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THE INFLUENCE OF DIFFERENT HOST PLANTS ON THE GREEN LEAFHOPPER *EMPOASCA DECIPIENS* PAOLI (HOMOPTERA: CICADELLIDAE) AND ITS PARASITOID *ANAGRUS ATOMUS* (L.) HALLIDAY (HYMENOPTERA: MYMARIDAE)

By

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Declaration

I, Rasmieh Al-Moaalem, hereby declare that the work presented in this thesis is my own work, which has not been submitted for a degree in any other university.

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I am solely responsible for the views expressed in this thesis and hope that this assembled information will provide a useful tool for students and scientists working on leafhoppers and their parasitoid.

Abstract

The green leafhopper *Empoasca decipiens* Paoli (Homoptera: Cicadellidae) has recently become a serious 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 promising biological control agent of *E. decipiens*. The objectives of this research were to assess the influence of host plants on the leafhopper and its parasitoid. Therefore, host plant acceptability and suitability for *E. decipiens* and effects of temperature on its life table parameters were studied. Additionally, the influence of several important vegetable host plants of the leafhopper on the development time and the rate of parasitism of *A. atomus* were investigated. Moreover, host discrimination behaviour of the parasitoid was examined.

The host plant acceptability of *E. decipiens* was studied in choice experiments using cucumber (*Cucumis sativus* L.), sweet pepper (*Capsicum annuum* L.), and tomato (*Solanum lycopersicum* L.). *Empoasca decipiens* significantly preferred cucumber for feeding and oviposition. The suitability of host plants for *E. decipiens* was determined based on the development time and the fitness of offspring, measured as weight and size of insects reared on each of the tested host plants. Development time of *E. decipiens* reared on broad beans was significantly shorter than on the other tested host plants. Moreover, the weight and size of the progeny was significantly higher on broad beans than on the other tested plants. The egg-adult development time, survivorship, and reproduction of *E. decipiens* were evaluated at three constant temperatures (16, 20 and 24 C), using broad beans as host plant. Development time for each larval instar was determined at 20 and 24 C, and at both temperatures tested the fifth larval instar had the longest duration. Fecundity and egg viability of *E.*

decipiens were studied at 16 and 20 C on broad beans. No significant differences in fecundity and egg viability between the two temperatures were recorded, and egg mortality was 7 and 9% at 16 and 20 C, respectively. Temperature significantly affected adult longevity, pre- and post-oviposition time but had no significant effect on fertility, oviposition period, larval mortality and sex ratio. The intrinsic rate of increase (r_m) and net reproduction rate (R_0) varied significantly among the three temperatures tested, and was highest at 20 C. Generation time was lower at 24 than that at 16 and 20 C, but doubling time was lower at 20 C than at the other two tested temperature regimes.

We additionally studied the interactions between *A. atomus* and host plants of *E. decipiens*, using broad beans, cucumber, and sweet pepper as model plants. The host plants did not significantly affect the egg-adult development, fertility, and rate of parasitism and sex ratio of A. anagrus. Development time was 16.17 ± 0.13, 16.05 ± 0.1 days on cucumber and sweet pepper, respectively. Fertility was relatively low, with a mean number of progeny of 14.57 ± 2.55 and 15.21 ± 2.53 per female on cucumber and sweet pepper, respectively. Pre-adult parasitoid mortality was higher on cucumber, but did not differ significantly from that on sweet pepper. Rearing of parasitoids on leafhoppers feeding on broad beans or cucumber did not significantly affect the rate of parasitism of *A. atomus* when subsequently released on leafhopper infested broad beans plants. However, pre-adult parasitoid mortality was significantly higher when parasitoid females used had been reared on leafhoppers on broad beans than on cucumber. Female *A. atomus* did not avoid neither self- nor conspecific superparasitism when released 1 or 24h after their first oviposition. In most cases superparasitism resulted in the death of the parasitoid eggs.

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1 General introduction

1.1 *Empoasca decipiens* and related species

The green leafhopper *Empoasca decipiens* Paoli (Hooptera: Cicadellidae) is a small cicada, which was first described by Paoli 1930 in Italy. The adult is scarcely 3-4 mm long (3 mm, Schmidt and Rupp 1997; 2-4 mm, Ossiannilsson, 1981). The male is smaller than the female (2.85 mm long), Koblet-Günthardt, 1975). The nymphs and adults are light green, sometimes the adults are of darker green colour. For identification of *Empoasca* spp., preparation of the male genitalia is necessary (De Long, 1971). Lately, Loukas and Drosopoulos (1992) developed an electrophoretic method for identification of *E. decipiens*.

Several species of the genus *Empoasca* are important pests of cultivated plants. The potato leafhopper *E. fabae* Harris is the most common insect pest found on field beans in Ontario, Canada (Whitfield and Ellis, 1976). It is also a key pest of alfalfa in the northeastern and northern central United States and southern provinces of Canada (Lamp, 1990). The polyphagous leafhopper *E. vitis* Goethe is a common pest in vineyards in central Europe (Schruft, 1987; Vidano *et al.*, 1987a).

1.2 Distribution and host plants

Empoasca decipiens is widespread in Europe, North Africa, and Central Asia (Ossiannilsson, 1981). Müller (1956) described *E. decipiens* as an economically non-important species in Central Europe; however, recently it has gained pest status in vegetables under protected cultivation in Germany (Schmidt and Rupp, 1997), the Netherlands and Great Britain (Helyer and Talbaghi, 1994) and in Bulgaria (Loginova, 1992). In Egypt, *E. decipiens* attacks cotton, beans, and potatoes (Habib *et al.*, 1972). In Turkey it has been reported on sesame (Kersting *et al.*, 1997), and on squash and cucurbits (Akkaya and Uygum, 2001). In Syria it is recorded on grapevine, cotton, cucurbits and sugar beet, though little is known on the economic importance of the pest (Silti & Ibrahim, 1991). Recently, population outbreaks have been noticed in Syria on many vegetables crops such as broad beans, cowpea, cucurbits, and also on some fruit trees like peach and apple.

1.3 Damage and feeding habits

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

Empoasca decipiens feeds on the vascular tissue, according to Schmidt and Rupp (1997) mainly on the phloem, whereas Koblet-Günthardt (1975) described it as parenchyma and phloem feeder.

Leafhoppers cause a specific damage symptom known as hopperburn in which leaf margins turn yellow, particularly at the leaf tip; these areas soon become necrotic, and the entire leaf may become yellow. Medler (1941) described plant injury by *E. fabae* as a combination of the feeding in vascular tissue and the action of saliva injected during the feeding process. This salivary secretion causes hypertrophy of the affected phloem cells, which in turn, causes an interruption of the translocation of photosynthetic materials from the leaves to a degree that causes plasmolysis of paranchymal cells and finally the resulting hopperburn. Symptoms of a leafhopper infestation sometimes appear very similar to ones caused by diseases or nutrient imbalances. Yet, according to Schmidt and Rupp (1997) no evidence of involvement of phytoplasma, pathogens commonly transmitted by leafhoppers, was found in plants infested by *E. decipiens*

1.4 Biology

Empoasca decipiens reproduces gonorchistic; one copulation is enough to fertilize all the eggs (DeLong, 1971). Adult females start to lay eggs after a pre-oviposition period, which may vary with climatic conditions from one to several days (Habib *et al.*, 1972). In several *Empoasca* spp., pre-oviposition period lasted from 1.5 to 10 days (Delong, 1938; Harries & Douglass, 1948; Nielson & Toles, 1968), but in hibernating species this may be several months. Eggs are inserted in the plant tissue mostly in the main vein of the leaf, the petiole and stem. The female produces up to 100 eggs during its lifetime, and lays them individually sub-epidermal on the underside of the leaves (Müller, 1956). Eggs are cylindrical, slightly curved, and of light green colour (Habib *et al.*, 1972). The development time of eggs varies according to temperature; Raupach (1999) reported that the development time of eggs was 28.3, 8.7, and 8.4 days at temperatures of 15, 28, 32.5°C respectively (Table 1.1).

Table1.1: Duration of the different developmental stages and the longevity of adult *Empoasca decipiens* (in days) at different temperatures (Raupach, 1999; Habib *et al.*, 1972).

Temperature (°C)	**Egg stage**	**Total larvae 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
32.5	8.40	10.49	*
35	8.19	10.84	*

* Values not determined. Data at 22, 27, and 29°C are from Habib *et al.* (1972), the other temperature values from Raupach (1999).

Empoasca decipiens passes through five nymphal instars; the development of nymphs takes 36.9, 11.7, 10.5 days at 15, 28, 32.5°C, respectively (Raupach, 1999) (Table 1). Nymphal development time varies also according to host plant species; at 29°C the development lasts 8.6 and 10.7 days on broad beans and tomata, respectively (Habib *et al.*, 1972). Likewise Raupach (1999) reported that at 30°C the development time of nymphs lasts 10.9 and 12.5 days on broad beans and eggplants, respectively. Longevity of adult can vary from one week (Habib *et al.*, 1972), to 148 days (Poos, 1932), depending on the climatic conditions and the host plant.

Empoasca decipiens overwinters as adults (Müller, 1957). According to Kersting *et al.* (1997) the sex ratio, as observed on yellow sticky traps, was 1.1:1 (male: female); the authors explained the higher proportion of males by their higher flight activity. However, the proportion of female *E. decipiens* in cotton in Egypt decreased from 100% in April to 50-22% in September (Hosny & El-Dessouki, 1967).

Studying the host plant preference in herbivores is particularly important when an oligo- and/or polyphagous pest species exhibits distinct differences in its feeding preference between host plants, indicating the potential feasibility of a host plant resistance (HPR) approach. Non-preference, also termed antixenosis, is one of the three types of mechanism of HPR (Painter, 1951, cited in Palaniswamy & Lamb, 1992). Raupach (1999) studied the host plant preference of *E. decipiens* for cucumber, tomato, sweet pepper, and eggplant. However in her experiments females laid no eggs because she had not considered the pre-oviposition period in *E. decipiens*. Yet Raupach (1999) could show that *E. decipiens* prefers to feed, though not significantly, on eggplants compared to the other tested host plants.

Kieckhefer and Medler (1964) hypothesized that information on oviposition preference would help to elucidate seasonal differences in population of the potato leafhopper *E. fabae*, especially during certain outbreak years, and therefore carried out experiments to determine the oviposition preference on broad beans, alfalfa, soybeans, and field peas. The leafhopper preferred broad beans for oviposition, followed by alfalfa, soybeans and field peas in descending order of preference. Moreover, young succulent alfalfa tissue was preferred over older lignified portions.

Jayaraj (1966) showed preference of *E. fabae* for feeding and oviposition on some varieties of castor beans. The preferred susceptible varieties doubled the quantities of some organic acids as the resistant one. The increase of organic acids resulted in increased respiratory activity of these plants, leading to an increase in the quantity of total nitrogen in the susceptible varieties.

Oviposition preference of the goldenrod stem galler *Eurosta solidaginis* (Fitch) (Dipt.: Tephritidae) was strongly influenced by plant genotype, but the presence of two other pests reduced the preference by 25%. The performance of stem galler were also significantly affected by goldenrod genotype, but in general was not affected by the presence of other herbivores early in the season. These results indicate that the preference-performance relationship of a herbivore for different host genotypes can be affected by the presence and/or feeding activity of other herbivores (Cronin *et al.*, 2001). Adati and Matsuda (2000) studied the effect of leaf surface wax on the feeding of the strawberry leaf beetle, *Galerucella vittaticollis* (Joannis) (Col.: Chrysomelidae), with reference to host plant preferences. They found that in some host plants such as *Rumex acetosa* L. (Polygonaceae: Polygonales) and *Fragaria ananassa* Duch. (Rosaceae: Rosales) which were preferred in choice tests, feeding was significantly decreased when the wax was removed.

1.5 Possibilities to control the pest

1.5.1Chemical control

Leafhoppers are generally susceptible to a broad range of pesticides. Thus using compounds like permethrin, carbaryl, and dimethoate can provide good control of e.g. *E. fabae* (University of California, Pest Management Guidelines, 2001). Older studies (i.e.. Zimmerman, 1948) reported good control of leafhoppers by sulphur dust or a pyrethrum-sulphur dust and satisfactory control by pyrethrum-talc dust. Combinations chloropyrifos and profenofos increased the effectiveness against *E. decipiens* (El-Nawawy *et al.*, 1983). However such treatments negatively affect natural enemies like

Scymnus interruptus (Goeze) (Col.: Coccinellidae), *Paederus alfierii* Koch (Col.: Staphylinidae) and *Chrysoperla carnea* (Steph.) (Neu.: Chrysopidae), thereby increasing the likelihood of secondary pest outbreaks. Insecticides applied against aphids are also effective against adults and nymphs of *E. decipiens*, but have only limited effect on the eggs, which are protected in the plant tissue, thus necessitating repeated applications (Anonym, 1998). The insect growth regulator buprofezin is recommended against *E. decipiens*; it has proved to effectively control the pest and cause little to no harmful effects on natural enemies in greenhouses (Helyer & Talbaghi, 1994), indicating the potential use of buprofezin in IPM programme against *E. decipiens*. However, buprofezin has no effect on adult leafhoppers.

