Biology and ecology of the predatory mite *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae)

ir. Isabelle Vantornhout

UNIVERSITEIT GENT

FACULTEIT BIO-INGENIEURSWETENSCHAPPEN

Biology and ecology of the predatory mite *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae)

ir. Isabelle Vantornhout

2006

ISBN 90-5989-131-7

Promotors: Prof. dr. ir. Luc Tirry
Department of Crop Protection, Laboratory of Agrozoology
Prof. dr. ir. Patrick De Clercq
Department of Crop Protection, Laboratory of Agrozoology

Dean: Prof. dr. ir. Herman Van Langenhove

Rector: Prof. dr. Paul Van Cauwenberge

ir. Isabelle VANTORNHOUT

BIOLOGY AND ECOLOGY OF THE PREDATORY MITE *IPHISEIUS DEGENERANS* (BERLESE) (ACARI: PHYTOSEIIDAE)

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:
Biologie en ecologie van de roofmijt *Iphiseius degenerans* (Berlese)(Acari: Phytoseiidae)

Please refer to this work as follows:
Vantornhout, I. 2006. Biology and ecology of the predatory mite *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae). PhD thesis, Ghent University, Ghent, Belgium.

ISBN 90-5989-131-7

VOORWOORD

Eindelijk is het zover! Het schrijven van dit voorwoord betekent het einde van een drukke, maar leerrijke periode. Eentje die startte op 5 januari 2000, de dag waarop de eerste populatie roofmijten werd geleverd. Drie weken later waren ze allemaal dood…. Een scenario dat zich, jammer genoeg, nog een aantal keer zou herhalen. Een Amerikaanse onderzoeker schreef me ooit: "Sometimes the animals do not cooperate." Ik heb het mogen ondervinden. Maar het is uiteindelijk allemaal goed gekomen. Naast een heleboel informatie over de biologie en ecologie van het beestje, ben ik onder meer ook te weten gekomen dat deze mijten hoge eisen stellen aan hygiëne, jonge cicaden gebruiken om paard te rijden, en occasioneel gevechten aangaan met schilderspenselen.

Bij de toestandkoming van dit werk heb ik ook kunnen rekenen op de steun en hulp van familie, vrienden en collega's. Hierbij wil ik hen graag bedanken.

Eerst en vooral wil ik mijn promotoren, Prof. dr. ir. Luc Tirry en Prof. dr. ir. Patrick De Clercq, bedanken. Zij hebben me de vrijheid gegeven om dit onderzoek uit te bouwen (en daarbij lokaal A102 om te bouwen). Bij hen kon ik altijd terecht met mijn vragen, zelfs op de drukste momenten. Hun kritische opmerkingen hebben zonder meer bijgedragen tot dit werk en werden door mij zeer gewaardeerd.

Prof. dr. N. De Pauw, Prof. dr. ir. M.-C. Van Labeke, dr. A. Minks, Prof. dr. F. Jacobs, dr. P. Grootaert en lic. G. Sterk wil ik danken voor hun bereidheid te zetelen in de lees- en examencommissie en voor hun suggesties en opmerkingen.

De (ex)-collega's van het laboratorium voor agrozoölogie verdienen zeker een bloemetje (zonder beestjes, uiteraard). Ik wil hen hierbij bedanken voor hun interesse, hun steun, de plantentips, de technische ondersteuning en vooral voor de aangename sfeer op het labo. Die vijf jaar en tien maanden zijn voorbij gevlogen.
Een vermelding verdienen ook de thesisstudenten op het labo die allen hebben bijgedragen tot dit werk: Hilde Leen, Kjell, Jan en Rebekka.

Ik mag de collega's op het "decanaat sensu largo" zeker niet vergeten. Bedankt voor de interesse, het goed *soigneren* van mijn dossier en de oppeptalk op maandagmorgen !

Verder wil ik ook al mijn vrienden bedanken voor hun steun en de nodige ontspanningsmomenten. Een speciaal woordje zou ik willen richten tot de vrienden uit de "P37": Diederik en Joeri. "*London, here we come*!".

En tot slotte, maar daarom niet in het minst, wil ik mijn ouders bedanken voor grote niet-aflatende steun de voorbije jaren. Bedankt lieve ouders, bedankt voor alles !

Isabelle Vantornhout
Gent, september 2006.

TABLE OF CONTENTS

It is difficult, if not impossible, to assess the potential of a predator as a biocontrol agent without knowledge of its feeding habit and developmental biology.

Momen, 1969

CHAPTER 1
INTRODUCTION, OBJECTIVES AND THESIS OUTLINE

1.1 INTRODUCTION

Biological control is considered as a key component of sustainable integrated pest management strategies that pursue reductions in the use of chemical pesticides. It is defined as the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making it less abundant and thus less damaging that it would otherwise be (Van Driesche and Bellows, 1996). Insects and mites belonging to different families have been frequently used as biological control agents of arthropods and molluscs (an overview is given by Van Driesche and Bellows (1996)).

The subject of this study, *Iphiseius degenerans* (Berlese) (Fig. 1.1), is a predatory mite belonging to the family Phytoseiidae. Phytoseiid mites are economically important predators of phytophagous mites and insects in greenhouse crops. Mass reared phytoseiid mites are commercially available and used, amongst others, against spider mite and thrips infestations in greenhouse crops (e.g., *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* McGregor, *N. cucumeris* (Oudemans). *Iphiseius degenerans* is used commercially in Belgium for thrips control in greenhouse crops since 1994.

Figure 1.1. Female and male *I. degenerans.*

Effectiveness of a predator in biological control depends on factors including the ability to develop to the adult stage using the host as food source, climatic adaptation, the lack of negative effects on other beneficials present in the same environment, a good rearing method, a high kill rate (high intrinsic rate of increase) and a good searching efficiency (van Lenteren and Woets, 1988). However, being a commercially available predatory mite, little information on the biology of *I. degenerans* is available in the literature.

1.2 OBJECTIVES OF THIS STUDY

Thorough knowledge on the biology of a predator is essential for its practical use. The objective of this study was to investigate some fundamental aspects of the biology of the predatory mite *I. degenerans.*

The research questions are:

1. What is the impact of food on the development, longevity and life table parameters of *I. degenerans*?
2. What is the maximum predation rate of *I. degenerans* on different natural prey species?
3. Does *I. degenerans* show a preference for a particular food source?
4. Is there an olfactory response involved when the predatory mite searches for prey?

1.3 THESIS OUTLINE

The purpose of the literature survey in **chapter 2** is to provide an overview of the information available on the predatory mite *I. degenerans*. The focus of this survey is on morphology, bionomics, predatory behaviour and practical use of *I. degenerans*.

The next three chapters explore some life history traits of the predatory mite. **Chapter 3** provides information on the egg size and body size of all life stages of the phytoseiid mite. This information is useful to distinguish the stages and this is needed in later

experiments. Further, in this chapter the mating behaviour of *I. degenerans* is described. **Chapter 4** examines the relationship between both diet and substrate, and the developmental biology of *I. degenerans*. We studied the possibility of the predatory mite to complete development to adulthood on five pollen species (pollen of almond, apple, castor bean, plum and sweet pepper pollen), four natural prey species (twospotted spider mite *Tetranychus urticae* Koch, western flower thrips *Frankliniella occidentalis* (Pergande), greenhouse whitefly *Trialeurodes vaporariorum* Westwood and green peach aphid *Myzus persicae* (Sulzer), a combination of *F. occidentalis* nymphs and castor bean pollen, and finally, two factitious prey species (Mediterranean flour moth *Ephestia kuehniella* Zeller and brine shrimp *Artemia franciscana* Kellogg). Subsequently, the influence of different substrates (artificial versus leaf arena) on the developmental performance was assessed. Life table parameters were studied in **chapter 5**. Fecundity, longevity and intrinsic rate of increase r_m were determined when the predatory mite was fed castor bean pollen, all life stages of *T. urticae*, *T. vaporariorum* eggs, *F. occidentalis* nymphs and *E. kuehniella* eggs. The VBA-macro written to calculate Jackknife values of r_m is described in appendix I.

Predation and prey preference of *I. degenerans* were analysed with respect to some economically important greenhouse pests, *F. occidentalis*, *T. vaporariorum*, and *T. urticae*. Three types of experiments were carried out: functional response tests, two choice preference tests and olfactometer tests. In **chapter 6** the functional response was quantified by measuring the prey consumed by adult females, which had been starved for 4 h, when different densities of eggs of *T. vaporariorum* or *T. urticae*, adult females of *T. urticae*, and first or second instars of *F. occidentalis* were offered. Appendix II describes the program code used to analyse the functional response data. The study in **chapter 7** was designed to evaluate prey selection in *I. degenerans*. Adult females were presented with varying ratios of two prey types (first or second instars of *F. occidentalis*, *T. vaporariorum* eggs or *T. urticae* eggs). The observed preference was discussed in relation to the predicted preference based on the individual functional response experiments. The influence of pollen on the predation rate of female predatory mites was also studied. Finally, in **chapter 8** the olfactory response of the predatory mites was investigated. Y-tube olfactometer experiments were carried out to investigate

the response of starved *I. degenerans* females towards odours emitted by clean leaves, pollen, *T. urticae* infested bean leaves, *F. occidentalis* infested bean leaves, and *T. vaporariorum* infested bean leaves.

The last chapter **(chapter 9)** presents a conclusion on the predatory abilities of *I. degenerans* as a biological control agent of greenhouse pests based on the results from the abovementioned studies.

CHAPTER 2
IPHISEIUS DEGENERANS (BERLESE): A LITERATURE REVIEW

2.1 INTRODUCTION

Iphiseius degenerans is a member of the family Phytoseiidae, which belongs to the order Mesostigmata (Gamasida). This species was first described as *Seius degenerans* Berlese, 1889, but was transferred to the genus *Iphiseius* by Berlese (1921). This genus is considered a problem taxon and has received a lot of attention of taxonomists. An overview of the taxonomic history of the genus and its species is given in Hansell and Chant (1973). Recently, Chant and McMurtry (2005) proposed *I. degenerans* as senior synonym for *Iphiseius martigellus* (El-Badry).

A survey of the literature revealed that about 130 studies on *I. degenerans* have been published since 1889. This is a relatively low number compared to the number of published records on other commercialized phytoseiid mites (e.g., *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus cucumeris* (Oudemans), *Amblyseius californicus* (McGregor)).

This chapter is intended to give an overview of the literature and to find the gaps in the knowledge already available on the predatory mite *I. degenerans*. The morphology, bionomics, predatory behaviour and practical use of *I. degenerans* will be discussed.

2.2 MORPHOLOGY

Phytoseiid mites are small, 300 to 600 µm in length, and whitish to brown in colour. The body of an adult phytoseiid mite is divided into two major regions, the gnathosoma bearing the mouthparts and the idiosoma bearing the legs. The idiosoma is covered by a dorsal shield and bears ventrally a number of shields. Setae, which are useful for classification, are present on both ventral and dorsal surfaces. Adult males usually are

smaller than the females. Males also have a different sclerotization of the ventral surface, possess sexual organs on the chelicerae, and usually have the sublateral setae inserted on the dorsal shield.

Iphiseius degenerans is a dark brown mite with a female basic body weight of 17.56 ± 0.27µg (Yao and Chant, 1990). The difference between the external anatomy of adult females and males is described in more detail in the next paragraphs. No detailed description of the immature stages was found in the literature.

2.2.1 Females

The idiosoma of a female *I. degenerans* is dorsally and laterally covered with a shield and has ventrally a number of smaller shields: a sternal, genital, ventral and an anal shield (Fig. 2.1 and 2.2).

The shield covering the dorsal and lateral surfaces is heavily sclerotized and is composed of two distinct parts: a dorsal shield and a marginal shield (Fig. 2.1).

The broadly ovate dorsal shield (length 430 - 470 µm and width 330 - 340 µm) is relatively smooth (Van der Merwe, 1968). It has 11 pairs of pores and 17 pairs of simple setae. These setae are arranged into three longitudinal rows: a dorsal series of six pairs, a median series of two pairs and a lateral series of nine pairs (4 pairs of prolateral and 5 pairs of postlateral setae) (Van der Merwe, 1968, Elbadry, 1970). The nomenclature of the setae used in the description of the predatory mite differs among authors (Fig. 2.1) (Evans, 1954; Rowell and Chant, 1979). The vertical setae D1 (j1) (30 - 34 µm) and L9 (Z5) (17 - 21 µm) are considerably longer than the others (5 – 8 µm).

In larvae and in protonymphs two distinct dorsal shields are present, but these fuse during the moult from protonymph to deutonymph (Rowell and Chant, 1978). According to Rowell and Chant (1978) not all dorsal setae are present in the larval stage. In the protonymphal stage Z1, Z5 and S5 are present as observable setae, but are only present as incipient setal nubs in the larva. Setae Z4 are homologous with the whip-like setae in the larvae.

The marginal shield is less heavily sclerotized, striated and surrounds the dorsal shield except in the region of the vertical setae. Two pairs of simple, minute setae (Mg1 and Mg2 or r3 and R1) are present on this shield (Elbadry, 1970).
The sternal shield is 70 - 76 µm long and 74-80 µm wide (Van der Merwe, 1968) and bears two pairs of pores and three pairs of simple setae (Fig. 2.2). A fourth pair of setae (the metasternal setae) is placed on small metasternal shields, in line with the middle of coxae III. The metasternal setae are added during the moult from protonymph to deutonymph (Rowell and Chant, 1978).

The anterior margin of the sternal shield is truncated and the posterior margin is provided medially with a distinct forked process extending to the level of the metasternal setae. The endopodals of coxae II are fused with the sternal shield forming its lateral margins and the conspicuous antero-lateral processes directed between coxae I and II. The remaining endopodals are weak and, with the exception of the posterior section to those bordering coxae IV, are difficult to detect.

The genital shield (width 110 - 118 µm) is wedge-shaped, the posterior margin being slightly convex. The one pair of genital setae is added during the moult from protonymph to deutonymph (Rowell and Chant, 1978). The transparent anterior part of the shield extends beyond the posterior margin of the sternal shield. The remaining sclerotized shield posterior to the genital shield is the ventrianal plate. This plate is fragmented into the ventral and the anal plate (Van der Merwe, 1963). The ventral shield is broadly rectangular, measuring 20 - 30 µm in length and 75 - 82 µm in width (Van der Merwe, 1968) and has three pairs of setae and a pair of large pores (Elbadry, 1970). This pair of pores is located posteriorly to this shield and caudally to the inner posterior pair of setae. The anal shield is 70 - 75 µm long and 76 - 80 µm wide. This shield bears three setae: the paired para-anal and the post-anal setae. Its anterior margin is excavated.

The ventral interscutal membrane in the region of the genital, ventral and anal shield is provided with four conspicuous pores and four pairs of simple setae. Setae JV4, ZV1 and ZV3 are added during the moult from protonymph to deutonymph (Rowell and Chant, 1978). The caudal pair (JV5) is of moderate length, 23 - 28 µm.

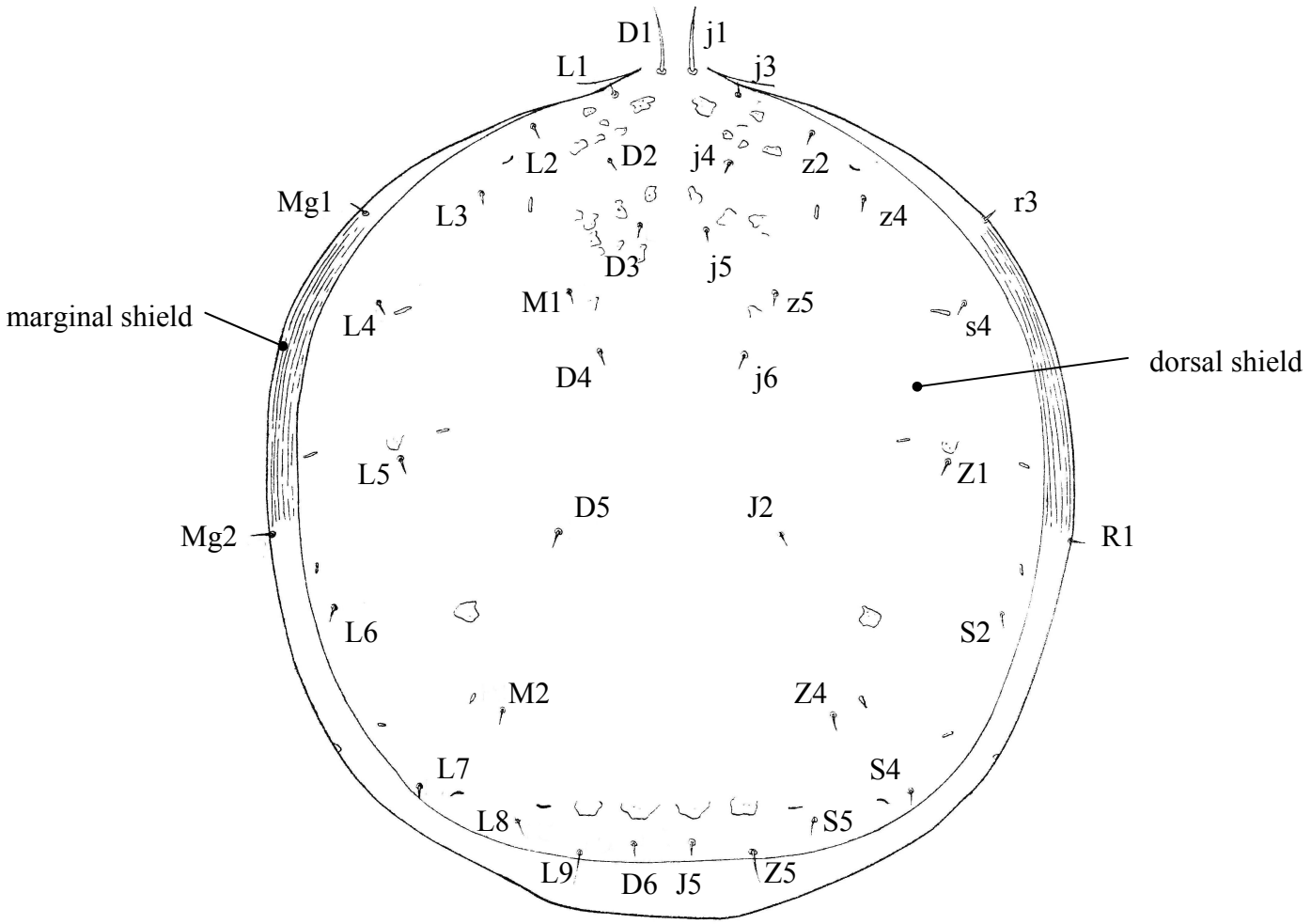


Figure 2.1. Dorsal and marginal shield of a female adult of *Iphiseius degenerans* (Evans, 1954).

Setal nomenclature according to Evans (1954): D, M, L, Mg (left), and to Rowell and Chant (1979): j, J, z, Z, s, S (right).

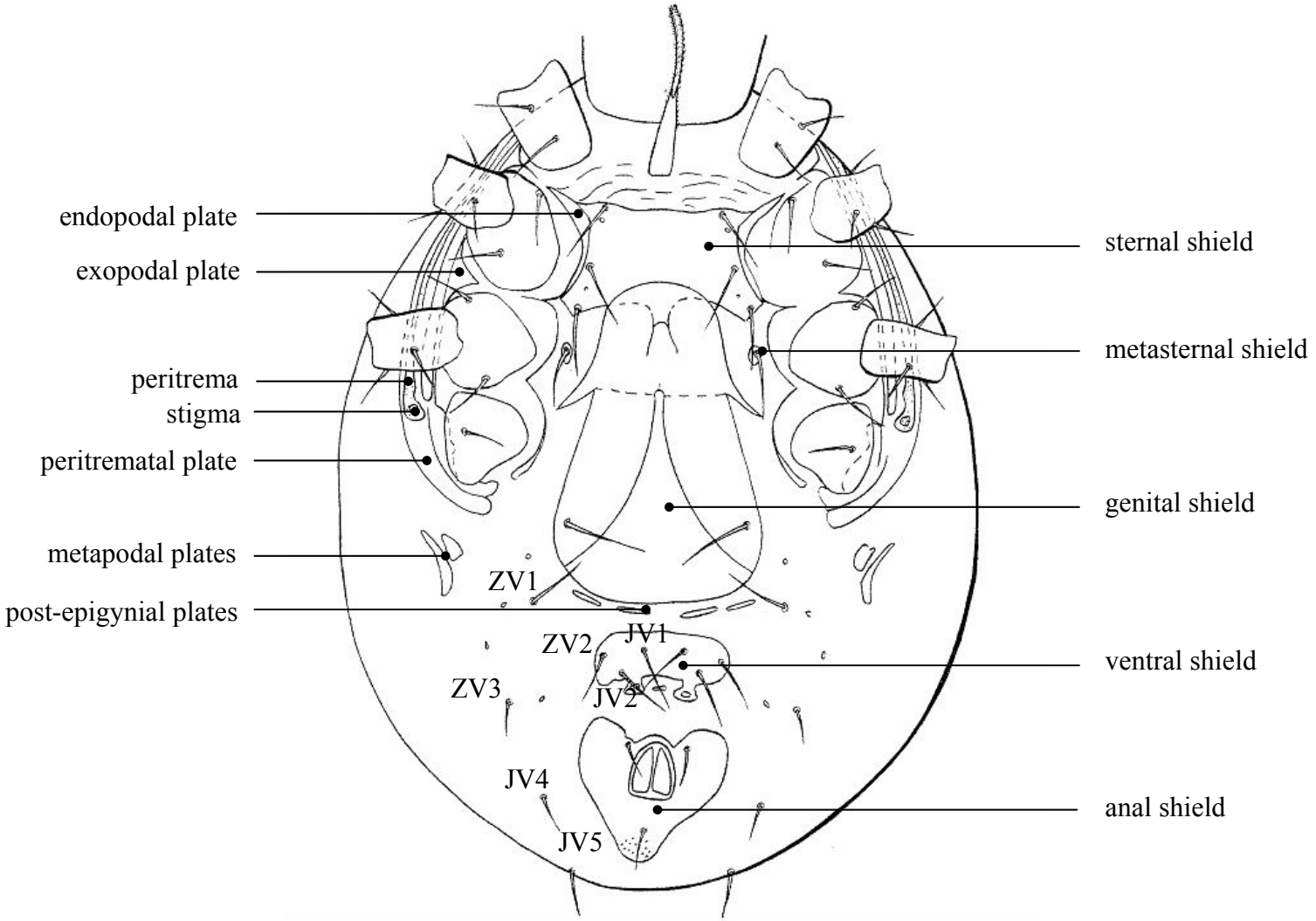


Figure 2.2. Venter of a female adult of *Iphiseius degenerans* (Evans, 1954).

On this membrane two pairs of metapodal plates, a pair of platelets lying on each side of the genital shield, are present (Elbadry, 1970). Posterior to the genital shield, a row of four (five) small-scattered plates, named the post-epigynial plates (Evans, 1954) is present.

The stigma is situated ventro-laterally in the region of the fourth intercoxal space. The peritreme is long and reaches almost as far as setae D1 (j1) on the dorsal shield. The peritrematal plate is fused posteriorly with the exopodal plate and extends a short distance around the posterior margin of coxa IV. This peritrematal-exopodal plate is not fused with the endopodal plate. Anteriorly, the peritrematal plate forms a complete chitinized band between the gnathosoma and the anterior margin of the dorsal shield. It is fused with the latter in the region of vertical setae.

The gnathosoma comprises a pair of pedipalps, a pair of chelicerae and a pair of stylets. Ventrally, the gnathosoma bears four pairs of setae. *Iphiseius degenerans* has a wide deutosternal groove (ca. 7 – 9 μm); this groove has seven rows of two denticles per row (Fig. 2.3a). The corniculi are strong and pointed distally. The epistome is weakly sclerotized and its anterior margin is smooth.

The pedipalps are composed of five free segments with the chaetotactic formula (2-3-6-14-16). The specialized setae on the palptarsus are two-pronged.

The general shape of the chelicerae is broadly triangular and blunt (Flechtmann, 1992b, Fig. 2.3b, c).

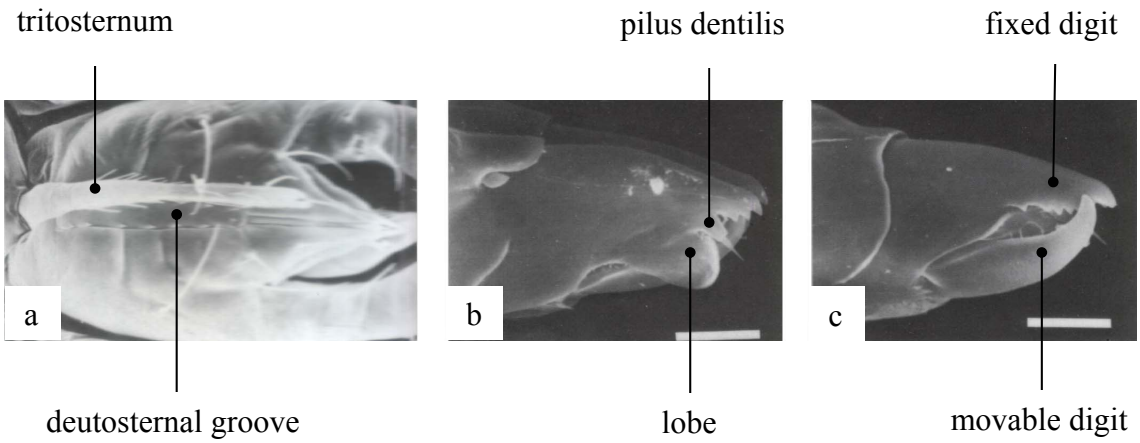


Figure 2.3. Gnathosoma of a female *Iphiseius degenerans*. a. Ventral side, b. Abaxial aspect of the chelicerae, c. paraxial aspect of the chelicerae (Flechtmann and McMurtry, 1992b).

The fixed digit of the chelicera is 28 - 30 µm long (Van der Merwe, 1968) and has 6 or 7 small teeth and a strong pilus dentilis on the inner half of its margin. An additional tooth is located proximal to the pilus dentilis. The fixed digit also bears a broadly rounded flangelike expansion (called a "lobe") that extends to the pilus dentilis (Flechtmann, 1992b). This lobe is produced into a spoonlike structure on the paraxial face of the digit. The movable digit is 28 - 30 µm long and bears a single tooth on its inner margin.

Adult *I. degenerans* have 4 pairs of long and slender legs. The first legs are 424 ± 2 µm long (Takafuji and Chant, 1976). Each leg has seven primary segments (coxa, trochanter, femur, genu, tibia, basitarsus and tarsus). All tarsi terminate in a praetarsus bearing a pulvillus and two claws. The genu, tibia and basitarsus of leg IV are each provided with a macroseta that is slightly swollen distally. Van der Merwe (1968) claims that these knobbed macrosetae are also present on genu II, genu III and on basitarsus IV. The remainder of the setation is composed of simple needle-like setae. The ontogenetic development of the leg setation has been described by Rowell and Chant (1978). The chaetotaxy of leg I, II and III is constant for the larval and protonymphal stages. Leg IV is not present in the larval stage and is added during the moult to protonymph. During the moult to deutonymph setae are added to the legs.

2.2.2 Males

The dorsal shield (353 – 376 µm long and 282 – 294 µm wide) of a male has nineteen pairs of setae: seta D1 (j1) is 28 - 31 µm long and seta L9 (Z5) 15 - 19 µm; the other setae are minute. The marginal shield is reduced to a narrow band extending posteriorly from the fusion point of the dorsal shield and the peritrematal plate. The setae-bearing portion of the marginal shield is now fused with the dorsal shield so that setae S1 (r3) and S2 (R1) are on the dorsal shield (Van der Merwe, 1968).

The sternal, metasternal and genital shields are fused to form a genitosternal shield, which extends from the anterior margin of coxae II to the middle of coxae IV. This shield bears five pairs of setae and three pairs of pores (Fig. 2.4). The male genital opening is situated on its anterior margin. The endopodals are strongly formed

throughout their length and are fused with the lateral margin of the genitosternal shield. They are produced into distinct processes directed towards the exopodal process I and the fused peritrematal-exopodal process surrounding the posterior margin of coxa IV (Evans, 1954).

The region posterior to coxae IV is almost entirely occupied by a large ventral shield and a smaller anal shield. The ventral shield measures 68 - 73 μm in length and 188 - 195 μm in width. It is strongly reticulated and provided with three pairs of setae situated in the posterior half of the shield. Four pairs of distinct pores are also present.

The anal shield, 49 - 53 μm long and 65 - 69 μm wide, bears three setae (Evans, 1954; Van der Merwe, 1968).

The stigma is situated ventro-laterally in the region of the fourth coxal space and the peritreme extends beyond the level of coxa I. The posterior portion of the peritrematal plate is fused with the exopodal and the ventral plates. Anteriorly it is strongly fused with the dorsal shield. The exopodal plate is similar in structure to that in the female and is not fused anteriorly with the peritrematal plate (Evans, 1954).The gnathosoma and pedipalps are essentially similar to those in the female. The fixed digit (24 μm long) has 4 or 5 small closely set teeth and a large pilus dentilis. The movable digit of the chelicerae is unidentated and is provided with a strong spermatophoral process, which is bent slightly ventrad and bilobed.

Legs are similar in shape as those of the females (Evans, 1954; Van der Merwe, 1968).

Phytoseiid mites, at this moment, are mainly identified based on their morphological characteristics. But because of their small size, identification is often difficult and requires a skilled taxonomist. According to Jeyaprakash and Hoy (2002) molecular identification could enhance the ability of researchers to identify phytoseiids encountered in the field and used in their studies. To distinguish between six commercially available phytoseiids among which *I. degenerans*, these authors investigated the mitochondrial 12S rRNA sequences of the predatory mites. These sequences were then used to design a “molecular ladder assay” that amplifies a diagnostically different sized DNA band from the phytoseiids using species-specific primers from the variable regions of the mitochondrial 12S rRNA gene.

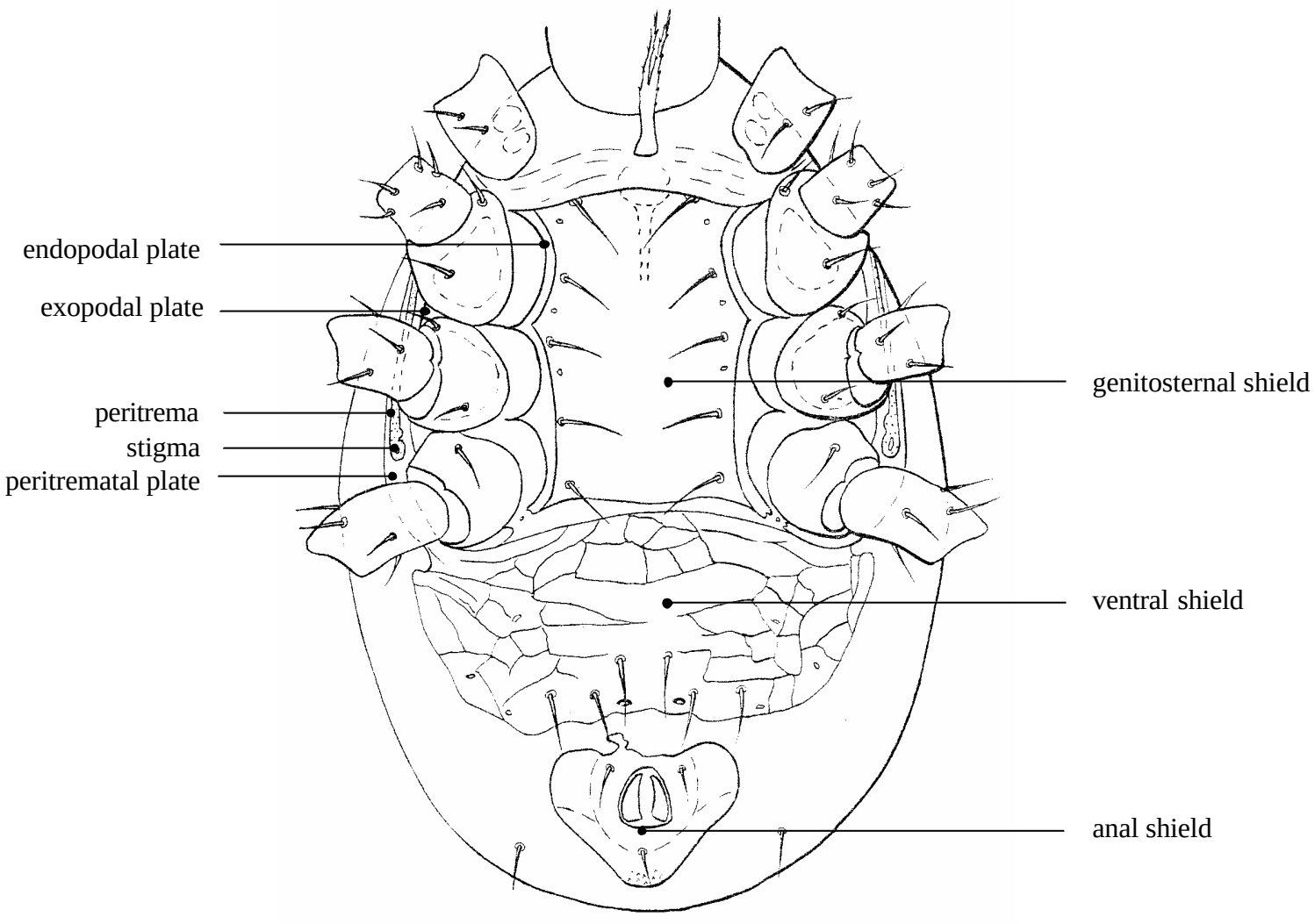


Figure 2.4. Venter of a male adult of *Iphiseius degenerans* (Evans, 1954).

2.3 DISTRIBUTION

Iphiseius degenerans occurs in different regions of Europe and Africa, and is also found in Asia (Fig. 2.5). Table 2.1 gives on overview of its natural distribution (country, plant species). Information on geographical distribution is based on surveys, carried out to characterize mites living in different regions and countries on different plant species.

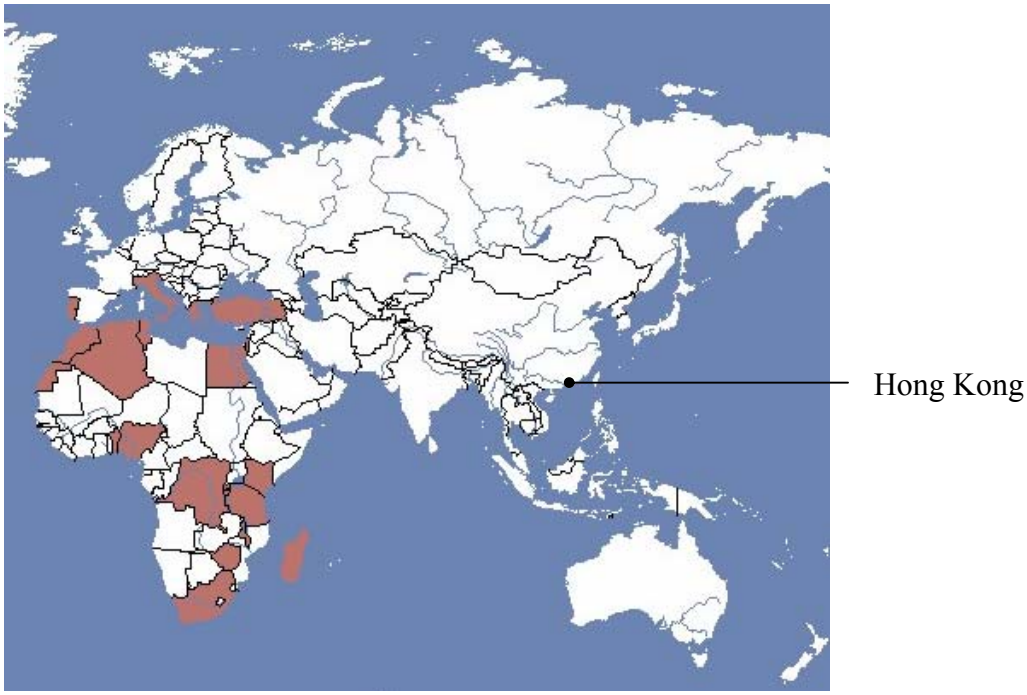


Figure 2.5. Natural distribution of *I. degenerans* in Europe, Africa and Asia.

