.: Delphacidae) eggs laid in the stems of cereals were invulnerable to attack by *A. atomus*, whereas eggs laid in leaf blades suffered high levels of parasitism, corroborating our results on the effect of plant structure on the rate of parasitism.

The observed decline in numbers of parasitized eggs older than six days is possibly due to the physiology of the host embryo. According to Cronin and Strong (1990a), well-developed older *P.*

marginata host embryos are more difficult to digest for *A. delicatus* than younger ones. However, due to the before mentioned methodological constraints, it cannot be clearly determined whether the observed differences in parasitism of younger and older *E. decipiens* eggs are the results of an avoidance of older embryos and/or a lower survival of immature *A. atomus* in old hosts.

Several biological characteristics of *A. atomus*, e.g. its short life cycle compared to its host and its ability to parasitize host eggs throughout the whole host development, indicate that the parasitoid can be an important mortality factor for *E. decipiens*. However, because of its comparatively low fecundity, high parasitoid release rates and/or combinations with other bio-control agents like predators and entomopathogenic fungi might be required for a successful control of *E. decipiens* in greenhouses. The release rates for *A. atomus* should also depend on the reproductive potential of the pest, the economic threshold and the parasitoids host finding behaviour. In ongoing studies we are investigating the searching behaviour and host discrimination ability of *A. atomus*, and the ability of the parasitoid to suppress leafhopper populations under greenhouse conditions.

3 SEARCHING AND OVIPOSITION BEHAVIOUR OF *ANAGRUS ATOMUS* ON FOUR HOST PLANTS OF ITS GREEN LEAFHOPPER *EMPOASCA DECIPIENS*

3.1 Abstract

Anagrus atomus L. is an important egg parasitoid of the green leafhopper *Empoasca decipiens* Paoli. In this study the ability of the parasitoid to locate and parasitize its host was investigated on four host plants of *E. decipiens*, i.e. broad beans (*Vicia faba* L.), sweet pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.) and French beans (*Phaseolus vulgaris* L.). For each plant species, the behaviour of the parasitoid was observed on plants with or without prior exposure to leafhopper. The searching and oviposition behaviour was mainly characterized by drumming, probing and resting. Parasitoids spent significantly less time on non-infested plants, and no probing behaviour was observed. On infested plants probing took on average <11 seconds. Frequency of resting behaviour was significantly greater on non-exposed than on exposed to leafhopper plants. Total foraging time was significantly longer on exposed than on non-exposed host plants, indicating that *A. atomus* females can efficiently discriminate between leaves with and without infestation, leaving non-infested leaves. Parasitism of *A. atomus* was influenced by the parasitoid density with the highest parasitism rate (63.99%) obtained at density of ten *A. atomus* females but the number of parasitized eggs per female and the searching efficiency decreased with the increasing of parasitoids density.

3.2 Introduction

The green leafhopper *Empoasca decipiens* Paoli (Homoptera: Cicadellidae) is a serious pest attacking vegetables and ornamentals in greenhouses and the field in The Netherlands, UK (Helyer and Talbaghi, 1994), Bulgaria (Loginova, 1992), Switzerland (Anonym., 1998aandb) and Germany (Schmidt and Rupp, 1997). *Empoasca decipiens* is polyphagous and attacks many field

crops including broad beans, cucumber, French beans, sweet pepper and many other vegetables (El-Dessouki and Hosny, 1969). Its feeding typically produces localized chlorosis in leaf tissue, and in extreme cases, affected leaves may become necrotic and drop from the plant. This feeding habit can lead to significant yield losses, both in terms of quantity and quality, particularly at high densities and when the fruits are punctured (Raupach et al., 2002). Currently, control strategies for leafhoppers mainly rely on the use of synthetic insecticides. The insect growth regulator Buprofezin has proved to effectively control *E. decipiens* with little to no harmful effects on natural enemies in greenhouses. However, it does not affect eggs and adults of leafhoppers (Helyer and Talbaghi, 1994). Chemical control of leafhoppers is difficult due to the lack of appropriated insecticides and yet unclear relationships between infestations and economic losses caused by many *Empoasca* spp. (Maixner et al., 1998). Moreover, insecticide applications often cause harmful side effects on beneficial organisms, particularly natural enemies like predators and parasitoids.

At present no well developed biological control strategies for *E. decipiens* are available. Research on the natural enemy complex of *Empoasca* spp. indicated that predators are not very likely to efficiently control the pest (Helyer and Talbaghi, 1994). The most promising candidate for augmentative biological control in greenhouses is the egg parasitoid *Anagrus atomus* L. (Hym.: Mymaridae) (Schmidt and Rupp, 1997; Cerutti et al., 1991). In several field surveys in southern Germany *A. atomus* proved to be the most common natural enemy associated with *E. decipiens* on vegetables (Rupp, 1999; Schmidt and Rupp, 1997). Egg parasitoids are often considered to be well-suited biocontrol agents since they attack the egg stage and can hence prevent further damage by the pests (Smith, 1996). However, at present little is known on the biology and ecology of *A. atomus*. Moreover, first releases of *A. atomus* on Reichenau island in southern

Germany in greenhouse cucumber production did not lead to a sufficient control of *E. decipiens* (Bünger et al., 2002). The reasons for this failure in biological control are yet poorly understood. Hence, a sound assessment of the efficacy of *A. atomus* as a potential biological control agent of *E. decipiens* is presently not possible because of the lack of crucial data on biology, ecology and behaviour of the parasitoid. Particularly more information on the temperature dependence, the host identification behaviour and the possible influence of the various host plants of *E. decipiens* on the biological control potential of *A. atomus* need to be gathered. The importance of chemical stimuli orienting the females to their host has been well documented for several egg parasitoids (Noldus and van Lenteren, 1985; Mattiacci et al., 1993). Parasitoids often use olfactory cues from their hosts and their host plants to successfully locate the hosts (e.g., Price et al., 1980; Vinson, 1981; Vinson et al., 1987; Tumilson et al., 1993). Moreover, the fitness of a searching parasitoid is a function of its host-finding ability; its efficiency as a biological control agent can thus be correlated to this behaviour (Godfray, 1994). Host plants of leafhoppers on which both host and parasitoid have been reared significantly affected several behavioural parameters of the closely related *A. nigriventris* Girault (Al-Wahaibi and Walker, 2000a&b; Honda and Walker, 1996), thus indicating possibilities to manipulate the oviposition preference of *A. atomus* by a careful selection of the host plants in mass rearing programs of the beneficial and/or for habitat management in greenhouses and the field. Hence the objectives of this study were to investigate in the laboratory the searching and oviposition behaviour of *A. atomus* as affected by different host plants of *E. decipiens*. Potential effects of parasitoid densities on parasitism and on the searching efficiency of *A. atomus* were also investigated.

3.3 Materials and Methods

3.3.1 *Insect colonies*

Leafhoppers were reared on broad beans *Vicia faba* L. following the protocol developed by Raupach et al. (2002). The culture of *A. atomus* was initiated from parasitoid pupae on primrose, *Primula vulgaris* Huds. (Primulaceae) leaves, obtained from English Woodland, the commercial supplier of *A. atomus* in the UK. The parasitoids were reared in a climatic chamber at 24°C, 65–70% relative humidity (RH), and a photoperiod of 16:8 h (L:D) on broad bean plants that previously had been infested with *E. decipiens* eggs. The plants were enclosed in a cylindrical ventilated plastic cage (32 cm tall, 13.5 cm diameter). Parasitoids used in all experiments had no previous oviposition experience (i.e., naïve) and were collected on the day of adult emergence.

3.3.2 *Searching behaviour of A. atomus on four host plants of E. decipiens*

Experimental plants

Four reproductive host plants of *E. decipiens* were used in the study, i.e., broad beans (*Vicia faba* L. [Fabaceae]), sweet pepper (*Capsicum annuum* L. [Solanaceae]), cucumber (*Cucumis sativus* L. [Cucurbitaceae]) and French beans (*Phaseolus vulgaris* L. [Fabaceae]). Experimental host plants were grown from commercially available seed material. Plants were grown in a greenhouse and no pesticides were applied. All plants were in their vegetative growth phase when used in the experiments. Two-weeks-old broad and French beans, and three-weeks-old cucumber and sweet pepper plants were used in the study.