1.5.2 Biological control agents

1.5.2.1 Enthomopathogenic fungi

The efficacy of five entomopathogenic fungal strains, including two *Metarhizium anisopliae* (Metsch.) strains, and one strain of *Beauveria bassiana* (Bals.), *Paecilomyces* fumosoroseus (Wize) and *Verticillium lecanii* (Zimm.) were investigated both under laboratory and greenhouse conditions. The result demonstrated that entomopathogenic fungi can be promising tool for control of the green leafhopper (Tounou, 2002).

1.5.2.2 Predators

Several predators like predatory bugs of the genus *Orius* (Hem.: Anthocoridae), dragonflies, predatory flies of the genus *Coenosia* (Dip.: Muscidae) have been reported as natural enemies of leafhoppers. Experiments carried out to test the efficacy of four predators, i.e. *Orius* spp., *C. carnea*, *Episyrphus balteatus* DeGeer (Dip.: Syrphidae), and *Adalia bipunctata* L. (Col.: Coccinellidae), for control *E. decipiens* gave no satisfactory results (Raupach, 1999). In addition, three *Coenosia* spp., predators of adult leafhoppers, had been previously tested as biocontrol agents in greenhouses. However, initial experimental releases under greenhouse conditions did not prevent high infestation levels of *E. decipiens* (Schmidt & Rupp, 1997). In addition, Günthhardt (1971) reported spiders as natural enemies of cicada. For instance *Anytis* spp. (Acari: Anytidae) and *Charletonia* spp. (Acari: Erythaedae) are important natural enemies of both *E. decipiens* and *E. vitis* (Vidano *et al.*, 1987b; Maixner *et al.*, 1998).

1.5.2.3 Parasitoids

Several *Anagrus* spp. (Hym.: Mymaridae) are important egg parasitoids of several *Empoasca* spp. Vidano *et al.* (1987) reported *A. atomus* Haliday as a natural enemy of *E. vitis*. *Anagrus atomus*, first described by Haliday in 1883, is a minute parasitic wasp. The adult is brown and 0.6 mm long, with a wingspan of 0.6 mm (Cooper, 1993). *Anagrus atomus* occurred spontaneously in greenhouses cultivated with cucumbers on the island Reichenau in southern Germany, but this natural occurrence did not suffice to prevent subsequent *E. decipiens* outbreaks (Schmidt & Rupp, 1997). *Anagrus atomus* was detected parasitizing eggs of the grape leafhopper *Arboridia adanae* (Dlabola) (Hom.: Cicadellidae) (Yigit & Erkilic, 1992). The authors suggested that the natural occurrence of *A. atomus* in conjunction with a second mymarid egg parasitoid, *Oligosita pallida* Kryger, was sufficient for controlling grape leafhoppers, particularly when rose leafhoppers *Edwardsiana rosae* (L.) on *Rubus* spp. and *Rose* spp. were available as alternative hosts during the winter.

Anagrus atomus is a polyvoltine species, that can parasitize leafhoppers eggs as long as the host embryo has not developed (Vidano *et al.*, 1987). The genus *Anagrus* is arrehnotek parthenogenetic (Cronin & Strong, 1990); thus unfertilised eggs yield only male offspring. Under favourable climatic conditions *A. atomus* reproduces continuously throughout the year (Cooper, 1993). Whereas the day length does not seem to influence the development of the parasitoid, the temperature is a crucial factor for the development of the wasp. Total development time varies between 16 and 21 days at temperatures between 18 and 24°C (Cooper, 1993).

Little is known on the efficacy of *A. atomus* for control of leafhoppers. Cooper (1993) reported that in 40% of the cases, releases of *A. atomus* in greenhouses in the UK resulted in sufficient control of *Hauptidia marocana* Melichar (Hom.: Cicadellidae), an important pest in the UK (Jacobson *et al.*, 1996) and France (Maisonneuve *et al.*, 1995), on tomatoes. Vidano *et al.* (1987) found that 50% of the eggs of *E. vitis* were parasitized by *A. atomus* in Italian vineyards.

At present *A. atomus* is the most promising biocontrol agent against *E. decipiens*. However, prior to any future implementation in greenhouses more data on the biology, ecology, and behaviour of this parasitoid is needed. Particularly the potential effects of different host plants of *E. decipiens* on the performance of *A. atomus* need to be thoroughly studied.

In a recent paper Al-Wahaibi and Walker (2000) reported how five host plants of the beet leafhopper *Circulifer tenellus* (Baker), i.e. sugar beets and four perennial weeds that harbour overwintering life stages of the leafhopper, affect the searching and oviposition behaviour and parasitization ability of the closely related egg parasitoid *A. nigriventris* Girault. *Circulifer tenellus* is a migratory insect, and is present on sugar beets on California from late spring to late summer. In fall it migrates to adjacent habitats and overwinters on several perennial plants.

In sugar beets *A. nigriventris* parasitizes *C. tenellus* eggs, and the rate of parasitism can reach very high levels. However, augmentative releases of the parasitoid in the overwintering habitat of *C. tenellus* failed to control the leafhoppers prior to their migration to sugar beets. Al-Wahaibi and Walker (2000) could clearly show, that the behaviour of *A. nigriventris* differed among the various host plant of *C. tenellus*, with certain host plants of the leafhopper being preferred and others not preferred by the parasitoid.

Similarly Lovinger *et al.* (2000) studied the influence of glandular trichoms on several alfalfa varieties on *A. nigriventris.* The authors revealed that glandular trichoms and their secretion can impede the movement of the parasitoid, thus negatively affecting the searching time and therefore weakening the efficacy of the parasitoid for control of potato leafhoppers.

1.5.3 Cultural control

The susceptibility of a plant to herbivorous insects may be affected by the associated vegetation. The practice of mixed cropping is believed to minimise crop damage. A study by Saxena and Basit (cited in Hedin, 1983) indicated that cotton can be protected from leafhopper infestations by intercropping with non-host plants such as castor beans or sponge gourd. Oviposition on cotton was reduced in both cases, but the mechanisms appear to be different. Volatiles from the castor bean plants reduced the number of leafhoppers landing on cotton, whereas sponge gourd was attractive to the leafhoppers. The insects oviposited on sponge gourd but emerging nymphs failed to develop on these plants and subsequently died. Time of planting can also influence the degree of injury caused by leafhoppers. According to Hosny and El-Dessouki (1968), a suitable sowing date can reduce the level of leafhopper infestations in cotton.

Crop residue management system has also an impact on leafhoppers. Removing crop residues resulted in a decreased density of potato leafhopper *E. fabae* on soybeans (Lam & Pedigo, 1998).

1.5.4 Host plant resistance

Plant breeding for resistance to leafhoppers relies on selecting plant genotypes, which have dense trichoms on the leaf surface. Trichoms on the leaf surface have basically three types of effects on insect behaviour. The first is simple impedance, the second is the physical trapping of pests by hooked hairs, and the third is stickiness caused by exudates from the glandular trichoms (Panda & Khush, 1995). However glandular trichoms secrete some secondary plant compounds like alkaloids, terpens, vlavonoids. These compounds act as antifeedants to some insects; tomatine for example is an antifeeding compound to the potato leafhopper *E. fabae* (Raman *et al.*, 1979, cited in Panda & Khush, 1995).

The breeding of hairy cotton in Africa and Asia for combating Indian cotton jassid *Amrasca biguttula* Ishida is one of the most prominent success stories in host plant resistance. In India, Husain and Lal (1940) (cited in Panda & Khush, 1995) developed hairy cotton varieties resistant to Indian cotton jassid, with dense trichoms on the leaf that deterred feeding and oviposition of the pest. By 1943, resistant varieties covered extensive areas, where previously Indian cotton jassids had seriously threatened cotton production. Likewise, soybean varieties with dense trichoms on the foliage can reduce oviposition and feeding of leafhoppers through antixenosis; oviposition is prevented mainly by impeding the ovipositor or proboscis from reaching the mesophyll or vascular bundles (Lee, 1983, cited in Panda & Khush, 1995).

In addition, increased tissue hardness and stems with small cross-sectional areas in *Medicago* clones resulted in restricted penetration of stylets and thus feeding of potato leafhoppers, and hence increased the resistance of plants to attack by these pests (Brewer *et al.*, 1986, cited in Panda & Khush, 1995).

1.6 Statement of the research problem

Recent outbreaks of the green leafhopper *E. decipiens* on vegetables and ornamentals in greenhouses caused significant economic losses, e.g. by considerably affecting the marketing value of tomatoes (Schmidt & Rupp, 1997).

Control measures must concert with biological control programmes already successfully in place against other important pests in greenhouses such as aphids, white flies and spider mites. Hence only environmental friendly control approaches like the use of host plant resistance (HPR) and/or augmentative release of natural enemies like *A. atomus* can assure sufficient control of *E. decipiens* without jeopardising the biological control of other important greenhouse pests. Although HPR may act additionally or synergistically with natural enemies, leading to a decrease of pest survival (Bottrell, 1998), specific plant characteristics that confer resistance to herbivores, like trichoms, may reduce the effectiveness of parasitoids by impeding their searching behaviour (Price *et al.*, 1980, cited in Lovinger *et al.*, 2000). Thus, understanding the response of a natural enemy to host plant characteristics is important for a successful integration of HPR and biological control in integrated pest management (IPM) (Lovinger *et al.*, 2000). Therefore the influence of different host plants of *E. decipiens* on the performance of the leafhopper and its parasitoid *A. atomus* need to be thoroughly investigated.

The intrinsic rate of increase is the only statistic parameter that adequately summarizes the physiological qualities of an animal relative to its capacity of increase (Andrewartha & Birch, 1954). Therefore, the intrinsic rate of increase and other life table parameters as affected by temperature have to be determined.

1.7 Objectives

The main goal of this research project was to study the influence of different host plants (i.e. broad bean, cucumber, tomato and sweet pepper) on the performance of *E. decipiens* and its parasitoid *A. atomus*, and it was divided into two parts.

In the first part the acceptability of adult *E. decipiens* of different host plant for oviposition and feeding, and the suitability of these host plants for the performance of the leafhoppers were studied. In addition, effects of different temperatures on various life table parameters of *E. decipiens* were investigated. In the second part of the study the influence of the host plants on the development time and rate of parasitism of *A. atomus* were examined. Additionally, host discrimination behaviour of the parasitoid was studied.

1.8 Research hypothesis

(i) *E. decipiens* shows preference for feeding and oviposition on certain host plants.

(ii) Temperature plays a critical role in determining the rate of development, survival, and reproduction of insect species (Messenger, 1964); thus temperature is a crucial factor affecting the development, fertility and longevity of *E. decipiens*

(iii) Preference for certain host plants or deferential rate of parasitism among different host plants by parasitoids attacking herbivores is common in egg parasitoids (Nordlund, 1994); hence the host plants of *E. decipiens* affect the performance and effectiveness of *A. atomus* against *E. decipiens*.

(iv) Avoidance of superparasitism in *A. atomus* is only possible when the parasitized leafhopper eggs can be recognized by the parasitoid female.

2. Host plant acceptability and suitability for the green leafhopper *Empoasca decipiens* and effect of temperature on its life table parameters

2.1 Abstract

The host plant acceptability of the green leafhopper *Empoasaca decipiens* Paoli was studied in choice experiments using cucumber (*Cucumis sativus* L.), sweet pepper (*Capsicum annuum* L.), and tomato (*Solanum lycopersicum* L.). *Empoasaca decipiens* significantly preferred cucumber for feeding and oviposition. The suitability of host plants for *E. decipiens* was determined based on the development time and the fitness of offspring, measured as weight and size of insects reared on each of the tested host plants. Development time of *E. decipiens* reared on broad beans was significantly shorter than on the rest of the tested host plants. Moreover, the weight and size of the progeny was significantly higher on broad beans was significantly than on the other tested plants. The egg-adult development time, survivorship, and reproduction of *E. decipiens* were evaluated at three constant temperatures (16, 20 and 24 C) using broad beans as a host plant. Development time for each larval instar was determined on broad beans at 20 and 24 C and at both temperatures tested the fifth larval instar had longest duration. Fecundity and egg viability of *E. decipiens* were studied at 16 and 20 C on broad beans. No significant differences in fecundity and egg viability between the two temperatures were recorded, and egg mortality was 7 and 9% at 16 and 20 C, respectively. Temperature significantly affected adult longevity, pre-oviposition and post oviposition time, but had no significant effect on fertility, oviposition period, larval mortality and sex ratio. The intrinsic rate of increase (r_m) and net reproduction rate (R_0) varied significantly

among the three temperatures and was highest at 20 C. Generation time was lower at 24 than that at 16 and 20 C, but doubling time was lower at 20 C.