Table 2.1. Geographic distribution of *I. degenerans*

Country	Plant species	Reference
Algeria	*Rubus ulmifolius* Schott	Athias-Henriot, 1957
Benin	*Manihot glaziovii* Müll.	de Moraes *et al.*, 1989
Burundi		de Moraes *et al.*, 1989
Canary Islands	*Ricinus communis* L.	Pande *et al.*, 1989
Cape Verde Islands	*Saccharum officinarum* L., *Trichilia emetica* Vahl., *Vitis vinifera* L., *Dolichos lablab* L.	Ueckermann, 1992
China (Hong Kong)	*Citrus* sp.	Swirski and Schechter, 1961
Egypt	*Citrus* sp.	El Badry, 1970
Greece	*Citrus* sp.	McMurtry, 1977; Papaioannou-Souliotis *et al.*, 1997
Israel	*Citrus* sp., *R. communis*, *Solanum vilosum* (L.) Mill., *Ficus sycomorus* L., *Psidium guajava* L., *Narcissus* sp.	Swirski and Amitai, 1961, 1984, 1990; Porath and Swirski, 1965; Rubin *et al.*, 1996; Palevsky *et al.*, 2003
Italy	leaves and moss, *Citrus* sp.	Berlese, 1892; McMurtry, 1977
Kenya	*Manihot esculenta* Crantz, *Carica papaya* L., *Albizzia alba*	Swirski and Ragusa, 1978; Skovgård *et al.*, 1993
Madagascar	*Fraxinus berlandieriana* DC., *Hibiscus rosa-sinensis* L., *Citrus limon* L., *C. papaya*, *Coffea arabica* L.	Blommers, 1976

Table 2.1. Geographic distribution of *I. degenerans* (continued)

Madeira	*Prunus domestica* L.	Carmona, 1962
Malawi	*C. papaya*	Munthali, 1987; De Moraes *et al.*, 1989; Zannou *et al.,* 2005
Morocco	*Citrus* sp.	McMurtry and Bounfour, 1989
Nigeria	Herbs	De Moraes *et al.*, 1989
Rwanda	*Musa* sp., *Citrus* sp., *Pennisetum purpureum* Schumacher, *Annona cherimola* P. Mill.	Pritchard and Baker, 1962
Sicily	*Citrus* sp., *Erythrina* sp., *Acanthus* sp.	Ragusa, 1986; Benfatto and Vacante, 1988; Ragusa and Tsolakis, 1995; Conti *et al.*, 2001;
South Africa	*Psidium guajava* L., *Hibiscus tiliaceus* L., *C. papaya*, *Gossypium* sp., *Morus* sp., *Sclerocarya* sp., *Erythrina caffra* Thunb., *Heeria paniculosa* (Sond.) Kuntze, *Jacaranda* sp., *Canna* sp., *Citrus* sp.	Van der Merwe, 1968; Catling, 1970; van den Berg, 1987;
Tanganyika (Tanzania)	*Coffea* sp.	Evans, 1954
Tunisia		Kreiter *et al.*, 2005
Turkey	*Citrus* sp.	Düzgünes, 1963
Zaïre	*Berlinia* sp., weeds	Pritchard and Baker, 1962; de Moraes *et al.*, 1989
Zimbabwe	*M. esculenta*, *Conyza sumatrensis* (Retz.) E. Walker, *Bidens pilosa* L.	Northcraft, 1987a,b

2.4 BIONOMICS

2.4.1 Development

2.4.1.1 Developmental stages

The life cycle of *I. degenerans* comprises five developmental stages: the egg, larva, proto- and deutonymph, and the adult stage.

2.4.1.2 Crucial factors for development and survival of immature stages

All mobile instars of *I. degenerans* feed, although feeding is not obligatory in all stages. The larvae feed facultatively and hence do not need food to moult into protonymphs but often will feed if food is available (Chittenden and Saito, 2001). Larvae have been observed feeding on protonymphs of *Tetranychus pacificus* McGregor (Takafuji and Chant, 1976).

Several conditions influence the development of immature stages including the availability and the kind of food, the presence of other predators in the environment, the oviposition strategy of female predators and climatic factors.

To continue development beyond the protonymphal stage, food is required. Takafuji and Chant (1976) reported that in absence of prey, protonymphs died within 1.5 days after moulting. de Courcy Williams *et al.* (2002, 2004b) studied the effect of the combination of food (all life stages of *Tetranychus urticae* Koch) and water availability on immature stages of different phytoseiid mites. When only water was available, *I. degenerans* immatures survived for 3.6 days; this is half of the developmental period required when food is also available (*i.e.*, 7 days). When deprived of food and water, the predator's survival time decreased to less than 2 days.

Various authors have assessed the developmental times for *I. degenerans*, in function of the type of food offered. These values are presented in Table 2.2. Food sources of plant origin, such as leaf tissue and extrafloral nectar of *Ricinus communis* L. are not sufficient to solely support development of the predator (van Rijn and Tanigoshi, 1999a,

1999b). On a diet of only pollen, however, *I. degenerans* is able to complete development to the adult stage. Literature reports on developmental times from egg to adult range from 5.2 to 8 days (Table 2.2).

Not every prey species and/or prey stage is suitable for complete development. Several authors tested numerous arthropod species as potential prey for *I. degenerans*. Sengonca and Drescher (2001) reported that there was no development on a diet of *Thrips tabaci* Lindeman nymphs. Ragusa and Tsolakis (1995) concluded the same when eggs and crawlers of the mealybug *Phenacoccus madeirensis* Green were offered as food. On a diet consisting of all stages of the tarsonemid mite *Polyphagotarsonemus latus* (Banks), *I. degenerans* completed development in 9 days. However, the tarsonemid mite is considered unfavourable food for the predatory mite since there was only 5% survival (McMurtry *et al.*, 1984). Although larvae of *I. degenerans* do not need food to survive to the protonymphal stage, Blaeser *et al.* (2002) reported a total developmental time of approximately 12 days, but also found a mortality of 25% in the larval stage when fed adult *T. urticae*. No explanation for this was given by the authors. Eggs of *T. pacificus* alone do not meet the requirements for normal development as the predator failed to develop beyond the protonymphal stage (Takafuji and Chant, 1976).

The duration of the immature stages depends also on food availability. The developmental times of the protonymphal and deutonymphal stages of *I. degenerans* decreased rapidly with increasing density of *T. pacificus* protonymphs, to finally reach a constant value (Takafuji and Chant, 1976, Eveleigh and Chant, 1981b). When offered 25 *T. pacificus* protonymphs per day, *I. degenerans* reached adulthood in 5.66 days (Takafuji and Chant, 1976). McMurtry (1977) reported a total developmental time of 8 days when the predatory mites were fed eggs and larvae of *T. pacificus*. *Iphiseius degenerans* needed 6.7 days to complete development when offered all stages of *T. urticae* (van Rijn and Tanigoshi, 1999a). Eveleigh and Chant (1981b) claim that *I. degenerans* protonymphs need 3 to 4 *T. pacificus* protonymphs a day to develop to the deutonymphal stadium and more than seven prey for maximum growth rate (minimum developmental time); deutonymphs need 4 to 5 prey for 100% survival and more than 9 prey for maximum growth rate. These needs are reflected in the higher number of prey killed by the deutonymphs. Underfed predators tend to become cannibalistic (Eveleigh and Chant, 1982d).

The study by Eveleigh and Chant (1982d) further revealed that the survival of *I. degenerans* nymphs was negatively affected by an increasing predator density, even when the number of prey killed per predator increased or remained unchanged. de Courcy-Williams *et al.* (2002) found that survival was high when immatures of the same species were allowed to interact with each other and there was little evidence of negative intraspecific interactions when food was available.

The survival of the juvenile predators is also affected by the oviposition strategy of the females (see also 2.4.2.1). Eveleigh and Chant (1982a) observed that *I. degenerans* females are unable to discriminate between patches of prey and hence do not distribute its progeny in relation to the distribution of prey. Females even oviposited in areas where prey was absent. In this way, the area over which prey and predator interact is reduced, and this affects the survival of the progeny. The survival will be highly dependent on the dispersal capacities of the immatures, which are more limited than those of the adults.

Humidity is also crucial for survival. The hatchability of predator eggs, which are the most vulnerable stage, strongly depends on of the relative humidity. Below 30% RH at 20 °C no eggs hatched whereas all eggs hatched at 82% RH. The egg stage duration was not significantly affected by humidity over the range of 60 - 82% RH (de Courcy Williams, 2004a). van Houten *et al.* (1993, 1995a) reported that the vapour pressure deficit at which 50% of the eggs hatch is 1.48 ± 0.21 kPa (corresponding with a relative humidity of 53 %). This is in accordance with the value of 56% found by de Courcy Williams *et al.* (2004a).

The effect of temperature on the development of the immature stages of *I. degenerans* when fed *T. urticae* was studied by Tsoukanas *et al.* (2006). Developmental thresholds for eggs, protonymphs, and deutonymphs were approximately the same (11.71, 10.11, and 11.28 °C, respectively) whereas that of the larval stage was found to be lower (7 °C) at 60% RH and a photoperiod of 16L:8D h.

Table 2.2 Developmental durations of the different stages of *I. degenerans* reported in the literature

Type of food	Duration (days)					Reference
	egg	larva	protonymph	deutonymph	egg-to-adult	
Pollen						
Hymenocyclus croceus					8	McMurtry, J.A., 1977
Maleophora crocea					6 - 7	McMurtry, J.A. *et al.*, 1984
Ricinus communis	1.92	0.88	1.10	1.33	5.2	van Rijn and Tanigoshi, 1999a
Vicia faba	2.06	0.88	1.14	1.12	5.2	van Rijn and Tanigoshi, 1999a
Arthropod prey						
Polyphagotarsonemus latus (all stages)					9	McMurtry, J.A. *et al.*, 1984
Tetranychus pacificus eggs and larvae					8	McMurtry, J.A., 1977
Tetranychus pacificus protonymphs	2.23 ± 0.63	0.96 ± 0.02	1.15 ± 0.01	1.32 ± 0.02	5.66 ± 0.03	Takafuji and Chant, 1976
Tetranychus urticae (all stages)	1.93	0.99	1.87	1.92	6.7	van Rijn and Tanigoshi, 1999a
Tetranychus urticae adults	ca. 3	ca. 3	ca. 6		ca. 12	Blaeser *et al.*, 2002
Arthropod prey and pollen						
Tetranychus neocaledonicus + *Bauchinia* sp. pollen or *Aloe chabaudii* pollen	7.3 (egg to deutonymph)					Blommers, 1976

2.4.2 Reproduction

2.4.2.1 Oviposition behaviour

The oviposition behaviour of *I. degenerans* is very complex and has been elucidated by Faraji *et al.* (2000, 2001, 2002a,b). In a sweet pepper crop, females of *I. degenerans* are usually found in flowers, where they feed on pollen and thrips nymphs (van Houten and van Stratum, 1995). Despite visiting flowers, females prefer to lay eggs on leaves. Eggs are laid in clusters inside domatia, which are parts of plants that have been modified to provide shelter to e.g., insects and mites (e.g., tiny pockets and hair tufts on the lower side of leaves). *Iphiseius degenerans* is not only capable to discriminate between eggs of interspecifics but also between closely and distantly related conspecifics, and prefers to add eggs to clusters of their own or closely related species (Faraji *et al.*, 2000). This oviposition behaviour is likely to be an adaptation to resist egg predation by other arthropods, including its thrips prey. The predatory mite avoids ovipositing at places where the risk of predation is high. Moreover, the predator uses the chemical cues of thrips to assess the predation risk, and consequently oviposits away from risky places. In addition, in domatia, eggs are probably less vulnerable to desiccation (Faraji *et al.*, 2001, 2002a,b).
Nevertheless, when eggs are laid close together sib-cannibalism can occur. This can be avoided in part by behavioural mechanisms such as variation in larval feeding behaviour, *i.e.*, non-feeding *I. degenerans* larvae (Chittenden and Saito, 2001). Faraji *et al.* (2002b) observed that newly hatched larvae leave the cluster and look for shelter in an unoccupied domatium.

When *I. degenerans* was subjected to short-day conditions (L10 (23 °C):D14 (16 °C)), no reproductive diapause was observed (van Houten *et al.*, 1993, 1995a). Wysoki and Swirski (1971) found active “post embryonic” stages on aboveground plants even in winter in Israel. Palevsky *et al.* (2003) also found *I. degenerans* the whole year round in Israeli citrus orchards. Also in winter, Ragusa (1986) found males and young stages in citrus orchards. He suggests that in Sicily this species is active the whole year round.

2.4.2.2 Female life span and fecundity

The mean longevity of adult females at 25 °C was 53 days when *I. degenerans* were provided with *T. pacificus*. Maximum longevity was obtained when 4 *T. pacificus* protonymphs were consumed by the predator per day (Takafuji and Chant, 1976). Diverse values are reported when the predator is fed with the twospotted spider mite *T. urticae*. The shortest lifespan is reported by Blaeser and Sengonca (2002); when fed 5 adult spider mites/day the predator lived 11.1 days. de Courcy Williams (2002, 2004a) reported a mean life span of 27.8 days when the predator was continuously fed a mixture of all life stages of *T. urticae*. These values are very low compared to the median life span (52.7 days) found by van Rijn and Tanigoshi (1999a) on a diet consisting of all stages of *T. urticae*. Sengonca and Drescher (2001) found that longevity was reduced with 50% when the phytoseiid was offered *T. tabaci* second instars (ca. 7.5 days) instead of *T. urticae* (15 days), possibly due to a lower nutritional value of the former prey. According to Blaeser and Sengonca (2002) there are no significant differences in female life span when the predator is offered *F. occidentalis* nymphs or *T. urticae* adults. According to Ramakers (1993), van Houten *et al.* (1995) and van Rijn and Tanigoshi (1999a) pollen has a great influence on the female life span. On broad bean pollen or castor bean pollen, a median life span of 41.8 or 44.3 days, respectively, was reported by van Rijn and Tanigoshi (1999a). Other plant sources can also have an important influence on the life span. Females can survive for several weeks on a diet of extrafloral nectar of castor bean alone; however, there is no reproduction. When added to a diet of castor bean pollen, the nectar can provide a contribution to the population growth by augmenting the oviposition and extending the longevity of females as compared to mites fed pollen alone. The oviposition rate significantly increased from 1.73 to 2.17 eggs/female.day when pollen plus nectar was provided, while the lifespan of the females increased with the length of the nectar feeding period (van Rijn and Tanigoshi, 1999b). Kennett and Hamai (1980) reported that *I. degenerans* is able to survive on an artificial diet, which was initially intended for rearing *Chrysoperla carnea* (Stephens) larvae, but predator cultures maintained on this diet gradually declined in viability within 2 - 3 months, probably due to nutritional deficiencies.

de Courcy William *et al.* (2004a) found no effect of humidity (between 60 and 82% RH) on the female adult life span, when food (a mixture of all life stages of *T. urticae*) was present continuously. When deprived of food and water, adult female mites were able to survive for 2 days; however, survival doubled when in the absence of food, free water was supplied (de Courcy Williams *et al.*, 2004a). van Rijn and Tanigoshi (1999b) observed that transition of well-fed females to a diet of only water or a leaf resulted in a mortality of 50% of the predators after approximately 4 days. Takafuji and Chant (1976) found that adult females expand their longevity in the absence of prey to 11 days by ingesting juices from a bean leaf. In contrast, according to Yao and Chant (1990) *I. degenerans* is not able to survive starvation for longer than 15 hours.

The life span of an adult female can be divided in a short pre-oviposition period (2.1 – 3 days) (Blommers, 1976; Takafuji and Chant, 1976; van Rijn and Tanigoshi, 1999a), followed by an oviposition period and finally a post-oviposition period. Takafuji and Chant (1976) reported an oviposition period of 30.8 ± 4 days and a post-oviposition period of 19.1 ± 6.1 days when the phytoseiid was offered *T. pacificus*. The oviposition period of *I. degenerans* took more than 50 days when fed *Tetranychus neocaledonicus* André together with the pollen of *Bauchinia* sp. or *Aloe chabaudii* Schönland (Blommers, 1976), while it lasted 58.3 ± 1.2 days when fed female *T. pacificus* (Yao and Chant, 1989). Kennett and Hamai (1980) registered an oviposition period of at least 21 days on a diet consisting of all stages of *T. urticae* and 18.9 days on an artificial diet initially intended for rearing *C. carnea*. In females fed *T. tabaci*, the duration of the oviposition period was reduced with 50% when compared with females fed spider mites (Sengonca and Drescher, 2001).

In the study of Takafuji and Chant (1976), the oviposition rate on *T. pacificus* females was relatively constant from day 4 to day 26 in the adult stage and then it gradually decreased. Twenty percent of the females died within their oviposition period. To complete oviposition, multiple matings were necessary. The daily egg mass produced by a female of *I. degenerans* fed *T. pacificus* females is about 39 % of its full adult body weight (Yao and Chant, 1990).

Eveleigh and Chant (1981b) reported that at least 8 protonymphs of *T. pacificus* per day are required to continue oviposition. Nwilene and Nachman (1996b) found that, at a prey density of 120 individuals/6 cm², an *I. degenerans* female had to eat 24 eggs, 13 protonymphs or 5 females of the cassava green mite *Mononychellus tanajoa* (Bondar) to produce a single egg.

In Table 2.3, oviposition rates of *I. degenerans* published up till now are listed, showing that the predator is able to reproduce on all kinds of food sources. Depending on the food source offered, oviposition rates range from 0 to 2.31 eggs/female.day. The different pollen species showed large differences in suitability as food for *I. degenerans*. Certain pollen species support the reproduction of the predatory mite similarly or even better than live prey (McMurtry, 1977; Ramakers and Voet, 1995; van Rijn and Tanigoshi, 1999a), whereas other pollen species are unsuitable for reproduction (e.g., cedar pollen). On *P. maderiensis* eggs and crawlers (Ragusa and Tsolakis, 1995) and on all stages of *P. latus* (McMurtry *et al.*, 1984), both immature survival and fecundity were low or nihil. Only a few studies report on the fecundity of *I. degenerans* when fed thrips species (*F. occidentalis* and *Scirtothrips citri* (Moulton), respectively). The highest oviposition was found when the predatory mite was fed *S. citri*, while fecundity was almost nihil when *F. occidentalis* nymphs were offered as food (Grafton-Cardwell *et al.*, 1999; Blaeser *et al.*, 2002). Moreover, Blaeser and Sengonca (2001) and Blaeser *et al.* (2002) observed that eggs of which the parental generation was fed *F. occidentalis* nymphs did not hatch. On a diet of spider mites, the number of eggs ranged from 0.31 to 2.24 eggs/female.day. The different values obtained on spider mites might be explained by the species and prey stage offered to the predatory mite (Table 2.3)

Phytoseiid mites are capable of responding to increasing prey populations by paralleling their numerical increase (Sabelis, 1985). The change in a predator's abundance in response to a changing prey density is named the numerical response (Solomon, 1949). According to Hassell (1966), predators can express two distinct types of numerical responses. First, there may be a change in reproductive and (or) survival rates of a predator with changing prey density; and second, there may be an aggregative response. The relationship between prey density and oviposition rate is named the reproductive

response (Nwilene and Nachman, 1996b). It has already been mentioned that immature predatory mites require a certain amount of prey within a certain stage in order to survive and develop successfully, and that successive predator stages require larger numbers of prey probably due to an increasing energy requirement (see 2.4.1.2). Takafuji and Chant (1976) studied the numerical response of adult *I. degenerans* and reported an increase in the rate of oviposition, up to a maximum level of approximately 2 eggs/female.day, as the density of *T. pacificus* adults increased. The oviposition of *I. degenerans* also increased with the number of *M. tanajoa* up to a maximum of 2 eggs/female.day (Nwilene and Nachman, 1996b). Eveleigh and Chant (1981b) reported that the mean daily oviposition rate of *I. degenerans* increased curvilinearly with increasing *T. pacificus* protonymph density.

Nwilene and Nachman (1996b) compared the reproductive response of *I. degenerans* with that of *Neoseiulus teke* (Pritchard and Baker). The reproductive response of *I. degenerans* to a change in *M. tanajoa* density was lower than that of *N. teke*. According to the authors this indicates that *I. degenerans* is less efficient in converting food energy into egg production. Eveleigh and Chant (1981b) and Yao and Chant (1990) came to the same conclusion when comparing *I. degenerans* and *P. persimilis* fed on *T. pacificus*. Moreover, Eveleigh and Chant (1981b) even concluded that at high *T. pacificus* protonymph densities, *I. degenerans* killed prey in excess of its needs for egg production and only partially consumed the majority of the prey killed.

By paralleling the numerical increase in response to an increasing prey density, the number of conspecific predators in the environment increases. An increasing predator density, however, does no influence the fecundity of *I. degenerans* as much as the prey density does (Eveleigh and Chant, 1982d). This is because the per capita consumption of prey is not affected by the predator density (Eveleigh and Chant, 1982e). Yao and Chant (1989) determined the egg production of *I. degenerans* when living alone or together with *P. persimilis*. They reported that the presence of heterospecific predators in the environment did not influence the reproductive rates as long as prey is abundant.

Table 2.3. Daily fecundity (eggs/female.day) of *I. degenerans* reared on different diets and substrates, as reported in the literature

Type of food	Daily fecundity	Substrate	Reference
Artificial diet	0.07	Parafilm M®	Kennett and Hamai, 1980
Water	0.02 ± 0.02	Green PVC	van Rijn and Tanigoshi, 1999a
Pollen			
Alnus rubra	1.55 ± 0.05	Green PVC	van Rijn and Tanigoshi, 1999a
Betula pubescens	2.12 ± 0.21	Green PVC	van Rijn and Tanigoshi, 1999a
Capsicum annuum	1.4 ± 0.1	Cucumber	van Houten *et al.*, 1995a
Cedrus libani	0.03 ± 0.03	Green PVC	van Rijn and Tanigoshi, 1999a
Corylus americana	1.21 ± 0.05	Green PVC	van Rijn and Tanigoshi, 1999a
Corylus avellana	1.96 ± 0.22	Green PVC	van Rijn and Tanigoshi, 1999a
Dendranthema x grandiflora	0.53 ± 0.05	Green PVC	van Rijn and Tanigoshi, 1999a
Echium angustifolium	0.66 ± 0.06	Green PVC	van Rijn and Tanigoshi, 1999a
Epilobium angustifolium	1.48 ± 0.15	Green PVC	van Rijn and Tanigoshi, 1999a
Epilobium angustifolium (bee collected)	0.40 ± 0.19	Green PVC	van Rijn and Tanigoshi, 1999a
Eucalyptus sp.	2.09 ± 0.15	Green PVC	van Rijn and Tanigoshi, 1999a
Fragaria x ananassa	1.30 ± 0.22	Green PVC	van Rijn and Tanigoshi, 1999a
Helianthus annuus	0.86 ± 0.09	Green PVC	van Rijn and Tanigoshi, 1999a
Hymenocyclus croceus	1.74 ± 0.09	*Persea indica*	McMurtry *et al.*, 1977
Juniperus sp.	0.00	Green PVC	van Rijn and Tanigoshi, 1999a
Maleophora crocea	2.05 ± 0.74	Lemon leaf	McMurtry *et al.*, 1984
Malus domestica	1.77 ± 0.11	Green PVC	van Rijn and Tanigoshi, 1999a
Mesembrianthemum sp.	1.47 ± 0.02	Green PVC	van Rijn and Tanigoshi, 1999a
Pinus sylvestris	0.43 ± 0.09	Green PVC	van Rijn and Tanigoshi, 1999a
Prunus armeniaca	2.01 ± 0.20	Green PVC	van Rijn and Tanigoshi, 1999a
Prunus avium	2.28 ± 0.13	Green PVC	van Rijn and Tanigoshi, 1999a

Table 2.3. Daily fecundity (eggs/female.day) of *I. degenerans* reared on different diets and substrates, as reported in the literature (continued)

Prunus domestica	1.24 ± 0.19	Green PVC	van Rijn and Tanigoshi, 1999a
Prunus dulcis	2.28 ± 0.16	Green PVC	van Rijn and Tanigoshi, 1999a
Pyrus communis	1.36 ± 0.10	Green PVC	van Rijn and Tanigoshi, 1999a
Ricinus communis	1.73 ± 0.07	Green PVC	van Rijn and Tanigoshi, 1999a
	> 2	Plastic	Ramakers and Voet, 1995
Rubus sp.	1.65 ± 0.11	Green PVC	van Rijn and Tanigoshi, 1999a
Salix babylonica	0.80 ± 0.02	Green PVC	van Rijn and Tanigoshi, 1999a
Typha angustifolia	1.98 ± 0.02	Green PVC	van Rijn and Tanigoshi, 1999a
Typha latifolia	1.94 ± 0.04	Green PVC	van Rijn and Tanigoshi, 1999a
Vicia faba	2.31 ± 0.15	Green PVC	van Rijn and Tanigoshi, 1999a
Arthropod prey			
Frankliniella occidentalis	0.09 ± 0.11	*Impatiens walleriana*	Blaeser *et al.*, 2002
	1.4 ± 0.2	Cucumber	van Houten *et al.*, 1995a
Mononychellus tanajoa eggs and juveniles	1.92 ± 0.15	*Manihot esculenta* L.	van Rijn and Tanigoshi, 1999a
Oligonychus coffeae	2-3	*Ricinus communis* L.	Blommers, 1976
Phenacoccus madeirensis	0.08	-	Ragusa and Tsolakis, 1995
Polyphagotarsonemus latus	0	Lemon	McMurtry *et al.*, 1984
Scirtothrips citri	1.96	Navel orange citrus	Grafton-Cardwell *et al.*, 1999
Tetranychus pacificus eggs and larvae	1.39 ± 0.2	*P. indica*	McMurtry, J.A., 1977
T. pacificus protonymphs	2.24 ± 0.05	-	Takafuji and Chant, 1976
T. urticae adults	0.31 ± 0.24	*I. walleriana*	Blaeser *et al.*, 2002
T. urticae all stages	1.17 ± 0.26	Blackberry	Kennett and Hamai, 1980
T. urticae eggs and juveniles	1.57 ± 0.13	Green PVC	van Rijn and Tanigoshi, 1999a
Arthropod prey + pollen			
Tetranychus neocaledonicus + *Bauchinia* sp. pollen or *Aloe chabaudii* pollen	1.5	-	Blommers, 1976

2.4.3 Intrinsic rate of increase

The intrinsic rate of natural increase (r_m) is a parameter frequently used to estimate the population growth. The value for r_m is calculated from the equation $\sum e^{-r_m x} l_x m_x = 1$ where l_x is the proportion of females surviving to age x and m_x is the mean number of female progeny per adult female at age x. The value is hence influenced by the reproduction rate, and developmental rate, which were discussed in the previous paragraphs.

van Rijn and Tanigoshi (1999b) estimated r_m values of *I. degenerans* when presented with broad bean pollen, castor bean pollen and *T. urticae* as food. They found r_m values ranging from 0.147 to 0.208 day^{-1}. Takafuji and Chant (1976) reported a value of 0.248 day^{-1} when the phytoseiid was fed *T. pacificus* females.

2.5 PREDATORY BEHAVIOUR

2.5.1 Foraging efficiency and distribution

Iphiseius degenerans is considered to be a generalist (type III predator) (McMurtry and Croft, 1997), feeding both on live prey and pollen. In search for its prey, the predator walks over the leaf surface holding its first pair of legs in front of its body. These legs are waved from side to side continuously, acting like antennae (Eveleigh and Chant, 1981c). The width of perception of *I. degenerans*, *i.e.,* the distance between the tips of the first pair of legs is 0.038 cm. The area the predator can traverse per unit of time is estimated to be 2.257 cm²/h.

Individual consumers spend most time in patches containing the greatest densities of prey; this is referred to as the aggregative response (Begon *et al.*, 1996). *Iphiseius degenerans* exhibits no aggregative response to high densities of the spider mite *T. pacificus*, it even tends to avoid areas covered with the webbing of this prey (Takafuji and Chant, 1976). The lack of aggregative response was also observed by Eveleigh and Chant (1982c). The predators randomly distributed their search effort among patches of different *T. pacificus* protonymphs densities. The number of visits, the length of the first

visit to each patch, and the amount of time per visit are also not influenced by prey density, and hence contribute to the random distribution of the search effort (Eveleigh and Chant, 1982c). This indicates that the predatory mites do not respond to the relative profitability of the patch (*i.e.,* the amount of prey a predator can collect during a given hunting time) (Eveleigh and Chant, 1982b; 1982c). This random distribution may be largely due to its searching pattern after prey captures. Eveleigh and Chant (1982g) reported that the predator exhibits area-restricted searching. This means that *I. degenerans* changes its searching behaviour after capturing a prey and tends to search near the point of last prey capture, regardless of the density and distribution of the prey. *Iphiseius degenerans* is slow in responding to temporal changes in prey distribution; it prefers instead to remain in the patch that was initially most profitable until most of the prey has disappeared (Eveleigh and Chant, 1982a). The predator also showed no response to changes in the spatial distribution of *M. tanajoa* (Skovgård *et al.*, 1993). According to the authors, the hunger level determines the dispersal of the predators. Such hunger-dependent behaviour may be a consequence of their high prey requirements and their low searching speed (Eveleigh and Chant, 1981b; 1982g), necessitating the adoption of a strategy to minimize the cost of frequent movement between patches.
The number of predators in a patch also did not greatly influence the distribution of the predator (Eveleigh and Chant, 1982b, d).

In patchy environments, due to predation by *I. degenerans* patches get exploited. As mentioned above, despite this exploitation, Eveleigh and Chant (1982b) observed little redistribution of the predator among the patches. Thus, the searching success of *I. degenerans* is independent of the spatial arrangement of the prey (Eveleigh and Chant, 1982b, 1982c). The predator density does not influence the searching efficiency in a patchy environment as there is no interference between predators (Eveleigh and Chant, 1982b): searching efficiency even showed a tendency to increase as predator density increased (Eveleigh and Chant, 1982e). According to these authors, this does probably happen in more complex environments, as the low dispersal capacity would prevent it from responding quickly to changes in the prey density. The searching activity of *I. degenerans* is increased by the stimulation of being contacted by a moving prey

(Takafuji and Chant, 1976). The searching time of *I. degenerans* protonymphs is density dependent: the higher the prey density the less time is spent searching for prey. According to Eveleigh and Chant (1982f) the number of prey killed per unit time by *I. degenerans* is not affected by prey distribution.

2.5.2 Feeding behaviour

When physical contact is made with a potential prey, recognition occurs immediately. The attacking of for instance spider mites starts with the predator touching the prey with tarsi I, then grasping it with legs II. The cuticle of the prey is cut with the chelicerae; the corniculi are partially introduced in the prey's body after cheliceral penetration. With the chelicerae and part of the hypostome inserted in the prey and after a short time of struggling, the prey is lifted from the substrate. The predatory mite then stands on its last two pairs of legs, orienting the body perpendicular to the substrate, either upright or inverted. Maintaining the grasp on its prey, but with the chelicerae and the hypostome no longer in contact with its prey, the predator turns the prey around several times before again drawing it to its gnathosoma and resuming feeding. Proteolytic enzymes are injected into the prey with at least some preoral digestion. By contraction of the pharynx muscles, the liquefied body contents of the prey are then sucked up (Flechtmann and McMurtry, 1992a). Considering the morphology of the chelicerae in relation to feeding, the same authors claim that *I. degenerans* in fact is a good pollen feeder. On the abaxial face of the fixed digit a large lobe which is produced into a "spoonlike" structure is present. When both chelicerae are at about the same level of protraction (retraction), a cavity is formed, dorsally and laterally closed by the chelicerae with their lobes and ventrally by the hypostome. The wide deutosternal groove is possibly also a modification associated with the intake of liquefied contents of the pollen grain core (Flechtmann and McMurtry, 1992a, b).

2.5.3 Prey spectrum

2.5.3.1 Spider mites

Up to the early 1990's, *I. degenerans* was usually studied as a natural enemy of spider mites.

Several studies under laboratory conditions have shown that *I. degenerans* is able to feed and reproduce on the cassava green mite *M. tanajoa,* with which *I. degenerans* is naturally associated in Kenya. Skovgård *et al.* (1993) found that the predator population increased in response to the growing cassava green mite population, but it failed to completely control *M. tanajoa* infestations. According to these authors, the ability of the predatory mite to control the cassava green mite was limited by the lack of spatial coincidence with the prey and predators and in particular, the fact that the predators stayed in the lower part of the canopy of the plants, while the phytophagous mites preferred the top. The study of Munthali (1989) suggests that *I. degenerans* is able to consume *M. progresivus* (Doreste) on cassava, at a ratio of 18 adult prey mites per day.

The generalist *I. degenerans* showed marked numerical responses when released in an avocado orchard to determine its effect on the avocado brown mite *Oligonychus punicae* (Hirst) (McMurtry *et al.*, 1984). Aponte *et al.* (1997) released several phytoseiid predatory mites, among which *I. degenerans*, to evaluate the effect on populations of the persea mite *O. perseae* in avocados. *Iphiseius degenerans* was unable to establish in the crop. This was apparently due to unfavourable environmental conditions and the inability to penetrate nests of the persea mite. Young female mites can kill over 10 *O. coffeae* females/day (Blommers, 1976).

Espino *et al.* (1988) used *I. degenerans* in a cucumber crop to control *T. urticae*. Blaeser and Sengonca (2001) found that *I. degenerans* adults devoured 0.5 *F. occidentalis* nymphs per day versus 4 *T. urticae* adults. The adult predators preferred *T. urticae* adults to thrips nymphs. The observations of Blommers (1976) indicate that *I. degenerans* is incapable of controlling *T. neocaledonicus* in the field. When offered *T. neocaledonicus* and pollen, predation dropped to less than 1 female per day; if pollen was withheld, the predation initially rose to 7 prey females in the first 24 hours, but then dropped. In laboratory trials, an *I. degenerans* female consumed 28.8 *T. pacificus*

protonymphs throughout the entire immature period; this number increased to a total of 1209 prey protonymphs during the pre-oviposition, oviposition, and post-oviposition periods. Eggs and larval stages of *T. pacificus* are equally consumed by the larvae but as the predator develops, its consumption of younger motile prey stages increases. This preference can be explained by the higher attacking activity of the predator when stimulated by movements of the prey (Takafuji and Chant, 1976).

Yao and Chant (1990) calculated that the total amount of food intake of *I. degenerans* during 24 hours using *T. pacificus* as prey was 33.1 µg. This prey species weighs almost as much as the predator.

Iphiseius degenerans does not consume captured prey completely and this feeding habit becomes more frequent as prey density increases (Takafuji and Chant, 1976).

2.5.3.2 Thrips

Laboratory, greenhouse and field experiments have been conducted to evaluate the potential of phytoseiid mites to reduce thrips populations in crops. Several phytoseiid mites including *I. degenerans* are potential predators of thrips species (van Lenteren and Loomans, 1998).

As *N. cucumeris* fails to control Western flower thrips, *F. occidentalis*, in sweet pepper during winter (probably caused by diapause and the low resistance of the predator's eggs to low humidity), van Houten *et al.* (1993, 1995a) searched for a non-diapausing thrips predator tolerant to low humidities. Out of 5 subtropical predatory mites, *I. degenerans* and *Amblyseius hibisci* (Chant) gave best results. During a 72h experiment under laboratory conditions (25 °C, 70% RH, L16:D8 h photoperiod, cucumber leaves), *I. degenerans* consumed 4.4 ± 0.5 first instars of *F. occidentalis* a day. In a number of reports, it was demonstrated that it is possible to control western flower thrips throughout the growing season with *I. degenerans* (van Houten and van Stratum, 1993, 1995). Even early in the growing season (January - March) the predatory mite established well and later on even displaced another thrips consuming mite, *N. cucumeris*. *Iphiseius degenerans* not only decimated thrips populations, there was also a good distribution, not only in the rows where it had been released but also in other plant

rows. In greenhouse experiments conducted by Opit *et al.* (1997) the predatory potential of *I. degenerans* on *Echinothrips americanus* (Morgan) in pepper and cucumber was assessed. The predatory mite did not reduce the thrips population significantly compared with the control. Valentin (1997) stated that *I. degenerans* barely preys on *E. americanus* nymphs, because of the larger size of the nymphs compared with other thrips species and thus a better defence mechanism of the prey.

The results of Brown *et al.* (1999) show that *I. degenerans* is able to prey on both *F. occidentalis* and *Heliothrips haemorrhoidalis* (Bouché), but that the predatory potential is influenced by both thrips and plant species. *I. degenerans* is more effective in killing *F. occidentalis* than *H. haemorrhoidalis*. On the plant species *Capsicum annuum* L. and *Dombeya acutangula* Cav. there was an effective decrease in the thrips population, where as the number of prey killed on *Crotalaria capensis* Jacq., *Tephrosia grandiflora* Aiton and *Saurauia nepaulensis* DC. did not differ significantly from the control mortality. Based on their field experiments, Chat-Locussol *et al.* (1998) concluded that *I. degenerans* did affect *F. occidentalis* populations in cucumber. Ten or 5 predatory mites per m² were introduced per week. Combined with *Orius majusculus* (Reuter) (5 predatory bugs/m²), it was even possible to delay the increase of the thrips population by one month. To improve thrips control in cucumber, van Rijn *et al.* (1999) showed that applying cattail pollen in the crop made the predator population increase more rapidly while the thrips population remained smaller compared with the control.

Grafton-Cardwell *et al.* (1999) evaluated the potential of augmentative releases of *I. degenerans* for reducing foliar damage caused by *S. citri* in citrus orchards. From a 5-day laboratory experiment, they concluded that *I. degenerans* is able to consume up to 5 citrus thrips nymphs per day. Ninety-six percent of the predators were able to survive on this prey and deposited 0.93 to 2.54 eggs per female per day. In commercial citrus nurseries, the predatory mite reduced the citrus thrips population and improved tree height and leaf numbers comparable with an abamectin insecticide treatment. In citrus orchards in Sicily, *I. degenerans* was found in association with the citrus thrips *Pezothrips kellyanus* (Bagnall), but it is still unclear whether the predator was responsible for natural control of the pest (Conti *et al.*, 2001a, 2001b, 2003). In laboratory and field predation tests on leek *I. degenerans* did not feed on *T. tabaci* nymphs (Rat-Morris, 1999).

2.5.3.3 Other prey species

Iphiseius degenerans was also found in association with the Japanese bayberry whitefly, *Parabemisia myricae* (Kuwana) in avocado and citrus orchards, preying upon larvae of this species (Swirski *et al.*, 1987). They also feed on eggs and nymphs of the citrus psylla *Trioza erytreae* (Del Guernica), although they seem to play a minor role in reducing the psyllid populations (Catling, 1970). In laboratory tests, *I. degenerans* preyed on the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) but the predation rate was dependent on the presence of pollen: when *Typha domingensis* Pers. pollen was added to the arena, survival of the predatory mite was much improved, but fewer citrus rust mites were killed (Palevsky *et al.*, 2003).