Experiment design

The experimental design was 4 x 2 factorial with the two factors being plant species (four) and prior exposure to leafhoppers (i.e., plants previously exposed to leafhoppers vs. non-exposed),

hence in total eight treatments. For each replicate two plants of each host plant species were used and one was exposed to adult *E. decipiens* by placing them over a 72-h period at 24°C in an adult leafhopper cage (46 x 46 x 122 cm) whereas the other was left non-exposed. Thereafter leaves were cut in discs and placed on potato dextrose agar (8 g/l) in a Petri dish (5.5 cm diameter). The size of the leaf discs was standardized to the Petri dish diameter. Each treatment was replicated 10 times. Naïve *A. atomus* females were collected at the day of their emergence. Before use female parasitoids were held in individual pairs with males in Eppendorf tubes (1.5 ml), containing a droplet of honey solution smeared through small holes on the topside of the tube, for 30 minutes to assure mating. One active female parasitoid, <24-h old, was released in the Petri dish and continuously observed under a binocular. The general behaviour of the parasitoids was examined and time on plants, duration of each probe, and resting time were recorded using a stopwatch. The observation began five minutes after the parasitoid had been introduced on the plant disc in the Petri dish and the wasp was observed for 15 minutes. After the observations, the leaves previously exposed to leafhoppers were incubated at 24°C for 12 days to check for presence/absence of leafhopper nymphs or parasitized eggs, the latter as indicated by their red color (Cooper, 1993).

Data recorded

The following behavioural parameters were recorded: (i) the duration of time off the plant (time that a parasitoid spent on the wall or lid of the Petri dish), (ii) the number and duration of probing (insertion of ovipositor in the plant tissue and the withdrawal), and (iii) resting, defined as non-searching behaviour.

Data analysis

Proportion of time on the plant, proportion of time probing, and number of probes were calculated for each treatment. Proportions were arcsine transformed before analysis. Two-way analysis of variance (ANOVA) was performed to test for the effect of the two main factors (plant species and previous exposure to leafhoppers) on the different variables (proportion of time spent probing, proportion of time spent on the leaf, number of probes and probes per minute).

3.3.3 Effect of *A. atomus* densities on parasitism and on searching efficiency

Potted *V. faba* plants were placed for 72-h in an adult leafhopper cage. The plants were then covered with a cylindrical cage (for details refer to the previous section) and kept in a climatic chamber at 24°C, 65-70% RH, and a photoperiod of 16:8 h (L:D). Naïve and mated female *A. atomus* were then released in the cylinder, permitting them to search for and parasitize host eggs hidden in the plant tissue. Five different parasitoid densities were tested, i.e. one, three, five, six and ten females per cylinder. After 12 days incubation period, leafhopper nymphs, unparasitized and parasitized eggs were collected and the rate of parasitism calculated. Egg densities and number of parasitized eggs per treatment were subjected to a log (1+x) transformation to homogenize the variance, and egg densities were used as covariant for parasitism of *A. atomus* in the ANOVA. Moreover the per capita searching efficiency of the parasitoid (A) was determined using the model developed by Hassell (1985): $A = (1/P) * \log(N/S)$ where *P* is the abundance of the searching parasitoids (i.e., parasitoid densities), *N* is the number of susceptible hosts (i.e., total host eggs in the plant), and *S* is the number of hosts surviving after parasitism (i.e., unparasitized). Each density was repeated five times.

3.4 Results

3.4.1 *General searching behaviour*

Two main distinct behavioural events could be distinguished in the searching and oviposition behaviour of *A. atomus*: (i) drumming the leaf surface with the antennae, and (ii) probing (i.e., insertion of the ovipositor in the plant tissue). While searching, drumming could be classified in two categories: (i) a rapid walk accompanied by low frequency antennal drumming, and (ii) a slow walking speed with high frequency of antennal drumming. In the latter category of drumming, the antennae remained very close to the leaf discs when an area was suspected to harbor leafhopper eggs. Most often the parasitoid examined several times the same area, which was then followed by probing. During short probes, frequently observed in this study, the ovipositor could be inserted partially or completely (to its length) before it was withdrawn. However, the wasps persistently returned to the same point and usually made several attempts of probing, generally lasting a few seconds.

3.4.2 *Interaction between host plant species and prior exposure to leafhoppers*

Results of the two-way ANOVA indicated for the dependent variables, which relate directly to searching and oviposition behaviour of the parasitoids (i.e., proportion of time on the plant, probing, and the number of probes per minute), no significant interactions between plant species and exposure conditions (Table 3.1). In all tested host plants, the prior exposure to leafhoppers strongly influenced the searching behaviour of *A. atomus*, especially for probing and resting.

Table 3.1 Probability levels from two-way Analysis of Variance for plant species, infestation status and their interactions

Dependent variables	Probability level		
	Plant species	Exposure status	Interactions
Variables based on total observation time			
Proportion of time on the plant	0.38	< 0.0001	0.49
Proportion of time probing	0.35	*	*
Proportion of time in non search activities	0.0044	< 0.0001	0.92
Number of probes per minute	0.82	*	*
Variables based on total on-plant time			
Proportion of time probing	0.35	*	*
Proportion of time in non search activities	< 0.0001	< 0.0001	0.062
Number of probes per minute	0.82	*	*

* No probe was observed on non-infested host plants; therefore no statistical comparison was made between exposed and non-exposed to leafhopper plants.

3.4.3 Effect of plant species and prior exposure on the searching behaviour of *A. atomus*

The behaviour of the parasitoids significantly differed between plants previously exposed and non-exposed to leafhoppers (Table 3.1). On exposed leaf discs parasitoids spent nearly all the observation time on the leaf discs, with no significant differences among the four tested host plants (Figure 3.1). In contrast, parasitoids spent significantly less time on the non-exposed leaf discs (pairwise comparison for each host plant separately, i.e. exposed vs. non-exposed, Table 3.1). Time spent on non-exposed leaf discs differed among the four host plants tested, with significantly less time spent on sweet pepper compared to broad bean leaf disc.

Table 3.2 Probe parameters of *A. atomus* on four different host plants previously exposed to *E. decipiens*

	Host plants			
	Cucumber	French beans	Sweet pepper	Broad beans
Total number of probes	13.80 ± 1.93	8.5 ± 4.28	9.22 ± 1.27	13.20 ± 4.29
Total probe duration (sec)	133.20 ± 40.84	62.75 ± 21.26	100. ± 18	126 ± 30.36
Proportion of time probing*	0.15 ± 0.045	0.07 ± 0.024	0.11 ± 0.02	0.075 ± 0.034
Number of probes/minute	7.33 ± 0.96	6.64 ± 2.02	6.15 ± 0.75	5.97 ± 0.91

*This parameter was calculated on total time spent on plant basis. No significant differences were found among the host plants ($P > 0.34$).

The number of parasitoid probes was significantly affected by the exposure status of the host plants, but no significant differences were found among the tested plant species. No probing was observed on non-exposed leaf discs. On all previously exposed leaf discs, the probe duration was less than 11 s and was not affected by the plant species (Table 3.2).

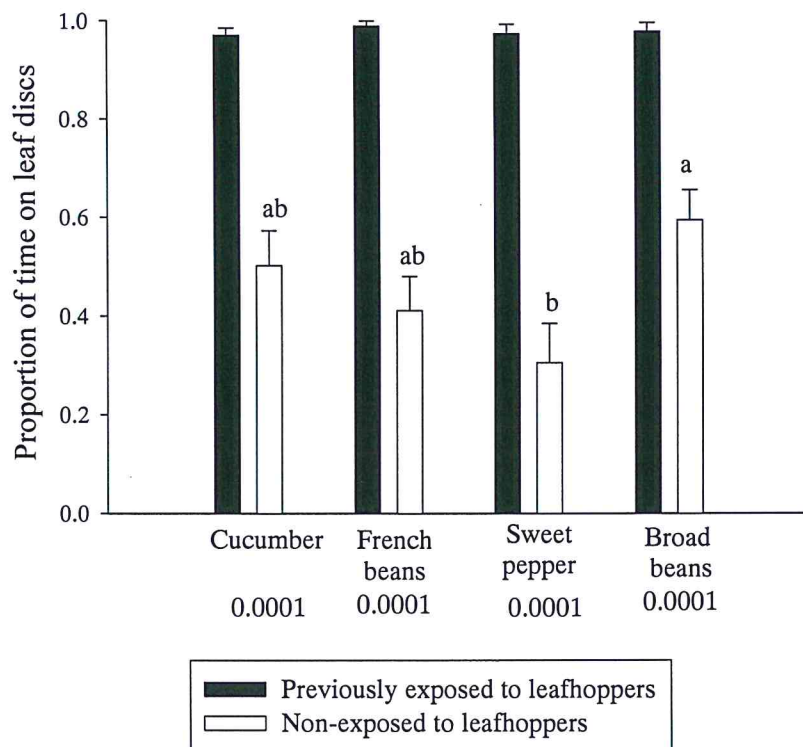


Figure 3.1 Proportion of the total observation time that *A. atomus* females spent on four host plants of *E. decipiens* with or without prior exposure to leafhoppers. Statistical differences among plant species were done separately for treatments with exposure (black bars) and without previous exposure (white bars) to leafhoppers. No significant differences between plant species were observed. Below each tested plant species the probability levels of the two-sample t-tests comparing leaf discs with (black bars) and without (white) previous exposure to leafhoppers infestation are given.