2.2. Introduction

Leafhoppers of the genus *Empoasca* (Homoptera: Cicadellidae) are worldwide serious pests of many crops, causing on attacked leaves a symptom known as hopperburn. This results from proteins or other substances in the insects' saliva which they secrete into the plant tissue while feeding; Other symptoms of phytotoxima include tip burn, wrinkling and cupping of leaves, marginal burning and stunting of smaller plants (Mau & Kessing, 2001a). In addition, leafhoppers cause serious damages when they feed on the fruits, often leading to malformation (Raupach, 1999).

The green leafhopper *Empoasca decipiens* Paoli is widespread in Europe, North Africa, west and central Asia. In Syria it is recorded on grapevine, cotton, cucurbits and sugar beet, though little is known on the economic importance of the pest (Silti & Ibrahim, 1991). In recent years outbreaks of *E. decipiens* have been reported on vegetables and ornamentals in European glasshouses (Schmidt & Rupp, 1997; Helyer & Talbaghi, 1994). Likewise, population outbreaks have been observed in Syria on many vegetable crops such as broad beans, cowpea, cucurbits, and also on some fruit trees like peach and apple. On peach, heavy infestation of *E. decipiens* on young trees caused early defoliation in 2001 (R. A-M., personal observation).

Studying the host plant preference in herbivores is particularly important when an oligo- and/or polyphagous pest species exhibits distinct differences in its feeding preference between host plants, indicating the potential feasibility of a host plant resistance (HPR) approach. Non-preference, also termed antixenosis, is one of the three types of mechanism of HPR (Painter, 1951, cited in Palaniswamy & Lamb, 1992).

Kieckhefer and Medler (1964) hypothesized that information on oviposition preference would help to elucidate seasonal differences in population of the potato leafhopper *E. fabae*, especially during certain outbreak years, and therefore carried out experiments to determine the oviposition preference on broad beans, alfalfa, soybeans, and field peas. The leafhopper preferred broad beans for oviposition, followed by alfalfa, soybeans and field peas in descending order of preference. Moreover, young succulent alfalfa tissue was preferred over older lignified portions. Jayaraj (1966) showed preference of *E. fabae* for feeding and oviposition on some varieties of castor beans. The preferred susceptible varieties doubled the quantities of some organic acids as the resistant one. The increase of organic acids resulted in increased respiratory activity of these plants, leading to an increase in the quantity of total nitrogen in the susceptible varieties.

Temperature plays a critical role in determining the rate of development, survival, and reproduction of insect species (Messenger, 1964). Raupach (1999) reported that the development time of eggs was 28.3, 8.7, and 8.4 days at temperatures of 15, 28, 32.5°C respectively.

The primary objective of this research project was and is to investigate the influence of different host plants on the performance of the leafhopper *E. decipiens* and the effect of temperatures on certain life table parameters of *E. decipiens*.

2.3. Materials and methods

2.3.1 *Empoasca decipiens* colony

A laboratory culture of *E. decipiens*, obtained from the Federal Biological Research Centre for Agriculture and Forestry (BBA) in Braunschweig, Germany, has been established at the Institute of Plant Disease and Plant Protection (IPP). The leafhoppers are continuously reared on broad beans (*Vicia faba* L. Cv. Weisskeimig/ Major) at 20 C and a photoperiod of L16:D18, following the protocol developed by Raupach (1999). Broad beans seeds were sown in small pots (12 cm in diameter). Ten to 15 plants were infested with *E. decipiens* by placing them in an oviposition cage (85x 60 x 60 cm). After seven days, the plants were then transferred (after gently shaking off all adult leafhoppers from the plants) to a clean rearing cage (same dimensions as before) for egg hatching. Freshly emerged nymphs were collected weekly and transferred to a new cage (same dimensions as before) with broad bean plants, where they were reared until adult stage. Under the before mentioned conditions a full generation cycle of *E. decipiens* requires approximately 30 days.

2.3.2 Experiment I: Host plant acceptability for feeding and oviposition of *E. decipiens*

The experiment was carried out in the greenhouse in cages (85x 60x 60 cm) in March 2003. Each cage contained one cucumber (*Cucumis sativus* L. Cv. Tyria), sweet pepper (*Capsicum annuum* L. Cv. Mazurka) and tomato (*Solanum lycopersicum* L Cv. Matina) plant of approximately the same height. The plants were randomly placed at an equal distance, measured from the centre of the cage, and five mated leafhopper females (12 days old) from the *E. decipiens* culture were released in each cage. The insects were left there for one week, and during this period the plants were observed twice a day and the feeding positions of the leafhoppers were recorded. Thereafter adult leafhoppers were removed from the cages and the plants were observed daily for egg hatching. The numbers of emerging nymphs on each host plant species were recorded for 20 days. The experiment was carried out with six replicates per host plant species.

2.3.3 Experiment II: Host plant suitability for *E. decipiens*

This experiment was conducted to test the effect of host plants, i.e. broad beans (*Vicia faba* L. Cv. Witkiem Major), cucumber (*Cucumis sativus* L. Cv. Tyria), cucurbits (Cucurbita pepo L.), and sweet pepper (*Capsicum annuum* L. Cv. Mazurka), on the development time of *E. decipiens*, and on the fitness of the F1, expressed as the weight and size of the insects reared on each of the tested host plants. Four pots of each host plant species were randomly placed in a separate cage (85x 60x 60 cm), and 20 females per cage were release for 24 hours. Thereafter the adults were removed and the cages were kept in a climatic chamber at 24°C, 65-70 relative humidity, 16:8 h (L: D). Cages were checked daily for the appearance of newly emerged adults; these were subsequently removed until no more adult *E. decipiens* emerged. Thereafter, adults were kept for 24 hours on a new plant to allow complete sclerotisation of the exoskeleton. Adults were then frozen at -20° C for later data recording. After defrosting, leafhoppers were sexed, and males and females were separated in groups of 5 individuals and the fresh weight was determined on an electronic balance (Sartorius AG, Göttingen, Germany) to the nearest 10^{-5} g. Thereafter the insects were dried in an oven at 80°C for three days, and then the dry weight was measured. Finally the head capsule width was recorded, as measured by the distance between the compound eyes, and the length of the apical radial cell of the right elytra was recorded of 20 randomly selected individuals of each sex from each host plant species (Larsen, 1990).

2.3.4 Experiment III: Influence of temperature on *E. decipiens*

2.3.4.1 Development time for each larval instar

Newly hatched larvae, recognisable by their red eyes (El-Kadi, 1974), were collected from the oviposition cage, and transferred to broad bean leaf discs placed on potato dextrose agar (8 g/ml) in a Petri dish (3 cm diameter). In each Petri dish one larva was placed and observed daily for ecdysis. The leaf discs had to be changed at least twice a week. This experiment was conducted at 20 and 24°C, 65-70 r.h., 16:8 L: D with 25 replicates per temperature regime. The development time was calculated according to the following formula:

$$\mathrm{D_i} = \frac{n\ x}{n},$$

where n_i is the number of individual emerging and x_i the time required for each individual to complete its development.

2.3.4.2 Fecundity and egg viability of *E. decipiens*

One newly emerged couple of *E. decipiens* were released in a Petri dish (19 cm diameter), containing a broad bean leaf, which was changed weekly until the death of the female. The experimental set-up was similar as in Miltz (2001), with the Petri dish transformed into arena. A plastic tube (4 cm long, 1cm diameter) was attached inside the

Petri dish to its sidewall, this tube served as water reservoir. Two holes were punched into the plastic reservoir in order to insert the leaf stem and the cannula, and two windows covered with nylon mesh were made in the lid of the Petri dish. The experiment was conducted at 16 and 20°C, with 30 replicates at each temperature regime. The replicates were split into two halves; in the first fertility and in the other fecundity of leafhoppers were determined. Since *Empoasca* spp. females lay their eggs in the plant tissue, the plant tissue was cleared and stained, using the methodology developed by Simonet and Pinkowski (1977), to locate eggs for determination of fecundity of *E. decipiens*. For this the leaf was removed from the petri dish, and each leaf was then boiled in a solution of lactophenol-acid fuchsin for 10 min and allowed to cool overnight. The lactophenol-acid solution consisted of 1 part distilled water, 1 part phenol, 1 part lactic acid and 2 parts glycerin. One g of acid fuchsin stain was added to 1 l of this solution. Excess stain was removed by rinsing with warm tap water, and thereafter the leaf was examined under a stereomicroscope for recording of the number of eggs. The fecundity was determined by releasing a newly emerged couple in a similar arena; the couple was transferred weekly to a new Petri dish, equipped with a new broad bean leaf, until the death of the female. The removed bean leaves were kept in the same Petri dishes until emergence of larvae at the same experimental conditions mentioned above.

2.3.4.3 Influence of temperature on life table parameters of *E. decipiens*

Development time from egg to adult, adult longevity, oviposition frequency and reproductive capacity of *E. decipiens* was studied at three constant temperature regimes. A newly emerged leafhopper couple was released into a cylindrical plant cage (32 cm height, diameter 13.5 cm), containing a two weeks old broad bean plant. The plants were changed weekly until the death of the female, and removed plants were kept in similar cages and were weekly controlled for egg hatching. Emerged larvae from each replicate were counted, and then transferred to new bean plants until emergence of all adults. Those were then counted and sexed. The experiment was conducted at 16, 20, and 24°C, 65-70% r.h., 16:8 h L: D, with 15 replicates for each temperature.

2.3.5 Statistical analysis:

Data on host plant acceptability for feeding and oviposition (Exp. I) were first arcsine transformed and then analysed by means of analysis of variance (ANOVA), using the general linear model (Proc GLM) of SAS to determine single or interaction effects of factors (SAS Institute, 1992). In case of significant *F*-values, means were separated with the Tukey test.

ANOVA, using the general linear model (Proc GLM) of SAS (SAS Institute, 1992), was also used in analysing the data on host plant suitability (Exp. II) and on the effect of temperature on *E. decipiens* (Exp. III). Mean separation was done using the Tukey test for development time on different host plants and at different temperatures, data on head and wing size, and fresh and dry weight among the different host plants, whereas the t-test was used for separation between females and males on each host plant, and t-test with Bonferroni probability adjustment was used for separation of means on pre-oviposition, oviposition and post-oviposition time.

Life table statistics were calculated according to Hulting *et al.* (1990), using the jacknife programme. Differences in r_m values among populations were calculated following the protocol of Dixon (1987) and compared with Newman-Keuls sequential test (Sokal and Rohlf, 1995) based on jacknife estimates of variance for r_m values (Meyer *et al.*, 1986).

2.4 Results

2.4.1 Host plant acceptability for feeding and oviposition of *E. decipiens*

2.4.1.1 Acceptability for feeding

On the first day of releasing the leafhopper, 50% of the test insects avoided the three plants and were found somewhere in the cage and not on the plants, whereas the rest of them showed similar acceptance to cucumber and sweet pepper but significantly less preference to tomato ($F_{20, 3} = 6.37$, $P \quad 0.0033$). The percentage of leafhoppers present on each of the tested host plants fluctuated during the one-week observational period (Figure 2.1). Host plant acceptance significantly differed among the three tested plants, with cucumbers significantly preferred by adult *E. decipiens*, followed by sweet pepper and tomato (Table 2.1).

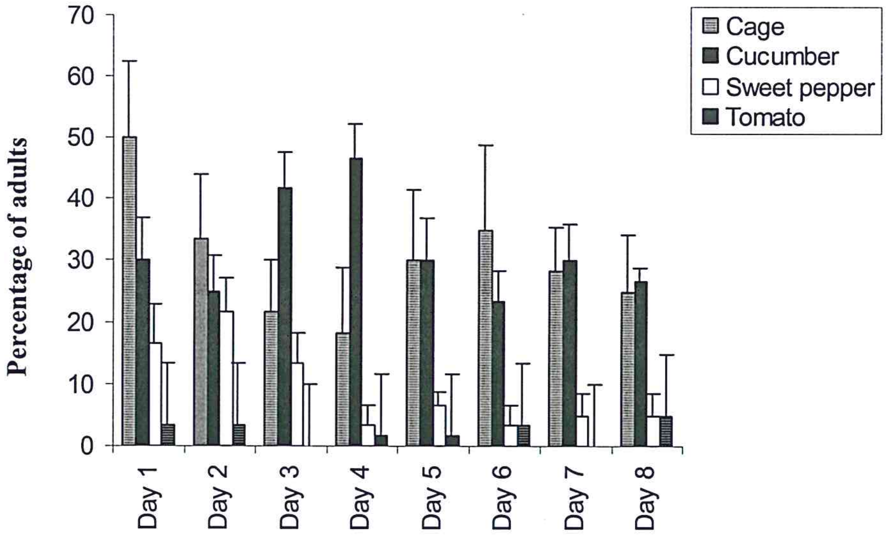

Figure 2.1: Host plant acceptance of *Empoasca decipiens* expressed as the proportion of adults observed on each of the tested host plants and off the plants in the cage (mean ± SE) over time.

2.4.1.2 Host plant acceptability for oviposition

Oviposition acceptance was compared by recording the mean number of emerged larvae from the tested host plants. Host plants significantly affected the egg laying preference of *E. decipiens* ($F_{15,2} = 19.87$, $P < 0.0001$). Significantly higher numbers of emerging larvae were recorded on cucumber, followed by sweet pepper and tomato (Table 2.1).