2.5.4 Functional response

To assess the (potential) role of *I. degenerans* in the biological control of phytophagous species, the type and the parameters (attack rate, handling time) of the functional response of the predator towards its prey can be indicative. The functional response is defined as the change in the predator's consumption rate in response to the density of the prey (Solomon, 1949). Holling (1966) studied the functional response of invertebrate predators in detail, and described three basic types of response curves: a type I response, which increases linearly to a plateau with increasing prey density; a type II response, which is a negatively accelerating rise to a plateau; and finally a type III response, which is an S-shaped rise to a plateau (see chapter 6 for more detailed information on functional responses).

Literature only reports on functional responses of *I. degenerans* to changes in the density of spider mite species (*T. pacificus*, *M. tanajoa*). The functional response models observed in these studies are predominantly type II (e.g., Takafuji and Chant, 1976; Eveleigh and Chant, 1981a,b, 1982f; Akpokodje *et al.*, 1990). Only the study of Nwilene and Nachman (1996a) reports type III responses for *I. degenerans* protonymphs and females preying on three stages of *M. tanajoa*. The experimental conditions, however, differed between studies.

The nutritional requirements of the predators, predator stage and age, the exposure time, and feeding history have an important effect on predatory behaviour and consequently, on the functional response (Eveleigh and Chant, 1981a). The effects of these factors are reflected in the values of the attack rate and the handling time. The attack rate increases from protonymphs to deutonymphs but is similar for deutonymphs and for adults. The handling time decreases as the predator stages becomes larger. For a given predator stage, the predator's attack rate declines and handling times increase as prey gets larger (Nwilene and Nachman, 1996a). Initial exposure to excess prey does not greatly affect the functional response of *I. degenerans* (Eveleigh and Chant, 1981a). According to Eveleigh and Chant (1981a, 1982c) and Nwilene and Nachman (1996a) the shape of the curve depends on the duration of the experiment in relation to the lifespan of the predator. Type II curves tend to become almost linear when the experimental period is extended from 3 to 24 hours (Eveleigh and Chant, 1981a). Takafuji and Chant (1976) reported that the initial increase in number of prey consumed per predator was almost linearly correlated with increasing *T. pacificus* density, and finally levelled off to a plateau. In their study, Nwilene and Nachman (1996a) suggested that the curve tends to become more sigmoid with experimental time. According to Nwilene and Nachman (1996a), who obtained another type of curve than Akpokodje *et al.* (1990) for the same predator and prey species, the size of the experimental arena also might affect the outcome of the functional response. Eveleigh and Chant (1982f) found that the overall shape of the functional response was not influenced by distribution of *T. pacificus* protonymphs (clumped, uniform or random), but that the estimates of the handling time and attack rate varied with prey distribution.

2.6 PRACTICAL APPLICATION

2.6.1 Release of *Iphiseius degenerans* in greenhouse crops

In 1994, the predatory mite *I. degenerans* was first commercialized in Belgium to control thrips in greenhouse crops (Guido Sterk, pers. comm.). This predatory mite has two advantages compared with another phytoseiid thrips predator, *N. cucumeris*: *I.*

degenerans has no diapause and it tolerates low relative humidity (Degheele *et al.*, 1997). In a sweet pepper crop an introduction of minimum 2000 predatory mites per ha is recommended, with a minimum of 20 mites per introduction point (Biobest, 2006; Koppert, 2006).

Iphiseius degenerans is known to be a generalist, using pollen as alternative food source. Hence, to support a predator population in the crop even before a pest is present, pollen can be used. Pollen of castor bean is known to be a high quality food source, equivalent to live prey and better than for instance pollen from oak and Cruciferae (Ramakers and Voet, 1995). As for *I. degenerans*, flowering castor bean plants cannot only be used for laboratory mass rearing (Nunnink, 1994, Ramakers and Voet, 1995) but also in an open rearing system (Ramakers and Voet, 1996). The combination of pollen and extrafloral nectar makes castor bean plants ideal rearing and banker plants (van Rijn and Tanigoshi, 1999b). The predators also tend to move from the banker plants into a sweet pepper crop, even in the absence of prey. The migration from leaf to leaf, or via plant supporting wires is far more important than migration via the soil (Ramakers and Voet, 1996). Ramakers and Voet (1995) recommend placing 15 bankers per hectare, and moving the plants every week to another part of the greenhouse. This is a cheap but slow method, applicable when no or very few thrips are present in the sweet pepper crop.

On preyless plants that produce little or no pollen (e.g., cucumber) establishment of the predator is poor (Ramakers and Voet, 1995). Spraying suspensions of bee-collected pollen on the cucumber plants makes them more attractive to the predators and allows them to establish and reproduce in the absence of thrips (Ramakers and Voet, 1993; Ramakers, 1995). van Rijn *et al.* (1999, 2002) showed that the addition of cattail pollen in a cucumber crop resulted in a fast growing predator population, and thus an increase in effectiveness of the predatory mite in controlling thrips. The thrips population remained small, despite the fact that the pest can also utilize pollen. This is because the predator population was strongly clustered on leaves with pollen, whereas the thrips concentrated on the young, top leaves.

2.6.2 Interaction among predators and their prey

Application of biological control agents in agricultural crops led to the replacement of simple tritrophic interactions by more complex food web interactions. A summary of these interactions is given in Janssen *et al.* (1998).

When *N. cucumeris* and *I. degenerans* are released at the same time in a sweet pepper crop, the latter shows a more rapid population increase and reaches higher population densities than *N. cucumeris*; finally, *I. degenerans* displaces the other predator. This is probably because *I. degenerans* visits more flowers and is more active on leaves than *N. cucumeris*, and thus is more likely to encounter thrips nymphs (van Houten and van Stratum, 1993, 1995). Wittmann and Leather (1997) found that *Orius laevigatus* (Fieber), a predatory bug, favours thrips over *I. degenerans*. These authors suggest that both predators can be used simultaneously in the biocontrol of the western flower thrips. Yao and Chant (1989) studied the interaction between *I. degenerans* and *P. persimilis* feeding on *T. pacificus*. When confined together on a single arena, *I. degenerans* out competed *P. persimilis*. According to the authors, the extermination of *P. persimilis* is caused by intraguild predation by *I. degenerans*. Female adults of *I. degenerans* females can eat 3.3 ± 1.4 *P. persimilis* eggs, 4.6 ± 0.4 larvae or 5.6 ± 0.4 protonymphs per day. On the other hand, eggs, larvae and protonymphs of *I. degenerans* are also prey for *P. persimilis*. However, predation of *I. degenerans* females on eggs, larvae or protonymphs of *P. persimilis* is significantly higher than that of *P. persimilis* on the corresponding stages of *I. degenerans*. Despite the possible interaction between the two predators, the presence of conspecifics or heterospecifics has no effect on the weight of *I. degenerans*, and hence on the food uptake. Both predators are even able to share meals (Yao and Chant, 1990).

van Schelt (1999, 2000) hypothesized that *I. degenerans* influences the performance of the predatory gall midge *Aphidoletes aphidimyza*, which is used for aphid control, by preying on its eggs. In semi-field tests on sweet pepper plants, he observed that approximately 40% of the midge's eggs were eaten.

According to Faraji *et al.* (2002b) cannibalism on eggs is rare in *I. degenerans*, but cannibalism on larvae by adults and nymphs can occur. Starved female *I. degenerans* were able to eat 3.5 ± 0.4 eggs, 4.1 ± 0.6 larvae or 2.0 ± 0.2 protonymphs of its own

species per day. However, when feeding on conspecific offspring females survived for maximum 8.9 days (Yao and Chant, 1989).

The interaction between *I. degenerans* and its prey *F. occidentalis* is of a special nature. All active stages of the thrips are capable of feeding on *I. degenerans* eggs, whereas the predatory mites only kill young thrips nymphs (Faradji *et al.*, 2001). According to Willemse (2002) the feeding on *I. degenerans* eggs is not a defence mechanism but rather a form of nutrition. Also, research by Janssen *et al.* (2003) indicated that thrips nymphs feed more on predator eggs when the host plants are of low quality (e.g., sweet pepper). Hence, on superior host plants the killing of eggs does not serve as food supplement, but has another purpose. Janssen *et al.* (2002) showed that thrips nymphs might discriminate between the eggs of a dangerous and of a harmless predator, killing more eggs of the dangerous predator. Because adult predatory mites avoid ovipositing near killed eggs, killing eggs of a predatory mite results in a deterring of adult predator populations and a reduction of the predation risk. According to Janssen *et al.* (1998) odours emitted by pest-infested plants affect the searching behaviour of the predators, which results in a different distribution of pests and predators over the plant. For instance, spider mites avoid leaves previously exposed to predatory mites (Grostal and Dicke, 2000). Janssen *et al*. (1988) studied the response of *I. degenerans* to different volatiles in an olfactometer. *Iphiseius degenerans* did not prefer thrips infested plants to clean plants. This behaviour was found somewhat puzzling as the predatory mite is employed as a thrips predator. But, when given the choice between thrips infested plants and spider mite infested plants, the predator preferred thrips infested plants. The choice for thrips infested plants here may be due to the repellence of plants infested with spider mites. The feeding history of the predatory mite may be the cause of the lack of an olfactory response (Janssen *et al.*, 1998). *Iphiseius degenerans* is normally reared on pollen (e.g., McMurtry and Scriven, 1965) and thus has no experience with thrips nymphs in most experiments. Conditioning on a specific herbivore may lead to a positive attraction (Janssen *et al.*, 1998). Furthermore, the hunger state also seems to affect the response. When well-fed, all stages of the predatory mite seem to be repelled by *T. pacificus* infested bean leaves. However, starved females are attracted to the same leaves (Dong and Chant, 1986). In contrast, according to Yao and Chant (1989), *I.*

degenerans responds to airborne cues of *T. pacificus*, and the percentage of the successful choices was unaffected by their starvation. However, in the same study well-fed females did lack a response to spider mite webbing.

2.6.3 Side effects of pesticides on *Iphiseius degenerans*

In order to utilize the phytoseiid mite in integrated pest management programs in several crops, it is essential to acquire information on the side-effects of pesticides to this predator. An overview of pesticides tested on *I. degenerans* is presented in Table 2.4. However, because each source employs a different methodology it is not possible to rank the pesticides according to their impact on the predatory mite.
Since *I. degenerans* is frequently found in citrus orchards, several authors evaluated the relative toxicity of pesticides used in citrus orchards. The phytoseiid seems to be more susceptible to insecticides and acaricides than *Panonychus citri* (Mc Gregor) and *T. pacificus*. However, azinphosmethyl and tricyclohexylhydroxytin are more toxic to the phytophagous mites, and there is no difference in susceptibility to hexakis (beta, beta-dimethyl(phenethyl)-distannoxane) (Jeppson *et al.*, 1975). Conti *et al.* (2004) concluded that the toxicity of azadirachtin, and abamectin and the mixture rotenone + pyrethrum, is not always negligible and can entail a reduction of the predator population.

In sweet pepper crops, aphids are often chemically controlled with pirimicarb. The LC50-value (Lethal Concentration 50) on the phytoseiid population is 91 mg a.i./l. This is far beneath the field dose of 250 mg a.i./l that is used in Dutch green pepper crops (van Houten and van der Staay, 1993). Stark *et al.* (1997) estimated the acute lethal concentrations and studied the population growth rate of *I. degenerans* after exposure to azadirachtin and dicofol. *Iphiseius degenerans* immatures were more susceptible to azadirachtin than the adults, but to dicofol an equal susceptibility was found. Both pesticides affected the instantaneous rate of increase; exposure to 250 ppm azadirachtin or 140 ppm dicofol caused an extinction of the population. The NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values for the instantaneous rate of increase are respectively 3.9 ppm and 7.8 ppm for azadirachtin and 17.5 ppm and 3.5 ppm for dicofol. The NOEC and LOEC values for reproduction

are 0.98 and 1.96 ppm for azadirachtin and 4.4 and 8.8 ppm for dicofol. These concentrations significantly reduced the reproduction, but there was no mortality. Ludwig and Oetting (2001) reported a high mortality (> 60%, direct effect) when the phytoseiid was exposed to azadirachtin and neem oil for 48 h, whereas treatment with insecticidal soap caused a lower mortality. They suggest that insecticidal soap or azadirachtin can be used in combination with *I. degenerans* by releasing mites after the application. If plants are treated with neem oil, predators should not be released until the residue has had a chance to dissipate. In laboratory trials, *I. degenerans* showed high mortality when exposed to six-day-old residues of avermectin b1 (0.3 ml/l water) and pyridaben (0.284 ml/l water). In greenhouse trials, these acaricides caused a lower mortality and had a shorter residual toxicity. Adult mortality of the predator was less than 10% when exposed to 6-day-old residues of avermectin b1 for 48 hours, while this was 44% for a 6-day-old residue of pyridaben. Thresholds (expressed as LT25) for pyridaben were estimated at 18 days for *I. degenerans* (Shipp *et al.*, 2000).

Both studies of Ludwig and Oetting (2001) and Shipp *et al.* (2003) indicate that the use of the entomopathogenic fungus *Beauveria bassiana* Balsamo is compatible with predatory mites. Ludwig and Oetting (2001) found that under greenhouse conditions, infection due to the entomopathogenic fungi *Verticillium lecanii* (Zimmermann) and *Metarhizium anisopliae* (Metchnikoff) should also be minimal for *I. degenerans*. PreFeRal, a microbial insecticide based on the entomopathogenic fungus *Paecilomyces fumosoroseus* (Wize) proved to be completely harmless for *I. degenerans*. Fenpropathrin, tebufenpyrad, and abamectin were used as positive toxic standards. The toxicity of endosulfan depends on the host plant and the compound results in a higher mortality on castor bean than on green bean plants (Sterk *et al.*, 1995).

Pesticides do not always kill the predators present in the crop, but can influence their foraging behaviour and reproduction. Brown *et al.* (2003) evaluated the effect of teflubenzuron (at 80 mg a.i./l) on *I. degenerans*. This insect growth regulator does not kill the predators, but has an effect on foraging behaviour, which seems to be plant species dependent. These authors observed that the predation on *F. occidentalis* is higher on untreated leaf disks of *D. acutangula* than on the leaf disks treated with teflubenzuron. However, *I. degenerans* caused a similar level of mortality in *F.*

occidentalis on untreated or treated leaf disks of *C. annuum*, *C. capensis* and *T. grandiflora*.

Table 2.4. Overview of the pesticides tested on *I. degenerans*

Pesticide	**Reference**
Abamectin	Conti *et al.*, 2004 Sterk *et al.*, 1995
Azadirachtin	Conti *et al.*, 2004; Stark *et al.*, 1997
Azinphosmethyl	Jeppson *et al.*, 1975
Biothion	Jeppson *et al.*, 1975
Chlorobenzilate	Jeppson *et al.*, 1975
Dicofol	Jeppson *et al.*, 1975 Stark *et al.*, 1997
Dimethoate	Jeppson *et al.*, 1975
Dioxathion	Jeppson *et al.*, 1975
Endosulfan	Sterk *et al.*, 1995
Fenpropathrin	Sterk *et al.*, 1995
Formetanate	Jeppson *et al.*, 1975
Hexakis (beta,beta-dimethyl(phenethyl)-distannoxane)	Jeppson *et al.*, 1975
Malathion	Jeppson *et al.*, 1975
Parathion	Jeppson *et al.*, 1975
Phosphamidon	Jeppson *et al.*, 1975
Propargite	Jeppson *et al.*, 1975
Pyrethrum	Conti *et al.*, 2004
Pyriproxyfen	Sterk *et al.*, 1995
Rotenone	Conti *et al.*, 2004
Tebufenpyrad	Sterk *et al.*, 1995
Teflubenzuron	Brown *et al.*, 2003
Tricyclohexylhydroxytin	Jeppson *et al.*, 1975

CHAPTER 3
MORPHOLOGY AND MATING BEHAVIOUR OF *IPHISEIUS DEGENERANS*

3.1 INTRODUCTION

Relatively few studies have provided detailed information on the life history of *I. degenerans*; therefore, the mating behaviour and some morphological features of the different life stages were documented.

An adult male waiting near or upon a female deutonymph which is ready to moult is a common phenomenon in phytoseiid mites. Mating usually takes place immediately after the final moult of the female. However, in some species a tendency to feed before mating was observed (Schulten, 1985). In Phytoseiidae two mating patterns occur: the "*Amblyseius-Typhlodromus* type" and the "*Phytoseiulus* type". The first pattern is characterized by the male mounting the dorsum of the female prior to the venter-to-venter mating position. In the latter pattern the male makes contact in a face-to-face position and then crawls underneath the female (Amano and Chant, 1978). According to the latter authors, *I. degenerans* follows the first type, but no detailed information is available.

After mating and insemination, females start laying eggs. All predatory mites of the cohort Gamasina pass through a larval stage and two nymphal stages (protonymph and deutonymph). The developmental stages are separated through moults. Knowledge on the size of the immature stages is helpful in recognising the different (mobile) life stages in a population at a glance. Literature, however, only reports on sizes of adult females and males of *I. degenerans*. Therefore, the sizes of eggs, larvae, protonymphs, female and male deutonymphs and adults were measured.

3.2 MATERIALS AND METHODS

3.2.1 Predator culture

A stock colony of *I. degenerans* was initiated in 2000 using mites obtained from Biobest NV (Belgium) and Koppert BV (The Netherlands), and was cultured in the laboratory for successive generations in a climatic cabinet at 25 ± 1 °C, 75 ± 5 % RH and a 16L:8D h photoperiod.

The mites were reared on a green plastic plate (20 x 25 x 0.3 cm) (Multicel, SEDPA, France) placed on top of a foam pad (20 x 25 x 4 cm) in a water containing plastic tray (30 x 40 x 7 cm) (Fig. 3.1). The edges of the Multicel plate were covered with absorbent paper immersed in the water in the tray, leaving an arena of 300 cm². The absorbent paper provided the mites with moisture and prevented them from escaping. Black sewing threads (ca. 5 cm long) served as oviposition substrates (van Rijn and Tanigoshi, 1999a). Pollen of castor bean (*Ricinus communis* L.) was added ad libitum every 3 days using a fine brush. To start new rearing cohorts, eggs were collected every other day.

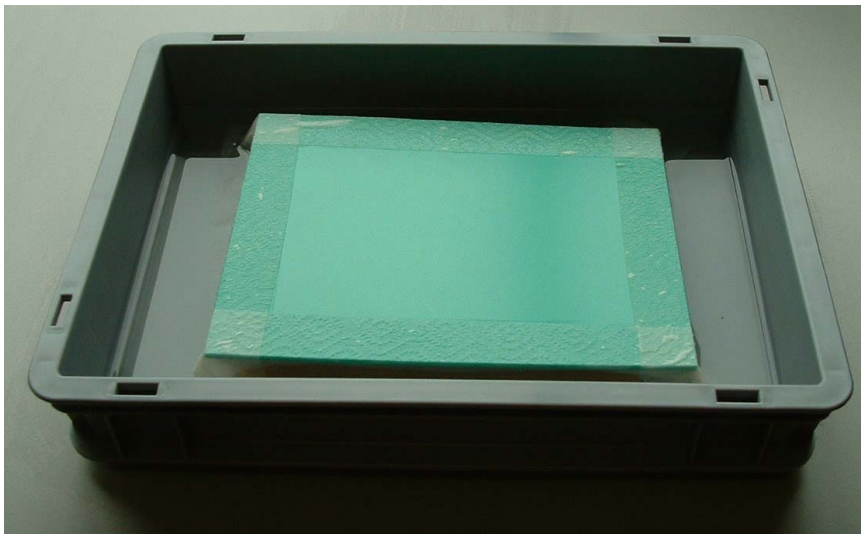


Figure 3.1. Rearing arena for *Iphiseius degenerans*.

3.2.2 Size of the different life stages

To measure the size of the different life stages of *I. degenerans*, eggs, larvae, protonymphs, deutonymphs, females or males were collected from rearing units maintained on castor bean pollen, almond pollen or apple pollen. Measurements were made with thirty dead or anaesthetised individuals using a dissection microscope and a graded ocular. Larvae, protonymphs and adults were killed by immersing them in boiling water (Flechtmann and McMurtry, 1992a). Deutonymphs, which were later still needed to determine sex, where anaesthetised with CO_2.

For larvae, nymphs and adults, length was measured in two ways: first from the posterior tip of the idiosoma to the anterior tip (thus without the gnathosoma), and second from the posterior tip of the idiosoma to the tip of the pedipalps. Width was measured at the broadest point of the idiosoma.

3.2.3 Mating behaviour of *Iphiseius degenerans*

Female and male deutonymphs were confined separately on small Multicel arenas. This prevented males from mating with newly moulted females prior to the start of the experiment. Within two days after the final moult, ten pairs were formed and observed under a binocular to which a video camera was connected. To measure the time required for the pre-mating behaviour and the duration of the first copulation, the predatory mites were continuously observed from their introduction into the arena until the end of the copulation event.

To determine the sex of the first egg laid, twenty additional pairs were formed. The first egg laid by each female was removed from the arena, transferred singly to another arena, allowed to hatch and reared on castor bean pollen until adulthood. Upon the last moult, sex was determined.

3.3 RESULTS

3.3.1 Size of the different life stages

In Table 3.1, dimensions of the different developmental stages of *I. degenerans* are presented.

Little variation was found in egg size. Eggs averaged 232 ± 1 µm in length and 175 ± 0 µm in width. In the larval and protonymphal stage it is not possible to distinguish between females and males. From the deutonymphal stage on, females and males can easily be distinguished based on their size. Female deutonymphs are on average 60 µm longer and 40 µm wider than male deutonymphs. The difference between females and males is more pronounced in the adult stage; females are on average 110 µm longer and 90 µm wider than males.

The gnathosoma shows little variation over the life stages. Length varies from 81 µm (adult male) to 114 µm (female deutonymph). In immatures the length of the gnathosoma equals about 25% of the total body length (idiosoma + gnathosoma), while this is about 18% in adults.

Table 3.1. Mean body size of the developmental stages of *I. degenerans* (µm ± SEM)

Developmental stage	***n***	**Idiosoma**		**Idiosoma + gnathosoma**
		Width	**Length**	**Length**
Larva	30	189 ± 2	251 ± 3	344 ± 4
Protonymph	30	208 ± 2	289 ± 3	394 ± 3
Deutonymph				
female	16	281 ± 6	360 ± 4	474 ± 6
male	14	242 ± 3	306 ± 9	415 ± 4
Adult				
female	30	362 ± 2	460 ± 2	553 ± 3
male	30	272 ± 2	350 ± 3	431 ± 4

3.3.2 Mating behaviour of *Iphiseius degenerans*

In preliminary tests, it was observed that *I. degenerans* males hover with their first two pairs of legs over the ophistosoma of a female deutonymph. However, not all males exhibited this behaviour.

Iphiseius degenerans displayed the "*Amblyseius-Typhlodromus* type" as already observed by Amano and Chant (1978). Figure 3.2 shows the behavioural steps in the "*Amblyseius – Typhlodromus* type" of pre-mating behaviour.

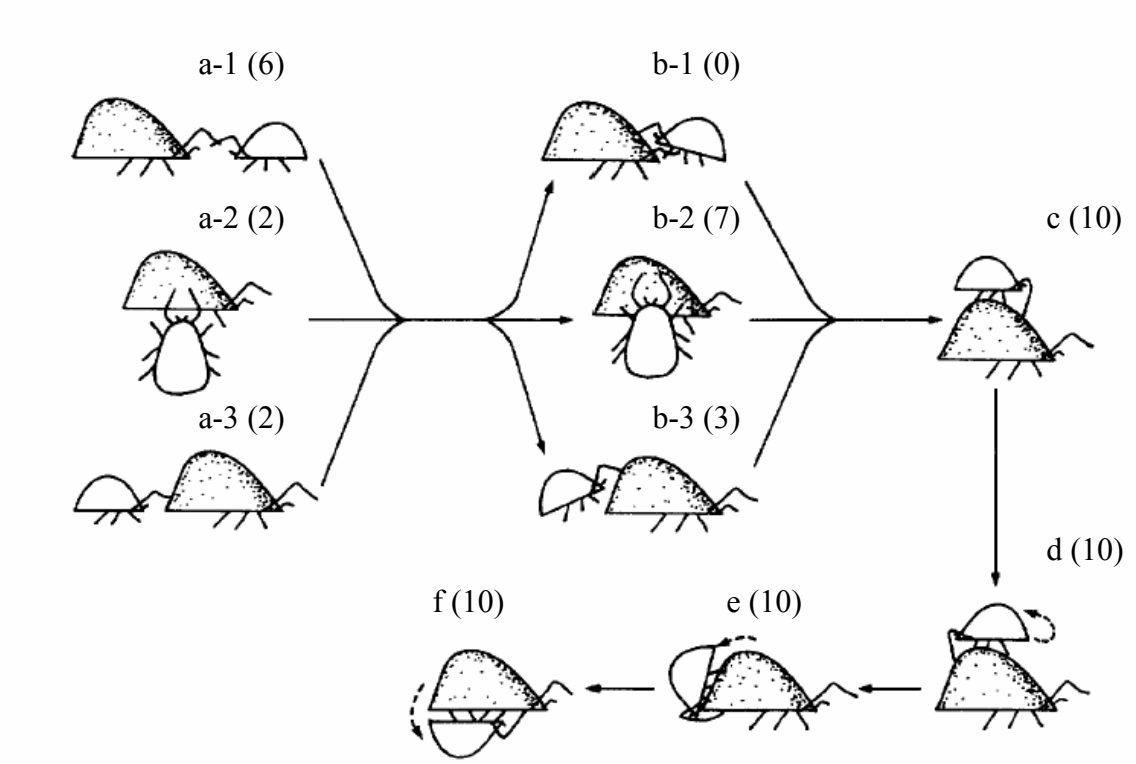


Figure 3.2. Diagram of mating behaviour in *I. degenerans*. The number in brackets indicates the total number of pairs observed in the present study in each category out of 10 replicates. Dotted larger animals represent females (drawing after Amano and Chant, 1978).

In 30% of cases, the female contacted the male. The male was approached from behind, after which the male turned around into a head-to-head position (Fig. 3.2, a-1). In the other cases the male approached the female from the side (a-2), from behind (a-3) or via the head (a-1) and then climbed on the dorsum of the female. Seven out of 10 males climbed on the females laterally (b-2) and 3 males climbed from behind (b-3). Once on

the dorsum of the female, the male spent wandering for an average of 19 ± 8 s, facing forwards and tapping the female with its first pair of legs (c). Finally the male turned 180° and crawled underneath the female via the rear end of the female (d-f). The time measured from the initial contact to the venter-to-venter position averaged 24 ± 8 s. The male was attached to the female by grasping it with the last two pairs of legs.
The length of time *in copula* was on average 48 ± 8 minutes (range 39 – 64 minutes). During copulation, the female was able to move around. The copulation mostly ended by the male leaving the female.

After a pre-oviposition period of a few days (see chapter 4) females started laying eggs. In 100% of cases the first egg of *I. degenerans* developed into a male.

3.4 DISCUSSION

3.4.1 Size of the different life stages

With the measurements of the eggs and the body size of *I. degenerans* immatures and adults presented in Table 3.1, it should be possible to enable the identification of a particular immature stage in a population.

Croft *et al.* (1999) determined the mean size of eggs of 13 phytoseiid mites. The length of these eggs varied from 184.5 to 243.5 µm. From those 13 phytoseiids only *Phytoseiulus persimilis* Athias-Henriot has larger eggs than *I. degenerans*.

Larvae, which are readily distinguishable by the fact that they have only 3 pairs of legs, average 251 µm in length and 189 µm in width. With these measurements larvae of *I. degenerans* are larger than the largest larvae (*i.e.,* those of *P. persimilis*) reported by Croft *et al.* (1999).

Adult phytoseiids are rarely longer than 500 µm. According to van der Merwe (1968) the dorsal shield of a female *I. degenerans* measures 430 to 470 µm in length and 330 to 340 µm in width; the dorsal shield of a male is smaller (353 to 376 µm in length, 282 to 294 µm in width). Our results correspond well with these values. Compared to other phytoseiids used for biological control, *I. degenerans* is among one of the largest mites. According to the measurements made by Croft *et al.* (1999) the length of the idiosoma

of *I. degenerans* exceeds that of *P. persimilis*. Idiosoma length of *Amblyseius andersoni* Chant, *Neoseiulus barkeri* (Hughes), *Neoseiulus cucumeris* (Oudemans), *Galendromus occidentalis* (Nesbitt), *Typhlodromus pyri* Scheuten, and *Euseius finlandicus* (Oudemans) varies from 350 to 420 µm, 350 to 380 µm, 395 to 400 µm, 340 to 350 µm, 300 to 340 µm and 340 to 355 µm, respectively (Karg, 1993).

3.4.2 Mating behaviour of *Iphiseius degenerans*

The "*Amblyseius-Typhlodromus* type" of pre-mating behaviour, is common to the majority of phytoseiid mites observed to date (Amano and Chant, 1986).

In *I. degenerans*, the females were often approached frontally as is also the case in *T. pyri* (Overmeer *et al.*, 1982), while in *Amblyseius potentillae* (Garman) the approach is more from the lateral side or from behind. In the present study, *I. degenerans* climbed mainly onto the female from the side. *Typhlodromus pyri* and *A. potentillae* both climb onto the female from behind. The time from initial contact to the venter-to-venter position is very variable. For *E. finlandicus*, the change between the position on top of the female and the mating position was almost instantaneous, and too short to be timed. The time during which the males stayed on the dorsum of the female and the duration of the copulation event were extremely variable in *Phytoseius macropilis* (Banks), and could take even up to 130 minutes. Males of *Typhlodromus pomi* (Parrott) wandered around for 9.2 minutes before switching to the mating position (Amano and Chant, 1986). Overmeer *et al.* (1982) reported that *A. potentillae* remained for a maximum of 2 minutes on top of the females, while *T. pyri* maintained this position for 10-15 minutes.

The time *in copula* varies among species, ranging from 45 minutes to more than 13 hours. The duration of the copulation of *E. finlandicus* (70 minutes) was less than one fifth of the copulation time noted for *T. pomi* (385 minutes). In *P. macropilis*, males were observed mating continuously with females for longer than 13 h, while others stayed in the mating position for only 45 minutes (Amano and Chant, 1986). Length of time *in copula* for *P. persimilis* and *Amblyseius bibens* Blommers was 149 and 145 minutes, respectively (Schulten *et al.*, 1978). Amano and Chant (1978) reported that *P. persimilis* and *A. andersoni* remained in the mating position for 131 and 185 minutes,

respectively. In *Amblyseius colimensis* Aponte and McMurtry the initial mating took 360 minutes, and the subsequent mating averaged 180 hours (Aponte and McMurtry, 1992). The duration of the initial copulation of *I. degenerans* observed in this study is relatively short compared to that of other phytoseiid mites.

Iphiseius degenerans starts to produce male progeny at the onset of the oviposition period. A sons-first pattern has been recorded for several other phytoseiid mites (e.g., *T. pomi*, *P. macropilis*, *E. finlandicus* (Amano and Chant, 1986), *P. persimilis* and *Amblyseius womersleyi* Schicha (Toyoshima and Amano, 1998)). According to Sabelis (1985) this pattern makes sense from an evolutionary point of view. The mother's fitness will be favoured if she ensures fertilization of her female progeny at the earliest possible moment, since reproduction in phytoseiids with paternal genome loss (pseudo-arrhenotoky) requires male gametes and thus, mating later in life would postpone reproduction (Sabelis, 1985).

CHAPTER 4

EFFECT OF POLLEN, NATURAL PREY AND FACTITIOUS PREY ON THE DEVELOPMENT OF *IPHISEIUS DEGENERANS*

4.1 INTRODUCTION

In determining the power of population increase of phytoseiid predators, the developmental time and survival of immature stages are crucial factors (Sabelis, 1985). Various authors assessed the development of phytoseiids on different kinds of food, but in the case of *I. degenerans* only a few publications on the developmental biology are available in the literature. In these studies, however, mainly pollen or tetranychid prey were offered as food (Takafuji and Chant, 1976; McMurtry, 1977; McMurtry *et al.*, 1984; van Rijn and Tanigoshi, 1999).

Considering the polyphagous character of this predator, the effect of 5 pollen species, four natural prey species (a mixture of *Tetranychus urticae* Koch life stages, nymphs of *Frankliniella occidentalis* (Pergande), *Trialeurodes vaporariorum* Westwood eggs, and nymphs of *Myzus persicae* Sulzer), a combination of *F. occidentalis* nymphs and *Ricinus communis* L. pollen, and 2 factitious prey species (*Ephestia kuehniella* Zeller eggs and *Artemia franciscana* Kellogg cysts) on the development of *I. degenerans* was determined. In addition, developmental performance of *I. degenerans* on different substrates (artificial versus leaf arena) was assessed. An artificial substrate (Multicel) was used in an attempt to standardize all experiments and to eliminate the influence of leaf feeding on the development of the predatory mite. Leaf arenas were used to investigate whether *I. degenerans* was able to develop on plant sap, to provide the natural prey with food or to serve as an oviposition substrate for prey.

This chapter is based on: Vantornhout, I., Minnaert, H.L., Tirry, L. and De Clercq, P. 2004. Effect of pollen, natural prey and factitious prey on the development of *Iphiseius degenerans*. BioControl 49: 627-644.

4.2 MATERIALS AND METHODS

4.2.1 Predator culture

Iphiseius degenerans was reared as described in chapter 3 (3.2.1 Predator culture).

4.2.2 Food sources

Pollen

Pollen from apple (*Malus domestica* Borkh.), almond (*Prunus dulcis* (Mill.) Webb) and plum (*Prunus domestica* L.) were purchased from Firman Pollen Co. (Washington, USA) and stored in the refrigerator (ca. 6 °C) during the experiments. Pollen from castor bean *R. communis* was collected from plants grown in a field plot at the Faculty of Bioscience Engineering of Ghent University. Flowers were collected, dried in an incubator at 37 °C and thoroughly shaken in a 50 µm mesh sieve to separate and collect the pollen. Pollen was stored in glass jars in the deep freeze (at –18 °C) for long term storage or in the refrigerator (ca. 6 °C) during the experiments. Flowers of sweet pepper (*Capsicum annuum* L. var. California Wonder) were collected; the anthers were cut and dried in an incubator at 37 °C. The pollen was separated, collected and stored as above.

Natural prey

Spider mites (*T. urticae*) were reared on green bean plants (*Phaseolus vulgaris* L. var. Prelude) in a climatic chamber at 30 ± 5 °C, 40 ± 5% RH and a 16L:8D h photoperiod. As *I. degenerans* is able to prey on all life stages of the twospotted spider mite, a mixture of different life stages of the mite was either offered to the predator on a small piece of green bean foliage or was brushed off directly onto the arena. To make sure all life stages remained present during the experiment, fresh prey of different life stages were added daily. On a bean leaf arena, spider mites were allowed to develop and produce webbing on the arena for 4 days before introduction of predatory mites.

The western flower thrips (*F. occidentalis*) was reared on green bean pods (*P. vulgaris*) and was kept in a climatic cabinet at 25 ± 1 °C, 75 ± 5 % RH and a 16L:8D h photoperiod. Either a mix of 1st and 2nd instar nymphs (10 nymphs per arena) or 10 first instar nymphs were offered to the predatory mites. Dead prey was removed daily from the arena, and replaced with fresh prey.

Adults of the greenhouse whitefly *T. vaporariorum* were collected from *Nicotiana glauca* Graham plants in greenhouses on the premises of the faculty. They were subsequently reared on green bean plants in a continuous culture in the laboratory. Whitefly eggs were obtained by transferring adults to a fresh bean leaf contained in a drum cell (Fig. 4.1). The drum cell consisted of a Plexiglas cylindrical ring (9 cm diameter, 3.5 cm high), a Plexiglas plate (9 cm diameter) and a mesh-covered ring (9 cm diameter). The ring has 7 ventilation holes (1 cm diameter) covered with nylon gauze. Each “drum” cell was placed on a plastic support that contained tap water. A primary bean leaf was placed in each “drum” cell with the stem hanging in the tap water of the support via a hole in the Plexiglas ring. To avoid scatter of the eggs over the whole leaf surface, the leaf was covered with a piece of cloth (Vileda®) in which a circle (diameter 4.5 cm) was cut. After 48 hours, adults were removed and the number of eggs was reduced to 100 by puncturing the excess with a needle.

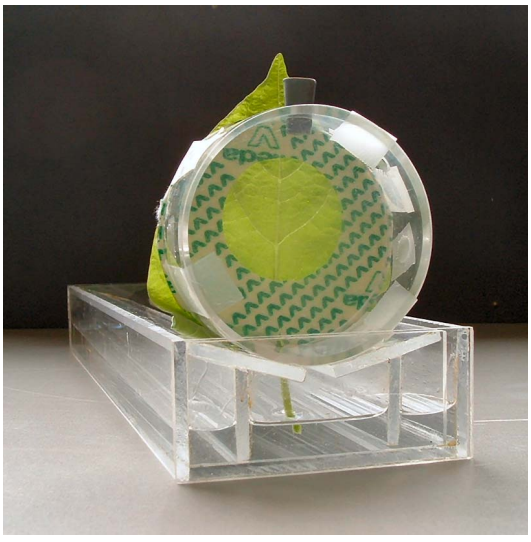


Figure 4.1. Drum cell.

Individuals of the green peach aphid *M. persicae* originated from INRA Antibes and were reared in the laboratory on pepper plants (*C. annuum* var. Cayenne Long Slim). A

mix of first and second instar nymphs were offered to the predatory mites (7 nymphs per arena). Every two days, aphid nymphs were removed and replaced with new ones.