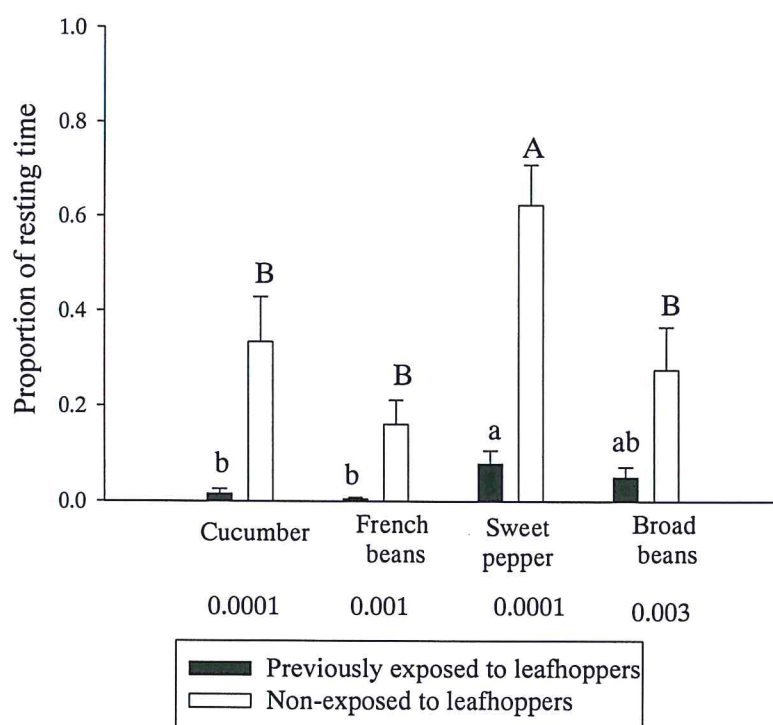


Figure 3.2 Proportion of resting of *A. atomus* females on different host plant species with or without prior exposure to leafhoppers. Columns represent means and error bars are standard errors of untransformed data. Statistical differences among plant species were done separately for treatments with (black bars) and without (gray bars) previous exposure to leafhoppers. Below each plant species' name is the probability level of two-sample t-test comparing exposed plants (black bars) and non-exposed plants (white) to leafhoppers for each host plant species. Capital letters compared non-exposed plant species and small letters exposed plant species.

On previously exposed leaf discs, parasitoid spent more than 92.21% of total on-leaf disc time searching actively for hosts and a maximum of 14.8% of searching time was used for probing. Subsequent incubation of previously exposed leaf discs showed that less than 20% (± 5) harbored 1-3 leafhopper eggs and only one parasitized egg was found in one cucumber leaf disc. Hence, the vast majority of probes were made in empty tissues.

Proportion of time based on the total time spent on leaf discs in which parasitoid were not engaged in searching activities (i.e. resting time) was significantly influenced by the host plant species and their exposure status (Figure 3.2). The proportion of time that parasitoid spent in resting was highest on sweet pepper, both for exposed and non-exposed leaf discs. Resting period differed significantly when previously exposed were compared with non-exposed leaf discs ($P < 0.003$). The parasitoid spent an average of 16.1% and 62.4% of total on-leaf disc time on non-exposed French beans and sweet pepper leaf discs, respectively, compared to 7.8% and 0.4% on exposed French beans and sweet pepper leaf discs, respectively. Thus, the parasitoid spent more time on, searched more, probed more and rested less on exposed than on non-exposed leaf discs.

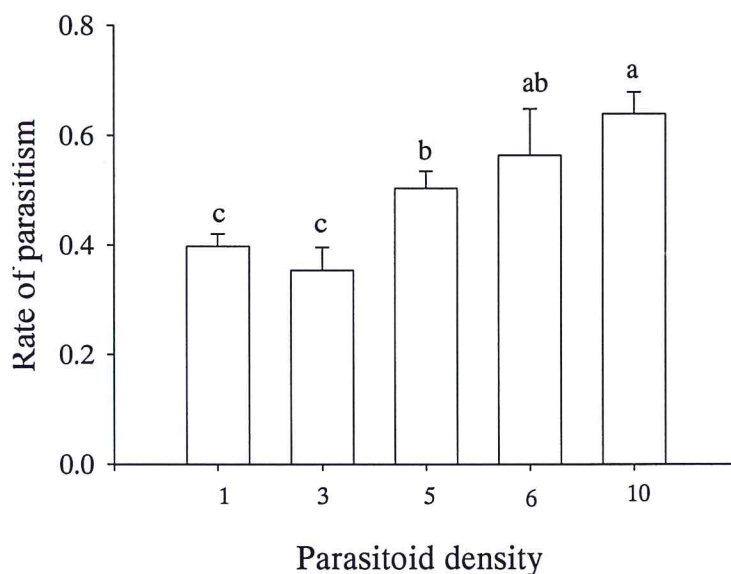


Figure 3.3 Effects of different *A. atomus* release densities on the rate of parasitism of *E. decipiens* eggs in *V. faba* plants. Bars followed by the same letter are not significantly different (ANOVA, $P < 0.05$)

3.4.4 Effects of *A. atomus* densities on parasitism and on their searching efficiency

The total number of parasitized eggs increased significantly with the number of released parasitoids ($F = 18.28$; $df = 4$; $P < 0.0001$), leading to high rates of parasitism at high parasitoid densities (Figure 3.3). With 63.99% the highest mean parasitism was recorded when ten females had been released. Analysis of covariance of the number of parasitized eggs as affected by the host density (i.e. total number of *E. decipiens* eggs) and the number of released parasitoids revealed that both factors were significant ($F = 13.63$; $df = 1$; $P = 0.0017$). The number of parasitized eggs per *A. atomus* female differed significantly ($F = 23.51$, $df = 4$; $P < 0.0001$) among the tested parasitoid densities. With increasing parasitoid densities the number of parasitized eggs/female dropped from 27.67 to 8.2 for density one and ten, respectively (Figure 3.4 II).

Plotting the searching efficiency against parasitoid density (Figure 3.4 I) revealed a decrease in searching efficiency with increasing parasitoid numbers, but ANOVA revealed no significant differences at all parasitoid densities greater than one at which density the searching efficiency was highest ($P > 0.5$).