Table 2.1: Host plant acceptance of *E. decipiens* expressed as the percentage of adults present on and number of larvae emerging from three vegetable species over time (mean ± SE).

Host plant	% adult *E. decipiens* (mean ± SE)	No. of larvae (mean ± SE)
Cucumber	31.67 ± 2.15a	15.67 ± 1.96a
Sweet pepper	9.38 ± 1.67b	4.83 ± 2.27b
Tomato	2.29 ± 0.74c	0.67 ± 0.21c

Means in the same column followed by different letters are significantly different at $P < 0.05$ (Tukey test)

2.4.2 Host plants suitability for *E. decipiens*

2.4.2.1 Development time

The tested host plants significantly affected the development time of female ($F_{88,\,3} = 38.55$, $P < 0.0001$) and male *E. decipiens* ($F_{71,\,3} = 39.42$, $P < 0.0001$). The significantly shortest and longest development time was on broad beans and sweet pepper, respectively (Table 2.2).

Table 2.2: Effect of four host plant species on the development time from egg to adult in *E. decipiens* (mean ± SE).

Host plants	Mean development time (in days ± SE)	
Broad beans	26.11 ± 0.13a	25.28 ± 0.13a
Cucumber	27.59 ± 0.22b	26.23 ± 0.09b
Cucurbits	28.23 ± 0.20bc	27.22 ± 0.36b
Sweet pepper	28.76 ± 0.24c	27.44 ± 0.19b

Means in the same column followed by different letters are significantly different at $P< 0.05$ (Tukey test).

2.4.2.2 Fitness of F1

2.4.2.2.1 Fresh and dry eight

The tested host plants significantly influenced the fresh ($F_{46, 3} = 40.43$, $P < 0.0001$) and dry weight ($F_{46, 3} = 40.24$, $P < 0.0001$) of the F1 adults. Male and female leafhoppers that had developed on broad beans were significantly heavier, both in terms of fresh and dry weight, as the ones from cucurbits, cucumber and sweet pepper (Figure 2.2 I-II). Fresh and dry weight of *E. decipiens* that originated from the three latter host plants did not differ significantly. For leafhoppers from cucurbits, cucumber and sweet pepper no significant differences in fresh and dry weight were recorded between the sexes, though females tended to be heavier. However, in *E. decipiens* from broad beans females were significantly heavier (dry and fresh weight) than males (Figure 2.2, I-II).

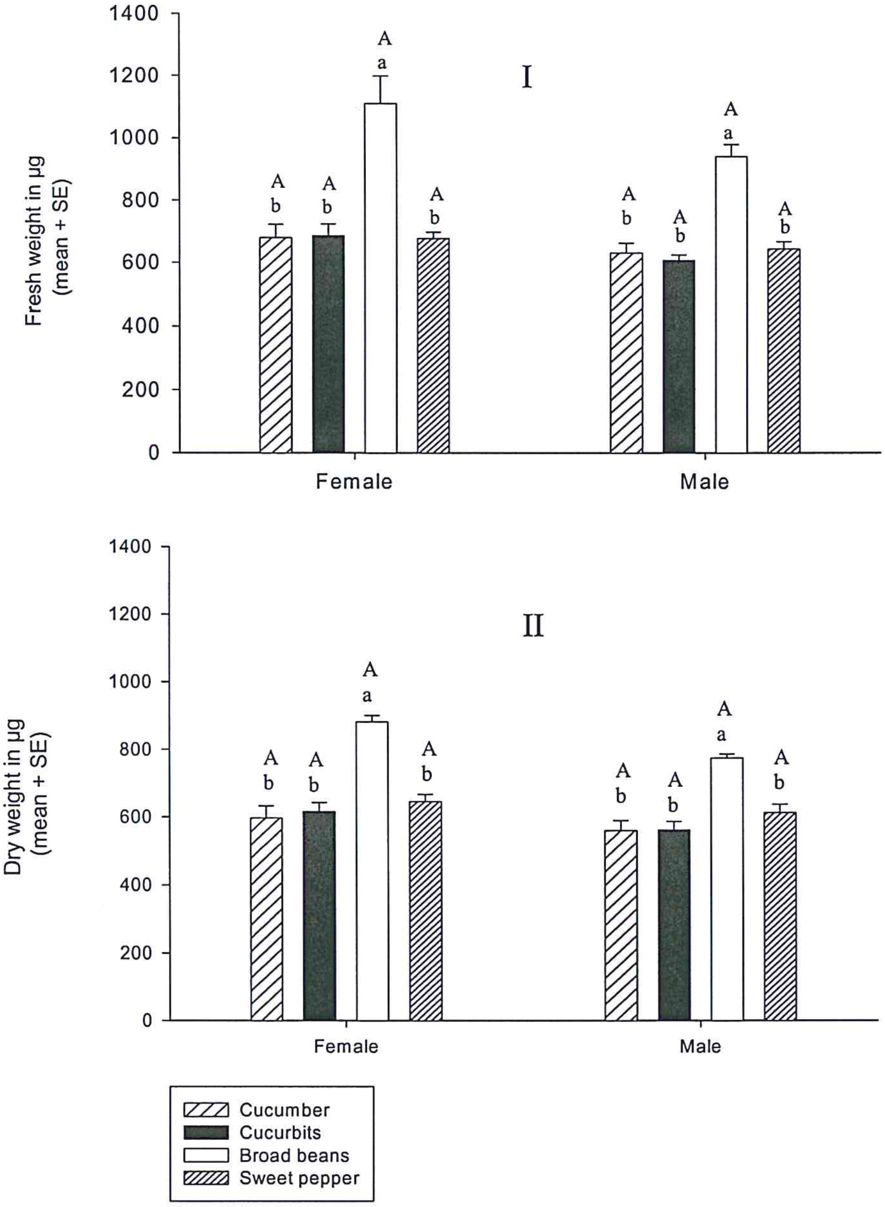

Figure 2.2: Effects of host plants on the fitness of F1 *Empoasca decipiens* in terms of fresh (I) and dry weight (II). Bars followed by different small letters indicate significant differences for the same sex, and capital letters between the sexes for the same host plant species (P = 0.05, Tukey test).

2.4.2.2.2 Head and ing

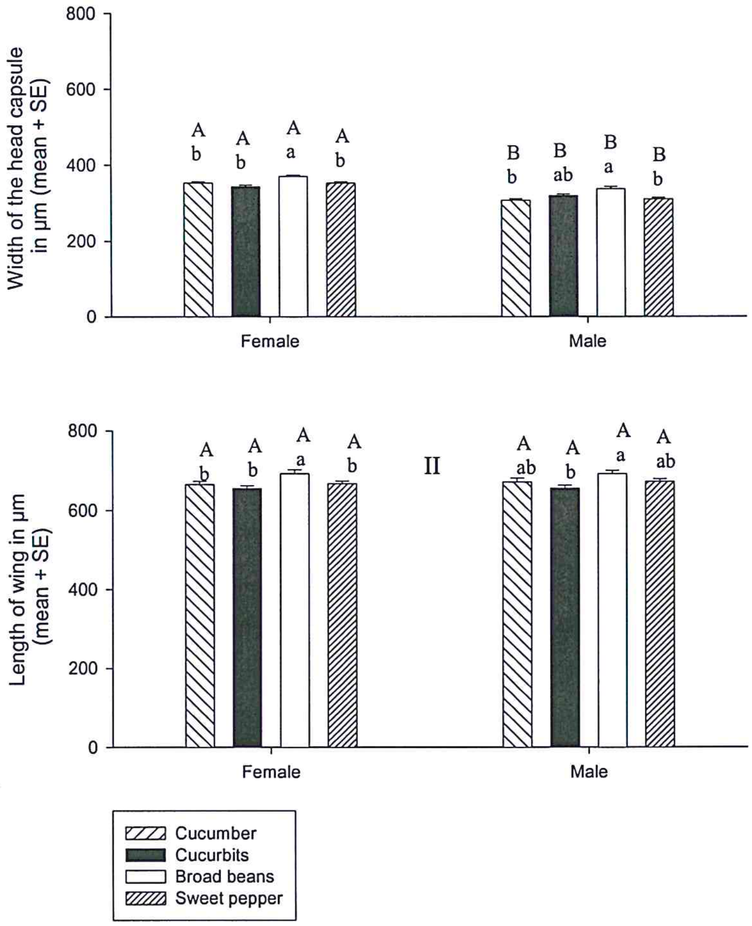

Figure 2.3: Effects of host plants on the fitness of F1 *Empoasca decipiens* in terms of width of head capsule (I) and length of wing cell (II). Bars followed by different small letters indicate significant differences for the same sex, and capital letters between the sexes for the same host plant species ($P = 0.05$, Tukey test).

Host plants significantly affected the head width in *E. decipiens* ($F_{152,3} = 13.27$, $P < 0.0001$). Except for male leafhoppers that had developed on cucurbits, in all other cases male and female leafhoppers from broad beans had significantly greater head width

(Figure 2.3, I). Moreover, in all host plants, females had significantly greater head width than males. Similarly, host plants significantly influenced the length of the apical radial cell of the right elytra ($F_{152, 3} = 8.22$, $P < 0.0001$). In female *E. decipiens* from broad beans it was significantly longer than in all other treatments, whereas in males only compared to leafhoppers from cucurbits (Figure 2.3, I-II). For this parameter no differences among the sexes were recorded.

2.4.3 Influence of temperature on *E. decipiens*

2.4.3.1 Development time for each larval instar

Empoasca decipiens develops over five larval instars. The development time for each larval instar at 20 and 24°C, separated for females and males, is presented in table 2.3. At 16°C development time for the individual larval instars could not be recorded. Temperature had a significant effect on the total larval development time ($F_{72,2} = 1958.99$, $P < 0.0001$), and decreased with increasing temperature. Total development time was significantly shorter at 16 than at 20 and 24°C ($p< 0.0001$), and at all temperatures total development time for male was shorter than for female *E. decipiens*. At 20 and 24°C development time of males were significantly shorter than for females in the fourth and fifth larval instars ($P < 0.0001$). Duration of the 5th larval instar was longest for both sexes at 20 and 24°C.

Table 2.3: Mean developmental time (in days +SE) of larval instars of *Empoasca decipiens* at 20 and 24°C and total development time at 16, 20 and 24°C.

		Mean development time (in days ± SE)		
		Temperatures (°C)		
Stage	Sex	16	20	24
L1		-	3.16 ± 0.11aA	2.52 ± 0.1bA
		-	3.40 ± 0.16aA	2.43 ± 0.14bA
L2		-	2.89 ± 0.13aA	2.40 ± 0.12bA
		-	3.00 ± 0.15aA	2.14 ± 0.10bA
L3		-	3.31 ± 0.10aA	2.12 ± 0.07bA
		-	3.30 ± 0.15aA	2.29 ± 0.16bA
L4		-	3.89 ± 0.17aA	2.52 ± 0.10bA
		-	3.40 ± 0.16aB	2.21 ± 0.11bB
L5		-	6.52 ± 0.20aA	5.28 ± 0.15bA
		-	6.00 ± 0.21aB	4.86 ± 0.10bB
Total		37.60 ± 0.75aA	19.79 ± 0.31bB	14.84 ± 0.17cC
		35.00 ± 0.32aB	19.10 ± 0.18bB	13.93 ± 0.13cC

Means within a row followed by different lower cases are significantly different, and means within a column, for a given development stage, followed by different upper cases are significantly different ($P < 0.05$, Tukey test).

2.4.3.2 Fecundity and egg viability of *E. decipiens*

No significant differences in fecundity and egg viability at 16 and 20°C were recorded ($P = 0.6232$) (Figure 2.4). Egg mortality was very low (7 and 9% at 16 and 20°C, respectively). Also fecundity and egg viability did not significantly differ between both temperatures (P = 0.1868). Oviposition lasted for nearly eight weeks at both temperatures, and insects tended to lay unfertile eggs toward the end of the oviposition period.

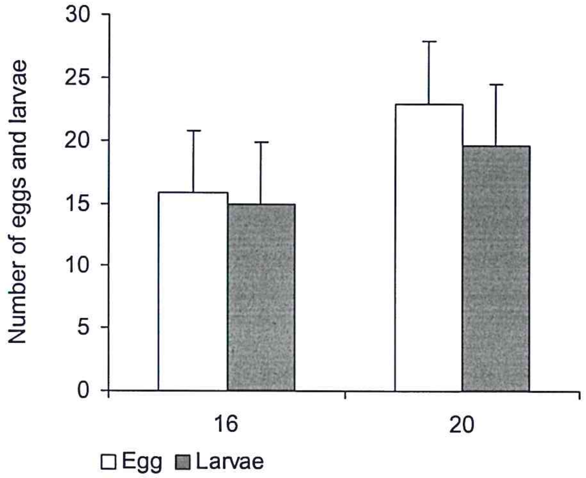

Figure 2.4: Fecundity and egg viability (mean ± SE) of *E. decipiens* at 16 and 20° C.