Factitious prey

Deep frozen eggs of the Mediterranean flour moth *E. kuehniella* were obtained from Koppert BV and stored in the deep freeze (at –18 °C). Eggs used in the experiments were thawed and kept in the refrigerator (ca. 6 °C) for about 7 days. Every 3 days the eggs were removed from the arena and new eggs were supplied (10 to 20 eggs per arena).

Cysts of the brine shrimp *A. franciscana* were obtained from the Laboratory of Aquaculture and Artemia Reference Centre, Ghent University, Belgium. Both dry encapsulated cysts and dry decapsulated cysts were used. Decapsulated cysts are cysts of which the outer alveolar layer is removed by washing in a hypochlorite solution (Van Stappen, 1996). The cysts were kept in the refrigerator during the experiments. Every 3 days the cysts were removed from the arena and new cysts were supplied (10 to 20 cysts per arena).

4.2.3 Experimental units

Artificial substrate

Each experimental unit consisted of a green Multicel plate (6 x 6 cm) placed on a 1 cm thick foam pad in a water-containing square petri dish (8 x 8 cm). The edges of the Multicel plate were covered with wet absorbent paper yielding an arena of approximately 12 cm². A 1 cm long black sewing thread served as a hiding place for the mites (Fig. 4.2 a).

Leaf substrate

This arena differs from the Multicel arena by the fact that in this experimental unit, the Multicel plate was replaced by a piece of sweet pepper leaf (*C. annuum* var. California Wonder) or bean leaf (*P. vulgaris* var. Prelude) cut to a similar size (6 x 6 cm) (Fig. 4.2.b).

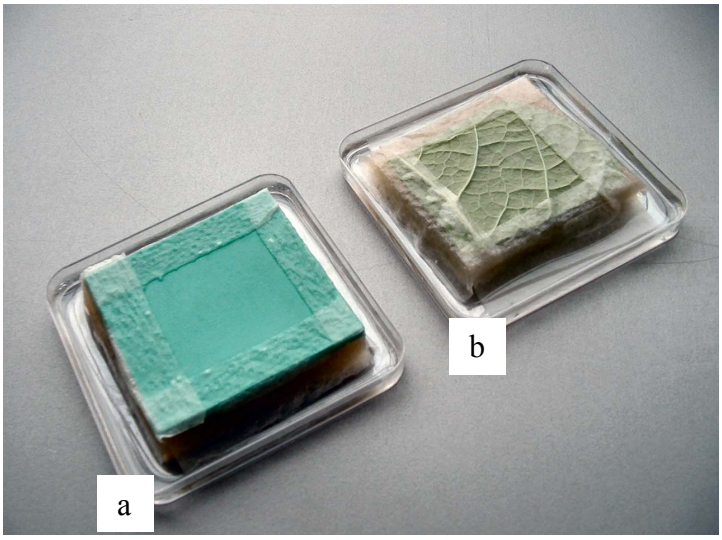


Figure 4.2. Experimental arenas: a. Artificial substrate, b. Leaf substrate.

4.2.4 Experiments

A series of experiments were carried out to determine the effect of diet and substrate on the development of *I. degenerans*.

First, the influence of the substrate (Multicel, sweet pepper leaf and bean leaf) on the development of *I. degenerans* in the absence of prey was assessed. Second, the predatory mite was reared on a Multicel arena and offered food from one of three categories: pollen (almond pollen, apple pollen, castor bean pollen, plum pollen and sweet pepper pollen), natural prey (*T. urticae* offered on green bean foliage, *T. urticae* brushed off onto the arena, and nymphs of *F. occidentalis* placed on the arena) or factitious prey (*Ephestia* eggs or *Artemia* cysts). Subsequently, on a bean leaf substrate *T. urticae*, *F. occidentalis* nymphs, *T. vaporariorum* eggs, *M. persicae* nymphs or a combination of *F. occidentalis* nymphs and castor bean pollen was offered to the

predatory mite. In a last series of experiments the effect of substrate (Multicel, bean leaf or sweet pepper leaf) on the development of the predatory mite when reared on three different diets was assessed. The selected diets were: castor bean pollen, the main food used in the mass rearing of *I. degenerans*, and two of its natural prey, *T. urticae* and *F. occidentalis*.

Development and survival of immature stages

To investigate the ability of *I. degenerans* to develop on the different kinds of food, one egg of the predatory mite was transferred to every experimental unit. Thirteen hours prior to the start of the experiments new black threads were placed in the predatory mite colony. From the eggs that were laid within 13 hours, thirty eggs were randomly chosen and transferred onto an experimental arena using a fine brush. There were 30 replicates per diet. When no food was added to the arena, the mites were able to consume water from the absorbent paper. In the experiments where food was presented to the predator, an excess of food was added as soon as the larvae emerged. The arenas were kept in a climatic cabinet at 25 ± 1 °C, 75 ± 5% RH and a 16L:8D h photoperiod. To obtain data on the duration of each developmental stage and on mortality and escape rates, observations were made every 12 hours until all individuals reached adulthood. The presence of exuviae on the arenas was used as a criterion for a successful moult to the next stage.

4.2.5 Data analysis

To avoid overestimation of the developmental duration of some stages, only the cases in which the eggs developed to adults were submitted to analysis. In experiments in which the number of obtained adults was less than 5 out of 30, no analyses were done. Developmental duration of the different life stages of the predatory mite was analysed using analysis of variance, followed by a multiple comparison test (Student Newman Keuls) at the $p = 0.05$ level (SPSS 12.0, SPSS Inc., 1989-2003).

4.3 RESULTS

The time spent by the males and females of *I. degenerans* in each developmental stage (egg, larva, protonymph and deutonymph) was affected by the tested diets and substrates.

4.3.1 Egg hatch

All eggs used in the experiments successfully hatched at the given climatic conditions. The mean developmental time of the egg stage (± SEM) of *I. degenerans* calculated over all replicates and independently of the food offered to the predatory mite was 2.41 ± 0.02 days, with no significant differences between males and females ($F = 0.5$, df = 368, $p = 0.5$). Although all eggs were harvested from the same colony maintained on castor bean pollen, there were significant differences in incubation time of eggs selected to initiate experiments (Tables 4.1 – 4.5).

4.3.2 Development of immature stages and survival in the absence of food

As expected, *I. degenerans* is not able to complete its development in the absence of food (Table 4.1). On all substrates larvae were able to develop into protonymphs, but then died as protonymphs 5 to 7 days after egg hatching. The predatory mites survived longer on a leaf arena than on a Multicel arena although significant differences between the arenas were only found in the egg and larval stage (eggs: $F = 5.4$, df = 56, $p < 0.01$; larvae: $F = 11.0$, df = 56, $p < 0.0001$; protonymphs: $F = 0.5$, df = 56, $p = 0.6$; total immature survival: $F = 0.8$, df = 56, $p = 0.4$).

Table 4.1. Development of immature stages of *I. degenerans* reared without food on three types of substrate

Substrate	n[b]	Stage duration (days)[a]			
		Egg	Larva	Protonymph	Egg – death
Multicel	25	2.37 ± 0.06a	1.02 ± 0.04a	1.55 ± 0.14a	4.94 ± 0.14a
Bean leaf	8	2.78 ± 0.10b	1.54 ± 0.16b	2.72 ± 0.30a	7.03 ± 0.40a
Sweet pepper leaf	24	2.53 ± 0.07a	1.03 ± 0.07a	2.98 ± 1.57a	6.55 ± 1.57a

[a]Means (± SEM) within a column followed by the same letter are not significantly different (Student Newman Keuls-test, $p > 0.05$)

[b]Number of individuals that reached the protonymphal stage (initial number = 30)

4.3.3 Effect of diet on development and survival of immature stages

When reared on pollen, tetranychid prey, thrips nymphs, whitefly eggs, aphids, brine shrimp cysts or flour moth eggs, developmental and survival rates varied as a function of food and substrate used (Tables 4.2 – 4.4).

All larvae developed to the protonymphal stage irrespective of diet. From the protonymphal stage on, effects of food were more pronounced.

For both females and males no development beyond the protonymphal stage was observed when 1st and 2nd instars of *F. occidentalis* or dry encapsulated cysts of the brine shrimp *A. franciscana* were offered (Table 4.2 and 4.3). Immature mortality recorded for abovementioned diets was 56.7% and 86.7% (*F. occidentalis* and dry encapsulated *A. franciscana* cysts, respectively); 43.3% and 13.3% of the individuals escaped from the arena, and were not retrieved. Immature mortality was highest in the protonymphal stage (40.0% and 73.3%, respectively). The highest escape rates were found among the larvae when fed thrips (26.7%); when fed dry encapsulated brine shrimp cysts 6.7% of the larvae and 6.7% of the deutonymphs escaped from the arena.

Table 4.2. Development of *I. degenerans* females reared on different diets on a Multicel arena

Diet	n[b]	Stage duration (days)[a]				
		Egg	Larva	Protonymph	Deutonymph	Total
Pollen						
Almond	12	2.31 ± 0.13ab	0.81 ± 0.08 a	1.49 ± 0.09abc	1.51 ± 0.06a	6.11 ± 0.11a
Apple	18	2.27 ± 0.04ab	0.94 ± 0.06a	1.46 ± 0.09abc	1.48 ± 0.08a	6.16 ± 0.08a
Castor bean	14	2.57 ± 0.07b	1.01 ± 0.00a	1.21 ± 0.07a	1.43 ± 0.08a	6.20 ± 0.09a
Plum	17	2.57 ± 0.06b	0.94 ± 0.04a	1.29 ± 0.09ab	1.52 ± 0.09a	6.32 ± 0.08a
Sweet pepper	12	2.39 ± 0.08ab	1.30 ± 0.06b	1.76 ± 0.24bc	1.58 ± 0.23a	7.04 ± 0.17b
Natural prey						
T. urticae on a bean leaf	11	2.25 ± 0.16ab	1.01 ± 0.04a	1.55 ± 0.08abc	1.39 ± 0.10a	6.21 ± 0.20a
T. urticae brushed off onto arena	15	2.30 ± 0.09ab	1.04 ± 0.06a	1.88 ± 0.15ca	1.63 ± 0.12a	6.85 ± 0.12b
F. occidentalis (1^{st} and 2^{nd} instars)	0	-	-	-	-	-
Factitious prey						
Decapsulated *Artemia* cysts	10	2.55 ± 0.08b	1.05 ± 0.09a	1.74 ± 0.15abc	2.41 ± 0.19b	7.76 ± 0.28c
Encapsulated *Artemia* cysts	0	-	-	-	-	-
E. kuehniella eggs	7	2.08 ± 0.11a	0.95 ± 0.01a	1.73 ± 0.11abc	2.26 ± 0.31b	7.02 ± 0.37b

[a]Means (± SEM) within a column followed by the same letter are not significantly different ($p > 0.05$, Student Newman Keuls).

[b]Number of individuals that reached adulthood; experiments were started with 30 eggs, data for emerging males are reported in Table 4.3.

Table 4.3. Development of *I. degenerans* males reared on different diets on a Multicel arena

Diet	n[b]	**Stage duration (days)**[a]				
		Egg	**Larva**	**Protonymph**	**Deutonymph**	**Total**
Pollen						
Almond	16	2.41 ± 0.09bc	0.98 ± 0.06ab	1.31 ± 0.11a	1.22 ± 0.08a	5.92 ± 0.08a
Apple	10	2.42 ± 0.08bc	0.95 ± 0.05ab	1.34 ± 0.08a	1.26 ± 0.09a	5.96 ± 0.09a
Castor bean	16	2.43 ± 0.09bc	0.88 ± 0.06a	1.37 ± 0.07a	1.22 ± 0.07a	5.91 ± 0.08a
Plum	9	2.53 ± 0.08bc	1.00 ± 0.00ab	1.29 ± 0.12a	1.28 ± 0.12a	6.09 ± 0.12a
Sweet pepper	6	2.60 ± 0.06c	1.12 ± 0.02b	1.85 ± 0.37ab	1.78 ± 0.31ab	7.35 ± 0.40b
Natural prey						
T. urticae on a bean leaf	14	2.15 ± 0.09ab	1.10 ± 0.06b	1.49 ± 0.08a	1.29 ± 0.09a	6.03 ± 0.10a
T. urticae brushed off onto arena	8	2.42 ± 0.08bc	0.99 ± 0.01ab	2.15 ± 0.16b	1.50 ± 0.15a	7.05 ± 0.26b
F. occidentalis (1^{st} and 2^{nd} instars)	0	-	-	-	-	-
Factitious prey						
Decapsulated *Artemia* cysts	17	2.52 ± 0.06bc	1.00 ± 0.00ab	1.58 ± 0.12a	2.28 ± 0.16c	7.38 ± 0.20b
Encapsulated *Artemia* cysts	0	-	-	-	-	-
E. kuehniella eggs	16	2.01 ± 0.07a	0.94 ± 0.01ab	1.90 ± 0.20ab	2.12 ± 0.16bc	6.97 ± 0.29b

[a]Means (± SEM) within a column followed by the same letter are not significantly different ($p > 0.05$, Student Newman Keuls).

[b]Number of individuals that reached adulthood; experiments were started with 30 eggs, data for emerging females are reported in Table 4.2.

On the remaining diets, development to the adult stage never took longer than 8.0 days. The adult stage was reached more rapidly when the predator was fed pollen (6.1 – 6.2 days and 5.9 – 6.0 days for females and males respectively), except for sweet pepper pollen and plum pollen, than when natural prey or factitious prey was offered (6.2 – 7.8 days and 6.0 – 7.4 days for females and males respectively).

In both sexes, significant differences in developmental periods were found between diets (females: $F = 12.5$, df = 115, $p < 0.0001$; males: $F = 11.5$, df = 111, $p < 0.0001$). Developmental times varied more as a function of diet in the protonymphal stage in females and, to a lesser extent, in the deutonymphal stage in males. In both females and males, mean total development time on almond, apple, castor bean and plum pollen was significantly shorter than on sweet pepper pollen. As for the diet consisting of *T. urticae*, development of the predator was affected by the manner in which the prey was offered. Development from egg to adult took significantly longer when a mixture of *T. urticae* life stages was directly brushed off onto the arena than when a bean leaflet infested with spider mites of all stages was supplied. In the factitious prey diets, dry decapsulated brine shrimp cysts resulted in a longer developmental time than Mediterranean flour moth eggs (7.8 and 7.4 days vs. 7.0 and 6.9 days for females and males respectively). In this case the difference between the two diets was only significant for *I. degenerans* females (Table 4.2). In seven out of nine diets, the total developmental time of the *I. degenerans* males was shorter than that of the females, with differences varying from 0.05 to 0.4 days. Male and female developmental times only differed significantly when castor bean pollen was supplied as food. Only in the case of sweet pepper pollen and twospotted spider mites brushed off onto a Multicel arena, did male development take slightly longer (max. 0.3 days).

During development, mortality and escape rates were strongly affected by diet. Figure 4.3 shows the mortality and escape rates recorded for the diets on which *I. degenerans* was able to complete its development. The diets are grouped per food category (pollen, natural prey and factitious prey), and within each category data are sorted from the shortest to the longest total developmental time. Mortality occurred when the predator was reared on sweet pepper pollen (26.7%), *Ephestia* eggs (20.0%), a mixture of *T. urticae* life stages brushed off onto the arena (10.0%) or supplied on a bean leaflet (6.7%). On all diets, except castor bean pollen, some juveniles (varying

from 3.3% to 13.3%) escaped from the Multicel arena. The highest percentages of escape were found when plum pollen, sweet pepper pollen or twospotted spider mites were supplied as food. Escape of the predatory mites occurred mostly in the larval stage.

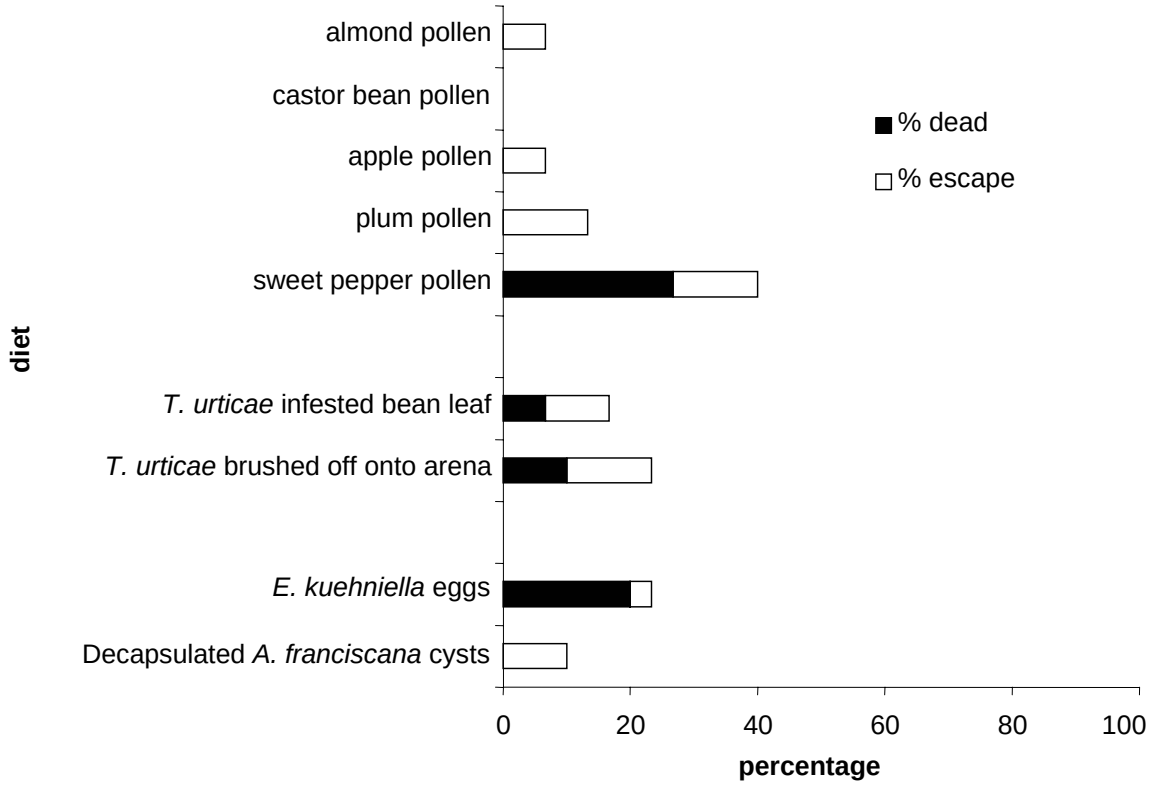


Figure 4.3. Mortality and escape rates of immature *I. degenerans* reared on different diets on a Multicel arena.

Developmental times of *I. degenerans* on different food sources offered on a detached bean leaf are reported in Table 4.4. On castor bean pollen, the predator reached adulthood sooner than on the other diets. When *F. occidentalis* nymphs were offered, only 4 (diet consisting of 1st instars) and 3 predatory mites (diet consisting of 1st and 2nd instars) out of 30 survived to adulthood. Adding castor bean pollen to the diet of 1st instar thrips increased the survival rate to 60%. No immature mites died when reared on this combination of thrips nymphs and pollen, whereas mortality ranged from 33.3 - 36.7% in the other diets consisting of thrips nymphs only.

Table 4.4. Development of *I. degenerans* reared on different diets on a bean leaf arena

Diet	***n*[b]**	**Stage duration (days)[a]**				
		Egg	**Larva**	**Protonymph**	**Deutonymph**	**Total**
Pollen						
R. communis	21	2.46 ± 0.10b	0.92 ± 0.09a	1.35 ± 0.09a	1.36 ± 0.12a	6.09 ± 0.11a
Natural prey						
T. urticae	8	2.53 ± 0.06b	1.04 ± 0.05a	2.07 ± 0.17c	2.41 ± 0.42b	8.05 ± 0.53c
F. occidentalis (1st instars)	4	2.51 ± 0.34	1.03 ± 0.01	1.97 ± 0.01	2.24 ± 0.14	7.75 ± 0.21
F. occidentalis (1st and 2nd instars)	3	2.57 ± 0.14	0.75 ± 0.16	2.77 ± 0.92	2.17 ± 0.74	8.26 ± 0.23
T. vaporariorum	20	2.63 ± 0.05b	1.02 ± 0.05a	1.75 ± 0.09b	1.70 ± 0.06a	7.11 ± 0.13b
M. persicae	15	2.16 ± 0.08a	1.09 ± 0.08a	2.26 ± 0.07c	1.86 ± 0.07a	7.38 ± 0.10b
Natural prey and pollen						
F. occidentalis (1st instars) and *R. communis*	18	2.70 ± 0.05b	0.96 ± 0.03a	1.66 ± 0.11ab	1.85 ± 0.09a	7.16 ± 0.16b

[a]Means (± SEM) within a column followed by the same letter are not significantly different ($p > 0.05$, Student Newman Keuls); means without a letter were excluded from analysis.

[b]Number of individuals that reached adulthood; experiments were started with 30 eggs.

Table 4.5. Effect of substrate on the development of *I. degenerans* when reared on different diets

Diet	**Substrate**	***n*[b]**	**Stage duration (days)[a]**				
			Egg	**Larva**	**Protonymph**	**Deutonymph**	**Egg -adult**
R. communis pollen	Multicel	30	2.49 ± 0.06a	0.94 ± 0.03a	1.29 ± 0.05a	1.32 ± 0.05a	6.05 ± 0.06a
	Bean leaf	21	2.46 ± 0.10a	0.92 ± 0.09a	1.35 ± 0.09a	1.36 ± 0.12a	6.09 ± 0.11a
	Sweet pepper leaf	25	2.32 ± 0.10a	1.09 ± 0.07a	1.38 ± 0.13a	1.54 ± 0.09a	6.34 ± 0.07b
T. urticae	Multicel + bean leaf	25	2.19 ± 0.09a	1.06 ± 0.04a	1.51 ± 0.06a	1.34 ± 0.07a	6.10 ± 0.10a
	Multicel	23	2.34 ± 0.06ab	1.02 ± 0.04a	1.97 ± 0.11b	1.58 ± 0.10a	6.92 ± 0.12b
	Bean leaf	8	2.53 ± 0.06b	1.04 ± 0.05a	2.07 ± 0.17b	2.41 ± 0.42b	8.05 ± 0.53c
	Sweet pepper leaf	1	2.77	0.98	2.00	1.58	7.33
F. occidentalis	Multicel	0	1.95 ± 0.08	1.37 ± 0.11	1.88 ± 0.42[c]	-	-
	Bean leaf	3	2.57 ± 0.14	0.75 ± 0.16	2.77 ± 0.92	2.17 ± 0.74	8.26 ± 0.23
	Sweet pepper leaf	2	2.77 ± 0.00	0.98 ± 0.00	2.75 ± 0.17	2.54 ± 0.20	9.04 ± 0.37

[a]Means (± SEM) within a column and a diet followed by the same letter are not significantly different ($p > 0.05$, Student Newman Keuls); means without a letter were excluded from analysis.

[b]Number of individuals that reached adulthood (initial number = 30).

[c]All individuals died in the protonymphal stage

The highest proportion of escaping mites (57%) was found in the diet consisting of 1st and 2nd instars of *F. occidentalis*, while 50% of the immatures escaped when offered 1st instar thrips nymphs. *Iphiseius degenerans* was able to develop on a diet of *T. vaporariorum* eggs or *M. persicae* nymphs. The obtained developmental times did not differ significantly from the values found when thrips nymphs and pollen were supplied as food. Immature mortality on a diet of aphid nymphs was higher than on whitefly eggs (38 versus 20%).

4.3.4 Effect of substrate on development and survival of the immature stages

Table 4.5 shows the effect of substrate used in the experimental unit on the development of *I. degenerans* on three diets. The developmental period from egg to adult on castor bean pollen averaged 6.0 days on a Multicel arena or a bean leaf, whereas on a sweet pepper leaf development took significantly longer (ca. 0.3 days).

A greater variation was observed when twospotted spider mites were provided as food. *Iphiseius degenerans* develops significantly faster when offered a spider mite infested bean leaf on a Multicel arena (indicated in Table 4.5 as Multicel + bean leaf). The longest developmental time (ca. 8.0 days) was recorded on the bean leaf substrate. When *T. urticae* was presented to the predator on a sweet pepper leaf, only 1 individual reached adulthood. Mortality of the predator reared on spider mites was 6.7% when an infested bean leaf was placed on a Multicel arena, 10.0% when the prey was brushed off onto the Multicel arena, 13.3% on a detached bean leaf arena and 36.7% on a detached sweet pepper leaf arena. Escape rates were 10.0%, 13.3%, 60.0% and 60.0%, respectively. When reared on spider mites, escape of the predatory mites was highest in the larval stage, whereas mortality occurred mostly in the protonymphal stage.

On a diet consisting of *F. occidentalis* nymphs, no development beyond the protonymphal stage was recorded when *I. degenerans* was reared on a Multicel arena. The predator lived on average for 5.2 days. Only 3 and 2 predatory mites reached adulthood on a detached bean leaf and sweet pepper leaf, respectively. On the detached bean leaf arena 56.7% of the predators escaped from the arena, and 33.3% died. Escape rates were higher in the larval stage (40.0%), whereas the mortality was highest in the

protonymphal stage (16.7%). Similar results were obtained on a sweet pepper leaf arena: 63.3% of the individuals escaped from the arena (53.3% as larvae) and 30.0% died as protonymphs. Here, no mortality was recorded in the larval and deutonymphal stages.

4.4 DISCUSSION

Developmental rates and immature survival of phytoseiid mites vary considerably depending on abiotic and biotic factors. Larvae of some phytoseiids, for example *Phytoseiulus longipes* Evans, *Neoseiulus cucumeris* (Oudemans), and *Neoseiulus californicus* (McGregor) (Badii and McMurtry, 1983; van Rijn and Tanigoshi, 1999; Chittenden and Saito, 2001) are able to develop into the protonymphal stage in the absence of food, but for further development, food such as natural prey or plant pollen is required. *Iphiseius degenerans* was unable to develop beyond the protonymphal stage when offered leaf tissue or water only. van Rijn and Tanigoshi (1999) and Chittenden and Saito (2001) reported similar results for the same species. Even when *I. degenerans* is able to feed on plant cells, the nutritional value of plant sap is insufficient to ensure full development. Porres *et al.* (1975) concluded that plant sap may be an alternative source of food and/or moisture for *Amblyseius hibisci* (Chant). However, Chant and Fleschner (1960) showed earlier that *A. hibisci* did not reproduce and that the lifespan was only slightly prolonged when compared with individuals living on water alone.

Pollen is considered to be favourite food for the immature stages of several phytoseiid mites. Development on pollen is reportedly completed within 6 – 7 days (Castagnoli and Simoni, 1990; Yue and Tsai, 1996; Broufas and Koveos, 2000). Particularly almond, apple and castor bean pollen yielded higher developmental rates and lower mortality, when compared with other diets. *N. cucumeris* also showed better immature survival and a shorter egg-to-adult period when reared on pollen (Castagnoli and Simoni, 1990). Our results indicate that almond pollen is even superior to castor bean pollen, which is used in our mass rearing of *I. degenerans*. van Rijn and Tanigoshi (1999) also reported a higher oviposition rate of *I. degenerans* on almond pollen than on *R. communis* (2.28 and 1.73 eggs/day, respectively). This suggests that almond pollen is

a better diet for mass rearing *I. degenerans*. The results on sweet pepper pollen seem surprising as *I. degenerans* is frequently found in sweet pepper flowers using pollen and/or thrips nymphs as food (van Houten and van Stratum, 1993). The developmental duration obtained on sweet pepper pollen is even higher than when *T. urticae* was supplied on a Multicel arena. van Rijn and Sabelis (1990) concluded that sweet pepper pollen is a determinant of *N. cucumeris* population size in periods of thrips scarcity.

In 7 of the nine diets offered on a Multicel arena, males developed slightly faster than the females (max. 9 h) (the difference was only significant in the diet consisting of castor bean pollen). Sabelis (1985) reported that egg-to-adult period of male phytoseiids is generally not shorter than that of females. However, in *Phytoseius hawaiiensis* Prasad and *N. cucumeris*, males develop more rapidly than the females, but in most species the difference between the developmental times of both sexes is not significant (e.g., Sanderson and McMurtry, 1984; Castagnoli and Simoni, 1990).

Generally, development of phytoseiid mites fed on tetranychids is completed within 5.4-8 days (Takafuji and Chant, 1979; Castagnoli and Simoni, 1990; Duso and Camporese, 1991; van Rijn and Tanigoshi, 1999; Kazak *et al.*, 2002). Our results fit well within that range. However, the developmental time was affected by two factors: the substrate used in the experimental unit, and the way in which the spider mites were offered to the predator on a Multicel arena. Shorter developmental times were obtained on Multicel than on leaf substrates. When spider mites were brushed from a leaf onto a Multicel arena, development of the predator took longer than when a bean leaf infested with spider mites was supplied. These differences might be caused by the webbing of the spider mites, as the adult stage was reached more rapidly on arenas on which less webbing was produced. On Multicel arenas, the webbing was produced around the black thread on the arena, and this was observed more frequently in experiments in which prey was brushed off onto the arena than when an infested bean leaf was supplied. As the bean leaf dried, prey wandered more frequently over the arena and thus was easier to seize by the predator, compared to prey in the webbing around the black thread. In experiments using leaf arenas, the whole area was covered with webbing, resulting in a longer developmental time of the predator. The escape rates on a leaf arena were 6 times higher compared to those on a Multicel arena. The webbing of *Tetranychus* spp. appears to be an impediment to foraging *I. degenerans*, and forces the

predator to leave the arena. This might explain the higher escape rates observed on leaf arenas. According to van Rijn and Tanigoshi (1999), *I. degenerans* and *N. cucumeris* showed a similar performance on pollen and on the twospotted spider mite, when the latter was offered without webbing. Sabelis and Bakker (1992) found that the phytoseiid mites that were better protected from the webbing of Tetranychidae had long setae in the medial and lateral position on the dorsal shield. *I. degenerans* may thus be less adapted to preying on spider mites, given that this species has short dorsal and lateral setae on the dorsal shield (Van der Merwe, 1968).

A diet consisting of eggs of the greenhouse whitefly *T. vaporariorum* was sufficient for the development of *I. degenerans*. When fed whitefly eggs the predator reached adulthood in 7 days, with a survival rate of 67%. These results are similar to those obtained when the predatory mites were fed on a combination of pollen and thrips nymphs. Attacks of *I. degenerans* on *Parabemisia myricae* (Kuwana) have been reported (Swirski *et al.*, 1987), but no further data on the predatory activity of *I. degenerans* against any whitefly species have been found in the literature. Several *Amblyseius*, *Typhlodromus* and *Euseius* species, however, have been reported as predators of the sweetpotato whitefly *Bemisia tabaci* (Gennadius). An overview of phytoseiid predators recorded feeding on *B. tabaci* is given by Gerling (2001) and Nomikou *et al.* (2001). Our results are consistent with those found in the literature. Developmental times of phytoseiids fed on immature stages of *B. tabaci* range from 7 to 9 days (Nomikou *et al.*, 2001; Meyerdirk and Coudriet, 1985, 1986). Meyerdirk and Coudriet (1986) reported that 47-65% of *E. scutalis* fed *B. tabaci* survived, whereas for *E. hibisci* a survival rate of 75% was recorded (Meyerdirk and Coudriet, 1985).

Iphiseius degenerans was able to complete development from egg to adult when fed on *M. persicae* nymphs. Fifty percent of the immatures survived to adulthood in 7.4 days. To our knowledge, development of *I. degenerans* when fed aphid nymphs has not been documented before. Nevertheless, the predatory mite has been recommended as a biological control agent for aphids (ATTRA, 2005).

Not all prey tested in this study was suitable for the development of *I. degenerans*. No development beyond the protonymphal stage occurred when the predator was fed 1st and 2nd instars of *F. occidentalis* on a Multicel arena. Also given the low number of adults obtained on a leaf substrate, it can be concluded that nymphs of *F. occidentalis*

are unfavourable food for immature *I. degenerans*. As this phytoseiid mite is commercialized as a thrips predator, these results seem surprising. Sengonca and Drescher (2001) reported similar observations with *I. degenerans* and 2nd instars of *Thrips tabaci* Lindemann as prey. For the commercialized thrips predator *N. cucumeris* developmental times ranging from 8.2 to 9.5 days have been found (Gillespie and Ramey, 1988; Castagnoli *et al.*, 1990; Castagnoli and Simoni, 1990). High immature mortality (37.83%) for the latter species has been reported by Castagnoli *et al.* (1990). The high percentages of escape and immature mortality on both Multicel and leaf arenas may be explained in part by the aggressive defence behaviour of the thrips nymphs. Larvae of *I. degenerans* escaped more from the arena than the other developmental stages. It is hypothesized that the less active larvae of the predator, compared to the other stages, are more disturbed by the wandering (and defence behaviour) of thrips nymphs and consequently flee to the absorbent paper in which they get stuck and die. It was also observed that the thrips nymphs (especially second instars) evade attacks, particularly of the smaller protonymphs, by jerking with their abdomen. In that way, protonymphs were deprived of food and died, explaining the high mortality in that stage. Bakker and Sabelis (1989) reported that thrips nymphs also evade attacks by predators by producing a drop of rectal fluid. Only when thrips nymphs are seized in the thoracic region, both jerking and droplet production are ineffective. Adding castor bean pollen to a diet of thrips nymphs resulted in longer developmental times compared to that on a diet consisting of pollen alone. The presence of pollen on the arena, however, reduced the mortality of *I. degenerans* immatures, but predators still escaped from the arena. As the thrips nymphs also used the pollen as food, the possibility of encountering thrips nymphs and as such being exposed to the defence behaviours of the thrips nymphs, still remained.

Iphiseius degenerans was able to develop to the adult stage on *E. kuehniella* eggs and decapsulated *A. franciscana* cysts, emphasizing the polyphagous character of the predatory mite and its ability to feed and develop on factitious foods. However, they do not constitute a good alternative for use in mass rearing of the predatory mite as these diets resulted in the longest developmental duration. In the literature no records were found in which phytoseiid mites were fed on *Ephestia* eggs or *Artemia* cysts. *Ephestia* eggs are frequently used as an alternative food source for natural enemies (examples in

Morrison, 1985; Schanderl *et al.*, 1988; Grenier *et al.*, 1989; Nicoli *et al.*, 1991; Vacante *et al.*, 1997).

On non-hydrated encapsulated *Artemia* cysts, *I. degenerans* failed to develop beyond the protonymphal stage, whereas full development did take place on decapsulated eggs. The lack of development may be explained by the fact that *I. degenerans* may not be able to pierce the alveolar layer of fresh *Artemia* cysts (see Van Stappen (1996) for detailed information on *Artemia* cysts). The ladybird beetle *Harmonia axyridis* (Pallas) (Hongo and Obayashi, 1997) and the anthocorid thrips predator *Orius laevigatus* (Fieber) (Arijs and De Clercq, 2001) are also able to develop on brine shrimp cysts. However, the developmental period of *O. laevigatus* nymphs was significantly shorter on decapsulated cysts than on encapsulated cysts.

In general, high escape rates were correlated with long developmental times (Fig. 4.3). This may indicate that when a food source is considered unfavourable, the predators start wandering in search of more suitable food. The high escape rates (especially of the larvae) and mortality of the immature stages (especially in the protonymphal stage) may also in part be explained by the experimental set-up. Plants provide more shelter for the larvae of *I. degenerans*. If the immature predatory mites are unable to seize prey such as spider mites or thrips nymphs, they can feed on an alternative food source such as pollen, or feed on prey that has been killed by an adult predator.

In conclusion, our study shows that *I. degenerans* is a generalist, able to develop on a wide range of natural and factitious foods.

Pollen has been shown to be an optimal food for the immature stages of several phytoseiid mites. The castor bean pollen we are using in our mass rearing may not result in the shortest developmental times, but is far easier to collect than the other pollen species used in this study.

Although *I. degenerans* is frequently found in the flowers of sweet pepper plants, sweet pepper pollen and thrips nymphs were found to be unfavourable food for immature development of the predator.

Further, *I. degenerans* may be not be able to cope with dense webbing of spider mites, but it may be effective in the initial phase of a spider mite infestation, when the leaves are not yet entirely covered with webbing.

The ability to develop on *T. vaporariorum* eggs and *M. persicae* nymphs implies the potential of the predator to control these pests, but further information on life history traits and prey preference is necessary.

As a next step in determining the effectiveness of *I. degenerans* as a biological control agent, further research will evaluate the ability of adult mites to feed and reproduce on different diets.

CHAPTER 5
INFLUENCE OF DIET ON LIFE TABLE PARAMETERS OF *IPHISEIUS DEGENERANS*

5.1 INTRODUCTION

There have been several studies on the immature development of *I. degenerans* (Takafuji and Chant, 1976; McMurtry, 1977; McMurtry *et al.*, 1984; van Rijn and Tanigoshi, 1999ab; Vantornhout *et al.*, 2004), and its reproductive characteristics (Takafuji and Chant, 1976; McMurtry, 1977; Kennett and Hamai, 1980; McMurtry *et al.*, 1984; van Houten *et al.*, 1995; Nwilene and Nachman, 1996). However, full life table studies were only performed by van Rijn and Tanigoshi (1999ab) on broad bean pollen, castor bean pollen, castor bean pollen and nectar, or the twospotted spider mite *Tetranychus urticae* Koch on an artificial substrate, and by Takafuji and Chant (1976) on the pacific spider mite *Tetranychus pacificus* McGregor on a paper substrate.

This study was conducted to determine the impact of different diets on some biological characteristics of the predatory mite. *Ricinus communis* L. (castor bean) pollen (the diet used for mass rearing *I. degenerans*), a mix of all stages of the spider mite *T. urticae* or of *F. occidentalis* nymphs (two natural prey species), *T. vaporariorum* eggs (potential natural prey species), and *Ephestia kuehniella* Zeller eggs (potential factitious food), were selected as food. In the diets consisting of natural prey, leaf arenas were used. In the case of spider mites both artificial and leaf arenas were used in order to investigate the influence of webbing on the life cycle parameters of the predatory mite. For the remaining diets only artificial arenas were used.