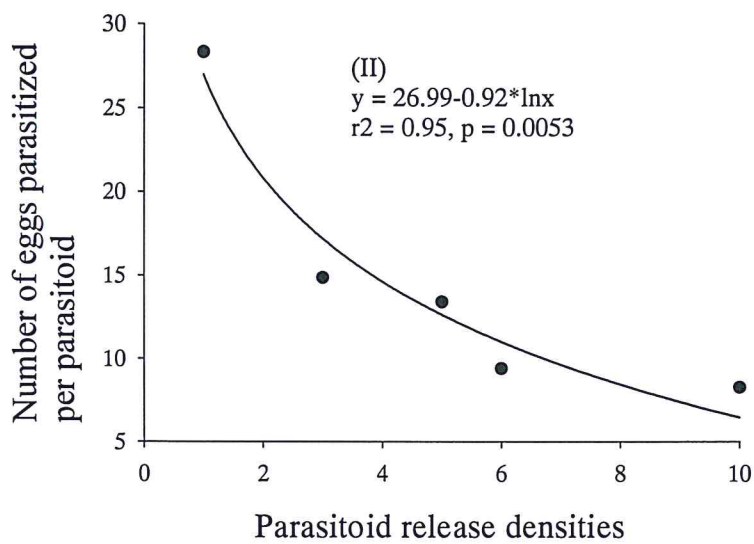
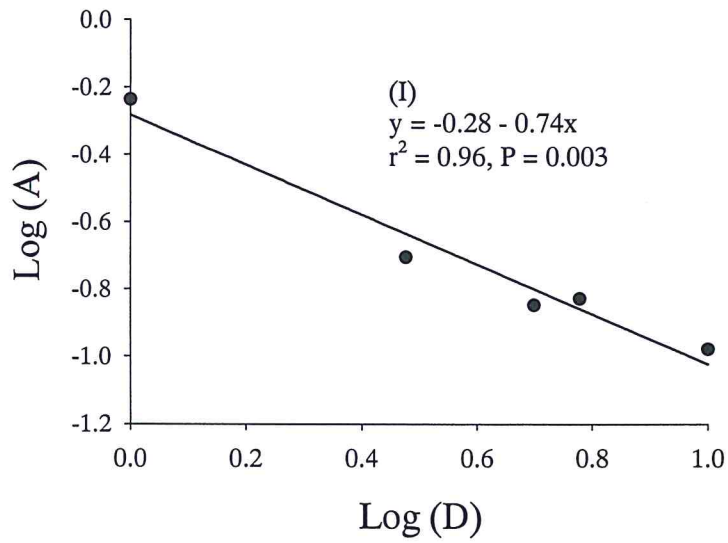


Figure 3.4 (I) Relationship between log transformed searching efficiency (A) and parasitoid density (D). (II) Number of parasitized eggs per female *A. atomus* as affected by different parasitoid densities

3.5 Discussion

All behavioural variables recorded in our study indicated that the previous presence of hosts elicited a host finding behaviour in *A. atomus*. Female *A. atomus* spent significantly more time on the leaf discs that had been previously exposed to leafhoppers than on non-exposed ones, indicating that the parasitoids can probably detect the presence of host traces. The likelihood of finding host eggs on plants that carry host traces is higher than if such stimuli are absent, and the females spend accordingly more time for searching on such leaf discs. Signals could originate from the host itself, e.g., honeydew, accessory gland secretions, or are results of the feeding and/or egg laying behaviour of the leafhoppers, i.e., feeding bumps or oviposition wounds (Cronin and Strong, 1990a; Moratorio, 1990; van Baaren et al., 1995; Conti et al., 1996). Feeding *E. decipiens* may contaminate the leaf surface with chemicals emanating from their bodies or the honeydew. Leafhopper honeydew is known to contain contact kairomones, which stimulates the searching response of egg parasitoids (Lou and Cheng, 2001). In our experiments probes were generally of short duration, and the great majority (95-98%) were performed in empty plant tissue, as revealed by the subsequent incubation of the leaf discs. In broad beans *E. decipiens* prefers to oviposit in stems than in leaves (Agboka, 2002). Short duration probes in empty plant tissue frequently occur in egg parasitoids attacking concealed hosts hidden in the plant tissue (Al-Wahaibi and Walker, 2000b), indicating that the perception of host cues on the plant surface does not suffice to locate host eggs imbedded in the plant tissue. Hence it seems that in *A. atomus* females initially perceive host cues with their antennae followed by probing with the ovipositor. The antennae might act as receiver for signals emanating from the surface while the ovipositor then detects the presence of host eggs in the plant tissue during probing. Another possible explanation of the observed probing in empty tissue could be that the wasps used in our experiments had no prior contact with hosts. Experience plays an important role in parasitoid

searching behaviour (e.g., Conti et al., 1997). However, we did not consider learning in our study because in biological control programs egg parasitoids are usually released as parasitized eggs close to emergence, since they are easier to store, handle, and release than adult wasps (Cooper, 1993; Maisonneuve et al., 1995; Bjorksten and Hoffmann, 1998). Hence there are few opportunities for behavioural manipulations of egg parasitoids during mass rearing, and innate host stimuli detection is probably more important for the host finding behaviour of egg parasitoids than learning (Al-Wahaibi and Walker, 2000a; Bjorksten and Hoffmann, 1995).

The tested host plant species had no effect on the searching behaviour of *A. atomus*, indicating that the parasitoids did not respond to additional stimuli of the plants when searching for hosts. The significantly longer resting time of female *A. atomus* on non-exposed compared to leave discs that originated from plants that had been previously exposed to leafhoppers further supports this hypothesis.

The rate of parasitism by *A. atomus* was significantly influenced by the density of the wasps, with the highest rate of parasitism recorded at the highest release rate. This suggests that increasing the numbers of *A. atomus* in mass releases could lead to a higher control efficacy. However, the parasitoids densities used in our study (1-10 females/0.1356 m²) were far higher than those previously tested under greenhouse conditions by Bünger et al. (2002) (i.e., 0.05-0.38 individuals/m²) or recommended by English Woodlands Biocontrol, the commercial supplier of *A. atomus* in the UK (i.e., 100 individuals/acre). In addition, even at the highest release density the rate of parasitism did not exceed 63.99%. This may be due to the generally low fecundity in *A. atomus* (Agboka, 2002) and possible interactions between females at higher release densities. Releasing more than six parasitoids per plant resulted in an overall increase in progeny, but a decrease in the number of parasitized eggs per female. Since many host eggs remained

unparasitized, this slight decrease in the multiplication rate might be attributed to lower parasitism efficiency with increased parasitoids mutual interference or superparasitism (Cronin and Strong, 1993) or unsuccessful probes within the plant tissue as observed in this study. Consequently the searching efficiency decreased with increasing parasitoids density.

In conclusion, *A. atomus* females can sense the previous presence of leafhoppers on plants and intensively search for hosts on such plants. The four tested host plants of *E. decipiens* did not affect the searching behaviour of *A. atomus*, suggesting that the parasitoids can be released on different host plants of *E. decipiens*. In ongoing laboratory and greenhouse experiments we are testing various release rates of *A. atomus* and are quantifying the impact of the parasitoid on the population dynamics of its host.

4 GENERAL DISCUSSION

The objective of this study with *A. atomus* was to assess its potential to control *E. decipiens* in greenhouses. Therefore, the rate of development and different life table parameters of the parasitoid were determined under three constant temperatures. Moreover, the response of *A. atomus* to different densities of leafhopper eggs and its ability to attack different host stages were investigated. Additionally, the host searching behaviour on four different host plants of *E. decipiens* and the effect of varying parasitoid densities on the rate of parasitism and the searching efficiency of *A. atomus* were examined. The results have elucidated some aspects of the life history of *A. atomus*.

Anagrus atomus can successfully develop and reproduce within a temperature range of 16-24°C, which corresponds well common climatic conditions in European greenhouses. Hence, the parasitoid can be present and active during almost the whole production period. Development time temperature dependence vary among *Anagrus* spp. For example, at 24°C, *A. takeyanus* Gordh required almost two weeks longer to emerge than *A. giraulti* Crawford (Meyerdirk & Moratorio, 1987). The thermal developmental requirements for *A. atomus* (251.2 day-degrees(DD)) are much lower than the 504 DD for *A. delicatus* Dozier (Cronin & Strong, 1990a) but higher than the 244.5 DD of *A. epos* Girault (Williams, 1984). The differences in thermal developmental requirements of these *Anagrus* spp. Probably reflect an adaptation to the development of their hosts. The lower development threshold in *A. atomus* is very close to that computed for *E. decipiens* (Raupach et al., 2002) within a temperature range of 15-28°C. Moreover, the thermal constant of *A. atomus* was 37.19% shorter than that estimated for *E. decipiens*. Thus, in terms of thermal requirements *A. atomus* can develop faster and built up faster populations than *E. decipiens*.