2.4.3.3 Influence of temperature on life table parameters of *E. decipiens*

Temperature had a significant effect on female longevity ($F_{36,2}$ = 7.07, P = 0.0026), and varied considerably among the three temperatures tested as well as within each temperature regime. Longevity decreased with increasing temperature. In average, females lived longer than males at all temperatures tested. Female survival and reproduction at the different temperatures are presented in Figure 2.5, A-C. The survival curve analysis revealed that adult survival was significantly affected by temperature (^{2} = 16.017; df = 2, P = 0.0003); thus the higher the temperature the earlier the numbers of surviving females started to decline.

Empoasca decipiens females started to lay eggs only after a pre-oviposition period, which was significantly influenced by temperatures ($F_{36,2}$ = 65.65, P<0.0001). However, the three temperatures tested had no significant effect on the total number of viable eggs laid and on the oviposition period, which lasted from 8-10 weeks (Table 2.4); by the middle of the oviposition period, the weekly number of eggs laid began to decrease gradually towards the end of the oviposition period.

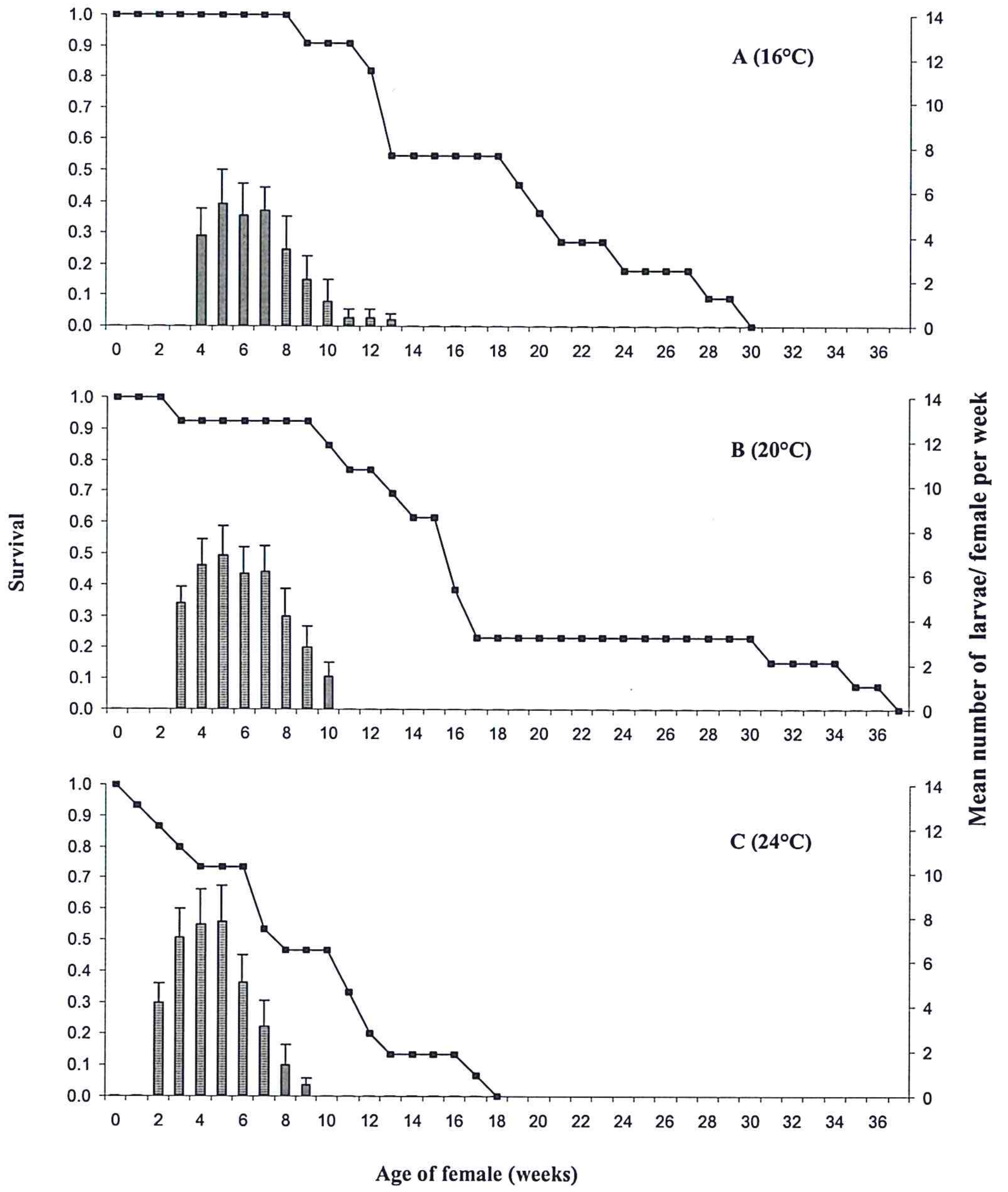

Figure 2.5: *Empoasca decipiens* survival and reproduction (mean ± SE) at 16°C (A), 20°C (B), and 24°C (C). Histogram is the number of larvae/week/female; line is survival curve of females.

Table 2.4: Adult longevity, number of emerged larvae, pre-oviposition, oviposition, and post-oviposition period, larval mortality and sex ratio (means ± SE) of *Empoasca decipiens* at three different temperatures.

	Temperatures (°C)		
	16	20	24
Longevity [1]	128 ± 14.5a	126 ± 19.4a	62 ± 9.4b
(min. – max.)	(18 - 193)	(8 - 267)	(8 - 127)
Longevity [1]	103 ± 23.9a	122 ± 23.5a	47 ± 7.2b
(min. – max.)	(7 - 194)	(19 - 263)	(8 - 127)
Emerged larvae [1]	27.5 ± 7.9a	39 ± 6.6a	37 ± 7.8a
(min. – max.)	(8 – 96)	(5 – 81)	(3 – 104)
Pre-oviposition [2]	22 ± 1.07a	13 ± 0.81b	10 ± 0.3c
(min. – max.)	(17 – 27)	(7 – 16)	(7 – 12)
Oviposition [2]	34 ± 4.51a	41 ± 5.47a	33 ± 4.67a
	(14 – 70)	(6 – 71)	(1 – 56)
Post-oviposition [2]	71.45 ± 11.38a	72 ± 15.52a	17.93 ± 5.34b
	(14 – 147)	(20 – 189)	(3 – 60)
Mortality (%)	17 ± 2.6a	12 ± 1.75a	13 ± 2.02a
Sex ratio [3]	53 ± 1.61a	52 ± 1.95a	50 ± 3.87a

[1] Means in the same row with different letters are significantly different (Tukey test $P < 0.05$); [2] means in the same row with different letters are significantly different (and t-test with Bonferroni adjustment, $P < 0.05$), [3] expressed as proportion of females.

The computed life table parameters for the three temperature regimes are presented in table 2.5. The net reproduction rate R_0 was significantly higher at 20 than at 16 and 24°C. Likewise, the intrinsic rate of increase (r_m) and the finite rate of increase were significantly higher at 20 than at 16 and 24. The mean generation time decreased with increasing temperature; however, the doubling time was shorter at 20 than at 16 and 24°C. The combined differences in egg-adult development, adult survivorship and fertility revealed strong differences in life table parameters of *E. decipiens*, especially in terms of r_m.

Table 2.5: Effect of three different temperatures on life table parameters of *Empoasca decipiens*.

Parameters*	Temperatures (°C)		
	16	20	24
r_m	0.014 ± 0.003c	0.043 ± 0.002a	0.026 ± 0.003b
R_0	4.67 ± 1.35c	20.28 ± 3.407a	4.78 ± 1.01b
G	112.65	70.94	60.62
DT	50.69	16.34	26.87
	1.01	1.04	1.03

* r_m = jackknife estimate of the intrinsic rate of increase (per capita rate of population growth), R_0 = net reproductive rate (female offspring per adult female), G = mean generation time (days), DT = doubling time (days) and = infinite rate of increase for population.

2.5 Discussion

The tested host plant species had a significant effect on feeding and oviposition in *E. decipiens*. Leafhoppers showed significant preference for feeding and oviposition on cucumber. In contrast, Raupach (1999) reported that *E. decipiens* had no significant feeding preference for eggplant, cucumber, sweet pepper, and tomato. However, in her study females failed to lay eggs because during the experiment the females were still in their pre-oviposition period. In tomatoes *E._decipiens* showed strong non-preference for feeding as well as for oviposition. This might be due to antifeeding compounds secreted by the glandular trichoms on the leaf surface of tomatoes; tomatine for example, a secondary compounds of glandular tichoms, act as antifeedants to potato leafhopper *E. fabae* (Raman *et al.,* 1979, cited in Panda and Khush, 1995). Moreover, Koblet-Günthardt (1975) failed to establish a culture of *E. decipiens* on tomatoes.

According to van Lenteren and Noldus (1990) shorter development time and greater total reproduction on a host reflect the suitability of the plant. Based on these criteria broad beans are the most suitable host plants for *E. decipiens*, since the development time of the leafhopper was significantly shorter on *V. faba* compared to other tested host plant species. These results corroborate earlier findings of Raupach *et al.* (2002) and (Koblet-Günthardt (1975), and indicate the superior host plant quality of broad beans for *E. decipiens*. Another reason for the short development time on broad beans could be the fact that the tested leafhopper strain had been mass reared on *V. faba* for more than six years. Reduced fitness of insects can be the result of various factors, including poor nutritional conditions of insect (Marden and Waage, 1990; Hakkarainen and Korpimakin,

1993) and small body size (Chapman, 1971; Anholt *et al.*, 1991; Reid and Roitberg, 1995; Blanckenhorn *et al.*, 1998). Thus the variation in fitness of leafhopper offspring, in terms of weight and size of the adults, from the different host plants might reflect differences in the nutritional quality of these host plants. In addition to habituation of our *E. decipiens* strain to broad beans, the recorded differences in fitness of leafhopper offspring may indicate a kind of antibiosis effect of the some of the tested host plants by producing smaller insects on apparently less suitable plants.

Fecundity of *E. decipiens* did not differ significantly between 16 and 20°C. In general the level of fecundity was rather low. The reason for this might have been that we offered only leaves as oviposition substrate, whereas *E. decipiens* females usually prefer to lay eggs in the stem of broad beans plants (Agboka, 2002). Egg mortality was low and did not significantly differ between 16 and 20°C.

Larval development time of *E. decipiens* on broad beans was significantly influenced by temperature; the shortest development time was recorded at 24°C. Similar results were obtained by Raupach (1999), though she recorded the total development time for female and male together whereas we recorded it for each larval instar and for each sex separately. Larval mortality was higher, though not significantly, at 16 than at 20 and 24°C. Raupach (1999) could show that the number of emerged adults was not related to the different temperature regimes but reflects the varying initial larval densities in the respective experimental units at the beginning of the experiment, suggesting that temperature has no significant effect on larval survival in *E. decipiens*.

Longevity of leafhoppers was significantly shorter at 24°C but did not differ between 16 and 20°C. However, longevity was highly variable at each temperature tested, with a maximum of 267 days at 20°C. In contrast Habib *et al.* (1972) reported that the longevity of male/female *E. decipiens* was 27/55.5, 15.5/38.8, 15.6/31.9 days at 19, 22 and 29°C, respectively. Moreover, in a study by Poos (1932) the longevity of *E. decipiens* did not exceed 148 days. In general, longevity of leafhoppers greatly differs between hibernating adults and during the summer months; during the latter period it mostly varies between a few days to 3-4 months (DeLong, 1971). At all temperatures tested female *E. decipiens* lived longer than males, which corresponds with results from a study on the beet leafhopper *C. tenellus* (Harries, 1971).

Empoasca decipiens females started to lay eggs only after a pre-oviposition period, which lasted on average 10, 13 and 22 days at 16, 20 and 24°C, respectively. Habib *et al.* (1972) reported that the pre-oviposition period varied from one to several days according to climatic conditions. In some leafhoppers species such as *E. fabae*, the pre-oviposition period lasts from 1.5 to 10 days, but it may extend for several months in hibernating adults (DeLong, 1971)

The oviposition period of individual females varied from a few days to up to two months at all temperatures tested. However, we recorded no significant differences in oviposition period among the different temperature regimes. Likewise there were no significant differences in fecundity among the different temperatures. In our study the average number of eggs laid, measured as number of emerged larvae, was 27, 39 and 37 larvae/female at 16, 20 and 24°C, respectively, whereas Müller (1956) reported that a female *E. decipiens* lays 100 eggs during her lifetime. In *E. fabae* the average fecundity is about 200 eggs per female (DeLong, 1938), and in the blackfaced leafhopper *Graminella nigrifrons* Forbes the lowest recorded fecundity is 16 eggs per female (Stoner, 1967).

The intrinsic rate of increase r_m and the net reproduction rate R_0 was significantly affected by temperatures and was higher at 20°C than at 20 and24°C. The reason for this could be the fact that our *E. decipiens* strain was originally collected near Braunschweig, northern Germany. The generation time was shorter at 24°C due to the shortest development time at this temperature; however, the doubling time was shorter at 20°C because of the higher intrinsic rate of increase at this temperature.