This chapter is based on: Vantornhout, I., Minnaert, H.L., Tirry, L. and De Clercq, P. 2005. Influence of diet on life table parameters of *Iphiseius degenerans.* Exp. Appl. Acarol. 35: 183-195.

5.2 MATERIALS AND METHODS

5.2.1 Predator culture

Iphiseius degenerans was reared as described in chapter 3 (3.2.1 Predator culture).

5.2.2 Food sources

In this study castor bean *R. communis* pollen, 1st and 2nd instars of the western flower thrips *F. occidentalis*, all stages of the spider mite *T. urticae*, eggs of the greenhouse whitefly *T. vaporariorum* and eggs of the Mediterranean flour moth, *E. kuehniella* were used as food sources. Rearing, handling and origin of these food sources have been described in chapter 4 (4.2.2 Food sources).

5.2.3 Experimental units

Both artificial arenas and leaf arenas (as described chapter 4, 4.2.3 Experimental units) were used in this study.

5.2.4. Experiments

Eggs from the stock colony were collected over a 12 h period and reared on a new artificial arena. To make sure all predatory mites had the same feeding history, immature stages in all treatments were fed on castor bean pollen until they reached adulthood. Newly moulted female mites (less than 24 h old) were transferred singly to either a Multicel or a leaf arena together with a male of the same age, and fed one of the food sources described above. Diet-substrate combinations used in the experiments were: castor bean pollen–Multicel arena, spider mites–Multicel arena, spider mites–bean leaf arena, thrips nymphs–sweet pepper leaf arena, whitefly eggs–bean leaf arena

and *Ephestia* eggs–Multicel arena. There were 30 replicates per combination of diet-substrate.

Every 7 days the predatory mites were transferred to a fresh arena. Males that died before their female partners were replaced.

Every replicate was observed daily until the death of the female. On each observation, the following parameters were recorded: interval (in hours) between the present and previous observation, number of dead and escaped females, and number of eggs laid per female.

The eggs produced by all females fed on a food source were pooled per daily observation and then spread over three new arenas. The resulting larvae were fed the same food as their parents. However, in experiments in which the parent generation was offered western flower thrips, the progeny was fed on castor bean pollen, because previous work (chapter 4) showed that western flower thrips nymphs are unfavourable food for immature development. On a sweet pepper leaf, only 7% of the predatory mites reached adulthood when presented with thrips nymphs. This low amount of adults obtained was due to a high degree of escape among the larvae of *I. degenerans* and a high mortality rate in the protonymphs (see chapter 4). Once the progeny reached adulthood, the survival, escape and sex ratio were calculated.

All experiments were conducted in a climatic cabinet at 25 ± 1 °C, 75 ± 5% RH and a 16L:8D h photoperiod.

5.2.5 Data analysis

Based upon the data obtained from the experiments described above, mean longevity, duration of the oviposition period, and fecundity (expressed as the number of eggs over the oviposition period) of female mites were calculated. Data from females that escaped from the arena or that drowned in the surrounding moist absorbent paper were excluded from these calculations. Given the high level of escape and mortality in the progeny of the predatory mite, the percentage of females was calculated as the number of daughters over the total amount of obtained adults in the progeny.

Second, the age-specific survival of the predatory mite in each treatment was described. To take the escape of females in account, survival was calculated by multiplying survival ratios over all previous observations. Hence, at each observation a survival ratio was calculated as *(N-D)/N*, where *N* is the number of mites alive at the previous observation, and *D* is the number of predators that died between two observations. To define the type of survivorship curve, the survival data were fitted to the three-parameter Gompertz function ($f(x) = a * \exp[-\exp(-(x - x_0)/b)]$), where *f(x)* is the survival at age *x*, and *a*, *b* and x_0 are the three parameters of the function. Parameter *a* determines the position of the asymptote, or the maximum value the survival reaches (*i.e., a* cannot exceed the value of 1). The rate at which the curve reaches this value is determined by parameter *b*. Parameter x_0 determines the position of the curve. Parameters were estimated using SigmaPlot 2002 (SPSS Inc., 1986-2001).

Third, life table parameters were calculated. The intrinsic rate of increase r_m was calculated from the equation $\sum e^{-r_m x} l_x m_x = 1$ where l_x is the proportion of females surviving to age *x* and m_x is the mean number of female progeny per adult female at age *x*. Other parameters calculated were the net reproductive rate R_0 (= $\sum l_x m_x$) or the mean number of daughters produced per female, the mean generation time *T* (= $(\ln R_0)/r_m$), and the doubling time *DT* (= $(\ln 2)/r_m$). To estimate the variance for r_m the Jackknife method was used (Meyer *et al.,* 1986). For this purpose a VBA-macro (MS Excel, see Appendix I) was written, based on the brief description of the technique in Hulting *et al.* (1990). The VBA-macro was tested for correctness, using the datasets in Hulting *et al.* (1990). Obtained Jackknife 'pseudo-values' of the intrinsic rate of increase were then transferred to SPSS 12.0 (SPSS Inc., 1989-2003) in which a multiple comparison test was used to evaluate the differences among the means.

Analysis of variance (ANOVA) was used to test for statistical differences in longevity, fecundity, and the Jackknife intrinsic rate of increase among diets. The ANOVA was followed by a multiple comparison test (Student Newman Keuls) at the *p* = 0.05 level (SPSS 12.0, SPSS Inc., 1989-2003).

5.3 RESULTS

5.3.1 Adult longevity and reproductive capacity

The results on adult female longevity and reproductive capacity are summarized in Tables 5.1 to 5.3 and Figure 5.1.

During the experiments a number of females escaped from the arena and were not retrieved. The percentage of females escaping ranged from 13.3% (castor bean pollen) to 76.7% (thrips nymphs).

The shortest female longevity (13.1 days) was found when the predator was fed on whitefly eggs. On the other diets the longevity ranged from 29.5 to 42.4 days (Table 5.1, $F = 11.4$, df = 91, $p \leq 0.0001$). Figure 5.1 shows the observed and simulated age-specific survivorship curves of *I. degenerans* reared on different diets. The Gompertz-function ($f(x) = a * \exp\left[-\exp(-(x - x_0)/b)\right]$) yielded a good fit to the data for each diet (simulated age-specific survivorship curve in Figure 5.1), with R^2 ranging from 0.905 to 0.987. Parameter estimates for this function are given in Table 5.2. The steep drop-off in the survivorship curve on a diet of castor bean pollen is indicated by the highest value of parameter *b*, whereas on the other diets the drop-off is less steep (as indicated by lower values of *b*). The x_0 value is highest on the diet consisting of thrips nymphs indicating that the position of this survivorship curve is more pronounced to the right, compared with the other curves.

Both observed and simulated age-dependent survivorship curves are described by a type I pattern in all cases. This pattern indicates a high survival at young and intermediate ages, followed by a steep drop-off in survival as individuals approach their maximum life span (Gotelli, 1995). The first 6 points of the observed survivorship curve indicate the survival of the immature stages. During the immature stage, in which predators were fed castor bean pollen, there was 100% survival (chapter 4). First adult mortality was observed from 1 (spider mites, Multicel arena) to 19 days (pollen) after moulting to the adult stage. The observed maximum female longevity on pollen, thrips nymphs, spider mites (artificial arena), spider mites (leaf arena), *Ephestia* eggs and whitefly eggs was recorded at 52, 75, 54, 52, 60, and 35 days respectively (Fig. 5.1).

Table 5.1. Female adult longevity and duration of the oviposition periods (days) of *I. degenerans* reared on different diets and substrates

Diet	**Substrate**	***n*[a]**	**Female longevity[b]**	**Pre-oviposition period[b]**	**Oviposition period[b]**	**Post-oviposition period[b]**
R. communis pollen	Multicel	26	36.7 ± 2.2bc	3.0 ± 0.1a	31.9 ± 2.1b	1.7 ± 0.8a
F. occidentalis nymphs	Sweet pepper leaf	7	40.8 ± 4.4c	4.6 ± 0.9b	35.3 ± 4.5b	0.9 ± 0.3a
T. urticae	Multicel	20	29.5 ± 2.5b	3.1 ± 0.2a	25.0 ± 2.3b	1.4 ± 0.7a
T. urticae	Bean leaf	14	35.1 ± 2.9bc	5.8 ± 0.5c	27.0 ± 3.0b	2.4 ± 0.8a
Ephestia eggs	Multicel	14	42.4 ± 2.8c	4.3 ± 0.4b	35.7 ± 3.1b	2.4 ± 0.8a
T. vaporariorum eggs	Bean leaf	11	13.1 ± 2.8a	2.9 ± 0.1a	9.0 ± 2.7a	1.3 ± 0.4a

[a]Number of females that died of a natural cause (initial number of females = 30)

[b]Means (± SEM) followed by the same letter are not significantly different (Student-Newman-Keuls–test, $p > 0.05$)

Table 5.2. Parameter estimates for the Gompertz function $f(x) = a * \exp[-\exp(-(x - x_0)/b)]$

Diet	**Substrate**	***n*[a]**	**Parameters**			R^2
			a	***b***	***x₀***	
R. communis pollen	Multicel	26	1	-10.2	48. 2	0.968
F. occidentalis nymphs	Sweet pepper leaf	7	1	-15.5	64.1	0.926
T. urticae	Multicel	20	1	-11.9	42.5	0.946
T. urticae	Bean leaf	14	1	-16.8	41.7	0.967
Ephestia eggs	Multicel	14	1	-10.4	57.6	0.987
T. vaporariorum eggs	Bean leaf	11	1	-11.0	33.2	0.905

[a]Number of females that died of a natural cause (initial number of females = 30)

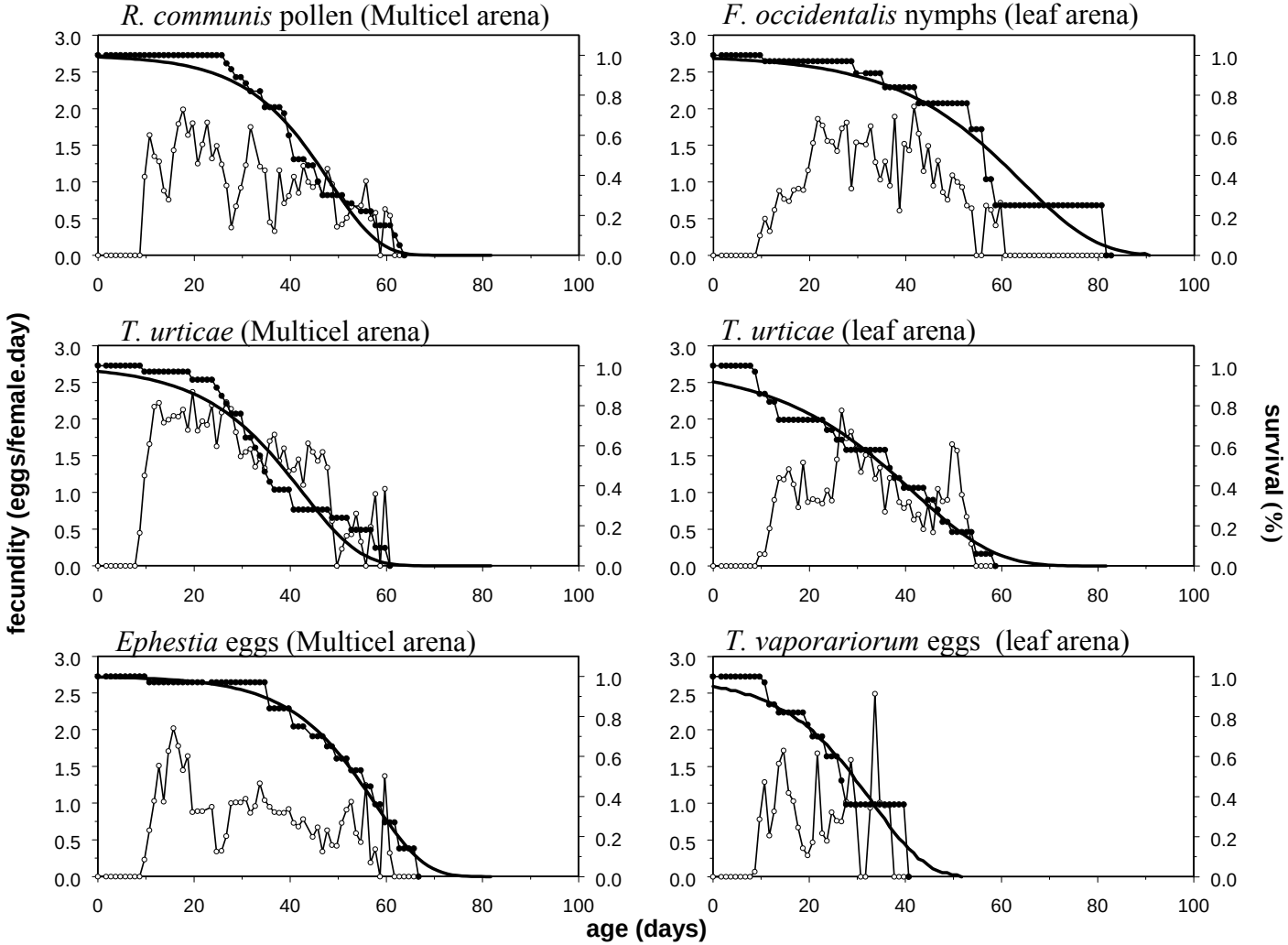


Figure 5.1. Age specific survivorship curve (solid dots), fitted survivorship curve (Gompertz function, solid line) and age specific daily fecundity (open dots) of *I. degenerans* reared on different diets and substrates.

After pairing *I. degenerans* males and females, the pre-oviposition period lasted 2.9 (whitefly eggs) to 5.8 days (spider mites, leaf arena) ($F = 12.5$, df = 91, $p < 0.0001$). No females died during the pre-oviposition period when fed pollen or whitefly eggs; when fed spider mites on a leaf arena 16.7% of the females died, while in the remaining diets only 3.33% died. The oviposition period lasted 9.0 (whitefly eggs) to 35.7 days (*Ephestia* eggs) (Table 5.1, $F = 10.1$, df = 91, $p < 0.0001$).

Mean egg production per female during the oviposition period was lowest for females reared on whitefly eggs (Table 5.3; $F = 8.99$, df = 91, $p < 0.0001$). The mean daily fecundity varied with female age. The main peaks in fecundity occurred on days 11-26 (pollen), 21-33 (thrips), 12-28 (spider mites, artificial arena), 26-38 (spider mites, leaf arena), 12-19 (*Ephestia* eggs), and 12-15 (whitefly eggs) (Fig. 5.1). On all diets, maximum daily oviposition did not exceed 2.5 eggs/female. Average daily oviposition ranged from 1 to 1.9 eggs/female, and was significantly higher on spider mites (artificial arena) than on the other diets ($F = 25.5$, df = 91, $p < 0.0001$). Over all the experiments in this study 4866 eggs were processed, of which 40% reached adulthood. Sixty percent of the obtained adults were female. The percentage of females in the live offspring per diet is given in Table 5.3. The survival rate of the progeny ranged from 7% (spider mites, leaf arena) to 61 % (pollen). The duration of the post-oviposition period varied between 1 and 2.5 days, and was independent of diet (Table 5.1, $F = 0.47$, df = 91, $p = 0.80$).

5.3.2 Life table parameters

Life table parameters of *I. degenerans* are summarized in Table 5.4. Significant differences between the intrinsic rates of increase were found ($F = 48.75$, df = 91, $p < 0.0001$).

The highest values of r_m (females/female.day) and R_0 (females/female) were found when the predatory mites were fed pollen (0.142 and 15.0, respectively), whereas the lowest values were recorded on spider mites (leaf arena) (0.015 and 1.53, respectively). When the predatory mites were fed *Ephestia* eggs or whitefly eggs, r_m and R_0 values

were higher than those found on spider mites (leaf arena), but lower than those obtained on the other diets.

Castor bean pollen resulted in the lowest generation time *T* (days) and doubling time *DT* (days) (19.0 and 4.88, respectively), whereas spider mites (leaf arena) yielded the highest values (29.6 and 46.2, respectively). Life table parameters obtained on spider mites (artificial arena) were similar to those on thrips.

Table 5.3. Total and daily fecundity (eggs/female), and offspring sex ratio (% females in the obtained adult progeny) of *I. degenerans* reared on different diets and substrates

Diet	**Substrate**	***n*[a]**	**Fecundity[b]**		**Sex ratio**
			Total	**Daily**	
R. communis pollen	Multicel	26	38.6 ± 3.1b	1.2 ± 0.1a	64
F. occidentalis nymphs	Sweet pepper leaf	7	42.6 ± 5.2b	1.3 ± 0.1a	40
T. urticae	Multicel	20	47.8 ± 4.0b	1.9 ± 0.1b	73
T. urticae	Bean leaf	14	32.9 ± 5.3b	1.1 ± 0.1a	62
Ephestia eggs	Multicel	14	36.9 ± 3.9b	1.0 ± 0.1a	53
T. vaporariorum eggs	Bean leaf	11	9.1 ± 2.5a	1.2 ± 0.1a	65

[a]Number of females that died of a natural cause (initial number of females = 30)

[b]Means (± SEM) followed by the same letter are not significantly different (Student-Newman-Keuls – test, $p > 0.05$)

5.4 DISCUSSION

Life table parameters are good indices of population growth under a given set of conditions. We demonstrated that diet had a significant effect on life cycle parameters of *I. degenerans*. The phytoseiid was able to survive and reproduce on all food sources offered. All r_m values found were within the range of values reported by McMurtry and Croft (1997) for generalist predators, *i.e.*, below 0.1 (*Typhlodromus pyri* Scheuten and *Phytoseius hawaiiensis* Prasad) up to 0.25 females/female.day (*I. degenerans*, *Amblyseius largoensis* (Muma), *Typhlodromalus limonicus* (Garman and McGregor) and *T. peregrinus* (Muma)).

Table 5.4. Life table parameters of *I. degenerans* reared on different diets and substrates

Diet	**Substrate**	n^a	r_m [bc]	**95% CI**[d]	R_0[e]	T[f]	DT[g]
Castor bean pollen	Multicel	26	0.142 ± 0.004d	0.135 - 0.150	15.0	19.0	4.88
Thrips nymphs	Sweet pepper leaf	7	0.114 ± 0.005c	0.101 - 0.127	14.5	23.5	6.08
Spider mites	Multicel	20	0.115 ± 0.003c	0.110 - 0.121	10.3	20.2	6.03
Spider mites	Bean leaf	14	0.015 ± 0.006a	0.002 - 0.028	1.53	29.6	46.2
Ephestia eggs	Multicel	14	0.073 ± 0.006b	0.061 - 0.086	5.02	22.0	9.49
Whitefly eggs	Bean leaf	11	0.056 ± 0.019b	0.015 - 0.098	2.36	16.2	12.4

[a]Number of females that died of a natural cause (initial number of females = 30)

[b]Means (± SEM) followed by the same letter are not significantly different (Student-Newman-Keuls-test, $p > 0.05$)

[c] intrinsic rate of increase in females/female.day

[d] 95% confidence interval of r_m

[e] net reproductive rate in females/female

[f] generation time in days

[g] doubling time in days

The demographic parameters of *I. degenerans* fed on castor bean pollen proved to be better than on any of the arthropod prey tested. The values we obtained (T = 19.0 days, r_m = 0.142 females/female.day, R_0 = 15.0 females/female) were lower than those reported by van Rijn and Tanigoshi (1999a) under comparable experimental conditions. In other studies it has also been shown that pollen is a good food source with respect to the fecundity of *I. degenerans* (e.g., Ramakers and Voet, 1995; van Rijn and Tanigoshi, 1999a).

When presented with thrips nymphs a high percentage of females escaped from the arena, with only 7 of the initial 30 females dying of a natural cause. This may be explained in part by the aggressive defence behaviour of the thrips nymphs. Hence, *I. degenerans* may encounter problems in catching this type of prey, and in search of more suitable prey it leaves the arena and gets stuck in the absorbent paper on the arena. Alternatively, *I. degenerans* may leave the arena to avoid egg predation by thrips nymphs. Janssen *et al.* (2003) showed that in presence of low quality plant food (e.g., sweet pepper) thrips nymphs feed more on *I. degenerans* eggs than they do on high quality plant food.

The oviposition rate observed on a mix of 1st and 2nd instars (*i.e.,* 1.3 eggs/female.day) is comparable with that on *F. occidentalis* first instars, as reported by van Houten and van Rijn (1995). On a diet of second instar citrus thrips (*Scirtothrips citri* (Moulton)), *I. degenerans* deposited 0.93 to 2.54 eggs/day during the first 5 days of their oviposition period (Grafton-Cardwell et al., 1998). For *Amblyseius cucumeris* (Oudemans), the number of eggs varied from 1.5 eggs/day on *F. occidentalis* nymphs to 1.87 eggs/female on first instar larvae of *Thrips tabaci* Lindeman (Gillespie and Ramey, 1988; Castagnoli and Simoni, 1990). The results from the previous chapter indicated that *I. degenerans* has great difficulty to develop to the adult stage on a diet of thrips nymphs; therefore, the eggs obtained from the parental generation were further reared on castor bean pollen. This must be taken into consideration when comparing the intrinsic rate of increase on *F. occidentalis* nymphs with the values obtained on the other diets in this study. In contrast to our findings, Blaeser and Sengonca (2001) were not able to calculate life table parameters for *I. degenerans*, because eggs produced by females fed on western flower thrips nymphs did not hatch. Life table parameters of *I. degenerans* preying on thrips nymphs were not found in the literature. For other

phytoseiids (e.g., *A. cucumeris*, *A. barkeri* (Hughes)) fed on *T. tabaci*, intrinsic rates of 0.154 females/female.day (Castagnoli and Simoni, 1990) and 0.22 females/female.day (Bonde, 1989) were reported.

The literature reports oviposition rates of *I. degenerans* fed Tetranychidae (*M. tanajoa, T. urticae, T. pacificus*) on different arenas (e.g., plastic, paper, leaves) (Takafuji and Chant, 1976; McMurtry, 1977; Kennett and Hamai, 1980; Nwilene and Nachman, 1996, van Rijn and Tanigoshi, 1999a). The oviposition rate we observed for *T. urticae* offered on a leaf substrate is lower (1.1 eggs/female.day) than the values reported in the literature, whereas that obtained here for spider mites on an artificial arena (1.9 eggs/female.day) is similar or higher.

The suitability of spider mites in our study depended on the substrate. When the phytoseiid preyed on *T. urticae* on an artificial arena, all observed life table parameters were superior to those obtained on a bean leaf arena. The inferior results on a leaf substrate may be due to the fact that *I. degenerans* has difficulty coping with the webbing of *T. urticae*. As was already described in chapter 4, on an artificial arena, the only webbing was produced around the black thread on the arena, and thus both predator and prey wandered more over the arena, resulting in a better capture of the prey. In experiments using leaf arenas the whole area was covered with webbing, reducing the mobility of the predator. Sabelis and Bakker (1992) found that phytoseiid mites that were better protected from the webbing of Tetranychidae had long setae in the medial and lateral position on the dorsal shield. *Iphiseius degenerans* may thus be less adapted to preying on spider mites, given that this species has short dorsal and lateral setae on the dorsal shield (Van der Merwe, 1968). The inferior values of the life table parameters may also be explained by the high escape rate of the progeny. van Rijn and Tanigoshi (1999a) reported an r_m value of 0.147 females/female.day when offering *T. urticae* infested bean leaves to *I. degenerans* on a PVC arena. On a diet of *T. pacificus* females, Takafuji and Chant (1976) found an r_m value of 0.248 females/female.day. For other phytoseiid mites (e.g., *Phytoseiulus persimilis* Athias-Henriot, *Amblyseius longispinosus* Evans, *Typhlodromus floridanus* (Muma)) that had been fed *T. urticae*, the r_m value ranged from 0.159 to 0.374 females/female.day (many references in Sabelis, 1985).

In the previous chapter it was shown that *I. degenerans* is able to complete its development when fed on *T. vaporariorum* eggs. Adult predatory mites are also able to feed and reproduce on a diet of whitefly eggs. The literature reports on life history traits of phytoseiids fed on immature stages of *T. tabaci*. Oviposition rates of mites that were offered immature stages of *B. tabaci* generally fluctuated between 0.1 and 2 eggs/female.day (Meyerdirk and Coudriet, 1985, 1986; Nawar and Sherif, 1993; Nomikou *et al.*, 2001 and references herein). Our findings fit well within that range. The value of r_m found for *I. degenerans* in the current study is smaller than the values reported for other phytoseiids feeding on whiteflies. Nomikou *et al.* (2001) reported r_m values ranging between 0.131 (*Typhlodromus athiasae* (Hirschmann)) and 0.215 females/female.day (*Euseius scutalis* (Athias-Henriot)).

The effect of *Ephestia* eggs, a widely used factitious food for insect predators, on the longevity and reproduction of *I. degenerans* has never been assessed before. Previous work indicated that *I. degenerans* is able to develop on *Ephestia* eggs (chapter 4). In the present study it was shown that the predatory mite is also capable to reproduce when fed eggs of the Mediterranean flour moth. Predatory mites fed *Ephestia* eggs lived longer than those fed spider mites (Multicel arena). The daily oviposition rate was similar to that on the other diets (except for spider mites on a Multicel arena). *Iphiseius degenerans* fed on *Ephestia* eggs had a higher growth potential than when fed on spider mites (leaf arena), but this did not exceed the growth potential on castor bean pollen (*i.e.,* the food source used for mass rearing *I. degenerans*) or on western flower thrips and spider mites (Multicel arena). The generalist *I. degenerans* is commercialized as a thrips predator in greenhouse crops (van Houten and van Stratum, 1993). Castor bean pollen appears to be by far the most suitable diet for mass rearing purposes. It results in rapid population growth, is easy to collect, and is much cheaper than for instance *Ephestia* eggs.

The polyphagous character of *I. degenerans* may have a positive and negative impact on its value as a predator in the field. A major advantage is that the predator is able to develop and reproduce on alternative prey when preferred food becomes scarce. As western flower thrips nymphs appear to be unfavourable food for development of *I. degenerans* (see chapter 4), the immature stages can feed on other prey or pollen present in the crop, whereas the adult mites may shift their focus to thrips nymphs. On the other

hand, the predator may prefer other food over thrips, which may in some situations impair its value as biocontrol agent targeting that pest. Further, the phytoseiid may exhibit a secondary effect on spider mites, but this is probably only the case when leaves are not yet entirely covered with webbing, since the predatory mites may not cope with dense webbing. Based on the low intrinsic rate of increase obtained when fed whitefly eggs, it is difficult to predict the value of *I. degenerans* as a whitefly predator. It is not likely that the predator will be able to control whitefly infestations in a crop, but instead will use whiteflies as an alternative food source when other prey becomes scarce. Life history traits of the predatory mite fed on the other immature stages of the whitefly need further investigation. The feeding behaviour of *I. degenerans* in the crop may be very complex due to its pollinivory. Pest control will only be improved if a decrease in predation rate due to feeding on pollen is compensated by a greater level of predation due to an increased predator population (McMurtry and Scriven, 1966; Wei and Walde, 1997). van Rijn *et al.* (2002) also suggest that supplying cattail pollen in a crop may promote thrips control by *I. degenerans*. Further research on the food preference of *I. degenerans* is warranted to understand the feeding ecology of the predator and its potential for augmentative biological control.

CHAPTER 6
FUNCTIONAL RESPONSE OF *IPHISEIUS DEGENERANS* TO DIFFERENT PREY SPECIES

6.1 INTRODUCTION

The determination of the functional response is an important aspect to unravel the dynamics of predator prey interaction. Solomon (1949) defined the functional response as the change in the number in prey consumed per predator to changes in prey densities. Holling (1961) suggested that there are three distinct types of functional response in invertebrate predators (Fig. 6.1):

- Type I, in which the attack rate of the predator increases linearly with prey density but then suddenly reaches a constant;
- Type II, in which the attack rate of the predator increases at a decreasing rate with increasing prey density until a constant is reached;
- Type III, in which the attack rate of the predator first increases at an increasing rate with prey density, but then decelerates towards a constant.

Three basic components of the functional response, *i.e.*, the length of prey exposure to the predator, the attack rate of the predator a and the prey handling time T_h exhibited by the predator alone, or together with a fourth component, the effects of hunger or egg complement, are sufficient to explain a Type II functional response. The handling time is the time in which the predator pursues, subdues, consumes and digests a single prey; the attack rate is the rate at which a predator searches its prey. A fifth component, the stimulation of the predator by each newly discovered prey, however, is necessary to explain the S-shaped curve of a Type III functional response (Holling, 1961).

Several studies have been carried out on the functional response of phytoseiid mites, mostly with spider mites as prey. A variety of functional response curves have been reported; most of these curves were Type II curves (e.g., Takafuji and Chant, 1976; Sabelis, 1985; Shipp and Whitfield, 1991; Fan and Petit, 1994; Badii *et al.*, 1999; Lester and Harmsen, 2002). Type I curves (linear rises of the functional response) were

reported by Takafuji and Chant (1976), Everson (1979) and Eveleigh and Chant (1981), while Type III curves were found by Nwilene and Nachman (1996a). Next to the classical types, other types of functional response curves were found or used by other authors:

- Type IV curves are dome-shaped curves which are characterized by a Type II curve followed by a decline in number of consumed prey at high densities (Takafuji and Chant, 1976; Castagnoli and Simoni, 1999);

- Type V curves, which are the reverse (*i.e.*, a second rise up to another plateau), and compound-curves (e.g., Type III-II curves) were reported by Eveleigh and Chant (1981) and Sabelis (1985).

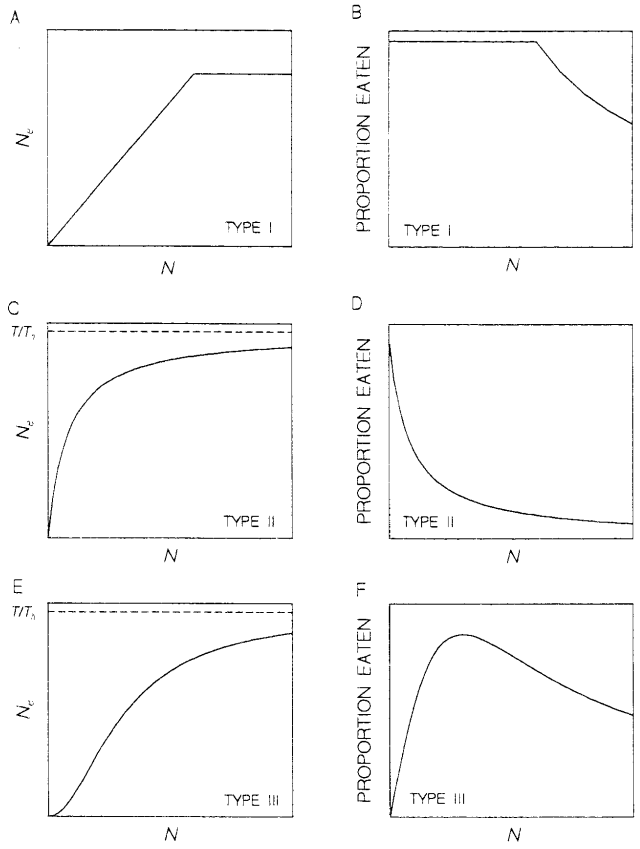


Figure 6.1. Three types of functional responses. The relationships between number of prey eaten (N_e) and number of prey present (N) are depicted in parts A, C, and E. The corresponding relationships between proportion eaten (N_e/N) and number of prey present (N) are depicted in parts B, D, and F (Juliano, 2001).

Despite the fact that *I. degenerans* has been commercialized as thrips control agent, the research on the functional response of the predator has until now been limited to spider mites as prey organisms. *Tetranychus pacificus* Koch as prey has been extensively studied by Eveleigh and Chant (1981) and Takafuji and Chant (1976), while Akpokodje *et al.* (1990) and Nwilene and Nachman (1996) assessed the functional response of *I. degenerans* on changing densities of the cassava green mite *Mononychellus tanajoa* Bondar.

The objective of this study was to determine the type(s) of functional response of *I. degenerans* females to densities of 1st and 2nd instars of the western flower thrips *Frankliniella occidentalis* (Pergande), eggs and adults of the twospotted spider mite *Tetranychus urticae* Koch, and eggs of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood under laboratory conditions. Further, the parameters of the functional response curves were estimated.

6.2 MATERIALS AND METHODS

6.2.1 Predator culture

Iphiseius degenerans was reared as described in chapter 3 (3.2.1 Predator culture).

6.2.2 Prey

The prey species tested in the functional response experiments were *T. urticae* eggs and adults, *T. vaporariorum* eggs and *F. occidentalis* 1st and 2nd instars. Descriptions of the rearing methods of these species are presented in chapter 4 (4.2.2 Food sources).

6.2.3 Experimental set-up

Functional response experiments were carried out in plastic containers (diameter: 4.5 cm, height: 1.5 cm) with a mesh-screened lid (100 µm). Arenas consisted of 4.5 cm diameter bean leaf discs placed upside down on a 1 cm layer of agar (1%). These leaf discs were punched out of primary bean leaves (Fig. 6.2).

The functional response on *T. urticae* eggs was determined at 8 different densities (1, 3, 5, 10, 20, 30, 40, 50 eggs/arena). Eggs were obtained by allowing adequate numbers of *T. urticae* females to oviposit on the leaf discs one or two days prior to the experiment. On the day of the experiment, the number of eggs was reduced to the designated level by puncturing superfluous eggs with a thin needle (diameter: 0.4 mm).

Figure 6.2. Experimental arenas used in the functional response experiments.

Predation on adult female *T. urticae* was measured at densities of 1, 3, 5, 10, 15, 20, 25, 30 and 40 females/arena. Prey mites were transferred directly from the stock culture to the experimental arena, after which predators were introduced immediately.

The densities of *T. vaporariorum* eggs tested were 1, 3, 5, 8, 10, 20, 30, 40, 60, and 100 eggs/arena. Leaf discs infested with eggs were obtained by confining adult *T. vaporariorum* to the lower side of bean leaves inside “drum” cells (see chapter 4 for a description, Fig. 4.1). After 48 hours, adults were removed and the number of eggs was reduced to the desired density by puncturing superfluous eggs with a needle. The leaf disc was then punched out and transferred to the experimental arena.

The functional response of *I. degenerans* attacking 1st or 2nd instars of *F. occidentalis* was determined at prey densities of 1, 3, 5, 8, 10, 15, 20, 30 and 40 prey individuals per arena. Prey was transferred directly from the stock culture to the experimental arena.

Before the start of the experiment, reproductively mature female predatory mites (8-12 days old), reared on castor bean pollen, were starved for 4 hours. One individual of the predator was introduced per arena, and every combination of prey density – predator was replicated 10 times.

The number of dead prey was recorded after 24 hours, during which consumed prey was not replaced (*i.e.*, prey depletion). Experimental arenas were maintained in a climatic cabinet at 25 °C, 75% RH and a 16L:8D h photoperiod.

6.2.4 Data analysis

Analyzing functional response data required two distinct steps. In a first step the shape of the functional response was determined by logistic regression analysis of the proportion of prey killed in relation to initial density ($\frac{N_e}{N_0}$). In this step a polynomial logistic model was fitted:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}$$

where N_e is the number of prey eaten, N_0 is the initial prey density and P_0, P_1, P_2 and P_3 are the parameters to be estimated. Regressions were obtained by starting with a cubic model, and deleting the highest order coefficients that were not significantly different from zero until all remaining coefficients in the model were significantly different from zero. A simple logistic model, which only contains P_0 and P_1, is the lowest order model that could be fitted. The sign of the linear parameter P_1 was used to distinguish between a Type II functional response and a Type III functional response. If $P_1 < 0$, the proportion of prey eaten declines monotonically with the initial number of prey offered, thus describing a Type II functional response. If $P_1 > 0$, the proportion of prey eaten is initially positively density dependent, describing a Type III functional response (Juliano, 2001).

In a second step, the parameters of the functional response (handling time T_h and attack rate a, respectively) were estimated and compared using a nonlinear least squares regression of the number of prey eaten versus prey density. Given that the experiments were conducted with prey depletion, Holling's disc equation ($N_e = \frac{aNT}{1 + aNT_h}$) could not be used.

Because it allows for prey depletion, the random predator equation of Rogers (1972) was used for Type II functional responses. The equation is as follows:

$$N_e = N_0 \{1 - \exp[a(T_h N_e - T)]\} \qquad \text{Equation 6.1}$$

with N_0 the initial density of prey, T_h the handling time, a the attack rate and T the total duration of the experiment, *i.e.,* 24 hours.

For Type III functional responses the equation reads:

$$N_e = N_0 \{1 - \exp[(d + bN_0)(T_h N_e - T)/(1 + cN_0)]\} \qquad \text{Equation 6.2}$$

with N_0 the initial density of prey, T_h the handling time, a the attack rate en T the duration of the experiment and *b, c, d* are constants from the function that relates a and N_0 in Type III functional responses: $a = (d + bN_0)/(1 + cN_0)$.

Parameters were obtained by fitting observed data to the models using the nonlinear least squares method with an iterative application of Newton's method. This step is needed because the equations 6.1 and 6.2. have N_e on both sides of the expression. (Juliano, 2001).

Logistic and nonlinear regressions were performed in SAS Learning Edition. (SAS Institute Inc., 2001). The program code is presented in Appendix II.

6.3 RESULTS

Parameter estimates from the logistic regression of proportion of prey eaten by *I. degenerans* over a 24 h period versus prey density are presented in Table 6.1.