Female parasitoids laid more eggs during the first days, indicating that egg production in *A. atomus* is proovigenic (Hokyo et al., 1966). However, the fecundity of *A. atomus* is low, a general trait in *Anagrus* spp. (Cronin & Strong, 1990a; Williams, 1984) so that only a limited number of eggs were laid even at increasing host densities. Consequently levels of parasitism in *A. atomus* are comparatively low, with an inverse density-dependence. Lessells (1985) also demonstrated, that parasitoids limited by their egg load, produce inverse density dependent parasitism. Similar response has been observed in *A. incarnatus* Haliday (Cronin & Strong, 1993). However, Liljeström and Virla (2001) studying the spatial parasitism of *A. flaveolus* Waterhouse in *Delphacodes kuscheli* Fennah (Homoptera: Delphacidae) showed that parasitism was directly density dependent. Likewise, Murphy et al. (1998) observed that the density of *Erythroneura elegantula* Osborn. (Homoptera: Cicadellidae) eggs positively influence the rate of parasitism in *A. epos*. In contrast, Cronin and Strong (1990b, 1993) found that spatial parasitism by *A. delicatus* and *A. optabilis* Perkins was independent of host density. They showed that the lack of host density response in the *A. delicatus* - *Prokelisia marginata* Van Duzee (Homoptera: Delphacidae) system is a consequence of *A. delicatus* dispersion from host clutches after laying only a small fraction of their eggs. In our study, *A. atomus* adults were caged on the same plant; this could have forced the parasitoid to search and parasitize more eggs on the same plant compared to a situation where the females are free to disperse.

However, these spatial density-parasitism relationships were recorded under rather experimental and thus possibly artificial conditions. Both temporal and environmental factors may obscure the true response of the parasitoids to host density (Hassell, 1985). In this study it was difficult to achieve a precise assessment of the impact of *A. atomus* and its ability to reduce leafhopper populations, because in *E. decipiens* it is impossible to determine the exact number of eggs laid in

the plant tissue. Moreover, the survival of *A. atomus* from eggs to pupae, the latter indicated by their red colour (Cooper, 1993), and egg mortality in *E. decipiens* could not be determined. The lack of these important life table statistics in *E. decipiens* renders a more detailed interpretation of the life parameters of *A. atomus* with the aim to assess the biocontrol potential of the mymarid rather difficult. More data on pest-parasitoid interactions are certainly needed. However, based on the here presented findings on *A. atomus* it can already be concluded, that due to the low fecundity of the parasitoid, high release rates and/or combinations with other bio-control agents like predators and entomopathogenic fungi are probably needed for a successful biological control of *E. decipiens* in greenhouses.

Releasing 1-10 female parasitoids resulted in increasing numbers of parasitized eggs, but the rate of parasitism did not exceed 63.99%. The number of female *A. atomus* that were released in the Plexiglas cylinders was higher than the 100 individuals/acre recommended by English Woodlands Biocontrol, the supplier of *A. atomus* in the UK, in case of heavy leafhopper infestations. Also Büniger et al. (2002) released considerably lower numbers of wasps in their experiments (i.e., 0.05-0.38 individuals/m²). However, a comparison of greenhouses releases with results of a laboratory study is difficult because the small size of the Plexiglas cylinder might have caused or enhanced interactions between the parasitoids (i.e., mutual interference and/or superparasitism) thereby reducing the rate of parasitism.

Under field conditions the release success not only depends on the number of parasitoids released but also on the dispersion of the wasps from the release point. The distribution of host eggs, the presence/or absence of host cues on the plants, which enhances the searching behaviour of *A. anagrus*, and the release method may be important factors influencing the rate of parasitism and thus the level of control. To improve the biocontrol potential of *A. atomus*, in future studies

the following release-related questions need to be addressed: (i) What is the mean distance of dispersal (spatial distribution) of *A. atomus* from the release point? If the distance is small and parasitoids cover only small patches when foraging, several closely adjacent points of release will be required to provide a sufficient coverage of the infested area. (ii) Which development stage of *A. atomus* (adults or pupae) should be released, and which vertical strata of the plants (and does this vary with host plants)? (iii) How do parasitoids respond to low host densities and/or a dispersed distribution of hosts? Answers to these questions should provide valid information on release frequency and optimum release density of *A. atomus*.

The distribution of leafhoppers within the plant can also affect the host location and parasitism of *A. atomus*, therefore affecting the efficiency of the parasitoids. In this study 59% of *E. decipiens* eggs were laid in the stems of broad beans although eggs were also found in the petioles and in the leaf limbs. Following the oviposition pattern of *E. decipiens*, *A. atomus* preferably parasitized eggs in the stems. Data from other mymarids suggest that the oviposition site of the host is an important determinant of whether an egg will be parasitized (Claridge and Reynolds, 1972; Ali, 1979). This pattern of oviposition behaviour of *A. atomus* and *E. decipiens* should be further studied in other host plants of the green leafhopper, since it might significantly affect the performance of the parasitoids after releases in greenhouses. Raatikainen (1967) found that *Javesella pellucida* (Fab.) (Homoptera: Delphacidae) eggs in cereal stems were invulnerable to attack by *A. atomus*, whereas eggs in leaf blades incurred high levels of parasitism. In oats, thin-walled stems had a higher incidence of parasitism than thick-walled stems, suggesting an inability of the wasp to detect or gain access to more deeply concealed hosts. However, Cronin (1991) found that the host plant provides no physical barrier to *A. delicatus* searching for *Prokelisia* spp. eggs concealed within the leaf. Because a refuge can exist in different forms (Udayagiri and

Welter, 2000) and can have a large effect on the host-parasitoid relationship, more emphasis should be placed on experimental design when testing for mechanisms of escape from parasitism.

Anagrus atomus successfully attacked and developed in 1-10 day-old eggs of *E. decipiens* but parasitism decreased in eggs older than six days. The decreasing suitability of older eggs is common in egg parasitoids and is probably due to the physiology of the well-developed older host embryos, which are more difficult to digest than younger ones (Cronin and Strong, 1990a) or to the hardening of the chorion of older eggs (Leibee et al., 1979).

The results of this study indicate that *A. atomus* females have the ability to detect the presence of their hosts and spent the majority of time on plants that were previously exposed to leafhoppers compared to 'clean' plants (i.e., without previous exposure). The behaviour in the mymarid may be result from chemical cues left by the host on the plants during feeding or ovipositing. Such cues could originate from the host itself, e.g., honeydew and/or accessory gland secretions. Leafhopper honeydew is known to contain contact kairomones, which stimulates the searching response of egg parasitoids (Lou and Cheng, 2001). On the other hand cues could result from the feeding and/or egg laying behaviour of the leafhoppers (i.e., feeding bumps or oviposition wounds (Cronin and Strong, 1990; Moratorio, 1990; van Baaren et al., 1995; Conti et al., 1996). The results of this study cannot give a final answer to these questions, but it can be hypothesized that *E. decipiens* contamination of the leaf surface with contact or volatile signal substances emanating from their bodies or honeydew may play a key role in host searching behaviour of *A. atomus*. The ability of *A. atomus* to respond to contact kairomones of its host *E. decipiens* would indicate a well developed host finding behaviour in the mymarid, which is a prerequisite for a successful use of an egg parasitoid in biological control. The recognition of *E. decipiens* infested

plants by *A. atomus* could increase time spent in the patch searching for hosts leading probably to high parasitism rate, therefore reducing the pest population.

5 REFERENCES

- Agboka, K., 2002. Life table study and searching behaviour of *Anagrus atomus* L. (Hymenoptera: Mymaridae), an egg parasitoid of the green leafhopper *Empoasca decipiens* Paoli (Homoptera: Cicadellidae). M.Sc. thesis, University of Hannover, Germany, 65 pp.
- Akkaya, A., Uygun, N. and K. Abak, 1999. Faunistic studies on harmful and beneficial insects on cucurbit vegetables in the Southeastern Anatolian region of Turkey. Proceedings of the First International Symposium on Cucurbits. Acta-Horticulturae, 492: 335-240.
- Al-Wahaibi, A.K. and G.P. Walker, 2000a. Searching and oviposition behaviour of a mymarid egg parasitoid, *Anagrus nigriventris*, on five host plant species of its leafhopper host, *Circulifer tenellus*. Entomologia Experimentalis et Applicata 96: 9-25.
- Al-Wahaibi, A.K. and G.P. Walker, 2000b. Oviposition behaviour of *Anagrus nigriventris*, an egg parasitoid of beet leafhopper, *Circulifer tenellus*. BioControl 45: 139-153.
- Anonym, 1998a. Pflanzenschutzmitteilungen Obst-, Reb- und Gemüsebau der Eidgenössischen Forschungsanstalt für Obst-, Wein- und Gartenbau 17/1998. FAW, Wädenswil, Switzerland.
- Anonym, 1998b. Pflanzenschutzmitteilungen Obst-, Reb- und Gemüsebau der Eidgenössischen Forschungsanstalt für Obst-, Wein- und Gartenbau 18/1998. FAW, Wädenswil, Switzerland.
- Beyer, A. H., 1922. Experiments on the biology and tripburn disease of the bean leafhopper, with methods of control, *Empoasca mali* Lebaron. Journal of Economic Entomology 15: 298-302.
- Bjorksten T.A. and A.A. Hoffmann, 1995. Effects of pre-adult experience on host acceptance in choice and non-choice tests in two strains of *Trichogramma*. Entomologia Experimentalis et Applicata 76: 49-58.
- Bjorksten, T.A. and A.A. Hoffmann, 1998. Plant cues influence searching behaviour and parasitism in the egg parasitoid *Trichogramma nr. brassicae*. Ecological Entomology 23: 355-362.