The result of this study cannot give a final answer to the question of host plants acceptability and suitability in *E. decipiens*, mainly because our leafhopper strain might have been habituated to broad beans. However, in future studies the host plant preference of *E. decipiens* should be tested under the field conditions using field populations of the leafhoppers. In ongoing field and laboratory experiments we are studying the host plant preference of *E. decipiens* originating from and reared on different host plants.

The intrinsic rate of increase of *E. decipiens* is lower than that calculated for *A. atomus* (Agboka, 2002), indicating that the egg parasitoid can be successfully used for biological control of the leafhopper. The release rate of *A. atomus* should depend on the reproductive capacity of the pest, the economic thresholds on different important vegetable hosts of *E. decipiens*, and the performance of the parasitoid as affected by the different host plants of the leafhopper. In ongoing study we are investigating the influence of host plants of *E. decipiens* on the rate of parasitism of *A. atomus* and the effects of the rearing history of the parasitoid on its performance.

3. The interaction between *Anagrus atomus* and host plants of *Empoasca decipiens* and host discrimination by the parasitoid

3.1 Abstract

Anagrus atomus L. (Hym.: Mymaridae) is an important egg parasitoid of the green leafhopper *Empoasca decipiens* Paoli. We studied the interactions between *A. atomus* and host plants of *E. decipiens*, using broad beans (*Vicia faba* L), cucumber (*Cucumis sativus* L.) and sweet pepper (*Capsicum annuum* L.) as model plants. The host plants did not significantly affect the egg-adult development, fertility, rate of parasitism and sex ratio of *A. anagrus*. Development time was 16.17 ± 0.13, 16.05 ± 0.1 days on cucumber and sweet pepper, respectively. Fertility was relatively low with a mean number of progeny of 14.57 ± 2.55 and 15.21 ± 2.53 per female on cucumber and sweet pepper, respectively. Pre-adult parasitoid mortality was higher on cucumber, but did not differ significantly from that on sweet pepper. Rearing of parasitoids on leafhoppers feeding on broad beans or cucumber did not significantly affect the rate of parasitism of *A. atomus* when subsequently released on leafhopper infested broad beans plants. However, pre-adult parasitoid mortality was significantly higher when parasitoids females used had been reared on leafhoppers on broad beans than on cucumber. Female *A. atomus* did not avoid neither self- nor conspecific superparasitism when released 1 or 24h after their first oviposition. In most cases superparasitism resulted in the death of the parasitoid eggs.

3.2 Introduction

Empoasca decipiens Paoli (Homoptera: Cicadellidae) is one of the most damaging leafhopper species in European greenhouses. The pest is widely distributed in central and southern Europe, North Africa, the Middle East and Central Asia (Ossiannilsson, 1981). Recent outbreaks of *E. decipiens* were reported in southern Germany (Schmidt and Rupp, 1997), the Netherlands and UK (Helyer and Talbaghi, 1994), in Bulgaria (Loginova, 1992) and in Switzerland (Anonym, 1998).

Empoasca decipiens is polyphagous herbivore and attacks many field crops, including broad beans, cucumber, sweet pepper and many other vegetables (El-Dessouki and Hosny, 1969). Both nymphs and adults of *E. decipiens* damage plants directly by sucking on the leaves and fruits (Helyer and Talbaghi, 1994). 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 *E. decipiens* mainly rely on the use of synthetic insecticides. The insect growth regulator Buprofezin has proved to effectively control *E. decipiens* nymphs 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). In general, chemical control of leafhoppers is difficult due to the lack of appropriate 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, and consequently disrupt the already existing successful biological control programs against other important greenhouse pests like white flies, leafminers and aphids. 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 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). *Anagrus atomus* can successfully develop and reproduce within a temperature range of 16-24°C, which corresponds well with common climatic conditions in European greenhouses; hence the parasitoid can be present and active during almost the whole production period (Agboka, 2002). The lower development threshold in *A. atomus* (Agboka, 2002) 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*, and, thus, in terms of thermal requirements *A. atomus* can develop faster and build up faster populations than *E. decipiens* (Agboka, 2002). However, 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, more detailed data on the biology, ecology and behaviour of the parasitoid is needed. Particularly, more information on the possible influence of the various host plants of *E. decipiens* on *A. atomus* will improve our understanding of the egg parasitoid and its potential for biological control of leafhoppers. Host plants of leafhoppers on which both hosts and parasitoids had been reared significantly affected the searching and oviposition behaviour and parasitization ability of the closely related mymarid egg parasitoid *A. nigriventris* Girault (Al-Wahaibi and Walker, 2000). Testing the effect of different parasitoid densities on the rate of parasitism showed, that the rate of parasitism by *A. atomus* increased with increasing release rates, though the proportion of parasitism did not exceed 64% (Agboka, 2002). This may be due to the generally low fecundity in *A. atomus* (Agboka, 2002), or to possible mutual interference among parasitoids or superparasitism (Croning and Strong, 1993). Hence the objective of this study was to investigate the possible influence of host plants of *E. decipiens* on the development time and rate of parasitism of *A. atomus*. Additionally, the host discrimination behaviour of the parasitoid was studied.

3.3 Materials and methods

3.3.1 Insect rearing

E. decipiens were reared on broad beans *Vicia faba* L. following the protocol developed by Raupach (1999). A culture of *A. atomus* was obtained from English Woodlands Biocontrol (UK). The parasitoids were reared at the Institute of Plant Diseases and Plant Protection (IPP), University of Hannover, following the protocol developed by Triapitsyn and Moratorio (1990) for the closely related *A. nigriventris*. Broad bean plants bearing leafhopper eggs were collected from the *E. decipiens* oviposition cage (85x 60x 65 cm) and placed in the insectary (24°C 2, 16:8 L: D). Each plant was then covered with a cylindrical cage (32 cm height, 13.5 cm) made of thin clear Plexiglas with two screened widows in the sidewall and a nylon mesh covering one top. Females *A. atomus* were released in the cage for oviposition, and 12 days later the plants were gently shaken to remove leafhopper nymphs that had emerged from unparasitized eggs. Thereafter broad bean stems and leaves were cut in small pieces containing individual parasitized egg and placed into 1.5 ml Eppendorf tubes with a piece of wet filter paper. The tubes were placed in the insectary (24°C 2, 16:8 L: D) and were daily checked for emergence.

3.3.2 Effect of leafhopper host plants on development time and fertility of *A. atomus*

Development time from egg to adult was studied using cucumber (*Cucumis sativus* L. Cv. Tyria [Cucurbitacae]) and sweet pepper (*Capsicum annuum* L. Cv. Mazurka [Solanacae]) as host plants for *E. decipiens*. Mated female *A. atomus* < 24 h old were individually released in the Plexiglas cylinders containing a cucumber or sweet pepper plant with true 4 leaves, that harboured *E. decipiens* eggs. Host eggs were obtained by previously exposing the plants to adult leafhoppers for 72 hours. Parasitoids were allowed to oviposit for 24 h. Thereafter, the development of the progeny was followed at 24°C, 65-70% relative humidity, 16:8 h L: D. Each host plant treatment was repeated 15 times. When the parasitized eggs started to turn red (Cooper, 1993), the stem and leaves were cut into individual pieces, harbouring a single parasitized egg, and each piece was then kept in an 1.5 ml Eppendorf tube until emergence of the adult parasitoid. Egg to adult development time were computed as

$$D_i = \frac{n\,x}{n},$$

where n_i is the number of individuals emerging and x_i the time required for each individual to complete its development.

To study the effect of host plants on the fertility of *A. anagrus*, one mated female wasp < 24 h old was released in a cylinder containing one of the tested host plants harbouring *E. decipiens* eggs and kept there until the death of the parasitoid. For each host plant 15 replicates were used, and the experiment was conducted at 24°C, 65-70% relative humidity, 16:8 h L: D. After 12 days leafhopper nymphs that had emerged from unparasitized eggs were counted, and plant parts harbouring parasitized eggs, as indicated by their red colour (Cooper, 1993), were cut in small pieces and kept in 1.5 ml Eppendorf tube until emergence of the F2. Emerging adults were counted and sexed, and the fecundity and the sex ratio of the F2 were calculated for each host plant species.

3.3.3 Effect of conditioning of *A. atomus* on the rate of parasitism

The objective of this experiment was to investigate whether host plants of *E. decipiens* on which the parasitoid had been reared would influence the performance of *A. atomus*. Hence, parasitoids were reared on *E. decipiens* eggs on cucumber and broad bean plants, and the emerging adults of *A. anagrus* were used in the experiments. Two treatments were tested, i.e. parasitoids reared on *E. decipiens* eggs on cucumber vs. broad bean plants. Two weeks old broad bean plants were placed for 72h in the leafhopper oviposition cage. The plants were then covered with a Plexiglas cylinder and kept in a climatic chamber at 24 C, 65-70% RH, and a photoperiod of 16:8 h (L: D). Subsequently, mated female *A. atomus* < 24 h old reared on *E. decipiens* eggs on cucumber or on broad bean plants were individually released and left in the cylinders until death. Each treatment was repeated 15 times. After 12 days the cylinders were removed and the number of *E. decipiens* nymphs that had emerged from unparasitized eggs were counted. Then plant parts harbouring parasitized eggs were cut in small pieces and kept in 1.5 ml Eppendorf tube until emergence of the F2. Emerging adults were counted and sexed. The rate of parasitism, the pre-adult parasitoid mortality and the sex ratio of the F2 were calculated for each treatment.

3.3.4 Host discrimination: Superparasitism

The objective of this experiment was to examine whether *A. atomus* female can discriminate between parasitized and unparasitized eggs. We used 2x2 factorial experimental design with the two factors being the time elapsed between the first and the second oviposition (i.e., 1 or 24 h) and the same or a different female (i.e., self- or conspecific superparasitism), hence in total four treatments. Host patches were created by confining 3-5 *E. decipiens* females to a broad bean leaf for 24 h. Leafhopper eggs in the leaf tissue were located using transmitted fiber-optic light under a stereomicroscope. The position of each egg was marked with a fine ink marker; leaf discs (3 cm diameter) ontaining 2-4 eggs were then excised using scalpel and placed on potato dextrose agar (8 g/ml) in a Petri dish (3 cm diameter). One mated *A. anagrus* female was then released into the Petri dish and was observed until its first oviposition in one of the marked leafhopper eggs. Thereafter the parasitized egg was marked and the parasitoid was removed and kept in a 1.5 ml Eppendorf tube. In the first treatment after one hour the same female was released in a Petri dish containing one parasitized egg and unknown number of unparasitized ones (1-3) leafhopper egg, and in the second treatment, a newly mated experienced female was released and in both treatments the ovipositional behaviour was observed. Similarly, after 24 h the same or a newly mated experienced female were released. In all experiments the observation lasted until the female had oviposited in one of the host eggs. Thereafter the female was removed and the Petri dishes were incubated at 24C until emergence of the F2.

3.3.5 Statistical analysis

Differences in development time of *A. anagrus* were analysed using Wilcoxon two-sided test (SAS Institute, 1990). Data on fertility, rate of parasitism, pre-adult mortality and on the effect of rearing history (i.e., conditioning) of the parasitoids on the rate of parasitism were analysed by means of analysis of variance (ANOVA), using the general linear model (Proc GLM) (SAS Institute, 1990). Data were transformed to arcsine values before analysis. In case of significant *F*-values means were separated with the Tukey test. Data on host discrimination behaviour were analysed by means of ^{2} test.

3.4 esults

3.4.1 Effect of host plants on development time and fertility of *A. atomus*

No significant differences in development time of *A. atomus* on *E. decipiens* eggs originating from leafhoppers having fed on cucumber and sweet pepper plants were recorded ($P = 0.1775$) (Table 3.1).

Table 3.1: Development time of *Anagrus atomus* on *Empoasca decipiens* reared on cucumber and sweet pepper.

Host pants	Mean development time (in days ± SE)		
Cucumber	16.6 ± 0.08	15.74 ± 0.2	16.17 ± 0.13
Sweet pepper	16.52 ± 0.11	15.57 ± 0.13	16.05 ± 0.1

The total number of *A. anagrus* offspring did not differ significantly between cucumber and sweet pepper (*P* = 0.8252) with 14.57 ± 2.55 and 15.21 ± 2.53 on cucumber and sweet pepper, respectively; the mean number of progeny per female was rather low. Pre-adult parasitoid mortality was higher on cucumber, but did not differ significantly from that on sweet pepper (*P* = 0.0582) (Figure 3.1). Likewise the rate of parasitism (*P* = 0.6749) (Figure 3.2) and the sex ratio of the offspring (P = 0.7367), expressed as the proportion of females, was not significantly affected by the host plants of *E. decipiens*.