For *F. occidentalis* first and second instars and *T. urticae* adults, the cubic and quadratic coefficients were non-significant and the linear term was negative, indicating a monotonic decrease in proportion of prey eaten versus prey density (Type II functional

response). A type II functional response was also found for *T. vaporariorum* eggs. For *T. urticae* eggs the best polynomial included significant linear, quadratic and cubic coefficients and the linear term was positive indicating a Type III functional response.
To fit a model (equation 6.1 or 6.2) to the data and to estimate functional response parameters (*i.e.*, attack rate *a* and handling time T_h), nonlinear least squares regression was used. Because the logistic regression indicated Type II functional responses for *F. occidentalis* first and second instars, spider mite adults and whitefly eggs, equation 6.1 was fitted. A significant test indicated a good overall fit of the models ($p < 0.001$). The models are depicted in Figure 6.3 (a, b, c, d).

Table 6.1. Maximum-likelihood estimates (± SEM) from the logistic regression of proportion of prey eaten by *I. degenerans* (N_e/N) as a function of initial density (N)

Prey species	**Parameter**[a]			
	Constant (P_0)	Linear (P_1)	Quadratic (P_2)	Cubic (P_3)
F. occidentalis	-0.501*	-0.062*	-	-
(1st instars)	(0.158)	(0.007)	-	-
F. occidentalis	-2.150*	-0.025*	-	-
(2nd instars)	(0.216)	(0.008)	-	-
T. urticae	-0.683**	-0.057**	-	-
adults	(0.175)	(0.007)	-	-
T. urticae	-2.819**	0.277**	-0.015**	0.0002**
eggs	(0.467)	(0.062)	(0.002)	(0.00003)
T. vaporariorum	2.813**	-0.094**	0.0005**	-
eggs	(0.173)	(0.0068)	(0.000)	-

[a] Parameters followed by * are significant at $p < 0.01$, ** $p < 0.0001$ (χ^2 test)

When fed thrips nymphs, spider mite females or whitefly eggs, the number of prey killed by a predator (N_e) increased at a decreasing rate until a plateau was reached. This indicates a type II functional response. Theoretically, an *I. degenerans* female could consume a maximum of 2.5 thrips nymphs or spider mite females, or 22 eggs of the greenhouse whitefly per day (24 h/T_h). The parameters describing the Type II functional responses are presented in Table 6.2. On all diets, the obtained handling times are significantly different from zero. Only the diets consisting of 2nd instars of *F. occidentalis* and *T. vaporariorum* eggs resulted in significant attack rates. In the other

cases the 95% confidence interval of *a* included 0, indicating that the attack rate is not significantly different from zero.

For the data on spider mite eggs, the logistic regression indicated a Type III functional response (Table 6.1, Fig. 6.3). In a first fit of equation 6.2, four parameters (T_h, *b, c,* and *d*) had to be assessed. Because all parameters were non-significant, the model was reduced by eliminating first *c* and eventually *d* from the equation. Finally a two-parameter model (with parameters *b* and T_h) was fitted, but although the model was significant, only *b* was significantly different from 0 (Table 6.2). Because this Type III functional response was described by a two-parameter model, the data were also fitted to a two-parameter Type II functional response. Fitting the data in equation 6.1 resulted in parameters $a = 0.0043 \pm 0.0026$ h^{-1} and $T_h = 2.772E\text{-}8 \pm 3.6344$ h. These parameters, however, were not significantly different from zero. Also, the residual sum of squares for the Type II model was greater than that for the Type III model, indicating that the latter model fits the data better.

To compare the Type II functional response for the different prey species, once again nonlinear least square regression was used. Since there were only two functional response models with significant parameters, only these two models were compared (*F. occidentalis* 2nd instars versus *T. vaporariorum* eggs). Statistical analyses indicated that, although the handling time for *F. occidentalis* was 9 times that for *T. vaporariorum*, there were no significant differences between handling time and attack rate.

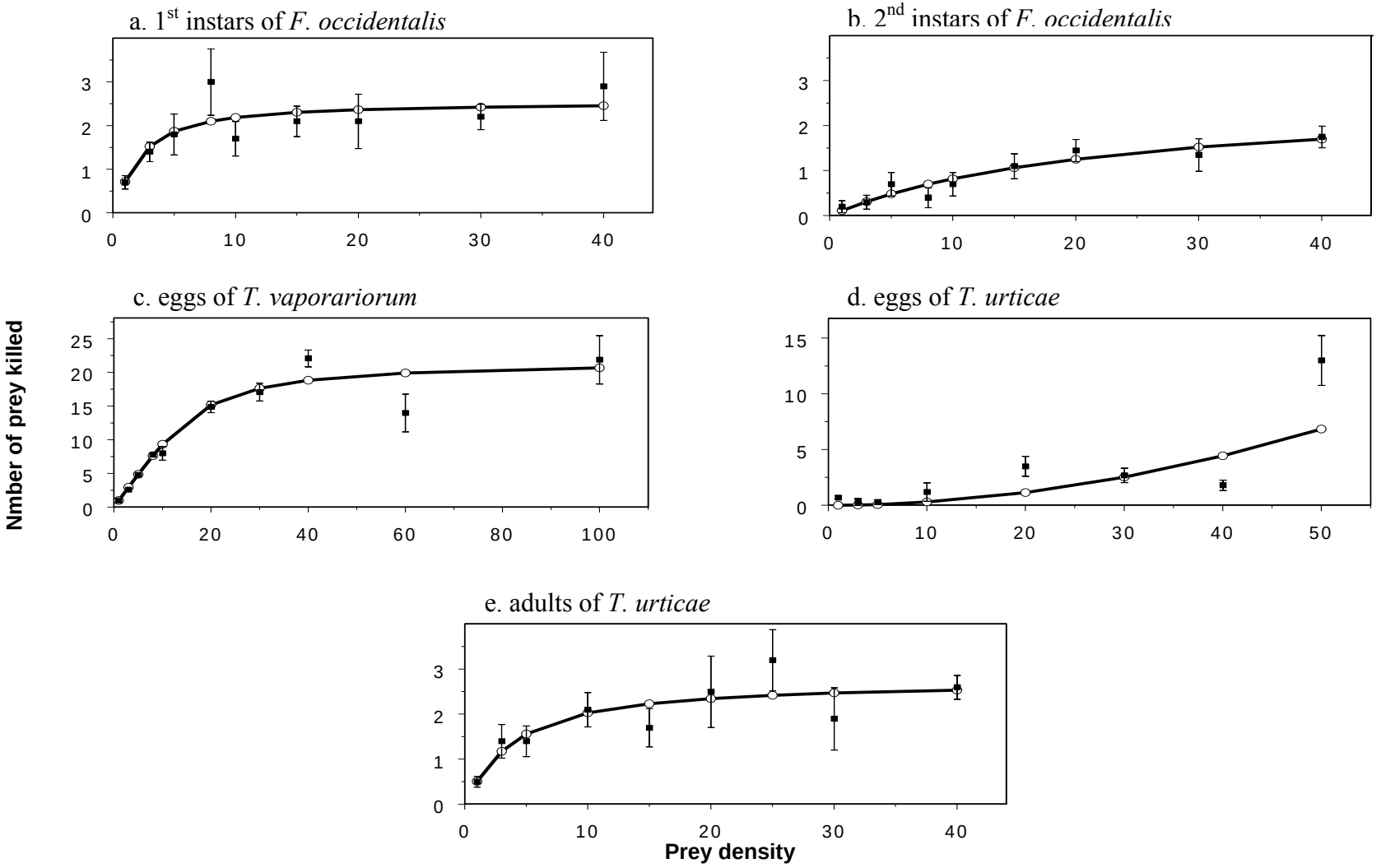


Figure 6.3. Functional responses of *I. degenerans* females to changing densities of different prey types. Number of prey killed determined in the experiments (solid squares), number of prey killed fitted (open dots).

Table 6.2. Parameter estimates (95% confidence interval) of the random predator equation (Rogers, 1972) for *I. degenerans* preying on different prey types

Prey species	***a***	T_h	***b***
F. occidentalis	0.073 ± 0.053	9.44 ± 1.21	-
(1st instars)	(-0.032 - 0.179)	(7.03 - 11.85)	-
F. occidentalis	0.0052 ± 0.0020	9.19 ± 2.98	-
(2nd instars)	(0.0012 - 0.0091)	(3.29 - 15.09)	-
T. urticae	0.036 ± 0.020	8.76 ± 1.19	-
adults	(-0.003 - 0.075)	(6.39 - 1.13)	-
T. urticae	-	1.27E-8 ± 1.89	0.00012 ± 0.00005
eggs	-	(-3.75 - 3.75)	(0.00002 - 0.00023)
T. vaporariorum	0.196 ± 0.079	1.10 ± 0.08	-
eggs	(0.039 - 0.353)	(0.93 - 1.27)	-

a: attack rate, *b*: parameter describing the relation between *a* and the initial prey density *N*, T_h: handling time

6.4 DISCUSSION

Logistic regression analysis revealed that the type of functional response exhibited by female adults of *I. degenerans* depends on prey species.

Iphiseius degenerans showed a Type II functional response when feeding on first or second instars of *F. occidentalis*, adult females of *T. urticae* or eggs of *T. vaporariorum*, and a Type III functional response when feeding on eggs of *T. urticae*. Although the logistic regression determined the shape of the functional responses of *I. degenerans* to be Type II or Type III and significant models could be fitted, the parameter estimates of these functional responses were not always significantly different from zero. The lack of significant parameters complicates ecological interpretation of the estimated functional responses. According to Juliano (pers. comm.) the best guide to distinguish Type II and Type III functional response is to observe the shape of the fitted curve. In the current study, the shapes of the Type II functional response models fitted the data very well. In all Type II response models a plateau appears to be reached at the higher prey densities. However, the number of prey consumed at this plateau was quite low. This low predation rate may be related to the long handling times and the low attack rates

estimated by the models. In two cases (*i.e.*, the diet consisting of 1st instars of *F. occidentalis* or adults of *T. urticae*), the attack rate was not significantly different from zero.

The plot of the average number of consumed *T. urticae* eggs versus initial prey density, however, indicated that a plateau is not yet reached at a density of 50 eggs per arena. It appears that the functional response is starting as a Type III functional response with a first plateau at density 20-30, followed by another Type III response at higher densities. To assess if the functional response of *I. degenerans* to changing densities of spider mites eggs is indeed a combined response, more experiments are necessary.

Based on the observations on the response of *I. degenerans* to changing densities of different prey types in our experiments, it can be concluded that this is a predator with a natural low predation rate. However, it should be taken into account that the experimental conditions used in this study might limit the predation rate of the predator (see below). In this aspect, it might be useful to conduct detailed studies on the behaviour of *I. degenerans* when attacking prey.

Different functional responses have been documented for phytoseiids feeding on different life stages of spider mites. Takafuji and Chant (1976) determined the functional response of *I. degenerans* females feeding on *T. pacificus* eggs. The number of consumed eggs rose almost linearly to a plateau. The predator was able to consume around 40 eggs in 24 hours, when 50 prey eggs were offered. Gotoh *et al.* (2004) summarized the daily prey consumption in adult females of acarophagous phytoseiid predator species found in the literature. The daily consumption of spider mite eggs ranged from 4.4 (*Phytoseiulus macropilis* Banks) to 33.7 (*Phytoseiulus persimilis* Athias-Henriot) at 25 °C. The authors reported a Type II response for *Amblyseius californicus* (McGregor), with a theoretical maximum consumption rate of 35 prey eggs. Fan and Petitt (1994) reported that the generalist *Neoseiulus barkeri* (Hughes) showed a Type II functional response to densities of different stages of the twospotted spider mite. A starved female could consume a theoretical maximum of 94 eggs, 98 larvae or 3 adult females of *T. urticae* per day at 25 °C. These values are higher than those obtained in the present study: at the highest prey density offered, *I. degenerans* consumed 13.4 eggs or 2.7 adults of *T. urticae*. Akpokodje *et al.* (1990) found that the functional response of larvae and females of *I. degenerans* attacking different

developmental stages of the cassava green mite *M. tanajoa* were all Type II. Maximum number of cassava green mite eggs and adults eaten by *I. degenerans* during a five-hour period were 13 and 3.6, respectively. In contrast, Nwilene and Nachman (1996) only found Type II curves for *I. degenerans* protonymphs attacking cassava green mite eggs and females. Females of this phytoseiid attacking eggs, protonymphs and female adults of *M. tanajoa* all showed Type III functional responses.

To our knowledge, no information on the functional response of *I. degenerans* to densities of the western flower thrips *F. occidentalis* is available in the literature. In this study, the predator exhibited a Type II functional response when feeding on first or second instars of *F. occidentalis*. In experimental conditions which closely resembled those in our study, Shipp and Whitfield (1991) described the functional response of *Amblyseius cucumeris* (Oudemans) when feeding on first instars of the western flower thrips. They reported Type II functional response curves for mated females. Estimated values of the attack rate and the handling time were 0.870 day^{-1} and 0.062 days, and 0.967 day^{-1} and 0.124 days on sweet pepper and cucumber leaf discs, respectively. The attack rate on 1^{st} instars of *F. occidentalis* found in the present study is about 2 times higher (0.0732 h^{-1} = 1.757 day^{-1}), while the handling time was 3 to 6 times longer (9.436 h = 0.393 days) than the values reported by Shipp and Whitfield (1991). *Amblyseius cucumeris* could consume a theoretical maximum of 16 or 8 thrips nymphs depending on the host plant species (sweet pepper or cucumber, respectively), whereas *I. degenerans* could consume no more than 3 first or second instars of *F. occidentalis* in our study.

In previous chapters, it has been shown that *I. degenerans* is able to feed and develop on eggs of the greenhouse whitefly *T. vaporariorum*. In the present study, the predatory mite exhibited a Type II response when offered eggs of *T. vaporariorum*. The literature reports no data on functional responses of phytoseiids feeding on different life stages of *T. vaporariorum*. According to Meyerdirk and Coudriet (1985) females of the phytoseiid mite *Euseius hibisci* (Chant) can consume an average of 4.5 *Bemisia tabaci* (Gennadius) eggs per female per day, with a range of 0 to 18.4. Nomikou *et al.* (2003) reported that both *Typhlodromus swirskii* (Athias-Henriot) and *Euseius scutalis* (Athias-

Henriot) killed more *B. tabaci* eggs and 1st instars than later stages. The predation rate on *B. tabaci* eggs by the two predatory mites was approximately 20 eggs per female per day. In the present study, *I. degenerans* could theoretically consume a maximum of 22 *T. vaporariorum* eggs per day which is very close to the values reported in above-mentioned studies.

Functional responses are usually measured to provide information on the suitability of the predator as a biological control agent. The functional response experiments in our study suggest that *I. degenerans* has limited predation capacity, particularly on *F. occidentalis* against which the predator is used in augmentative biological control in protected cultivation.

The information obtained in laboratory tests, however, is not easily extrapolated to field conditions. Functional responses measured in the laboratory describe a short-term behavioural phenomenon; the experiments last a short time relative to the life span of the predator (Murdoch, 1973). According to Berry *et al.* (1988) several problems are associated with estimating functional responses in the laboratory. Predators are not allowed to leave a patch in search of higher densities where prey location is more efficient, and as a consequence predators consume more prey at low density than they would probably do in nature. This may result in Type II functional responses when the underlying response is really Type III (Van Lenteren and Bakker, 1976). The functional response of a phytoseiid mite may be affected by the size of the experimental arena (Akpokodje *et al.*, 1990) or by plant species (Shipp and Whitfield, 1991; Skirvin and Fenlon, 2001). Due to consumption by predators or reproduction by ovipositioning prey, changes in prey densities may occur. Also, the length of the experimental period may be critical (Eveleigh and Chant, 1981).

In this study, functional response was assessed with one predator and one prey species. Prey preference and switching behaviour are additional factors to be considered when evaluating a predator. Although *I. degenerans* is released for control of *F. occidentalis*, this predator is a generalist (Croft and McMurtry, 1997) and little is known on its prey preference and switching behaviour. In the next chapters these two factors will be studied.

CHAPTER 7
EVALUATING THE PREY PREFERENCE OF *IPHISEIUS DEGENERANS* UNDER LABORATORY CONDITIONS

7.1. INTRODUCTION

Iphiseius degenerans has received attention mainly as a natural enemy of thrips and spider mites and only in a few studies the predation on other prey species has been addressed (Catling, 1970; Swirski *et al.*, 1987; Palevsky *et al.*, 2003). In previous chapters, it was shown that *I. degenerans* is able to develop, reproduce and feed on different prey species which they are likely to encounter in the field.

In all these experiments, the predatory mites were confined singly with one prey species and were not allowed to leave the arena in search for a more favourable prey. Additional knowledge on prey preference might be useful in understanding the predator's behaviour in the field. A predator exhibits preference for a prey type when the proportion of that prey in the predator's diet is higher than its proportion in the predator's environment (Begon, 1996). Preference for a prey type, however, is not always constant. Prey preference can change depending on the relative presence of the available prey types. This behaviour is called "switching". As a particular prey species declines in numbers, partly by predation by the predator, the predator switches the greater proportion of its attacks to another prey that has become the most abundant (Murdoch, 1969). Switching behaviour in phytoseiid mites (e.g., *Phytoseiulus persimilis* Athias-Henriot, *Euseius finlandicus* (Oudemans)) has been observed by Blackwood *et al.* (2001), but further little information is available.

In this study, the prey preferences of *I. degenerans* were assessed. Predation of adult female predatory mites was measured in two-choice leaf disc experiments. Different ratios of prey species were offered to examine the preference of *I. degenerans* and the predator's ability to adjust its feeding behaviour depending on the relative abundances of each prey species. The obtained results were compared with the preference predicted using the results from the functional response experiments (chapter 6).

7.2 MATERIALS AND METHODS

7.2.1 Predator culture

Iphiseius degenerans was reared as described in chapter 3 (3.2.1 Predator culture).

7.2.2 Prey

The prey species tested in the food preference experiments were eggs of *T. urticae* and *T. vaporariorum,* and 1st and 2nd instar nymphs of *F. occidentalis*. Descriptions of the rearing methods of the insects and mites used in the experiments are presented in chapter 4 (4.2.2 Food sources).

7.2.3 Experimental set-up

To examine the prey preference of *I. degenerans*, adult females (8 – 12 days post-maturation) were given a choice between two prey types. Single female predatory mites were starved for 4 hours and then placed on a bean leaf disc in a plastic container.

A detailed description of the way prey infested leaf discs were obtained and of the experimental arena, is given in chapter 4 (4.2.2 Food sources) and chapter 6 (6.2.3 Experimental units), respectively. Prey was offered to the predator in pair-wise combinations:

- combination 1: 1st instars of *F. occidentalis* vs. 2nd instars of *F. occidentalis*
- combination 2: 1st instars of *F. occidentalis* vs. eggs of *T. vaporariorum*
- combination 3: 1st instars of *F. occidentalis* vs. eggs of *T. urticae*
- combination 4: eggs of *T. vaporariorum* vs. eggs of *T. urticae*

First analysis of prey preference showed that *I. degenerans* had a significant preference for 1st instars of *F. occidentalis*. For that reason no combinations were made with 2nd instars of *F. occidentalis* and eggs of *T. vaporariorum* or *T. urticae*.

The total amount of prey items in each combination was 60. For each combination three prey density ratios were tested (1:1, 1:2 and 2:1), and for each ratio there were 20 replicates.

In addition, the influence of castor bean pollen on predation rates on abovementioned prey types was tested. Bean leaf discs were infested with 20, 30, or 40 prey individuals and dusted with castor bean pollen. Single female predatory mites were starved for 4 hours and then transferred onto the bean leaf disc in a plastic container. Twenty replicates were used per prey density.

Also, the predation rate was assessed when the predatory mite was offered only one prey species. Single female predatory mites were starved for 4 hours and then transferred onto the bean leaf disc infested with 20, 30, or 40 prey individuals. There were 20 replicates per prey density.

The number of dead prey was recorded after 24 hours. Dead prey were not replaced during the experimental period. Experimental arenas were maintained in a climatic cabinet at 25 °C, 75% RH and a 16L:8D h photoperiod.

7.2.3 Data analysis

Predation

The mean predation rate was analysed using analysis of variance, followed by a multiple comparison test (Student Newman Keuls) at the $p = 0.05$ level (SPSS 12.0, SPSS Inc., 1989-2003).

Preference index

Different methods are available to measure and express prey preference. An overview of these methods is given by Cock (1978). In the current study preference was analysed by application of Manly's Beta-index (1972, 1974):

$$\beta_i = \frac{\log\left(\frac{r_i}{A_i}\right)}{\sum_{s=1}^{K} \log\left(\frac{r_s}{A_s}\right)}$$

where β_i is the measurement of the predator's preference for prey belonging to class i, r_i and r_s are the number of surviving prey belonging to prey class i and s, A_i and A_s are the initial number of prey belonging to prey class i and s, and K is the number of different prey classes (in the experiments conducted during this study $K = 2$).

This index was chosen because it is appropriate to use in situations where both prey species are offered simultaneously and are not replenished throughout the experiment (Cock, 1978).

The index assigns preference values from 0 (preference for class 2) to 1 (preference for class 1), where 0.5 represents no preference.
The β-value was calculated per replicate and averaged to obtain a mean β-value for each prey ratio. Mean β-values were considered significant when the 95% confidence intervals did not overlap $\beta = 0.5$.

Switching behaviour

To evaluate the capacity of *I. degenerans* to switch between two prey species (frequency-dependent preference), two different methods were used.

First, β -values were compared using analysis of variance (ANOVA). Second, β -values were fitted to Manly's linear model of frequency-dependent selection (Manly, 1973):

$$\beta = a + b\rho$$

where a is the intercept, b the slope and $\rho = A_1/(A_1+A_2)$ or the proportion of prey of class 1 out of the total number of prey offered to the predator. In this model, parameter b can be regarded as the measurement of switching capacity between the two prey species. A positive slope of the regression line indicates that preference for prey 1 increases as the proportion of prey 1 offered to the predator increases. A negative slope suggests that preference for prey 1 decreases as the proportion of prey 1 offered increases. A horizontal line suggests frequency-independent behaviour.

Predicting prey preference using the functional response models

According to Cock (1978), the parameters of the individual prey type functional responses can be used to predict prey preference when both prey types are presented together to the predator. The null hypothesis in this method assumes that the predator's response remains constant in the presence of either prey type individually and both types together. Deviations from the predicted values would be caused by a change in one of the searching parameters due to either a change in search strategy, or the selection and/or rejection of disproportionate numbers of prey (switching).

To predict prey preference from functional response models five steps were involved:

(1) functional response experiments for each prey separately were performed (Chapter 6)

(2) the attack rate a and the handling time T_h were estimated using the random predator equations for functional response (Rogers, 1972):

$$N_e = N_0\{1-\exp[a(T_h N_e - T)]\} \text{ (Type II)} \qquad \text{Equation 7.1}$$

$$N_e = N_0\{1-\exp[(d+bN_0)(T_h N_e - T)/(1+cN_0)]\} \text{ (Type III)} \qquad \text{Equation 7.2}$$

with N_0 the initial density of prey, T_h the handling time, a the attack rate and T the duration of the experiment and b, c, d are constants from the function that relates a and N_0 in Type III functional responses: $a = (d + bN_0)/(1 + cN_0)$.

(3) two functional response equations were combined to describe the two-prey interaction (Lawton *et al.*, 1974):

$$N_{e1} = N_{01}\{1 - \exp[-a_1(T - T_{h1}N_{e1} - T_{h2}N_{e2})]\} \quad \text{Equation 7.3}$$

$$N_{e2} = N_{02}\{1 - \exp[-a_2(T - T_{h2}N_{e2} - T_{h1}N_{e1})]\}$$

with N_0 the initial density of prey, T_h the handling time, a the attack rate and T the duration of the experiment for prey 1 and 2, respectively.

(4) the predation of *I. degenerans* over a range of densities of the two prey species together was examined;

(5) the proportion of prey type I within the diet was plotted against the proportion of prey type I present in the environment, and the observed preference was compared with the predicted preference.

7.3. RESULTS

Predation rates of *I. degenerans*, expressed as the number of prey killed per 24 hours, are presented in Table 7.1.

The results indicate that when two prey types were offered simultaneously *I. degenerans* preyed on both types of prey. The presence of a second food source influenced the number of prey eaten. Compared with the control (no second food source offered), the predator consumed less prey in the presence of pollen, although this reduction was not always significant. The reduction in prey consumption in the presence of pollen ranged from 31% to 90%. In some combinations, adding a second prey species to the environment of the predator reduced the number of the first prey species consumed by the predator, although this reduction was not always significant (Table 7.1). One exception is the combination of *T. urticae* eggs vs. 1st instars of *F. occidentalis*; here the presence of thrips nymphs led to a significantly higher consumption of *T. urticae* eggs compared with the number of spider mite eggs preyed upon when offered alone to the predator.

Table 7.1. Mean number of prey consumed (± SEM) by *I. degenerans* in 24 h when prey is offered alone or together with a second food source at three different prey ratios (prey 1: prey 2)[b]

Prey 1	**Prey 2**	**Number of prey 1 killed[a]**		
		Prey ratio		
		1:2	**1:1**	**2:1**
F. occidentalis 1st instars	No second prey	2.00 ± 0.37a	2.65 ± 0.36b	3.80 ± 0.49a
	T. urticae eggs	2.90 ± 0.47a	2.32 ± 0.38b	3.50 ± 0.42a
	T. vaporariorum eggs	1.95 ± 0.37a	2.05 ± 0.48ab	3.20 ± 0.55a
	R. communis pollen	1.25 ± 0.30a	1.05 ± 0.21a	2.60 ± 0.31a
		$F = 2.43$	$F = 3.19$	$F = 1.22$
		df = 96	df = 97	df = 97
		$p = 0.053$	$p < 0.05$	$p = 0.31$
F. occidentalis 2nd instars	No second prey	1.45 ± 0.45b	1.35 ± 0.36a	1.75 ± 0.24a
	F. occidentalis 1st instars	2.22 ± 0.49b	0.74 ± 0.28a	1.41 ± 0.45a
	R. communis pollen	0.40 ± 0.11a	0.50 ± 0.14a	1.13 ± 0.22a
		$F = 8.70$	$F = 2.59$	$F = 1.00$
		df = 57	df = 58	df = 52
		$p < 0.01$	$p = 0.08$	$p = 0.37$

Table 7.1. Mean number of prey consumed (± SEM) by *I. degenerans* when prey is offered alone or together with a second food source at three different prey ratios (prey 1: prey 2) (Continued)

T. urticae eggs	No second prey	3.50 ± 0.90b	2.70 ± 0.65a	1.80 ± 0.46a
	F. occidentalis 1st instars	6.71 ± 0.94c	6.10 ± 1.08b	10.4 ± 1.51b
	T. vaporariorum eggs	2.00 ± 0.84ab	2.40 ± 0.61a	1.25 ± 0.28a
	R. communis pollen	0.35 ± 0.30a	0.60 ± 0.32a	0.50 ± 0.31a
		$F = 12.0$	$F = 10.4$	$F = 30.2$
		df = 77	df = 73	df = 75
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
T. vaporariorum eggs	No second prey	14.9 ± 0.85c	17.1 ± 1.31c	22.1 ± 1.26d
	F. occidentalis 1st instars	5.90 ± 0.90b	9.75 ± 1.47b	9.60 ± 1.39b
	T. urticae eggs	2.44 ± 0.52a	2.13 ± 0.52a	2.67 ± 0.57a
	R. communis pollen	6.40 ± 0.83b	7.45 ± 0.98b	15.3 ± 1.67c
		$F = 41.7$	$F = 22.6$	$F = 38.4$
		df = 75	df = 74	df = 77
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

[a]Means (± SEM) within a column and a first prey species followed by the same letter are not significantly different (Student Newman Keuls-test, $p > 0.05$)

[b]When two arthropod prey types are offered simultaneously, the total number of prey items offered equals 60; when one prey type is offered without a second prey, or with *R. communis* pollen, the total number of prey is 20 (ratio1:2), 30 (ratio 1:1) or 40 (ratio 2:1)

Overall, the consumption by *I. degenerans* was very low (Table 7.2). In treatments with two prey types, 60 prey individuals were offered of which a minimum of 3.65 (1st instars vs. 2nd instars of *F. occidentalis*, ratio 1:2) and a maximum of 13.3 (*T. vaporariorum* eggs vs. *T. urticae* eggs, ratio 1:2) prey items were consumed.

Within a prey combination, the total number of prey eaten remained constant (combination 1: $F = 2.52$, df = 53, $p = 0.09$; combination 2: $F = 1.02$, df = 59, $p = 0.37$; combination 4: $F = 0.36$, df = 48, $p = 0.70$), except in the third combination (1st instars of *F. occidentalis* – *T. urticae* eggs) in which the mean total number of prey consumed at ratio 1:2 differed significantly from that consumed at ratio 1:1 ($F = 4.03$, df = 58, $p < 0.05$).

Table 7.2. Mean number of prey consumed (± SEM) out of a total of 60 prey individuals for different prey combinations and prey ratios

Combination	**Prey ratio**[a]		
	1:2	**1:1**	**2:1**
Combination 1			
F. occidentalis (1st instars)	3.65 ± 0.63a	3.35 ± 0.41a	5.00 ± 0.63a
F. occidentalis (2nd instars)			
Combination 2			
F. occidentalis (1st instars)	11.55 ± 1.45a	11.80 ± 1.68a	9.10 ± 1.28a
T. vaporariorum eggs			
Combination 3			
F. occidentalis (1st instars)	13.30 ± 1.56b	8.42 ± 1.16a	10.21 ± 0.84ab
T. urticae eggs			
Combination 4			
T. vaporariorum eggs	3.69 ± 0.60a	4.53 ± 0.89a	4.67 ± 1.04a
T. urticae eggs			

[a] Means (± SEM) within a row followed by the same letter are not significantly different (Student Newman Keuls-test, $p > 0.05$)

To evaluate the preference of *I. degenerans* for the various prey species, Manly's index of preference was calculated (Manly, 1973, 1974). According to Murdoch (1969), prey preference is best determined when both prey species are offered in equal amounts. In the first three combinations, 1st instars of *F. occidentalis* were selected as prey 1; thus, an index above 0.5 suggests preference for 1st instars of *F. occidentalis*; in the fourth combination, whitefly eggs were chosen as prey 1. The preference indices for each combination of prey species offered to the predator are presented in Table 7.3.

First, preference was evaluated when both prey types were offered in equal numbers (ratio 1:1 in Table 7.3). In combination 1 (first instars vs. second instars of *F. occidentalis),* the predator showed a significant preference for first instars of the thrips. When first instars of *F. occidentalis* were offered together with eggs of *T. vaporariorum* (combination 2), there was a significant preference for the latter. In combinations 3 and 4, *I. degenerans* preferred *T. urticae* eggs over first instars of the western flower thrips or eggs of the greenhouse whitefly; however, since the 95% confidence interval included 0.5 these preferences are not significant.

Table 7.3. Values of Manly's preference index β (95% confidence interval) for *I. degenerans* females offered different combinations of prey in different ratios

Combination	**Prey ratio**[a]		
	1:2	**1:1**	**2:1**
Combination 1			
F. occidentalis 1st instars	0.73b	0.84b	0.48a
F. occidentalis 2nd instars	(0.54 – 0.92)	(0.74 – 0.94)	(0.33 – 0.64)
Combination 2			
F. occidentalis 1st instars	0.30a	0.18a	0.29a
T. vaporariorum eggs	(0.20 – 0.40)	(0.10 – 0.26)	(0.14 – 0.44)
Combination 3			
F. occidentalis 1st instars	0.39a	0.35a	0.25a
T. urticae eggs	(0.25 – 0.53)	(0.20 – 0.50)	(0.13 – 0.37)
Combination 4			
T. vaporariorum eggs	0.74a	0.46a	0.72a
T. urticae eggs	(0.59 – 0.89)	(0.22 – 0.70)	(0.54 – 0.90)

[a] Means (95% CI's) within a row followed by the same letter are not significantly different (Student Newman Keuls-test, $p > 0.05$)

Second, preference indices were compared among prey ratios within each combination. In combinations 2 to 4, the observed preference of *I. degenerans* was constant over the whole range of density ratios of the two prey species offered (Table 7.3, Fig. 7.1). Significant changes in β only occurred in combination 1, where *F. occidentalis* nymphs of different instars were offered together (F = 6.42, df = 53, $p < 0.01$), indicating switching behaviour.

The relationship between β-indices within a prey combination could be described by Manly's linear model of frequency-dependent selection (Manly, 1973): $\beta = a + b\rho$. The results are plotted in Figure 7.1 (solid lines), while parameters of the models are shown in Table 7.4. The estimate of b was only significant in combination 1 (first instars and second instars of *F. occidentalis)*, which indicates switching. The linear model had a negative slope, indicating a negative switching behaviour, with a significantly decreased preference for first instars of *F. occidentalis* in response to an increased abundance of first instars.

Table 7.4 Parameter estimates (± SEM) of Manly's linear model of frequency-dependent selection

Combination	**Parameter**[a]	
	a	***b***
Combination 1		
F. occidentalis 1st instars	1.06 ± 0.17*	-0.74 ± 0.33*
F. occidentalis 2nd instars		
Combination 2		
F. occidentalis 1st instars	0.26 ± 0.12*	-0.01 ± 0.24
T. vaporariorum eggs		
Combination 3		
F. occidentalis 1st instars	0.55 ± 0.14*	-0.44 ± 0.27
T. urticae eggs		
Combination 4		
T. vaporariorum eggs	0.65 ± 0.21*	-0.01 ± 0.39
T. urticae eggs		

[a] Parameter estimates followed by * are significantly different from zero ($p < 0.05$, linear regression)

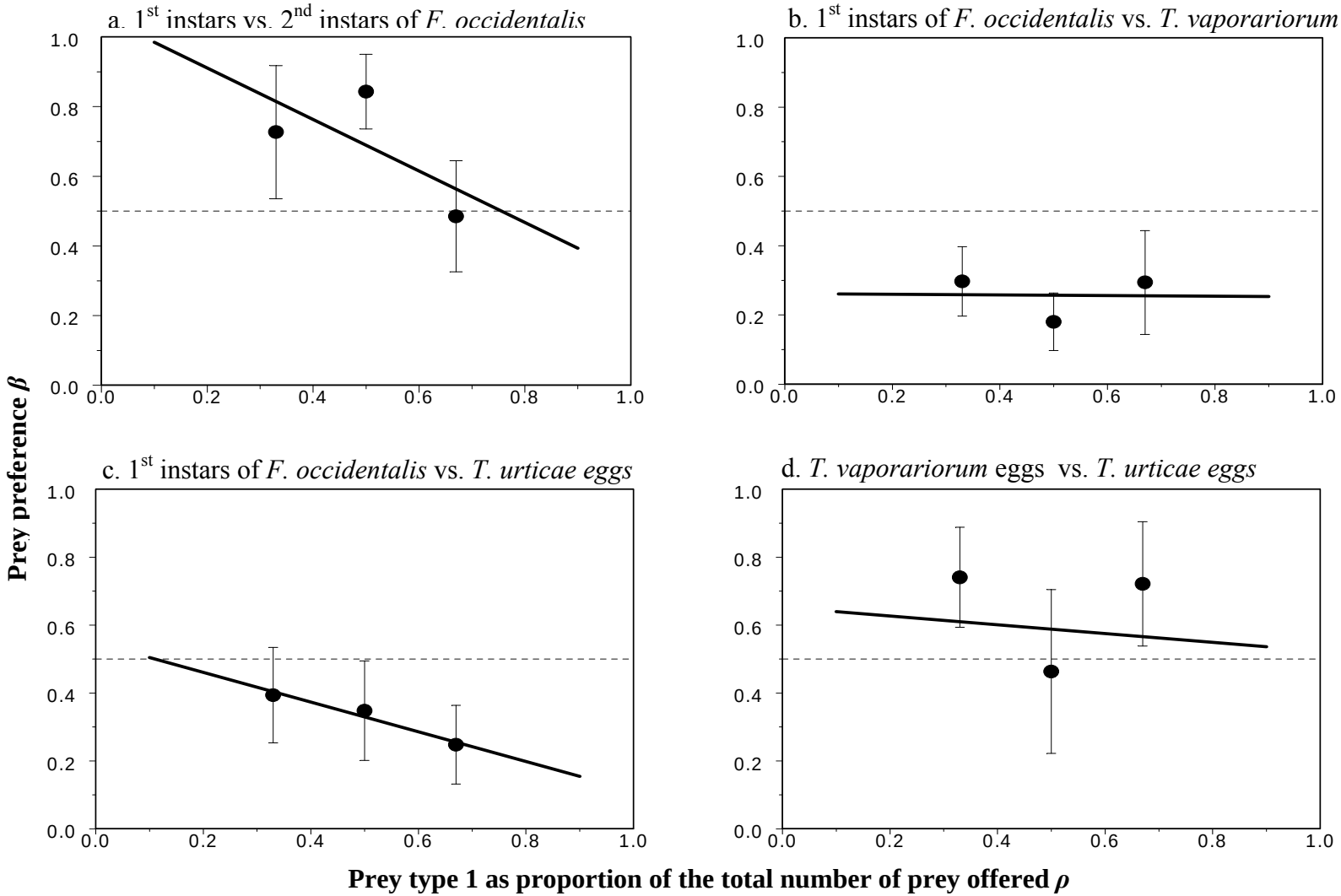


Figure 7.1. Prey preference β with 95% confidence intervals (solid dots). Bold solid lines represent Manly's linear model of frequency-dependent selection (Manly, 1973): $\beta = a + b\rho$

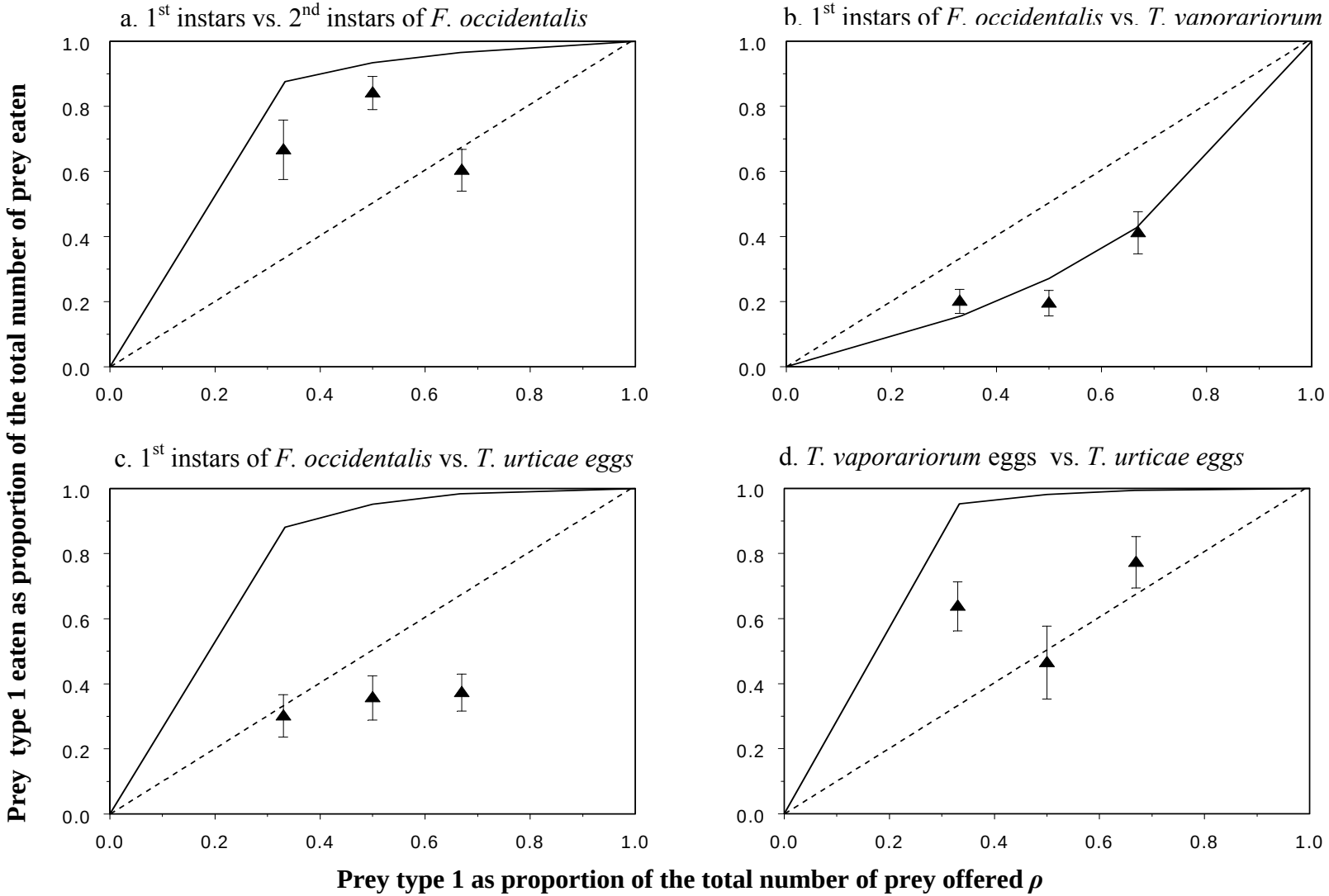


Figure 7.2. Predation of *I. degenerans* upon different prey species: observed proportion of prey 1 in the diet (± SEM) (solid triangles). The solid line shows the predicted preference based on the individual functional response curves. The dotted line is the no-preference line

The parameters of the individual functional responses were used to predict consumption of prey when both prey types are presented together. Using the 2-prey interaction (equation 7.3), the number of prey consumed was predicted for different prey types and different prey densities. Results were compared with the observed predation over the same range of densities (Fig. 7.2). In each case the predicted and observed proportion of prey type 1 in the diet are plotted against the proportion of prey type 1 of the total initial prey density (60 prey individuals).