- Bosco-D, A. Arzone, 1991. Studies on the oophagous parasitoids of *Lindbergina aurovittata* (Douglas) and *L. spoliata* (Horvath) (Homoptera Auchenorrhyncha). Redia 1: 147-162.
- Bünger V. I., Liebig, H.-P. and C. P. W. Zebitz, 2002. Die Biologische Kontrolle der Baumwollzikade *Empoasca decipiens* Paoli (Homoptera: Cicadellidae) in Gewächshausgurken. Gesunde Pflanzen, 3-4: 105-110.
- Campbell, A., B.D. Frazer, N. Gilbert, A.P. Gutierrez and M. Mackauer, 1974. Temperature requirements of some aphids and their parasites. Journal of Applied Ecology 11: 431-438.
- Carter, W. C., 1939. Injuries to plants caused by insect toxins. Botanic Review 5: 273-327.
- Cerutti, F., J. Baumgärtner, and V. Delucchi, 1990. Research on the grape vine ecosystem in Tessin: III. Biology and mortality factors affecting *Empoasca vitis* Goethe (Homoptera, Cicadellidae, Typhlocybinae). Mitteilungen-der Schweizerischen-Entomologischen-Gesellschaft 63:43-54.
- Cerutti, F., J. Baumgärtner. and V. Delucchi, 1991. The dynamics of grape leafhopper *Empoasca vitis* Göthe populations in southern Switzerland and the implications for habitat management. Biocontrol Science and Technology 1: 177-194.
- Claridge, M.F. and W.J. Reynolds, 1972. Host plant specificity, oviposition behaviour, and egg parasitism in some woodland leafhoppers of the genus *Oncopsis* (Hemiptera: Homoptera: Cicadellidae). Transactions of the Royal Entomological Society of London 124: 149-166.
- Conti, E., W.A Jones, F. Bin and S.B. Vinson, 1996. Physical and chemical factors involved in the host recognition behaviour of *Anaphes iole* Girault, an egg parasitoid of *Lygus hesperus* Knight (Hymenoptera: Mymaridae; Heteroptera: Miridae). Biological Control 7: 10-16.
- Conti, E., W. A. Jones, F. Bin and S.B. Vinson, 1997. Oviposition behaviour of *Anaphes iole*, an egg parasitoid of *Lygus hesperus* Knight (Hymenoptera: Mymaridae; Heteroptera: Miridae). Annals of the Entomological Society of America 90: 91-101.

- Cook, R. M. and S. F. Hubbard, 1977. Adaptive searching strategies in insect parasites. *Journal of Animal Ecology*, 46: 115-125.
- Cooper, S., 1993. The biology and application of *Anagrus atomus* (L.) Haliday. *Bulletin OILB-SROP* 18(8): 42-43.
- Cronin, J.T. and D.R. Strong, 1990a. Biology of *Anagrus delicatus* (Hymenoptera: Mymaridae), an egg parasitoid of *Prokelisia marginata* (Homoptera: Delphacidae). *Annals of the Entomological Society of America* 83: 846-854.
- Cronin, J.T. and D.R. Strong, 1993. Parasitoid interactions and their contribution to the stabilization of Auchenorrhynca populations. In "Planthoppers: their ecology and management" R. F. Denne and T.C. Perfect (eds.). Chapman and Hall, New York. pp. 799
- Cronin, T.T. and D.R. Strong, 1990b. Density-independent parasitism among host patches by *Anagrus delicatus* (Hymenoptera: Mymaridae): experimental manipulation of hosts. *Journal of Animal Ecology* 59: 1019-1026.
- Cuperus, G.W., E.B. Radcliffe, D.K. Barnes and G.C. Marten, 1983. Economic injury levels and economic thresholds for potato leafhopper (Homoptera: Cicadellidae) on alfalfa in Minnesota. *Journal Economic Entomology* 76: 1341-1349.
- Dahlman, D.L. and E.T. Hibbs, 1967. Response of *Empoasca fabae* to tomatine, solanine, leptine I; matidine solanidine and demissidine. *Annals of Entomological Society of America* 60: 732-740.
- DeLong, D. M., 1938. Biological studies of leafhopper, *Empoasca fabae* as a bean pest. United States Department of Agriculture Technical Bulletin 618: 1-60
- DeLong, D. M., 1971. The bionomics of leafhoppers. *Annual Review of Entomology* 16: 179-210.

- Demichelis, S. and A. Manino, 1995. Electrophoretic detection of dryinid parasitoids in *Empoasca* leafhoppers. *Journal of applied Entomology* 119: 8, 543-545.
- Dixon, A.F.G., 1987. Parthenogenetic reproduction and the rate of increase in aphids, pp. 269-285. In Minks, A. K. And Harrewijn, P. (eds.), *aphids their biology, natural enemies and control*. Elsevier, Amsterdam.
- Elden, T. C. and M. McCaslin, 1997. Potato leafhopper (Homoptera: Cicadellidae) resistance in perennial glandular-haired alfalfa clones. *Journal of Economic Entomology* 90:842-847.
- El-Dessouki, S.A. and M.M. Hosny, 1969. Host plants, symptoms of infestation and certain characteristics of *Empoasca* ssp. (Jassidae) on cotton plants in Cairo Area, U.A.R. *Zeitschrift für Angewandte Entomologie* 63: 272-281.
- El-Nawawy, A.S., I. Abd-el-Rahman, M.A. Ashry, A. Hosny and A. Belal, 1983. Effect of mixtures of a foliar fertilizer and each of several insecticides on sucking pests and their predators in cotton fields. *Mededelingen-van-de-Faculteit-Landbouwwetenschappen,-Rijksuniversiteit-Gent* 48: 1, 117-127
- Fenton, F.A. and A. Hartzell, 1920. The life history of the potato leafhopper (*Empoasca mali* Le Baron). *Journal of Economic Entomology* 13: 400-408.
- Fenton, F.A. and A. Hartzell, 1923. Bionomics and control of the potato leafhopper, *Empoasca mali* Lebaron. *Iowa Agricultural Experimental Station Research Bulletin* 78:377-440.
- Flock, R.A., R.L. Doult, R.C. Dickson and E.F. Laird, 1962. A survey of beet leafhopper egg parasites in the Imperial Valley, California. *Journal of Economic Entomology* 55: 277-281.
- Fricke, A., U. Mandau, A. Schubach and R. Uhte, 1999. Datensammlung für die Betriebsplanung im Intensivgemüsebau, vol 7: pp 61. Institut für Gemüse-und Obstbau der Universität Hannover (Eds.).

- Godfray, H.C.J., 1994. Parasitoids. Behavioural and evolutionary Ecology. Princeton University Press, Princeton, NJ 473 pp.
- Gonzales, A. L. and J. A. Wyman, 1991. Effect of varying potato to leafhopper (Homoptera: Cicadellidae) population densities on snap bean yield. *Journal of Economic Entomology* 84: 644-649.
- Graham, P. H., 1978. Some problems and potentials of field beans (*Phaseolus vulgaris* L.) in Latin America. *Field Crop Research* 1: 295-317.
- Granovsky, A.A., 1930. Differentiation of symptoms and effect of leafhopper feeding on histology of alfalfa leaves. *Phytopathology* 20: 121.
- Günthart, H., 1971. Beitrag zur Kenntnis der Kleinzikaden (Typhlocybinæ, Hom., Auch.) der Schweiz. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 43: 218-224.
- Habib, A., A. Badawi and F. Herakly, 1972. Biology study on certain species of leafhoppers, (Hemiptera-Cicadellidae) in Egypt. *Zeitschrift für Angewandte Entomologie* 71: 171-178.
- Haliday, A.H., 1883. An essay of the classification of the parasitic hymenoptera of Britain, Which correspond with the *Ichneumones minuti* of Linnaeus, *Entomological Magazine*, London, 1: 333-3550.
- Harries, F.H. and J.R. Douglass, 1948: Bionomic studies on the beet leafhopper, *Ecological Monograph*, 18: 45-79.
- Hassell, M.P. and R. M. May, 1974. Aggregation in predators and insect parasitoids and its effect on stability. *Journal of Animal Ecology* 43: 567-594.
- Hassell, M.P., 1985. Insect natural enemies as regulating factors. *Journal of Animal Ecology* 54: 323-334.
- Helyer, N.L. and A. Talbaghi, 1994. Evaluation of Buprofezin against Green Leafhopper (*Empoasca decipiens*). *Tests of Agrochemicals and Cultivars* 15: 8-9.