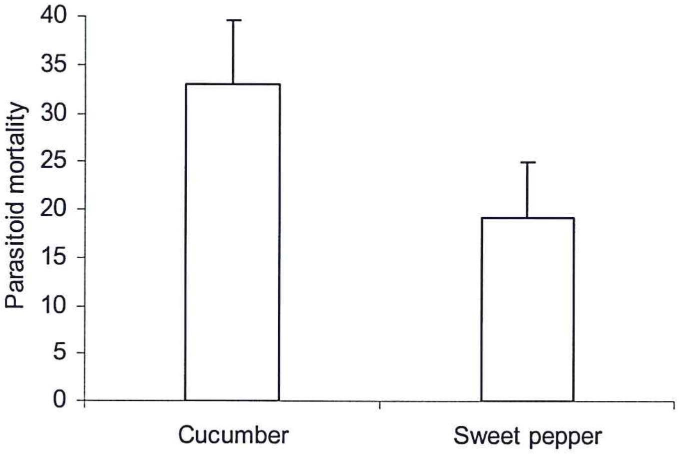

Figure 3.1: Effect of host plants of *Empoasca decipiens* on the pre-adult mortality of *Anagrus atomus*

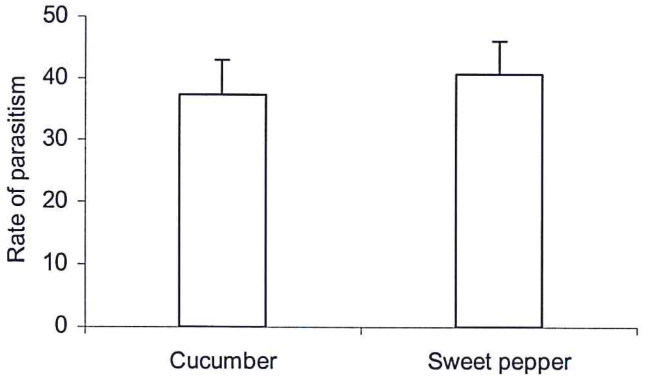

Figure 3.2: Effect of host plants of *Empoasca decipiens* on the rate of parasitism of *Anagrus atomus*.

3.4.2 Effect of conditioning of *A. atomus* on the rate of parasitism

The rate of parasitism of *A. atomus* on *E. decipiens* on broad beans was not significantly affected by the rearing history of the parasitoids (P = 0.6353) (Figure 3.3), i.e. females that had been reared on broad beans did not perform better than those originating from cucumbers. However, pre-adult mortality in *A. anagrus* was significantly higher when parasitoids originating from broad beans were exposed to leafhopper eggs on *V. faba* ($F_{28,1}$= 6.10, P = 0.0199) (Figure 3.4).

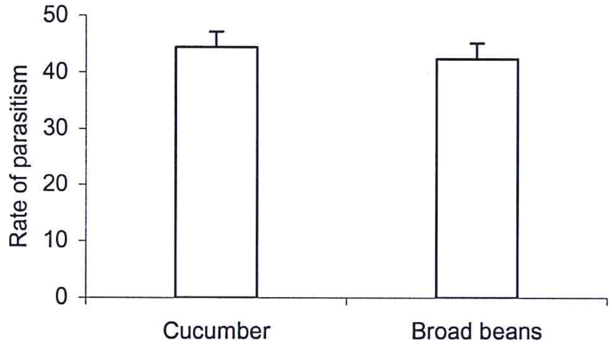

Figure 3.3: Effect of rearing history (i.e., conditioning) of *Anagrus atomus* on the rate of parasitism of *Empoasca decipiens* eggs on two different host plants.

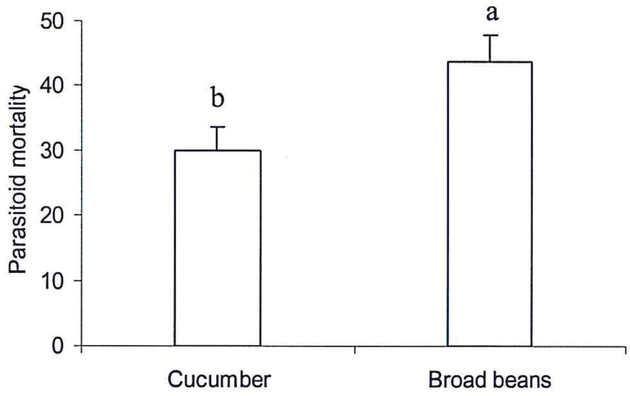

Figure 3.4: Effect of conditioning of *A. atomus* on the parasitoid pre-adult mortality

3.4.3 Host discrimination: Superparasitism

Female *A. atomus* were not able to discriminate between host eggs, which had been previously parasitized by the same female or by a different female irrespective of the time that had elapsed after the initial oviposition (Table 3.2). When females were released 24 h after the first oviposition, in 60 and 66.3% of the observed cases, the same and a conspecific female superparasitized eggs, respectively. When the females were released one hour after the initial oviposition, self- and conspecific superparasitism were less frequently, with 25 and 50% of the superparasitized leafhopper eggs, respectively. The rate of emergence from superparasitized eggs was very low: Out of 26 superparasitized eggs in the four treatments only five male and three females emerged. Thus in the majority of the cases superparasitism led to the death of parasitoids in the *E. decipiens* eggs.

Table 3.2: Response of *Anagrus atomus* females to parasitized and unparasitized *Empoasca decipiens* eggs at two time intervals after the initial oviposition.

		% Oviposition	
	Host eggs	Same female	Different female
1 hours	Parasitized	25	50
	Unparasitized	75	50
		$^{2} = 1.169$ $P = 0.2796$ n = 18	
24 hours	Parasitized	60	66.3
	Unparasitized	40	33.7
		$^{2} = 0.1435$ $P = 0.7048$ n = 30	

3.4 Discussion

The host plants of herbivores are not neutral substrates upon which interacting herbivores-natural enemies occur. Both the dynamics as well as the outcome of a particular herbivore-natural enemy interaction may vary with the herbivore's host plant species, or even genotypes within these species, and understanding of such variations is central to the study of tritrophic interactions (Hare, 2002). For example, development time of *Microplitis croceipes*Cresson (Hym.: Braconidae), a larval parasitoid of *Heliothis virescens* (Fab.) (Lep.: Noctuidae), increased on certain soybean genotypes (Powell *et al.*, 1984). However, in our study host plant species of *E. decipiens* did not significantly affect the development time of *A. atomus*. Likewise, fertility of *A. atomus* did not differ between sweet pepper and cucumber but was lower compared to parasitoids reared on *E. decipiens* on broad beans (Agboka, 2002). The latter might be due to lower number of leafhopper eggs within the plant tissue of cucumber and sweet pepper plants than in broad beans. In general *V. faba* is considered to be a superior host plant for *E. decipiens* (Koblet-Guenthardt, 1975). Orr *et al.* (1985) recorded reduced total fecundity of the scelionid egg parasitoid *Telenomus chloropus* Thomson when it parasitized *Nezara viridula* (L.) (Hem.: Pentatomidae) eggs on certain genotypes of soybean. Preference for certain host plants or differential rate of parasitism among different host plants by parasitoids attacking herbivorous insects are widespread phenomena in egg parasitoids (Nordlund, 1994). However, in this study the rate of parasitism did not differ between both host plants tested, and, additionally, did not differ from that previously reported by Agboka (2002) on broad beans. In contrast Al-Wahaibi and Walker (2000) observed differences in rate of parasitism of *A. nigriventris* on *C. tenellus* on different host plants, indicating that the parasitoid showed a preference for certain host plants. Stiling (1980) found high levels of parasitism for an *Anagrus* sp. associated with three *Eupteryx* leafhopper species on stinging nettle, yet found little or no parasitism on the surrounding weedy vegetation harbouring *Eupteryx* spp. In *Empoasca* spp. it is not possible to clearly define the number of eggs laid in the plant tissue. Hence it is possible that the higher rate of parasitism on broad beans compared to sweet pepper and cucumbers was due to higher egg density on *V. faba*. Yet, we conducted our experiments under no-choice conditions; thus parasitoids were not able to switch to more preferred plants. Consequently in the field the rate of parasitism of *A. atomus* on different host plants of *E. decipiens* might be different than that observed in this study.

The level of parasitism by *A. atomus* did not differ between parasitoids emerging from parasitized *E. decipiens* eggs on broad beans or cucumber. Contrary to that Al-Wahaibi and Walker (2000) recorded higher rates of parasitism by *A. nigriventris* on *C. tenellus* on sugar beets if parasitoids had been reared on this host plant species. In our study, the pre-adult parasitoid mortality was significantly higher when the parasitoid had been reared on broad beans. The higher mortality could have been due to superparasitim, suggesting increased searching and host locating activities of *A. atomus* reared on broad beans. In general host searching by parasitoids is enhanced on plant species from which they had emerged (Godfray, 1994). Herard *et al.* (1988) hypothesised that plant-derived chemicals are present in the cocoon of the developing parasitoid and that contact with these chemicals at time of eclosion increase the responsiveness of the adult parasitoid to certain plant odours.

Host discrimination refers to a parasitoid's ability to distinguish between parasitized and non-parasitized hosts (van Lenteren, 1981). Such ability is often based on the marking of host eggs after oviposition, using chemical markers, which deter other females from attacking this host (Godfray, 1994). Some parasitoid species use only external markers like the scelionid egg parasitoid *Trissolcus basalis* (Wollaston) of *N. viridula*; the female marks the egg after oviposition by dragging the ovipositor over the egg in a figure-eight pattern (Weber *et al.*, 1996). In our experiments we detected no marking behaviour of parasitized eggs by *A. anagrus*. Hence *A. atomus* is not able to discriminate between parasitized and unparasitized eggs, neither in case of conspecific superparasitism, nor in self-superparasitism, although self- and conspecific superparasitism were less frequent when the females were released one hour after the first oviposition. The parasitids showed some degree of ovipositional restrain towards parasitized eggs. However, had we exposed the parasitized eggs to the parasitoid for a longer period of time, a higher rate of superparasitism may have resulted due to the breakdown of ovipositional restrain. Whether this is because the parasitoid produces no markers or the produced markers were, for unknown reasons, not perceived remains to be thoroughly investigated. However, marking of hosts is not in all cases advantageous since the female needs to spend time and energy in applying the mark, and is giving away information to its competitors; consequently, competitors may use these information not only to avoid parasitism of the marked host, but also to superparasitise (van Alphen and Visser, 1990). For instance Croning and Strong (1993) observed that *A. delicatus* Dozier (Hym.: Mymaridae), an egg parasitoid of *Prokelisia marginata* (van Duzee) (Hom.: Delphacidae), did not avoid parasitizing hosts that had been recently parasitized by conspecific wasps; superparasitism was largely a random process, and increased with the ratio of parasitized to unparasitized hosts. On the contrary, Moratorio (1990) reported that *A. mutans* Walker and *A. silwoodensis* Walker avoided a second oviposition in eggs of *Dicrantopis hamata* (Boheman) (Hom.: Delphacidae), i.e. the female drills its ovipositor into the parasitized host egg and then withdraws it. Such behaviour was also observed in this study, but just in two replicates. Rejection of parasitized hosts after ovipositor insertion has been reported in a number of parasitoid species (Fisher, 1961; van Lenteren, 1976). Wylie (1965) suggested that secretions from the poison reservoir deposited during egg laying might render the host unacceptable to another female. The probability of superparasitism is strongly influenced by the encounter rate with parasitized hosts. It is often observed in laboratory experiments that parasitoids confined with their hosts will commence to superparasitise once all unparasitised hosts have been attacked (Godfray, 1994). However, in *A. atomus* in the great majority of the observed cases, the female oviposited in the first egg it encountered, no matter whether this egg was non-parasitised or had been previously parasitised by itself or a conspecific female. Long considered as the result of mistakes made by imperfect animals, superparasitism is nowadays recognized as adaptive in a number of different situations (van Alphen *et al.*, 1990). Visser *et al.* (1992) showed with evolutionary stable strategy models that superparasitism is an adaptive strategy for a solitary parasitoid if the female depletes a patch with a number of competing conspecifics. In our study, females searched alone in patches (i.e. without direct competition with conspecifics), and in such a situation self-superparasitism is always considered to be maladaptive (Visser *et al.*, 1990). However, we also observed self- and conspecific superparasitism when non-parasitised hosts were present. Thus, in

A. atomus superparasitism might be related to a lack of host discrimination of parasitized eggs. Self-superparasitism can be adaptive when the host encapsulates the parasitoid eggs. In such a case superparasitism can have a higher pay-off than oviposition in an unparasitized host because encapsulation of the first egg weakens the host defence and consequently the second egg might evade encapsulation (van Alphen *et al.*, 1990). Nothing is known regarding possible egg encapsulation in *A. atomus*. Moreover, in our study superparasitism led to the death of both parasitoid eggs in most of the observed cases. In general, superparasitism often leads to an increase in parasitoid mortality (e.g. Ryan, 1971; Vinson and Iwantsch, 1980). It can cause premature host mortality via the increased burden for the host, i.e. more than one parasitoid, and hence lead to high mortality in parasitoid offspring (van Lenteren, 1981). Cannibalism in hatched parasitoid larvae could be another reason for high offspring mortality. For instance Witsack (1973) reported that only one *A. incarnatus* Haliday adult emerged from superparasitized *Anakelisia fasciata* Kirchbaum (Hom.: Delphacidae) eggs because of cannibalism among parsitoid larvae.