Iphiseius degenerans showed a preference for 1st instars over 2nd instars of *F. occidentalis* at two ratios (Fig. 7.2a, ratio 1:2 and 1:1). Based on the individual functional response curves, there should be preference for 1st instars over all ratios. The deviation between predicted and observed preference indicated that there was a slight change in the predator behaviour when the two prey stages were offered simultaneously. The same can be concluded when comparing predicted and observed preference when whitefly eggs and spider mite eggs were offered (Fig. 7.2d). Here the observed value at ratio 1:1 differed from the predicted one. Figure 7.2b shows that there is a preference for *T. vaporariorum* eggs over 1st instars of *F. occidentalis*. This preference is predicted from the separate functional response curves. The outcome of choice between 1st instars of *F. occidentalis* and *T. urticae* eggs is depicted in Figure 7.2c. *Iphiseius degenerans* had a preference for spider mite eggs at ratio 1:1 and 2:1, although the functional response experiments predicted the contrary.

7.4. DISCUSSION

When a prey type was offered in combination with pollen, predation on the prey was affected. In three out of four combinations, predation on arthropod prey was significantly reduced in the presence of pollen. This concurs with the results of Wei and Walde (1997) who looked at the effect of the presence of *Typha latifolia* L. pollen on the functional response of *Typhlodromus pyri* Scheuten to *Panonychus ulmi* (Koch). However, in their study, the effect on predation was limited. At a density of 30 prey individuals offered, the predation rate with pollen was approximately 80% of that when no pollen was present. McMurtry and Scriven (1966) also concluded that the consumption of *Oligonychus punicae* (Hirst) by *Amblyseius hibisci* (Chant) decreased

when pollen was added, with an average reduction of 60.4 and 25.7% depending on the quantity of pollen offered ('high' or 'low'). Feeding on alternative food like pollen has received a lot of attention, regarding the persistence of the predator population in the field and consequently its role in biological control. According to Wei and Walde (1997) the enhancement of biological control due to the presence of pollen should be most likely when the primary prey is preferred more than pollen (thus minimizing the reduction in predation rate), but the alternative food is also of high quality, thus maximizing the numerical response. In the case of *I. degenerans*, the presence of pollen as an alternative food source is likely to reduce the predation on thrips nymphs, spider mite eggs and whitefly eggs.

Prey preference of phytoseiid mites has been evaluated when the predators were offered immature life stages of con- and heterospecifics (Schausberger, 1999; Walzer and Schausberger, 1999), different life stages of one prey species (Takafuji and Chant, 1976, Fernando and Hassell, 1980; Clements and Harmsen, 1993; Blackwood, 2001) or life stages of different prey species (Clements and Harmsen, 1993; Smith *et al.*, 1996). *Typhlodromus caudiglans* Schuster preferred active larvae and protonymphs over eggs and adult females of *P. ulmi* (Clements and Harmsen, 1993). Smith *et al.* (1996) tested the preference of five phytoseiid mites to eggs and immatures of *Mononychellus tanajoa* (Bondar) and *Mononychellus caribbeanae* McGregor. None of the predators showed any preference based on the consumption of eggs, but they did show a preference for *M. tanajoa* based on the consumption of mobile immatures.

In the present study, preference for a particular prey species was determined by estimating Manly's preference index (1973) and was predicted from the individual functional responses (Cock, 1978). Although *I. degenerans* is considered a generalist predator (McMurtry and Croft, 1997), the predator showed a clear preference in at least two cases. It showed selectivity towards 1st instars over 2nd instars of *F. occidentalis* when equal numbers of the two prey stages were present. A significant preference was also observed for *T. vaporariorum* eggs over 1st instars of *F. occidentalis*. The lack of preference for thrips in our experiments is surprising as the predator is used as a biological control agent of thrips. The preference for whitefly eggs over first instars of

F. occidentalis is not clear. Immobile stages may be preferred as the predator does not need to spend extra energy for catching the prey. On the other hand, for mobile prey, there is an increased probability of an encounter. Thrips nymphs, however, are known to be aggressive and able to evade attacks by predators (Bakker and Sabelis, 1989). The choice for *T. vaporariorum* eggs over thrips nymphs concurs with the results from the experiments on developmental time (chapter 4), revealing that a diet of whitefly eggs resulted in a faster developmental time (7.11 days) and a higher survival of the immatures (66.7 %) than did a diet of thrips nymphs (7.75 days and 13 % respectively). In contrast, in terms of reproductive success, thrips nymphs are a better choice than whitefly eggs. The intrinsic rate of increase of the predatory mite when fed thrips nymphs was about two times higher than that on a diet of whitefly eggs (0.114 vs. 0.056 females/female.day). Whether phytoseiid mites select the best prey species in terms of reproductive success was investigated by Dicke *et al.* (1990). They found that the prey preference of *Amblyseius* (= *Euseius*) *finlandicus* (Oudemans) for *Aculus schlechtendali* (Nalepa) could be understood in terms of reproductive success, whereas preferences shown by *T. pyri* and *Amblyseius potentillae* (Garman) could not.

When confronted with different ratios of the tested prey types, the overall trend is that there is no switching behaviour in *I. degenerans*. The only exception to non-switching was the combination with 1st and 2nd instars of *F. occidentalis*. *Iphiseius degenerans* exhibited negative switching, with a significantly decreased preference for 1st instars of *F. occidentalis* in response to an increased abundance of first instars. Blackwood *et al.* (2001) reported on switching behaviour in five phytoseiid mite species. Adult females of *E. finlandicus* and *P. persimilis* exhibited a slightly negative prey-stage switching, feeding more on *T. urticae* larvae in response to increased abundance of prey eggs. In contrast, positive switching occurred in adult females of *T. pyri*.

The preferences shown by *I. degenerans* can be summarized as follows: *T. vaporariorum* eggs - *T. urticae* eggs > 1st instars of *F. occidentalis* > 2nd instars of *F. occidentalis*. In 3 out of 4 combinations tested, the preference was generally predicted well by the individual functional response curves. This means that the searching

behaviour of female *I. degenerans* was the same when confronted with a single or two prey species. One exception was found when the predator was simultaneously offered 1st instars of *F. occidentalis* and *T. urticae* eggs. The individual response curves predicted a preference for 1st instars of *F. occidentalis*, but in choice tests the predatory mite was observed to prefer *T. urticae* eggs, although this preference was not significant at all ratios tested. This discrepancy may be explained by a change in predatory behaviour when both species were offered together. As mentioned above, the reason why eggs are preferred is not clear. To investigate this, observing phytoseiid mites in a patch with two or more prey species would probably provide some more information on the foraging behaviour of *I. degenerans*.

Investigation of the searching behaviour of *I. degenerans* would be helpful in understanding abovementioned results. The orientation of the predatory mite in an olfactometer might provide useful information for this purpose. The olfactory response of *I. degenerans* females to infested bean leaves with different prey species will be studied in the next chapter.

CHAPTER 8
OLFACTORY RESPONSE OF *IPHISEIUS DEGENERANS* TOWARDS ODOURS FROM PLANTS, POLLEN AND PREY

8.1 INTRODUCTION

In the previous chapter prey preference of *I. degenerans* was assessed in two-choice experiments. When arriving in a prey patch with one or more prey stages or species, the predator has to make foraging decisions (Dicke, 1988). Chemical communication, both between arthropods and between plants and arthropods, plays a very important role in the behaviour of an arthropod predator. Chemical information can originate from the herbivore, its food, herbivore-associated organisms or from interactions between these sources (Vet and Dicke, 1992). Some plants have been found to produce and emit volatile infochemicals in response to attacks by herbivores. These infochemicals are released in large amounts and the blend composition is specific for a plant species or genotype, and for the herbivore species or instar damaging the plant (Vet and Dicke, 1992; Dicke and Vet, 1999). This mechanism is a mode of indirect plant defence. Indirect plant defences bypass the direct defence route against the second trophic level (the herbivore) by promoting the effectiveness of the third level (the herbivore's natural enemies) (Sabelis *et al.*, 1999). Research conducted over the past two decades has shown that these volatiles can be used by predatory heteropterans and mites, and by parasitoids to find their prey (e.g., Dicke, 1988; Dickens, 1999; Lo Pinto *et al.*, 2004; McGregor and Gillespie, 2004; Gardiner *et al.*, 2005). The use of these infochemicals by natural enemies of herbivores is presumed to increase predation efficiency by reducing searching time and increasing attack rate (Dicke and Vet, 1999). Phytoseiid mites can discriminate between different herbivore species on the same host plant. Apparently, they prefer the volatiles related to one prey species over volatiles related to another species (Dicke *et al.*, 1988).

The interaction between plants, spider mites and phytoseiid predators is well documented. The trithrophic system consisting of Lima bean plants (*Phaseolus lunatus* L.) – *Tetranychus urticae* Koch – *Phytoseiulus persimilis* Athias-Henriot has received considerable attention (e.g., Sabelis and Van de Baan, 1985; Dicke, 1988; Shimoda and Dicke, 2000; Maede and Takabayashi, 2001; De Boer *et al.*, 2005).
To obtain more information on the prey location behaviour of *I. degenerans*, the ability of the predator to detect volatiles from infested bean leaves was assessed. This was done by presenting female predatory mites odours from uninfested bean leaves (*Phaseolus vulgaris* L.), and leaves originating from plants infested with *Frankliniella occidentalis* (Pergande), *T. urticae* or *Trialeurodes vaporariorum* Westwood, or leaves dusted with *Ricinus communis* L. pollen in two-choice experiments using a Y-tube olfactometer.

8.2 MATERIALS AND METHODS

8.2.1 Predator culture

Iphiseius degenerans was reared as described in chapter 3 (3.2.1 Predator culture).

8.2.2 Preparation of infested bean leaves

Four types of food sources were used in the trials: bean leaves infested with *T. urticae*, *T. vaporariorum*, or *F. occidentalis* or dusted with pollen of *R. communis*. Descriptions of the rearing methods of the insects and mites, and of the collection of pollen used in the experiments are presented in chapter 4 (4.2.2 Food sources)

To obtain leaves infested by *T. urticae*, 150 adult spider mites per leaf were introduced onto fresh bean plants with two primary leaves. Plants were placed in a climatic chamber at 30 ± 5 °C, 40 ± 5% RH and a 16L:8D h photoperiod to allow spider mites to establish a population with mixed life stages.

Whitefly infested bean leaves were obtained by placing fresh bean plants into the stock culture of *T. vaporariorum* (25 ± 1 °C, 50 ± 5% RH and a 16L:8D h photoperiod). Leaves bearing eggs, larvae, pupae and adult whiteflies were used in the experiments.

First and second instars of *F. occidentalis* were gently brushed off from a green bean pod onto fresh bean plants. Infested plants were kept in the laboratory at 25 ± 1 °C, 50 ± 5% RH and a 16L:8D h photoperiod. When the typical damage by thrips (cell damage and drops of excrements) was visible, leaves were used in the olfactometer trials.

In the trials with pollen, the odour source consisted of uninfested bean leaves dusted with approximately 0.2 g of castor bean pollen per leaf.

In each replicate, four primary bean leaves were used as odour source.

8.2.3 Olfactometer

To test the attraction of *I. degenerans* towards volatiles of different odour sources, a Y-tube olfactometer was used (Fig. 8.1). The olfactometer consisted of a central glass tube (diameter: 4 cm, length: 15 cm) with two arms (diameter: 4 cm, length 11 cm). The side arms were extended with 21 cm long glass tubes and connected with screw caps (SVL42, diameter: 4.2 cm). These extensions contained two smaller glass tubes, between which a metal mesh was placed.

Air was blown into the olfactometer by a pump (KNF Neuberger pump, type N035 AN 18) and run through Tygon® tubing. Air was then filtered through activated charcoal (Whatman ® Carbon Cap ™) and split into two air streams (3 l/min). The flow rate was adjusted by flow meters (Aalborg ®). Conditioning was done by leading the air stream through glass gas wash bottles filled with distilled water, and was then directed to two similar bottles that contained the odour sources. The resulting humidified odour flows were then introduced into the two arms of the Y-tube olfactometer. The air left the olfactometer through the central tube.

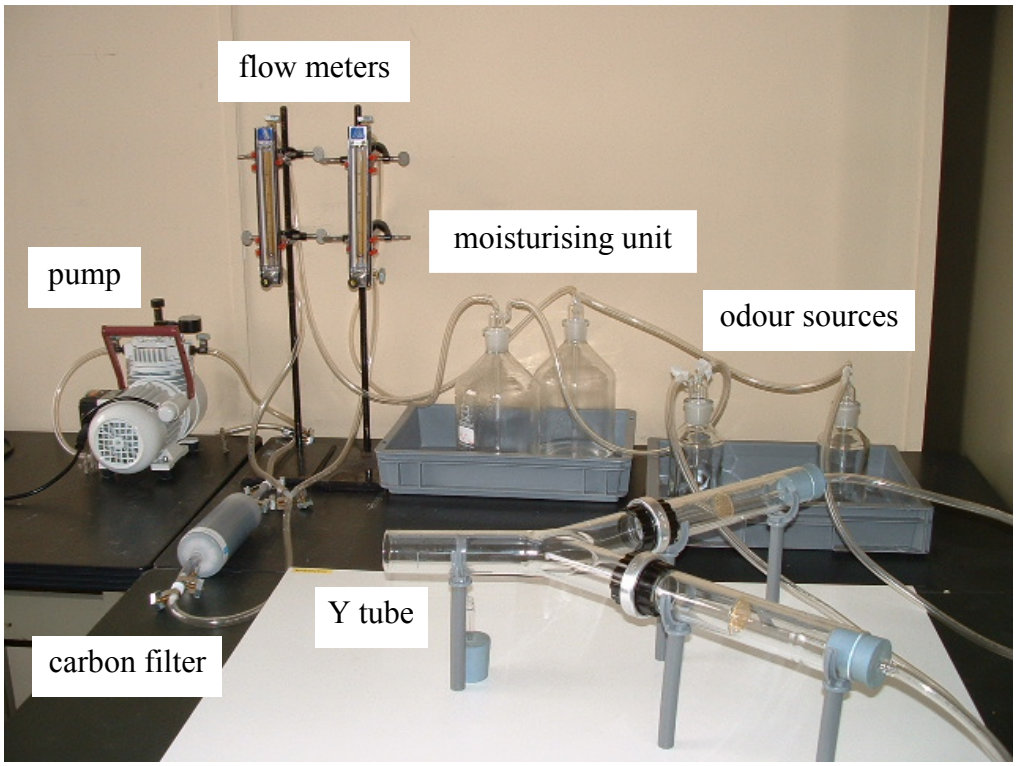


Figure 8.1. Y-tube olfactometer.

To provide a runway on which predatory mites could walk during the experiments, a Y-shaped iron wire was inserted in the Y-tube. A metal mesh fitted into the SVL42 connection supported the wire at the end of the Y-tube arms. The base of the wire was turned downwards and rested at the bottom of the Y-tube.

8.2.4 Experimental set-up

Individual females were introduced on the iron wire and had to move upwind. At the fork, they had to make a choice between the two odour sources. When the predator reached the end of one of the olfactometer arms (*i.e.*, the black SVL42 connection, Fig. 8.1.), the choice for the respective odour source was recorded. Each female was observed for 5 min. Predators that did not make a choice within this time span were recorded as “no choice”.

In the olfactometer, females were offered a choice between following combinations:

- Clean bean leaves vs. bean leaves dusted with castor bean pollen
- Clean bean leaves vs. *T. urticae* infested bean leaves
- Clean bean leaves vs. *F. occidentalis* infested bean leaves
- Clean bean leaves vs. *T. vaporariorum* infested bean leaves
- Bean leaves dusted with castor bean pollen vs. *T. urticae* infested bean leaves
- Bean leaves dusted with castor bean pollen vs. *F. occidentalis* infested bean leaves
- Bean leaves dusted with castor bean pollen vs. *T. vaporariorum* infested bean leaves
- *T. urticae* infested bean leaves vs. *F. occidentalis* infested bean leaves
- *T. urticae* infested bean leaves vs. *T. vaporariorum* infested bean leaves
- *F. occidentalis* infested bean leaves vs. *T. vaporariorum* infested bean leaves

For each combination of odour sources, thirty females were used. All predators were 8-12 days post-maturation, reared on castor bean pollen and starved for 4 hours prior to the start of the experiment. Each female was used only once.

After 15 replicates, the odour sources were uncoupled and connected to the other arm of the Y-tube to compensate for unforeseen asymmetry in the set-up. After each set of 30 replicates the glass tubes and odour sources were rinsed with acetone and the Tygon® tubing with water.

8.2.5 Data analysis

The results of each experiment were analyzed for statistical significance by a chi-square test (SPSS 12.0, SPSS Inc., 1989-2003). The null hypothesis was that predators exhibited a 50:50 distribution over the two odour sources.

8.3 RESULTS

The proportion of *I. degenerans* females responding to infested bean leaves is shown in Figure 8.2.

Iphiseius degenerans females were significantly more attracted to clean bean leaves compared to odours from thrips infested leaves ($\chi^2 = 6.53$, $p < 0.05$). No preference was observed for leaves infested with spider mites or whiteflies ($\chi^2 = 0.14$, $p = 0.71$; $\chi^2 = 0.00$, $p = 1$). The predator did not discriminate between clean bean leaves and leaves dusted with pollen ($\chi^2 = 0.13$, $p = 0.72$).

When offered a choice between odours from pollen-dusted leaves and prey-infested leaves, the predatory mite showed no preference for either one of the odour sources ($\chi^2 = 0.00$, $p = 1$; $\chi^2 = 0.53$, $p = 0.47$; $\chi^2 = 0.00$, $p = 1$). When presented with leaves infested with thrips and leaves infested with spider mites, females were significantly more attracted to the thrips-infested leaves ($\chi^2 = 6.53$, $p < 0.05$). No attraction nor rejection was observed when *I. degenerans* was offered a choice between whitefly infested and spider mite infested leaves, and whitefly infested and thrips infested leaves ($\chi^2 = 1.20$, $p = 0.27$; $\chi^2 = 0.13$, $p = 0.72$).

The number of non-responding females was generally low. Only when spider mite infested leaves and uninfested leaves were presented, two predator females did not make a choice within five minutes.

8.4 DISCUSSION

In this study an olfactometer was used to investigate the response of *I. degenerans* to volatiles from prey infested bean leaves. *Iphiseius degenerans* females were offered a choice between odours from uninfested bean leaves and leaves originating from plants infested with either *F. occidentalis*, *T. urticae* or *T. vaporariorum*, or leaves dusted with *R. communis* pollen.

Female predatory mites that had been reared on castor bean pollen did not show a response to pollen odours. Nonetheless, *I. degenerans* is often found in flowers feeding on pollen and thrips (van Houten and van Stratum, 1995). Based on our results it remains unclear whether the predator is attracted to the flowers due to the presence of pollen or thrips. It has been demonstrated that pollen emit odours than can be used by insects to find this food source (Kirk, 1985; Cook *et al.*, 2002).

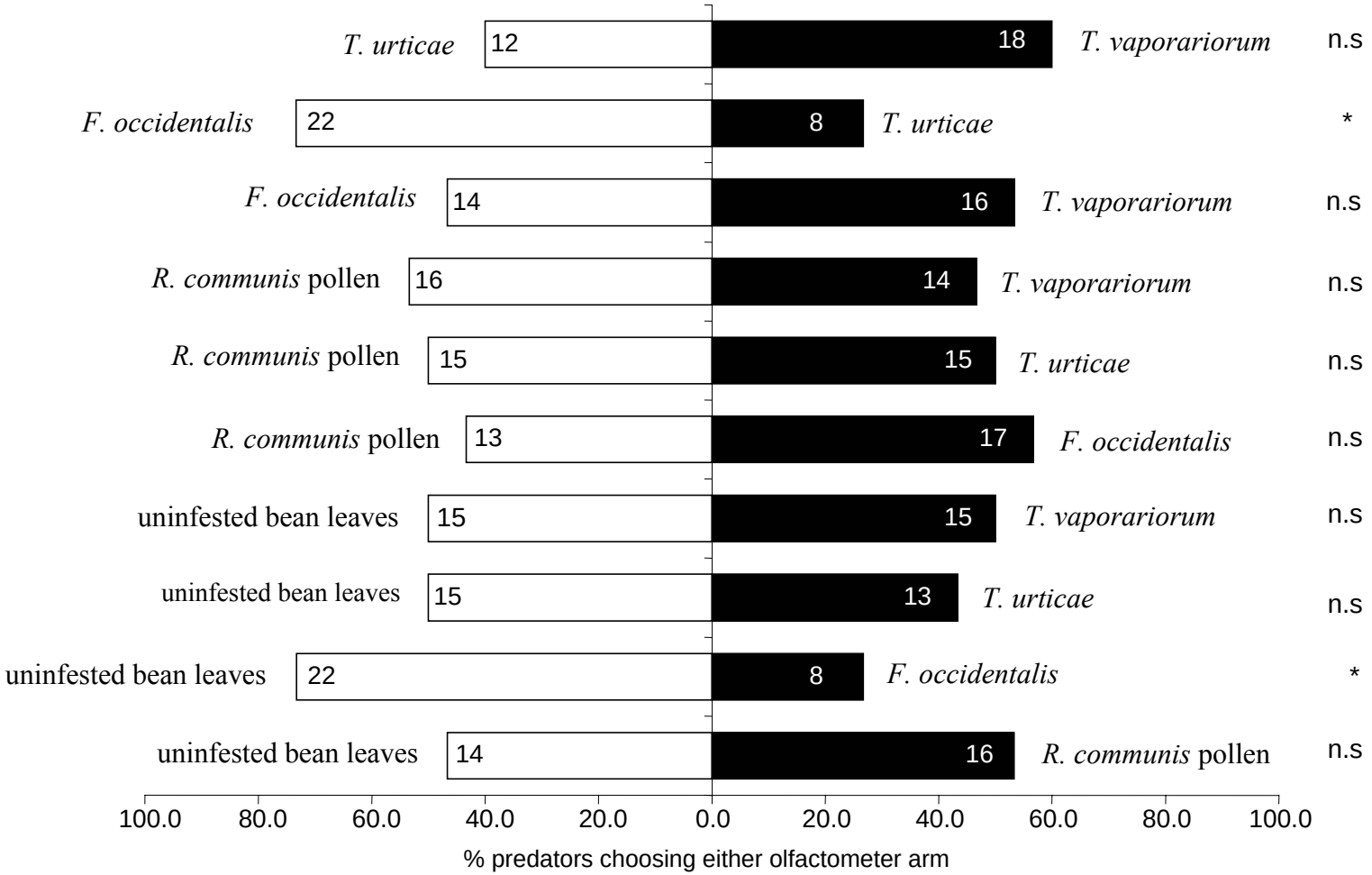


Figure 8.2. Response of *I. degenerans* females in a Y-tube olfactometer. Numbers in bars indicate the number of females choosing for either olfactometer arm. n.s : $p > 0.05$, * : $p < 0.05$.

The olfactometer experiments showed that the predators did not discriminate between odours from spider mite infested, whitefly infested, or pollen dusted bean leaves and clean bean leaves. However, female predators preferred clean leaves over thrips infested leaves. Our results correspond with those obtained by Janssen *et al.* (1998) who investigated the response of three phytoseiid predators, including *I. degenerans*, to odours emanating from infested cucumber plants. They reported that both *P. persimilis* and *N. cucumeris* were attracted to plants with thrips, while *I. degenerans* showed no preference for plants infested with thrips over clean leaves. The specialist *P. persimilis* was also attracted to spider mites, *N. cucumeris* showed neither attraction nor avoidance and *I. degenerans* avoided plants infested with spider mites.

The predator did not discriminate between leaves with spider mites and leaves with whiteflies, and between leaves with thrips and leaves with whiteflies. However, *I. degenerans* showed a strong preference for leaves with thrips over spider mite infested leaves. This was also reported by Janssen *et al.* (1998). According to these authors, the reason why thrips-infested plants were preferred over plants with spider mites, was probably because the predator was repelled by plants with spider mites. But this was not shown in our data, since repellence was only observed when the predatory mite was offered a choice between leaves infested with *F. occidentalis* and *T. urticae*.

It would be interesting to know whether the preferences exhibited in the Y-tube trials also occur when prey species are present in the same prey patch. This can be investigated by comparing results from olfactometer studies, prey preference studies or electrophoretic analysis of the gut content of predators. Dicke (1988) showed that *Typhlodromus pyri* Scheuten preferred *Panonychus ulmi* (Koch) to *Aculus schlechtendali* (Nalepa) based on the olfactory response. This conclusion was confirmed by the electrophoretic analysis of the gut content of *T. pyri* (Dicke and De Jong, 1988). Results of the present study show that *I. degenerans* preferred *F. occidentalis* to *T. urticae* and although not significant, somewhat more females oriented towards *T. vaporariorum* than towards *T. urticae*. There was no clear response when *F. occidentalis* and *T. vaporariorum* were presented together. These findings do not concur with the results of the two-choice predator experiments (chapter 7). When equal amounts of these prey species were offered simultaneously in two-choice leaf disc

experiments, the predator did not discriminate between *T. vaporariorum* and *T. urticae*, but preferred eggs of *T. urticae* or *T. vaporariorum* over *F. occidentalis* first instars. In olfactometer tests, preference for *F. occidentalis* can solely be based on olfactory information, whereas in predation experiments also tactile stimuli may influence predator response. It has already been mentioned that thrips nymphs (especially second instars) evade attacks by jerking with their abdomen (chapter 4) and can also evade attacks by predators by producing a drop of rectal fluid (Bakker and Sabelis, 1989). Alternatively, Dong and Chant (1986) mentioned that the searching efficiency of *I. degenerans* may be more reliant on tactile information. Takafuji and Chant (1976) showed that *I. degenerans* became more active and the searching activity was stimulated when prey bumped into resting *I. degenerans*.

The responses measured in the present study may have been affected by several factors. *Iphiseius degenerans* was reared on castor bean pollen and had no experience with either of the prey species. Further, the hunger level of the phytoseiid may also influence the response. According to Dong and Chant (1986), food deprivation and life stage of *I. degenerans* affected its response to *Tetranychus pacificus* McGregor prey mites. Only starved females showed a positive response, while well-fed adults and protonymphs did not. In the present study, females were starved for 4 h. Longer starvation times may result in a different response. However, preliminary tests showed that fitness of the predator may be affected by starvation times longer than 24 h.

Gardiner *et al.* (2005) reviewed numerous factors related to the condition of the host plant or the predator affecting the attraction of a phytoseiid predator to plants damaged by spider mites. Beside the hunger level of the predator and the predator's rearing history, within-plant variation in volatile production, presence of other (conspecific or heterospecific) arthropods, the abundance of prey, the amount of light and water received by the plant and the infection of the predator with a pathogen are also factors which may play a role in the attraction of the predator. To assess the importance of these factors in the olfactory response of *I. degenerans*, however, further study is necessary.

CHAPTER 9
GENERAL DISCUSSION, CONCLUSION AND PERSPECTIVES

The western flower thrips, *Frankliniella occidentalis* (Pergande) became one of the major crop pests in European greenhouses since its introduction in Europe in 1983 (van Lenteren and Loomans, 1998). A variety of predators, entomopathogenic fungi, nematodes and parasitoids of thrips are known (an overview is given by Sabelis and van Rijn (1997) and van Lenteren and Loomans (1998)). Several of these natural enemies proved to be good enough to control thrips, but only under specific conditions (van Lenteren and Loomans, 1998). For example, *Amblyseius cucumeris* (Oudemans) and *Orius* spp. are successfully introduced for thrips control in greenhouses in spring and summer (Van den Meiracker and Ramakers, 1991). Unfortunately, in winter, both species enter a reproductive diapause induced by short day conditions. Also, eggs of *A. cucumeris* are vulnerable to low air humidity.

In 1995, van Houten *et al.* reported on experiments that aimed at selecting new phytoseiid predatory mites which were able to control *F. occidentalis* year round. In these experiments, the rates of predation and oviposition on a diet of 1st instars of *F. occidentalis*, the rate of oviposition on a diet of sweet pepper pollen, the reproductive diapause incidence under short day conditions and the egg-hatching success at different ambient humidities was studied on cucumber leaf discs. Based on their results, the authors suggested that *Iphiseius degenerans* (Berlese) and *Amblyseius hibisci* (Chant) were the most promising candidates for biological control of the western flower thrips.

A survey of the literature, presented in the second chapter of this study, revealed that information on *I. degenerans* is relatively scarce. For instance, no records were found on life table parameters and functional responses when the predator was fed *F. occidentalis*. Also, there was no information found on the prey preference of the predatory mite. According to van Lenteren and Woets (1988) the ability to develop to the adult stage on the host, climatic adaptation, the lack of negative effects on other beneficials present in the same environment, a good rearing method, a high kill rate (high intrinsic rate of increase) and a good searching efficiency are essential criteria for preintroductory evaluation of natural enemies for biological control in greenhouses, and

yet little or no information on these criteria was available for *I. degenerans*. It can be concluded that, although *I. degenerans* has been used for years as a biological control agent, it is not clear how *I. degenerans* interacts with its primary target, the western flower thrips *F. occidentalis*.

This study was undertaken to elucidate interactions of *I. degenerans* with prey species the predator is likely to encounter in the crop (*F. occidentalis, Tetranychus urticae* Koch and *Trialeurodes vaporariorum* Westwood*).* Following research questions were addressed during this study:

1. What is the influence of food on the development, longevity and life table parameters of *I. degenerans* ?
2. What is the maximum predation rate of *I. degenerans* on different natural prey species?
3. Does *I. degenerans* show a preference for a particular food source?
4. Is there an olfactory response involved when the predatory mite searches for prey?

To provide an answer to these questions, studies on *I. degenerans* were carried out under laboratory conditions of 25 ± 1 °C, 75 ± 5% RH and a 16L:8D h photoperiod.

Life history traits of the predatory mite (*i.e*., development, fecundity, longevity, life table parameters) were explored in relation to different food sources in chapters 4 and 5. Previous studies reported on the immature development of *I. degenerans*, but in most of these studies pollen or tetranychid prey was offered as food (Takafuji and Chant, 1976; McMurtry, 1977; McMurtry *et al.*, 1984; van Rijn and Tanigoshi, 1999ab). Reproductive characteristics were reported by Takafuji and Chant (1976), McMurtry (1977), Kennett and Hamai (1980), McMurtry *et al.* (1984), van Houten *et al.* (1995) and Nwilene and Nachman (1996), however, full life table studies were only performed by van Rijn and Tanigoshi (1999ab) on broad bean pollen, castor bean pollen, castor bean pollen and nectar, or on the twospotted spider mite *Tetranychus urticae* Koch on an artificial substrate, and by Takafuji and Chant (1976) on the pacific spider mite *Tetranychus pacificus* McGregor on a paper substrate. As mentioned before,

information on developmental rates and life table parameters is crucial to fully appreciate the potential of a predator for use in augmentative biological control.
Iphiseius degenerans is considered a generalist predator (Croft and McMurtry, 1997). The present study revealed that its polyphagous character is not restricted to prey that the predator is likely to encounter in the field, but is extended to factitious prey such as cysts of the brine shrimp *Artemia franciscana* Kellogg. Its polyphagous character may both have a positive and negative impact on its value as a predator in the field.

One positive impact, as discussed in chapter 4, is the ability to develop on alternative prey. *Frankliniella occidentalis* nymphs, the primary target of *I. degenerans*, appeared to be unfavourable food for immature development. A high percentage of *I. degenerans* individuals were observed to escape the experimental arenas when the adult predators were presented with *F. occidentalis* nymphs (chapter 5). It was hypothesized that predatory mites may start wandering in search of more suitable food, explaining the high escape and mortality rates when thrips nymphs were supplied as prey. According to Lester and Harmsen (2002), a predator stage that cannot gain nutrition from a prey population may be considered a weak link in the food chain. This situation, however, may be alleviated should there be alternative prey available to any weak-link stage. *Iphiseius degenerans* is able to develop on nymphs of the green peach aphid *Myzus persicae* (Sulzer), the spider mite *T. urticae*, and eggs of the whitefly *T. vaporariorum* within 8 days. Adult predatory mites were able to feed and reproduce when fed these natural prey species, although population growth differed among these diets. The use of pollen as an alternative food source by phytoseiid mites has received considerable attention. Supplying pollen may promote pest control if the decrease in predation rate due to feeding on pollen (as observed in chapter 7) is compensated by a greater level of predation due to an increased predator population (McMurtry and Scriven, 1996; Wei and Walde, 1997). Offering castor bean pollen in combination with *F. occidentalis* nymphs reduced the mortality of the *I. degenerans* immatures, but predators still fled from the arena. Castor bean pollen offered solely to *I. degenerans* did not cause mortality, nor did the predatory mites tend to escape from the arena. Compared with the diets consisting of *T. urticae* and *T. vaporariorum* eggs, the diet of castor bean pollen resulted in the lowest generation time and doubling time.

In conclusion, due to its polyphagous character *I. degenerans* can use alternative prey to build up a population in the crop. But how effective is the predator population in controlling the target pest population? Literature reports neither on predation rates nor on prey preference of *I. degenerans*.