- Hokyo, N., K. Kiritani, F. Nakasaji and M. Shiga, 1966. Comparative biology of the two scelionid parasites of *Nezara viridula* (L.) (Hemiptera: Pentatomidae). *Applied Entomology and Zoology* 1: 94-102.
- Honda, J.Y. and G.P. Walker, 1996. Olfactory response of *Anagrus nigriventris* (Hym.: Mymaridae): effects of host plant chemical cues mediated by rearing and oviposition experience. *Entomophaga* 41: 3-13.
- Hulting, F.L., D.B. Orr and J.J. Obrycki, 1990. A computer program for calculating and statistical comparison of intrinsic rates of increase and associated life table parameters. *Entomologist* 73: 601-612.
- Jacobson, R.J., Chambers, R.J. and J.C. van Lenteren, 1996. Control of glasshouse leafhopper (*Hauptidia maroccana*: Homoptera, Cicadellidae) within an IPM programme in protected tomatoes. *Bulletin OILB-SROP* 19: 67-70.
- Johnson, H.W., 1934. Nature of injury to forage legumes by potato leafhopper. *Journal of Agricultural Research* 49: 379-406.
- Kersting, U., Baspinar, H., Uygun, N. and S. Satar, 1997. Comparison of two sampling methods for leafhoppers (Homoptera, Cicadellidae) associated with sesame in the east Mediterranean region of Turkey. *Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz* 70: 131-135.
- Koblet-Günthardt, M., 1975. Die Kleinzikaden *Empoasca decipiens* Paoli und *Euptrix atropunctata* Goetze (Homoptera: Auchenorrhyncha) auf Ackerbohnen (*Vicia faba* L.). Anatomische und physiologische Untersuchungen. PhD. Thesis, University of Zurich, Switzerland.
- Kornegay, J. L and C. Cardona, 1990. Development of an appropriate breeding scheme for tolerance to *Empoasca krameri* in common bean. *Euphytica* 47:223-231

- Kühne, S., 1998. Open rearing of generalist predators: A strategy for improvement of biological pest control in greenhouses. *Phytoparasitica* 26: 277-281.
- Lamp, W.O., G.R. Nielsen and G.P. Dively, 1991. Insect pest induced losses in alfalfa: Patterns in Maryland and implications for management. *Journal of Economic Entomology* 84: 610-618.
- Leibee; G.L., B.C. Pass and K.V. Yeargan, 1979. Developmental rate of *Patsson lameerei* (Hymenoptera: Mymaridae) and the effect of host egg age on parasitism. *Entomophaga* 24: 345-348.
- Lessells, C. M., 1985. Parasitoid foraging: should parasitism be density-dependent? *Journal of Animal Ecology* 54: 27-41.
- Liljesthröm, G. G. and E. Virla, 2001. Spatial density dependent egg parasitism of *Delphacodes kuscheli* (Homoptera: Delphacidae) by *Anagrus flaveolus* (Hymenoptera: Mymaridae) in Tuncumán province, Argentina. *Egg parasitoid News* 13: 10.
- Loginova, E., 1992. Some new pests of glasshouse crops in Bulgaria and their control by an IPM program. *Bulletin OEPP/EPPO* 22: 357-361.
- Lou, Y. and J. Cheng, 2001. Host recognition kairomone from *Sogatella furcifera* for the parasitoids *Anagrus nilaparvatae*. *Entomologia Experimentalis et Applicata* 101: 59-67.
- Lovinger, A., D. Liewehr and W.O. Lamp, 2000. Glandular Trichomes on Alfalfa Impede Searching Behaviour of the Potato Leafhopper Parasitoid. *Biological Control* 18: 187-192.
- Maisonneuve, J.C., J. Blum and L.R. Wardlaw, 1995. Contre la cicadelle de la tomate en serre. Un nouvel auxiliaire: *Anagrus atomus*. *Phytoma* 471: 24-27.
- Maixner, M., Reinert, W. and A. Weber, 1998. Insect parasitoids and mite parasites of leafhoppers and planthoppers (Auchenorrhyncha) in vineyards. *Bulletin OILB-SROP* 21: 75-

- Mattiacci, L., S.B. Vinson, H.J. Williams, J.R. Aldrich and F. Bin, 1993. A long-range attractant kairomone for egg parasitoids *Trissolcus basalis* (Wol.), isolated from defensive secretion of its host, *Nezara viridula*. *Journal of Chemical Ecology* 19: 1067-1181.
- Medler, J. T. 1941. The nature of injury to alfalfa, caused by *Empoasca fabae* Harris. *Annals of Entomological Society of America* 34:439-450.
- Messenger, P.S., 1964. The influence of rhythmically fluctuating temperatures on the development and reproduction of the spotted alfalfa aphid, *Therioaphis maculata*. *Journal of Economic Entomology* 57: 71-76.
- Meyer, J.S., C.G. Ingersoll, L.L. McDonald and M.S. Boyce, 1986. Estimating uncertainty in population growth rate: jackknife vs. bootstrap techniques. *Ecology* 67: 1156-1166.
- Meyerdirk, D.E. and M.S. Moratorio, 1987. Seasonal population density of *Anagrus giraulti* (Hymenoptera: Mymaridae), an egg parasitoid of *Circulifer tenellus* and *Empoasca* spp. (Homoptera: Cicadellidae). *Journal of Economic Entomology* 80: 362-365.
- Meyerdirk, D.E. and N.A. Hessein, 1985. Population dynamics of the beet leafhopper, *Circulifer tenellus* (Baker), and associated *Empoasca* spp. (Homoptera: Cicadellidae) and their egg parasitoids on sugar beets in southern California. *Journal of Economic Entomology* 78: 346-353.
- Missouri Botanical Garden: Integrated Pest Management (IPM) Information, 1999. Deciduous Trees and Shrubs, Insects. <http://www.mobot.org/MOBOT/hort/ipm/leafhop.html>. Accessed: November 2000
- Moratorio, M.S., 1990. Host finding and oviposition behaviour of *Anagrus mutans* and *A. silwoodensis* (Hymenoptera: Mymaridae). *Environmental Entomology* 19: 142-147.

- Müller, H.J., 1956. Homoptera. Auchenorrhyncha. Zikaden, pp. 150-306. *In*: Sorauers Handbuch der Pflanzenkrankheiten. Tierische Schädlinge an Nutzpflanzen. 5. Band, 2. Teil, 3. Lieferung. H. Blunck (Hrsg.). Paul Parey, Berlin und Hamburg, 5. Auflage:1956.
- Murphy, B. C., J.A. Rosenheim, R.V. Dowell and J. Granett, 1998. Habitat diversification tactic for improving biological control: parasitism of the western grape leafhopper. *Entomologia Experimentalis et Applicata* 87: 225-235.
- Nielson, M. W. And S. L. Toles, 1968. Observations on the biology of *Acinopterus angulatus* and *Aceratagallia curvata* in Arizona. *Annals of Entomological Society of America*, 61:54-56.
- Noldus, L.P.J.J. and J.C. van Lenteren, 1985. Kairomone for the egg parasite *Trichogramma evanescens* Westwood. II. Effect of contact chemicals produced by two of its hosts, *Pieris brassicae* L. and *Pieris rapae* L. *Journal of Chemical Ecology* 11: 793-800.
- North American alfalfa Improvement Conference (NAAIC), 1998. Standard tests to characterized pest resistance in alfalfa cultivars (<http://www.naaic.org/stdsts/potatolh.htm> on August 20, 2001).
- Ossiannilsson, F., 1981. The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavia* 7(2).
- Paoli, G., 1930. Caratteri diagnostici dell *Empoasca* e descrizione di nuove specie (Diagnostic characteristic of *Empoasca* and description of new species). *Toscanna Società di Scienze Naturali Attività* 39: 64-75.
- Price, P.W., C.E. Bonton, P. Gross, B.A. McPheron J.N. Thompson and A.E. Weiss, 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11: 41-65.
- Raatikainen, M., 1967. Bionomics, enemies and population dynamics of *Javesella pellucida* (F.) Homoptera: Delphacidae. – *Annales Agriculturae Fenniae* 6: 1-147