In conclusion, *A. atomus* females can successfully parasitise *E. decipiens* eggs on the tested host plants, indicating that the parasitoid can be successfully released on different host plants of the leafhopper. However, because of the low rate of parasitism, combined releases with other bio-control agents such as enthomopathogenic fungi might be required for successful control of *E. decipiens*. Yet the release rate of *A. atomus* needs to be determined first; too low release rates might lead to insufficient control levels, whereas too high rates of release might result in an increase in superparasitism. High levels of superparasitism, as observed in this study, are possibly due to confining the parasitoid females in a small arena where the encounter rate with parasitized eggs is high, leaving the females no chance to switch to another host patch. Hence, in future studies superparasitim should be assessed by dissecting randomly collected *E. decipiens* eggs from plants previously exposed to *A. atomus* under greenhouse condition. In case similarly high levels of superparasitism as recorded in our experiments would be observed, then *A. atomus* populations should be screened for strains/biotypes that have the ability to avoid superparasitism.

4.1 General discussion

The objective of this research was to assess the influence of host plants of *E. decipiens* on the leafhopper and its parasitoid *A. atomus*. Therefore the acceptability of different host plants for oviposition and feeding of adult *E. decipiens*, and the suitability of these host plants for the performance of the leafhopper, as well as the effects of the different temperatures on various life table parameters of *E. decipiens* were studied. Additionally, the influence of the host plants on the development time and rate of parasitism of *A. atomus* were investigated. Moreover, the host discrimination behaviour of *A. atomus* was examined. The tested host plant species had a significant effect on feeding and oviposition in *E. decipiens*. Leafhoppers showed significant preference for feeding and oviposition on cucumber. In contrast Raupach (1999) reported that *E. decipiens* had no significant feeding preference for eggplant, cucumber, sweet pepper, and tomato. In her study, on tomatoes *E. decipiens* showed a strong non-preference for feeding as well as for oviposition. This might have been due to anti-feeding compounds secreted by the glandular trichoms on the leaf surface of tomatoes; tomatine for example, a secondary compounds of glandular tichoms, act as an anti-feedant for the potato leafhopper *E. fabae* (Raman *et al.,* 1979, cited in Panda and Khush, 1995). Moreover, Koblet-Günthardt (1975) failed to establish a culture of *E. decipiens* on tomatoes.

According to van Lenteren and Noldus (1990) shorter development time and greater total reproduction on a host reflect the suitability of the plant. Based on these criteria broad beans are the most suitable host plants for *E. decipiens*, since the development time of the leafhoppers was significantly shorter on *V. faba* compared to other tested host plant species in this study. These results corroborate earlier findings by Raupach *et al.* (2002) and Koblet-Günthardt (1975), and indicate the superior host plant quality of broad beans for *E. decipiens*. Another reason for the short development time on broad beans could be the fact that our tested leafhopper strain had been mass reared on *V. faba* in the laboratory for more than six years. Fecundity of *E. decipiens* did not differ significantly between 16 and 20°C. In general the level of fecundity was rather low. The reason for this might have been that we offered only leaves as oviposition substrate, whereas *E. decipiens* females usually prefer to lay eggs in the stem of broad beans plants (Agboka, 2002). Egg mortality was low and did not significantly differ between 16 and 20°C.

Larval development time of *E. decipiens* on broad beans was significantly influenced by temperature; the shortest development time was recorded at 24°C. Similar results were obtained by Raupach (1999), though she recorded the total development time for female and male together whereas we recorded it for each larval instar and for each sex separately. Larval mortality was higher, though not significantly, at 16 than at 20 and 24°C, respectively. Raupach (1999) could show that the number of emerged *E. decipiens* adults was not related to the different temperature regimes tested, but reflects the varying initial larval densities in the respective experimental units at the beginning of the experiment, suggesting that temperature has no significant effect on larval survival in *E. decipiens*.

In our study longevity of leafhoppers was significantly shorter at 24°C but did not differ between 16 and 20°C. However, longevity was highly variable at each temperature tested, with a maximum of 267 days at 20°C. In contrast Habib *et al.* (1972) reported that the longevity of male/female *E. decipiens* was 27/55.5, 15.5/38.8, 15.6/31.9 days at 19, 22 and 29°C, respectively. Moreover, in a study by Poos (1932) the longevity of *E. decipiens* did not exceed 148 days. In our experiments, at all temperatures tested female *E. decipiens* lived longer than males, corresponding with results of a study on the beet leafhopper *C. tenellus* (Harries, 1948, cited in DeLong, 1971). *Empoasca decipiens* females started to lay eggs only after a pre-oviposition period, which lasted on average 10, 13 and 22 days at 16, 20 and 24°C, respectively. Habib *et al.* (1972) reported that the pre-oviposition period varied from one to several days, according to climatic conditions. In some leafhoppers species, such as *E. fabae*, the pre-oviposition period lasts from 1.5 to 10 days, but may extend for several months in hibernating adults (DeLong, 1971). The oviposition period of individual females varied from a few days to up to two months at all temperatures tested. However, we recorded no significant differences in oviposition period among the different tested temperature regimes. Likewise, there were no significant differences in fecundity among the different temperatures. In our study the average number of eggs laid, measured as number of emerged larvae, was 27, 39 and 37 larvae/female at 16, 20 and 24°C, respectively, whereas Mueller (1956) reported that a female *E. decipiens* lays up to 100 eggs during her lifetime. The intrinsic rate of increase r_m and the net reproduction rate R_0 was significantly affected by temperatures and was higher at 20°C than that at 16 and 24°C. The reason for this could be that the *E. decipiens* strain used in our study was originally collected near Braunschweig, northern Germany. Due to the shortest development time at 24°C, the generation time was shorter at this temperature than that at the other two tested temperature regimes; however, the doubling time was shorter at 20°C because the intrinsic rate of increase was higher at this temperature.

In our study host plant species of *E. decipiens* did not significantly affect the development time of *A. atomus*. Likewise, fertility of *A. atomus* did not differ between sweet pepper and cucumber, but was lower compared to parasitoids reared on *E. decipiens* eggs on broad beans (Agboka, 2002). The latter might be due to lower number of leafhopper eggs laid in the plant tissue of cucumber and sweet pepper plants than in broad beans. In general *V. faba* is considered to be a superior host plant for *E. decipiens* (Koblet-Guenthardt, 1975). Orr *et al.* (1985) recorded reduced total fecundity of the scelionid egg parasitoid *Telenomus chloropus* Thomson when it parasitized *Nezara viridula* (L.) (Hem.: Pentatomidae) eggs on certain genotypes of soybean. In our study, the rate of parasitism did not differ between both host plant species tested (i.e. sweet pepper and cucumber), and, additionally, did not differ from that previously reported by Agboka (2002) on broad beans. In contrast, Al-Wahaibi and Walker (2000) observed differences in rate of parasitism of *A. nigriventris* on *C. tenellus* on different host plants, indicating that the parasitoid showed a preference for certain host plants. Stiling (1980) found high levels of parasitism for an *Anagrus* sp. associated with three *Eupteryx* leafhopper species on stinging nettle, yet found little or no parasitism on the surrounding weedy vegetation harbouring *Eupteryx* spp. In *Empoasca* spp. it is not possible to clearly define the number of eggs laid in the plant tissue. Hence it is possible that the higher rate of parasitism on broad beans compared to sweet pepper and cucumbers was due to higher egg density on *V. faba*. Yet, we conducted our experiments under no-choice conditions; thus parasitoids were not able to switch to more preferred plants. Consequently in the field the rate of parasitism of *A. atomus* on different host plants of *E. decipiens* might be different than that observed in this study.

The level of parasitism by *A. atomus* did not differ between parasitoids emerging from parasitized *E. decipiens* eggs on broad beans or cucumber. Contrary to that Al-Wahaibi and Walker (2000) recorded higher rates of parasitism by *A. nigriventris* on *C. tenellus* on sugar beets if parasitoids had been reared on this host plant species. In our study, the pre-adult parasitoid mortality was significantly higher when the parasitoid had been reared on broad beans. The higher mortality could have been due to superparasitism, suggesting increased searching and host locating activities of *A. anagrus* reared on broad beans. In general host searching by parasitoids is enhanced on plant species from which they had emerged (Godfray, 1994). Herard *et al.* (1988) hypothesised that plant-derived chemicals are present in the cocoon of the developing parasitoid and that contact with these chemicals at the time of eclosion increase the responsiveness of the adult parasitoid to certain plant odours.

Host discrimination refers to a parasitoid's ability to distinguish between parasitized and non-parasitized hosts (van Lenteren, 1981). In our experiments we detected no marking behaviour of parasitized eggs by *A. atomus*. Hence *A. atomus* is not able to discriminate between parasitized and unparasitized eggs, neither in case of conspecific superparasitism, nor in self-superparasitism, although self- and conspecific superparasitism were less frequent when the females were released one hour after the first oviposition. Whether this observation was caused by the absence of ovipositional markers in *A. atomus*, or whether the markers were, for unknown reasons, not perceived remains to be investigated. However, in 25% of the replicates of the 'one hour-same female' treatment, female *A. atomus* initially probed and subsequently rejected the parasitized egg and oviposited into the unparasitized egg. Once the same or a conspecific female had successfully oviposited into a parasitized or unparasitized egg, the experiment was terminated. Possibly, extending the observation period might have led to superparasitism in cases where the same female after one hour rejected to superparasitize *E. decipiens* eggs because of increasing ovipositional pressure of the female parasitoid.

Croning and Strong (1993) observed that *A. delicatus* Dozier, an egg parasitoid of *Prokelisia marginata* (van Duzee) (Hom.: Delphacidae), did not avoid parasitizing hosts that had been recently parasitized by conspecific wasps; in their study superparasitism was largely a random process, and increased with the ratio of parasitized to unparasitized hosts. On the contrary, Moratorio (1990) reported that *A. mutans* Walker and *A. silwoodensis* Walker avoided a second oviposition in eggs of *Dicrantopis hamata* (Boheman) (Hom.: Delphacidae). In his experiment, the female drilled its ovipositor into the parrasitized host egg and then withdrew it. Such kind of behaviour was also observed in our study, but just in few replicates. Rejection of parasitized hosts after ovipositor insertion has been reported in a number of parasitoid species (Fisher, 1961; van Lenteren, 1976). The probability of superparasitism is strongly influenced by the encounter rate with parasitized hosts. It is often observed in laboratory experiments that parasitoids confined with their hosts will commence to superparasitise once all unparasitised hosts have been attacked (Godfray, 1994). However, in *A. atomus* in the great majority of the observed cases, the female oviposited in the first egg it encountered, no matter whether this egg was non-parasitised or had been previously parasitised by itself or a conspecific female. Long considered as the result of mistakes made by imperfect animals, superparasitism is nowadays recognized as adaptive in a number of different situations (van Alphen *et al.*, 1990). Visser *et al.* (1992) showed with evolutionary stable strategy models that superparasitism is an adaptive strategy for a solitary parasitoid if the female depletes a patch with a number of competing conspecifics. In our study, females searched alone in patches (i.e. without direct competition with conspecifics), and in such a situation self-superparasitism is always considered to be maladaptive (Visser *et al.*, 1990). However, we also observed self- and conspecific superparasitism when non-parasitised hosts were present. Thus, in *A. atomus* superparasitism might be related to a lack of host discrimination of parasitized eggs. Self-superparasitism can be adaptive when the host encapsulates the parasitoid eggs. In such a case superparasitism can have a higher pay-off than oviposition in an unparasitized host because encapsulation of the first egg weakens the host defence and consequently the second egg evade encapsulation (van Alphen *et al.*, 1990). Nothing is known regarding egg encapsulation in *A. atomus*. Moreover, in our study superparasitism led to the death of both parasitoid eggs in most of the observed cases. Very often superparasitism leads to an increase in parasitoid mortality (e.g. Ryan, 1971; Vinson and Iwantsch, 1980). It can cause premature host mortality via the increased burden for the host, i.e. more than one parasitoid, and hence lead to high mortality in parasitoid offspring (van Lenteren, 1981). Cannibalism in hatched parasitoid larvae could be another reason for high offspring mortality. For instance Witsack (1973) reported that only one *A. incarnatus* Haliday adult emerged from superparasitized *Anakelisia fasciata* Kirchbaum (Hom.: Delphacidae) eggs because of cannibalism among parsitoid larvae. In conclusion, *A. atomus* can be successfully released on different host plants of the leafhopper. However, because of the low rate of parasitism, combined releases with other bio-control agents such as enthomopathogenic fungi might be required for successful control of *E. decipiens*. Yet the release rate of *A. atomus* needs to be determined first; too low release rates might lead to insufficient control levels, whereas too high rates of release might result in an increase in superparasitism.

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