- Raupach, K., 1999. Untersuchungen zur Biologie und zum Schadpotential der Zwergzikade *Empoasca decipiens* Paoli (Homoptera, Auchenorrhyncha: Cicadellidae (Jassidae)) einem neuen Schädling an Gemüse unter Glas. Diplomarbeit, Institut für Pflanzenkrankheiten, Universität Hannover.
- Raupach, K., Borgemeister, C., Hommes, M., Poehling, H.M. and M., Sétamou, 2002. Effect of temperature and host plants on the bionomics of *Empoasca decipiens* Paoli (Homoptera, Auchenorrhyncha: Cicadellidae) Crop Protection 21 (2), 113-120.
- Rice, M.E., 1996. Leafhopper on the increase. Publication IC-476. Integrated Crop Management, Iowa State University Extension Service, Ames.
- Roda, A.L., D.A. Landis and M.L. Coggins, 1997. Forage grasses elicit emigration of adult potato leafhopper (Homoptera: Cicadellidae) from alfalfa-grass mixtures. Environmental Entomology 26: 745-153.
- Rupp, J., 1999. Zikadenbekämpfung an Gurken auf der Insel Reichenau 1998. Gemüse 3/99: 172-173.
- SAS Institute, 1992. SAS user's guide: statistics, version 6th edition. SAS Institute, Cary, NC.
- Schmidt, U. and J. Rupp, 1997. Zikadenschäden an Gurke auf der Insel Reichenau. Gemüse 12/97: 691-692.
- Schmidt, U. and J. Rupp, 1997. Zikadenschäden an Gurke auf der Insel Reichenau. Gemüse 12/97: 691-692.
- Schruff, G., 1987. Die Rebenzikade - Auftreten, Bedeutung und Bekämpfung. Obstbau Weinbau 24: 42-44.
- Smith, F. F. And F.W. Poos, 1931. The feeding habit of some leafhoppers of the genus *Empoasca*. Journal of Agricultural Research 53: 267-285.

- Smith, S.M., 1996. Biological control with *Trichogramma*: advances, successes and potential of their use. *Annual Review of Entomology* 41: 375-406.
- Soika, G. and G. Labanowski, 1996. Species composition of leafhoppers (Cicadellidae) on annual ornamental plants cultivated for seed, *Zeszyty Naukowe Instytutu Sadownictwa-I-Kwiaciarnictwa-w-Skiemiewicach* 3: 153-166.
- Sokal, R.R. and J.F. Rohlf, 1995. *Biometry. The principles and practice of statistics in biological research*, 3rd ed. Freeman, New York.
- Triapitsyn, S.V. 1998. *Anagrus* (Hymenoptera: Mymaridae) egg parasitoids of *Erythroneura* spp. and other leafhoppers (Homoptera: Cicadellidae) in North American vineyards and orchards: A taxonomic review. *Trans. American Entomological Society* 124, 77-112.
- Triapitsyn, S.V. and M.S. Moratorio, 1998. Host associations of *Anagrus nigriventris* Girault (Hymenoptera: Mymaridae) and techniques for its rearing under insectary conditions. *Mitteilungen der BBA* 356: 185-191.
- Tumilson, J.H., T.C. Turlings and W.J. Lewis, 1993. Semiochemically mediated foraging behaviour in beneficial parasitic insects. *Archives of Insect Biochemistry and Physiology* 22: 385-391.
- Udayagiri, S. and S.C. Welter, 2000. Escape of *Lygus hesperus* (Heteroptera: Miridae) eggs from parasitism by *Anaphes iole* (Hymenoptera: Mymaridae) in strawberries: Plant structure effects. *Biological Control* 17: 234-242.
- Van Baaren, J., J.P. Nenon and G. Bovin, 1995. Comparison of oviposition behaviour of solitary and gregarious parasitoids (Hymenoptera: Mymaridae). *Journal of Insect Behaviour* 8: 671-686.
- Van Driesche, R.G., 1983. Meaning of "percent parasitism" in studies of insect parasitoids. *Environmental Entomology* 12: 1611-1622.

- van Schoonhoven, A., G.J. Hallman and S.R. Temple, 1985. Breeding for resistance to *Empoasca kraemeri* Ross and Moore in *Phaseolus vulgaris* L., pp. 405-422. In: L.R. Nault and J.G. Rodriguez (eds.): The leafhoppers and planthoppers. John Wiley and Sons Inc., New York.
- Vidano, C. and A. Arzone, 1988. Natural enemies of *Zyginidia pullula* (Rhynchota: Auchenorrhyncha. 6th Auchenorrhyncha Meeting. Turin, Italy, September 6-11, 1987. Proceedings [edited by Vidano, C.; Arzone, A.]. 1988, 581-590.
- Vidano, C., A. Arzone and A. Alma, 1987a. Investigations on Auchenorrhyncha accused or suspected to be noxious to vine in Italy, pp. 87-95. In: R. Cavalloro (ed.): Integrated pest control in viticulture: Proceedings of a meeting of the EC Experts' Group, Portoferraio, 26-28 Sept. 1985. A.A. Balkema, Rotterdam.
- Vidano, C., A. Arzone and C. Arnó, 1987b. Researches on natural enemies of viticolous Auchenorrhyncha, pp. 97-101. In: R. Cavalloro (ed.): Integrated pest control in viticulture: Proceedings of a meeting of the EC Experts' Group, Portoferraio, 26-28 Sept. 1985. A.A. Balkema, Rotterdam.
- Vietmeier, A., Hommes, M. and H.-P. Plate, 1996. Einige wichtige Vertreter der räuberischen Blumenwanzen (Heteroptera: Anthocoridae) und ihre Eignung zur biologischen Schädlingsbekämpfung. Mitteilungen der BBA, Heft 325.
- Vinson, S.B., 1981. Habitat location. In: Nordlund, D.A., Jones, R.L., and Lewis, W.J. (eds.), Semiochemicals, Their Role in Pest Control, John Wiley and Sons, New York, pp. 51-68.
- Vinson, S.B., G.W. Elzen and H.J. Williams, 1987. The influence of volatile plant allelochemicals on the third trophic level (parasitoids) and their herbivorous host. In: Insects-plants (V. Labeyrie, G. Fabres, T.D. Lachaise, eds.) Junk publ. Dordrecht, 540 pp.
- Wardlow, L.R. and A. Tobin, 1990. Potential new additions to the armoury of natural enemies for protected tomatoes. Bulletin SROP/WPRS 13(5): 225-227.

- Whitefield, G. H. and C. R. Ellis, 1976. The pest status of foliar insects on soybeans and white beans in Ontario. *Proceedings of Entomological Society of Ontario* 107: 47-55.
- Williams, D.W., 1984. Ecology of the blackberry-leafhopper-parasite system and its relevance to California grape agroecosystems. *Hilgardia* 52: 1-33.
- Williams, L. and T.E. Martinson, 2000. Colonization of New York vineyards by *Anagrus* spp. (Hymenoptera: Mymaridae: Overwintering biology, within-vineyard distribution of the wasp, and parasitism of Grape leafhopper, *Erythroneura* spp. (Homoptera: Cicadellidae), eggs. *Biological control* 18, 136-146.
- Yigit-A, and L. Erkilic, 1992. Studies on bio-ecology and control of grape leafhopper (*Arboridia* (= *Erythroneura*) *adanae* Dlab.) (Homoptera: Cicadellidae) in southern Anatolia region. *Zirai-Mucadele-Arastirma-Yilligi*. 22-23: 25-28.
- Zimmerman, E. C., 1948. *The Insects of Hawaii* Vol. 4, Homoptera: Auchenorrhynca. University of Hawaii Press, Honolulu. 268pp.