Functional response experiments, together with numerical response experiments are regarded as key components in the selection of predators. Based on these experiments, it is possible to determine the theoretical maximum number of prey consumed, which may give an indication of the number of predators that have to be introduced in the crop to control a pest population. As demonstrated in chapter 6, *I. degenerans* is a predator with a low natural predation rate. The predator showed a Type II functional response when feeding on 1st or 2nd instars of *F. occidentalis*, adult females of *T. urticae* or eggs of *T. vaporariorum*, and a Type III functional response when feeding on eggs of *T. urticae*. Theoretically, an *I. degenerans* female could consume a maximum of 2.5 spider mite females, or 22 eggs of the greenhouse whitefly per day. The plateau in the Type III functional response was not yet reached in the present study, but at the highest density offered, the predatory mite consumed ca. 13 *T. urticae* eggs per day. *Amblyseius cucumeris* could consume a theoretical maximum of 16 or 8 first instars nymphs of *F. occidentalis* depending on the host plant species (sweet pepper or cucumber, respectively) (Shipp and Whitfield, 1991), whereas *I. degenerans* could consume no more than 3 first or second instars of *F. occidentalis* in the present study.

It is worth mentioning here that the absence of standardized protocols to conduct and analyze these functional response experiments complicated the interpretation and comparison of results. Berry *et al.* (1988) summarized several problems that are associated with estimating functional responses in the laboratory (e.g., predator movement out of a patch, size of the experimental arena, …). In the present study, difficulties were encountered when analysing the functional curves; there was a discrepancy between the conclusions based on the statistical analysis and those based on the visualized functional response curves.

A disadvantage of its polyphagous character may be that the focus of *I. degenerans* may shift towards other (pest)species present in the crop. Prey preference tests conducted in the laboratory may indicate what prey species is preferred (chapter 7). Again, this

information is not available in the literature. In the study of van Houten *et al*. (1995) it is assumed that *I. degenerans* prefers first instars of *F. occidentalis* based on the smaller size of this prey type, without testing this assumption in two-choice preference tests. Based on two-choice preference tests performed in the present study, the preferences shown by *I. degenerans* can be summarized as follows: *T. vaporariorum* eggs - *T. urticae* eggs > 1st instars of *F. occidentalis* > 2nd instars of *F. occidentalis*.

Given the low intrinsic rate of increase obtained when fed whitefly eggs, it is difficult to predict the value of *I. degenerans* as a whitefly predator. It is not likely that the predator will be able to control whitefly infestations in a crop, but instead will use whiteflies as an alternative food source when other prey becomes scarce. The phytoseiid may exhibit a secondary effect on *T. urticae*, but this is probably only the case when leaves are not yet entirely covered with webbing, as the predatory mites may not cope with dense webbing. This is supported by the high escape rate of the progeny from the experimental arena and the high doubling time of the predator population when *I. degenerans* was reared on spider mites.

The preference was generally predicted well by the individual functional response curves, except when the predator was offered 1st instars of *F. occidentalis* and *T. urticae* eggs simultaneously. The individual functional response curves predicted a preference for 1st instars of *F. occidentalis*, but the predatory mite was observed to prefer *T. urticae* eggs, although this preference was not significant at all prey density ratios tested. This discrepancy may be explained by a change in predatory behaviour when both species were offered together.

The preference for 1st instars over 2nd instars of *F. occidentalis* changed with the density ratio of offered thrips instars. *Iphiseius degenerans* exhibited negative switching, with a significantly decreased preference for 1st instars of *F. occidentalis* in response to an increased abundance of 1st instars.

Prey preference of the predatory mite was further analyzed in olfactometer experiments (chapter 8). Predators did not discriminate between odours from spider mite infested, whitefly infested, or pollen dusted bean leaves and clean bean leaves. However, female predators preferred clean leaves over thrips infested leaves. The results from the olfactometer tests did not correspond with the results of the two-choice experiments on leaf arenas. *Iphiseius degenerans* did not discriminate between leaves

with spider mites and leaves with whiteflies, and between leaves with thrips and leaves with whiteflies. However, it showed a strong preference for leaves with thrips over spider mite infested leaves. The olfactory responses of the predator remain unclear as the preference for thrips infested leaves and the repellence for spider mite infested leaves was not shown in all combinations with one of these prey species present. Predators reared on *R. communis* pollen did not show a response to pollen odours in olfactometer experiments.

Based on the results of the laboratory experiments (*i.e.*, its polyphagous character, the inability of immature predators to develop when offered thrips nymphs, the high escape rate when prey is considered suboptimal for development and reproduction, the low predation rate and the absence of a strong preference for certain prey species), it would be expected that *I. degenerans* is not a successful thrips predator. Nevertheless, *I. degenerans* has been used for many years in agricultural practice. Since the laboratory experiments carried out in the current study could not explain the control potential of this phytoseiid predator, further research may be warranted to fully understand its role in the regulation of arthropod pest populations. For instance, video monitoring of predatory mites when foraging and electrophoretic analysis of the gut content may elucidate the preference behaviour of *I. degenerans.* Also, it would be imperative to study how the behaviour of *I. degenerans* in the laboratory is reflected in more complex field situations, by conducting more field realistic experiments.

Recently, a new predatory mite has been commercialized in Europe for control of thrips populations: *Amblyseius* (= *Typhlodromips*) *swirskii* (Athias-Henriot). *Amblyseius swirskii* is expected to replace *A. cucumeris* as the standard phytoseiid mite against thrips (Bolckmans *et al*., 2005). Further, according to Nomikou *et al*. (2001, 2002) this predatory mite can also be used as a biological control agent of the tobacco whitefly *Bemisia tabaci* Gennadius. Like *I. degenerans,* this generalist predatory mite exhibits no diapause and thus can be used for year-round control. But again, very little information on the biology and predatory properties of this mite is available. It might be worthwhile to study biological characteristics and predatory behaviour of *A. swirskii* in depth, in order to gain insight in its capacities as a predator and in order to compare these

properties with those of *I. degenerans* in order to find out if *A. swirskii* can eventually be a valuable alternative to *I. degenerans* in augmentation biological control.

SUMMARY

The subject of this study, *Iphiseius degenerans* (Berlese), is a predatory mite belonging to the family Phytoseiidae. Phytoseiid mites are economically important predators of phytophagous mites and insects in greenhouse crops. *Iphiseius degenerans* is used commercially in Belgium for thrips control in greenhouse crops since 1994.

A survey of the literature revealed that information on *I. degenerans* is relatively scarce. For instance, no records were found on life table parameters and functional responses when the predator was fed *F. occidentalis*. Also, there was no information found on the prey preference of the predatory mite. Nevertheless, these biological parameters are, among others, essential criteria for preintroductory evaluation of natural enemies for biological control in greenhouses.

It can be concluded that, although *I. degenerans* has been used for years as a biological control agent, it is not clear how *I. degenerans* interacts with its primary target, the western flower thrips *Frankliniella occidentalis* (Pergande).

Laboratory studies were undertaken to elucidate interactions of *I. degenerans* with prey species the predator is likely to encounter in the crop (*F. occidentalis, Tetranychus urticae* Koch and *Trialeurodes vaporariorum* Westwood).

In the first experimental part of the study, life history traits of the predatory mite (*i.e.*, development, fecundity, longevity, life table parameters) were explored in relation to different food sources.

The experiments conducted in this part revealed that its polyphagous character is not restricted to prey that the predator is likely to encounter in the field, but is extended to factitious prey such as cysts of the brine shrimp *Artemia franciscana* Kellogg. This polyphagous character may both have a positive and negative impact on its value as a predator in the field.

Frankliniella occidentalis nymphs, the primary target of *I. degenerans*, appeared to be unfavourable food for immature development. A high percentage of *I. degenerans*

individuals were observed to escape the experimental arenas when the adult predators were presented with *F. occidentalis* nymphs. It was hypothesized that predatory mites may start wandering in search of more suitable food, explaining the high escape and mortality rates when thrips nymphs were supplied as prey. *Iphiseius degenerans* is able to develop on nymphs of the green peach aphid *Myzus persicae* (Sulzer), the spider mite *T. urticae*, and eggs of the whitefly *T. vaporariorum* within 8 days. Adult predatory mites were able to feed and reproduce when fed these natural prey species, although population growth differed among these diets. The intrinsic rate of natural increase (r_m) varied between 0.015 and 0.115 females/female.day. The diet consisting of *T. urticae* offered on a Multicel arena resulted in the highest population growth whereas the diet of *T. urticae* brushed of onto a bean leaf resulted in the slowest population growth.

The use of pollen as an alternative food source by phytoseiid mites has received considerable attention. Supplying pollen may promote pest control if the decrease in predation rate due to feeding on pollen is compensated by a greater level of predation due to an increased predator population (McMurtry and Scriven, 1996; Wei and Walde, 1997). Offering castor bean pollen in combination with *F. occidentalis* nymphs reduced the mortality of the *I. degenerans* immatures, but predators still fled from the arena. Castor bean pollen offered solely to *I. degenerans* did not cause mortality, nor did the predatory mites tend to escape from the arena. Compared with the diets consisting of *T. urticae* and *T. vaporariorum* eggs, the diet of castor bean pollen resulted in a higher population growth (r_m = 0.142 females/female.day).

In conclusion, due to its polyphagous character *I. degenerans* can use alternative prey to build up a population in the crop. But how effective is the predator population in controlling the target pest population? Literature reports neither on predation rates nor on prey preference of *I. degenerans*.

The predatory behaviour and prey preference were examined in the second experimental part of this study.

Functional response experiments indicated that *I. degenerans* shows a Type II functional response when feeding on 1st or 2nd instars of *F. occidentalis*, adult females of *T. urticae* or eggs of *T. vaporariorum*, and a Type III functional response when feeding on eggs of *T. urticae*. These experiments also indicated that this is a predator

with a low natural predation rate. Theoretically, *I. degenerans* could consume no more than 3 first or second instars of *F. occidentalis*, 2.5 spider mite females, or 22 eggs of the greenhouse whitefly per day. The plateau in the Type III functional response was not yet reached in the present study, but at the highest density offered, the predatory mite consumed ca. 13 *T. urticae* eggs per day.

A disadvantage of its polyphagous character may be that the focus of *I. degenerans* may shift towards other (pest) species present in the crop. Prey preference tests conducted in the laboratory may indicate what prey species is preferred. Based on two-choice preference tests performed in the present study, the preferences shown by *I. degenerans* can be summarized as follows: *T. vaporariorum* eggs - *T. urticae* eggs > 1st instars of *F. occidentalis* > 2nd instars of *F. occidentalis*.

Given the low intrinsic rate of increase obtained when fed whitefly eggs, it is difficult to predict the value of *I. degenerans* as a whitefly predator. It is not likely that the predator will be able to control whitefly infestations in a crop, but instead will use whiteflies as an alternative food source when other prey becomes scarce. The phytoseiid may exhibit a secondary effect on *T. urticae*, but this is probably only the case when leaves are not yet entirely covered with webbing, as the predatory mites may not cope with dense webbing. This is supported by the high escape rate of the progeny from the experimental arena and the high doubling time of the predator population when *I. degenerans* was reared on spider mites.

The preference was generally predicted well by the individual functional response curves, except when the predator was offered 1st instars of *F. occidentalis* and *T. urticae* eggs simultaneously. The individual functional response curves predicted a preference for 1st instars of *F. occidentalis*, but the predatory mite was observed to prefer *T. urticae* eggs, although this preference was not significant at all prey density ratios tested. This discrepancy may be explained by a change in predatory behaviour when both species were offered together.

The preference for 1st instars over 2nd instars of *F. occidentalis* changed with the density ratio of offered thrips instars. *Iphiseius degenerans* exhibited negative switching, with a significantly decreased preference for 1st instars of *F. occidentalis* in response to an increased abundance of 1st instars.

Prey preference of the predatory mite was further analyzed in olfactometer experiments. Predators did not discriminate between odours from spider mite infested, whitefly infested, or pollen dusted bean leaves and clean bean leaves. However, female predators preferred clean leaves over thrips infested leaves. The results from the olfactometer tests did not correspond with the results of the two-choice experiments on leaf arenas. *Iphiseius degenerans* did not discriminate between leaves with spider mites and leaves with whiteflies, and between leaves with thrips and leaves with whiteflies. However, it showed a strong preference for leaves with thrips over spider mite infested leaves. The olfactory responses of the predator remain unclear as the preference for thrips infested leaves and the repellence for spider mite infested leaves was not shown in all combinations with one of these prey species present. Predators reared on *R. communis* pollen did not show a response to pollen odours in olfactometer experiments.

Based on the results of the laboratory experiments (*i.e.*, its polyphagous character, the inability of immature predators to develop when offered thrips nymphs, the high escape rate when prey is considered suboptimal for development and reproduction, the low predation rate and the absence of a strong preference for certain prey species), it would be expected that *I. degenerans* is not a successful thrips predator. Nevertheless, *I. degenerans* has been used for many years in agricultural practice. Since the laboratory experiments carried out in the current study could not explain the control potential of this phytoseiid predator, further research may be warranted to fully understand its role in the regulation of arthropod pest populations.

SAMENVATTING

De Californische trips *Frankliniella occidentalis* (Pergande) werd geïntroduceerd in Europa in 1983 en is sindsdien één van de belangrijkste plagen in kasteelten (van Lenteren en Loomans, 1998). Een groot aantal natuurlijke vijanden van trips (predators, entomopathogene schimmels, nematoden en parasitoïden) zijn reeds gekend; een overzicht van deze natuurlijke vijanden werd gegeven door Sabelis en van Rijn (1997) en van Lenteren en Loomans (1998). Een aantal van deze natuurlijke vijanden zijn in staat om trips succesvol te bestrijden, maar enkel onder specifieke omstandigheden. *Amblyseius cucumeris* (Oudemans) en *Orius* spp. bijvoorbeeld worden succesvol uitgezet in kasteelten tijdens de lente en de zomer (Van den Meiracker Ramakers, 1991). In de winter gaan deze tripsbestrijders echter in een reproductieve diapauze (winterrust) onder invloed van korte dag omstandigheden. Een bijkomend probleem is dat de eitjes van *A. cucumeris* gevoelig zijn voor lage luchtvochtigheden.

van Houten *et al*. (1995) rapporteerden over een studie die als doel had roofmijten te selecteren die *F. occidentalis* het hele jaar rond kunnen bestrijden. In deze studie werden de predatie en eiafleg voor een aantal roofmijten gevoed met eerstestadiumnimfen van *F. occidentalis*, de eiafleg wanneer de roofmijten gevoed werden met paprikapollen, de reproductieve diapauze onder invloed van korte dag omstandigheden en de ontluiking van de eitjes onder invloed van verschillende relatieve vochtigheden bestudeerd. Op basis van de bekomen resultaten, suggereerden de onderzoekers dat *Iphiseius degenerans* (Berlese) en *Amblyseius hibisci* (Chant) veelbelovende kandidaten voor de biologische bestrijding van de Californische trips zijn.

Literatuuronderzoek, voorgesteld in het eerste hoofdstuk van voorliggende studie, wees echter uit dat er weinig informatie over *I. degenerans* beschikbaar is. Er zijn bijvoorbeeld geen gegevens voorhanden over de levenstabelparameters en de functionele responsen wanneer de roofmijt gevoed wordt met *F. occidentalis*. Verder is er ook weinig of geen informatie beschikbaar over de voedselpreferentie van de roofmijt.

Volgens van Lenteren en Woets (1988) zijn er een aantal essentiële criteria die bij de evaluatie van natuurlijke vijanden voor biologische bestrijding in kasteelten nader bestudeerd moeten worden: de mogelijkheid van de predator om te ontwikkelen tot het adultstadium wanneer ze gevoed worden met de prooi, de aanpassing aan klimatologische omstandigheden, de afwezigheid van negatieve effecten op andere natuurlijke vijanden in het gewas, een gemakkelijke kweekmethode, een grote capaciteit om prooien te doden (hoge intrinsieke groeisnelheid) en een goede zoekefficiëntie. Voor *I. degenerans* zijn deze gegevens schaars.
Op basis van deze vaststelling kan geconcludeerd worden dat, alhoewel *I. degenerans* reeds jaren gebruikt wordt als een natuurlijke vijand van de Californische trips *F. occidentalis*, het niet duidelijk is hoe de roofmijt interageert met dit plaaginsect .

Het doel van deze studie was de interacties tussen *I. degenerans* en een aantal plaagorganismen (*F. occidentalis*, *Tetranychus urticae* Koch en *Trialeurodes vaporariorum* Westwood) op te helderen. De onderzoeksvragen die hierbij gesteld werden, waren:

- Wat is de invloed van verschillende voedselbronnen op de ontwikkeling, levensduur, en levenstabelparameters van *I. degenerans*?
- Wat is de maximale hoeveelheid prooien die *I. degenerans* kan consumeren?
- Heeft *I. degenerans* een voorkeur voor een bepaalde voedselbron?
- Spelen olfactorische prikkels een rol wanneer de roofmijt op zoek gaat naar voedsel?

Om een antwoord op bovenstaande vragen te formuleren, werden een aantal experimenten uitgevoerd in het laboratorium bij 25 ± 1 °C, 75 ± 5% RV en een licht/donkercyclus van 16 uur licht en 8 uur duisternis.

De biologische karakteristieken van de predator (ontwikkeling, fecunditeit, levensduur, levenstabelparameters) werden bestudeerd in relatie met verschillende voedselbronnen in hoofdstukken 4 en 5. In vorige studies over de ontwikkeling van *I. degenerans* werden meestal pollen of spintmijten aangeboden als prooi (Takafuji en Chant, 1976; McMurtry, 1977; McMurtry *et al.*, 1984; van Rijn en Tanigoshi, 1999ab). Kenmerken over de reproductie werden gerapporteerd door Takafuji en Chant (1976),

McMurtry (1977), Kennett en Hamai (1980), McMurtry *et al.* (1984), van Houten *et al.* (1995), en Nwilene en Nachman (1996), maar volledige levenstabellen werden enkel opgesteld door van Rijn en Tanigoshi (1999ab) wanneer de roofmijt gevoed werd met bonenpollen, wonderboompollen, wonderboompollen en nectar, of met kasspint *T. urticae* op een plastic substraat, en door Takafuji en Chant (1976) wanneer de spintmijt *Tetranychus pacificus* McGregor werd aangeboden op een papieren substraat. Zoals eerder vermeld is de informatie over ontwikkelingstijden en levenstabelparameters cruciaal om het vermogen van de predator bij de biologische bestrijding van plaaginsecten en –mijten volledig te begrijpen. *Iphiseius degenerans* wordt aanzien als een generalist (Croft en McMurtry, 1997). The huidige studie toonde echter aan dat het polyfage karakter van de roofmijt zich niet beperkt tot prooien die de predator kan tegenkomen in het gewas, maar ook voeding op onnatuurlijke voedselbronnen inhoudt zoals de cysten van het pekelkreeftje *Artemia franciscana* Kellogg. Dit polyfage karakter kan echter zowel een positieve als negatieve invloed hebben op de waarde van de predator in natuurlijke systemen.

Een voordeel, bediscussieerd in hoofdstuk 4, is het vermogen van de roofmijt om zich te ontwikkelen op alternatieve prooien. De nimfen van *F. occidentalis*, de belangrijkste plaag waartegen *I. degenerans* wordt uitgezet, zijn immers geen goede voedselbron om de ontwikkeling van de roofmijt te ondersteunen. Naast het niet kunnen voltooien van de ontwikkeling tot het adultstadium, werd ook opgemerkt dat een groot percentage roofmijten die *F. occidentalis* nimfen kregen aangeboden, vluchtten van de arena. Verondersteld werd dat deze vluchtende roofmijten op zoek gaan naar een andere, meer geschikte voedselbron. Volgens Lester en Harmsen (2002) is het predatorstadium dat niet in staat is een prooipopulatie te gebruiken voor verdere ontwikkeling, een zwakke schakel in de voedselketen. Deze predators kunnen eventueel wel verder ontwikkelen wanneer ze zich voeden met een alternatieve prooi.
Iphiseius degenerans kan zich ontwikkelen tot het adultstadium in 8 dagen wanneer de mijt gevoed wordt met nimfen van de perzikluis *Myzus persicae* (Sulzer), de spintmijt *T. urticae* en eitjes van de kaswittevlieg *T. vaporariorum*. Volwassen roofmijten zijn in staat om zich te voeden en voort te planten wanneer ze deze prooien aangeboden krijgen, maar de populatiegroei is afhankelijk van de prooisoort. Het gebruik van pollen

als een alternatieve voedselbron voor roofmijten heeft reeds veel aandacht gekregen. Het aanbieden van pollen kan de plaagbestrijding in het gewas helpen, maar enkel indien de daling van het aantal geconsumeerde prooien als gevolg van het zich voeden op pollen (zoals geobserveerd werd voor *I. degenerans* in hoofdstuk 7) gecompenseerd wordt door een stijging in de prooiconsumptie als gevolg van de toegenomen predatorpopulatie (McMurtry en Scriven, 1996; Wei en Walde, 1997). In de huidige studie werd waargenomen dat door het gezamenlijk aanbieden van wonderboompollen en *F. occidentalis* nimfen de mortaliteit van de onvolwassen roofmijten daalde, maar dat er nog steeds roofmijten wegvluchtten van de arena. Ter vergelijking, wanneer wonderboompollen als enige voedselbron werd aangeboden, dan werd er noch mortaliteit noch ontsnapping waargenomen. Vergeleken met de diëten bestaande uit *T. urticae* en *T. vaporariorum* eieren, resulteerde het dieet bestaande uit wonderboompollen in de laagste generatietijd en verdubbelingstijd.

Samenvattend, door zijn polyfage karakter kan *I. degenerans* alternatieve voedselbronnen gebruiken om in een gewas een populatie op te bouwen. Maar hoe effectief is deze predatorpopulatie in het bestrijden van de beoogde prooipopulatie? De wetenschappelijke literatuur rapporteert noch over predatie noch over voedselpreferentie van *I. degenerans.*

Experimenten over de functionele respons en de numerieke respons worden gezien als belangrijke componenten bij de selectie van predators. Op basis van de resultaten uit dit type van experimenten is het mogelijk om het maximaal aantal prooien dat een predator per dag kan consumeren, te bepalen. Deze waarde kan een indicatie geven van het aantal predators dat geïntroduceerd moet worden in het gewas om een goede plaagbestrijding te garanderen. In hoofdstuk 6 werd geargumenteerd dat *I. degenerans* een predator met een lage predatiecapaciteit is. De roofmijt vertoonde een Type II functionele respons als reactie op toenemende prooidensiteiten van eerste- of tweedestadiumnimfen van *F. occidentalis*, volwassen wijfjes van *T. urticae* of eieren van *T. vaporariorum*, en een Type III functionele respons bij voeding op *T. urticae* eieren. Theoretisch kon een *I. degenerans* wijfje maximaal 2,5 wijfjes van de spintmijt, of 22 eieren van de kaswittevlieg per dag consumeren. Het plateau in de Type III

functionele respons werd niet bereikt, maar bij de hoogste prooidensiteit consumeerde *I. degenerans* ca. 13 *T. urticae* eieren. *Amblyseius cucumeris* kon een theoretisch maximum van 16 of 8 eerstestadiumnimfen van *F. occidentalis* consumeren (op paprika en boon, respectievelijk) (Shipp en Whitfield, 1991), terwijl *I. degenerans* niet meer dan 3 eerste- of tweedestadiumnimfen van deze prooi per dag kon consumeren in de huidige studie.

Bij de proeven over de functionele respons dient er ook opgemerkt te worden dat door het niet bestaan van gestandaardiseerde protocollen om deze experimenten uit te voeren en te analyseren, de interpretatie en vergelijkingen van de bekomen data bemoeilijkt worden. Berry *et al*. (1988) haalden al een aantal problemen aan die het bepalen van de functionele respons van een predator compliceren, zoals bijvoorbeeld de (on)mogelijkheid van een predator om de arena te verlaten, en de grootte van de experimentele arena. In de huidige studie was de moeilijkheid bij het analyseren en interpreteren van de resultaten te wijten aan het verschil in conclusies enerzijds gebaseerd op de statistische analyse en anderzijds op de visuele voorstelling van de functionele respons.

Een nadeel van het polyfage karakter van de roofmijt, is dat de focus van *I. degenerans* kan verschuiven naar andere (plaag)insecten en -mijten aanwezig in het gewas. Het bestuderen van de voedselpreferentie in het laboratorium kan aangeven welke prooi de predator zal verkiezen in het gewas (hoofdstuk 7). Informatie over de voedselpreferentie van *I. degenerans* is echter ook niet aanwezig in de literatuur. In de studie van van Houten *et al.* (1995) wordt verondersteld dat *I. degenerans* eerstestadiumnimfen van *F. occidentalis* prefereert omdat dit het kleinste stadium is. Voedselpreferentietesten die deze veronderstelling staven, werden echter nog niet uitgevoerd. Op basis van de resultaten uit hoofdstuk 7 kan de prooivoorkeur van *I. degenerans* als volgt worden samengevat: *T. vaporariorum* eieren - *T. urticae* eieren > eerstestadiumnimfen van *F. occidentalis* > tweedestadiumnimfen van *F. occidentalis*. Gebaseerd op de kleine waarde van de intrinsieke groeisnelheid (hoofdstuk 5), is het echter moeilijk om het potentieel van *I. degenerans* als biologische bestrijder van wittevliegen te voorspellen. Hoogstwaarschijnlijk is de roofmijt niet in staat om wittevliegaantastingen in het gewas onder controle te houden, maar kan wittevlieg wel als een alternatieve voedselbron

gebruikt worden. *Iphiseius degenerans* kan een secundair bestrijdingseffect op *T. urticae* genereren, maar dit is waarschijnlijk slechts het geval wanneer de bladeren nog niet volledig met spinsel zijn bedekt. De roofmijt lijkt immers moeilijkheden te hebben met de dichte webben van de spintmijt. Deze vaststelling is gebaseerd op het feit dat er een hoog percentage roofmijten vluchtten van de arena en er een hoge verdubbelingstijd van de populatie werd waargenomen wanneer roofmijten werden gevoed met het kasspint.

De voorkeur werd over het algemeen goed voorspeld door de individuele functionele responscurven, behalve wanneer gelijktijdig eerstestadiumnimfen van *F. occidentalis* en eieren van *T. urticae* werden aangeboden. De individuele responscurven voorspelden een voorkeur voor eerstestadiumnimfen van *F. occidentalis,* maar observaties wezen uit dat de roofmijt *T. urticae* eieren prefereerden, hoewel deze voorkeur niet bij alle geteste verhoudingen significant was. Deze discrepantie kan erop wijzen dat *I. degenerans* zijn zoekgedrag aanpast wanneer de twee prooisoorten tegelijkertijd worden aangeboden.

De voorkeur voor eerste- boven tweedestadiumnimfen van *F. occidentalis* wijzigde wanneer de verhouding van de aangeboden tripsnimfen veranderde. *Iphiseius degenerans* vertoonde negatieve switching; dit duidt op een verminderde voorkeur voor eerstestadiumnimfen van *F. occidentalis* als reactie op een verhoogde proportie van dit prooitype in het aangeboden dieet.

De voedselpreferentie werd verder geanalyseerd in olfactometerexperimenten (hoofdstuk 8). *Iphiseius degenerans* was niet in staat onderscheid te maken tussen de geuren afkomstig van bonenplanten aangetast door spintmijten of wittevliegen, bonenplanten bestoven met wonderboomstuifmeel en onaangetaste bonenbladeren. *Iphiseius degenerans* verkoos wel onaangetaste bladeren boven bladeren aangetast door tripsen. *Iphiseius degenerans* maakte geen onderscheid tussen bladeren met spintmijten en bladeren met wittevliegen, en tussen bladeren met tripsen en bladeren met wittevliegen, maar vertoonde wel een sterke voorkeur voor bladeren met tripsen boven bladeren met spintmijten.

De olfactorische respons bleef onduidelijk aangezien de voorkeur voor bladeren aangetast door tripsen en de afkeer voor bladeren met spintmijten niet in alle combinaties met één van deze prooien werd aangetoond.

Roofmijten gekweekt op wonderboompollen vertoonden geen reactie op geurstoffen afkomstig van deze pollensoort in de olfactometerexperimenten.

Gebaseerd op de resultaten van de voorgestelde laboratoriumexperimenten (het polyfage karakter van de roofmijt, het onvermogen om zich tot het adultstadium te ontwikkelen wanneer tripsnimfen worden aangeboden als voedsel, het hoge percentage mijten dat vlucht wanneer de aangeboden prooi niet geschikt is voor een optimale ontwikkeling en reproductie, de lage prooiconsumptie en het ontbreken van een sterke voorkeur voor bepaalde prooisoorten), kan er verwacht worden dat *I. degenerans* geen succesvolle tripspredator is. Niettemin wordt *I. degenerans* al vele jaren gebruikt in de landbouwpraktijk. Aangezien het, op basis van de laboratoriumexperimenten uit de huidige studie, niet mogelijk is om het bestrijdingspotentieel van deze roofmijt te verklaren, kan verder onderzoek belangrijk zijn om de rol van de roofmijt bij de bestrijding van plagen volledig te begrijpen. Met behulp van videomonitoring en de elektroforetische analyse van de darminhoud van *I. degenerans* kan het voorkeursgedrag verder uitgeklaard worden. Het is ook noodzakelijk om na te gaan hoe het gedrag dat *I. degenerans* vertoont in het laboratorium zich manifesteert in meer complexe natuurlijke omstandigheden, door het uitvoeren van "semi-field" of veldexperimenten.

Onlangs werd een nieuwe roofmijt geïntroduceerd op de markt in Europa, namelijk *Amblyseius* (= *Typhlodromips*) *swirskii* (Athias-Henriot). Deze roofmijt zou *A. cucumeris*, die nu standaard wordt gebruikt in de biologische bestrijding van trips, moeten vervangen (Bolckmans *et al.*, 2005). Volgens Nomikou *et al.* (2001, 2002) kan deze roofmijt ook als biologische bestrijder van de tabakswittevlieg *Bemisia tabaci* Gennadius worden gebruikt. Net zoals *I. degenerans* gaat deze roofmijt niet in diapauze onder invloed van korte dag omstandigheden en kan ze bijgevolg het hele jaar door uitgezet worden. Maar opnieuw is er zeer weinig fundamentele informatie over de biologie en de predatiecapaciteit van deze mijt beschikbaar. Het is bijgevolg lonend om de biologische kenmerken en de predatiecapaciteit van *A. swirskii* diepgaand te bestuderen om een volledig beeld te krijgen van het potentieel van deze roofmijt. Deze eigenschappen kunnen met die van *I. degenerans* vergeleken worden om te bepalen of

A. swirskii ook een waardevol alternatief voor *I. degenerans* bij de biologische bestrijding kan zijn.

REFERENCES

Akpokodje, G.O., Odebiyi, J.A., Oschieng, R.S. and Herren, H.R. 1990. Functional responses of *Neoseiulus idaeus* and *Iphiseius degenerans* (Acarina: Phytoseiidae) feeding on the cassava green mite, *Mononychellus tanajoa* (Acarina: Tetranychidae). Mitt. Schweiz. Entomol. Ges. 63: 327-335.

Amano, H. and Chant, D.A. 1978. Mating behaviour and reproductive mechanisms of two species of predacious mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius andersoni* (Chant) (Acarina: Phytoseiidae). Acarologia XX: 196-213.

Amano, H. and Chant, D.A. 1986. Laboratory studies of the feeding habits, reproduction and development of three Phytoseiid species, *Typhlodromus pomi*, *Phytoseius macropilis* and *Amblyseius* finlandicus (Acari: Phytoseiidae), occurring on abandoned apple trees in Ontario, Canadia. Exp. Appl. Acarol. 2: 299-313.

Aponte, O. and McMurtry, J. 1992. Mating behaviour and reproductive mechanisms of *Amblyseius colimensis* Aponte and McMurtry (Acari: Phytoseiidae). Bol. Entomol. Venez. N.S. 7: 1-12.

Aponte, O., Hoddle, M. and Heraty, J. 1997. Biological control of pests on avocado in California. California Avocado Research Symposium, 13-18.

Arijs, Y. and De Clercq P., 2001. Rearing *Orius laevigatus* on cysts of the brine shrimp *Artemia franciscana*. Biol. Control 21: 79-83.

Athias-Henriot, C. 1957. Phytoseiidae et Aceosejidae (Acarina: Gamasina) d'Algérie. I. Genres *Blattisocius* Keegan, *Iphiseius* Berlese, *Amblyseius* Berlese, *Phytoseius* Ribaga, *Phytoseiulus* Evans. Bull. Soc. Hist. Nat. Afr. Nord 48: 319-352.

ATTRA - National Sustainable Agriculture Information Service. 2005. Greenhouse IPM: Sustainable Aphid Control. http://www.attra.org/attra-pub/gh-aphid.html#app1

Badii, M.H. and McMurtry, J.A. 1983. Effect of different foods on development, reproduction and survival of *Phytoseiulus longipes* (Acarina: Phytoseiidae). Entomophaga 28: 161-166.

Badii, M.H., McMurtry, J.A. and Flores, A.E. 1999. Rates of development, survival and predation of immature stages of *Phytoseiulus* longipes (Acari: Mesostigmata: Phytoseiidae). Exp. Appl. Acarol. 23: 611-62.

Bakker, F.M. and. Sabelis, M.W. 1989. How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomol. Exp. Appl. 50: 47-51.

Begon, M., Harper, J.L., Townsend, C.R. 1986. Ecology. Blackwell Scientific Publications, Oxford, UK, 876p.

Benfatto, D. and Vacante, V. 1988. Osservazioni sul controllo chimico dei fitofagi degli agrumi: acarofauna utile e indifferente. Annali dell'Istituto Sperimentale per l'Agrumicoltura XX-XXI: 181-196.

Benfatto, D., Conti, F., Frittitta, C., Perrotta, G., Raciti, E. and Tumminelli, R. 2000. Risultati di prove di lotta contro il nuovo tripide degli agrumi *Pezothrips kellyanus* (Bagnall). ATTI Giorn. Fitopatol. 1: 381-386.

Berlese, A. 1889. Acari, Myriopoda et Scorpiones hucusque in Italia reperta. Fasc. 54: 9. Cited in Evans (1954).

Berlese, A. 1921. Acari, Myriopoda et Pseudoscorpiones… Indice Sinonimico. Redia, 14: 95. Cited in Evans (1954).

Berry, J.S., Holtzer, T.O., Innis, G.S. and Logan, J.A. 1988. Simple order of prey preference technique for modelling the predator functional response. Exp. Appl. Acarol. 5: 207-224.

Biobest. 2006. http://www.biobest.be

Blackwood, J.S., Schausberger, P. and Croft, B.A. 2001. Prey-stage preference in generalist and specialist phytoseiid mites (Acari: Phytoseiidae) when offered *Tetranychus urticae* (Acari: Tetranychidae) eggs and larvae. Environ. Entomol. 30: 1103-1111.

Blaeser, P. and Şengonca, C. 2001. Laboruntersuchungen zur Prädationsleistung von vier *Amblyseius* Raubmilbenarten gegenüber *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) und *Tetranychus urticae* Koch (Acari: Tetranychidae) als Beute. Gesunde Pflanzen 53: 218-223.

Blaeser, P., Sitjar, M.L. and Şengonca, C. 2002. Laboruntersuchungen zur Entwicklung, Lebensdauer und Reproduction von vier *Amblyseius*-Raubmilbenarten bei Ernährung mit *Tetranychus urticae* Koch (Acari: Tetranychidae) und *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Nachrichtenbl. Deut. Pflanzenschutzd. 54: 307-311.

Blommers, L. 1976. Some Phytoseiidae (Acarina: Mesostigmata) from Madagascar, with descriptions of eight new species and notes on their biology. Bijdragen tot de Dierkunde 46: 80-106.

Bolckmans, K., van Houten, Y. and Hoogerbrugghe, H. 2005. Biological control of the tobacco whitefly (*Bemisia tabaci)* and of western flower thrips (*Frankliniella occidentalis)* with the phytoseiid predatory mite *Amblyseius swirskii* Athias-Henriot, 1962. International Symposium of Biological Control of Arthropods, September 12 to 16, 2005*,* Davos, Switzerland.

Bonde, J. 1989. Biological studies including population growth parameters of the predatory mite *Amblyseius barkeri* (Acarina: Phytoseiidae) at 25 °C in the laboratory. Entomophaga 34: 275 – 287.

Broufas, G.D. and Koveos, D.S. 2000. Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae). Environ. Entomol. 29: 743-749.

Brown, A.S.S., Simmonds, M.S.J. and Blaney, W.M. 1999. Influence of species of host plants on the predation of thrips by *Neoseiulus cucumeris*, *Iphiseius degenerans* and *Orius laevigatus*. Entomol. Exp. Appl. 92:283-288.

Brown, A.S.S., Simmonds, M.S.J. and Blaney, W.M. 2003. Influence of a short exposure to teflubenzuron residues on the predation of thrips by *Iphiseius degenerans* (Acari: Phytoseiidae) and *Orius laevigatus* (Hemiptera: Anthocoridae). Pest Management Science 59: 1255-1259.

Carmona, M.M. 1962. Contribuição para o conhecimento dos ãcaros das plantas cultivadas em Portugal. Agronomia Lusitana 24: 5-20.

Castagnoli, C. and Simoni, S. 1990. Biological observations and life table parameters of *Amblyseius cucumeris* (Oud.) (Acarina: Phytoseiidae) reared on different diets. Redia LXXIII: 569-583.

Castagnoli, M. and Simoni, S. 1999. Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). Exp. Appl. Acarol. 23: 217-234.

Castagnoli, M., Del Bene G., Gargani E. and Simoni S. 1990. Possibilita di controllo biologico di *Thrips tabaci* Lind. e *Frankliniella occidentalis* (Pergande) (Thys. Thripidae) con *Amblyseius cucumeris* (Oud.) (Acarina Phytoseiidae). Redia LXXIII: 53-61.

Catling, H.D. 1970. The bionomics of the South African citrus psylla *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). 4. The influence of predators. J. Entomol. Soc. sth. Africa 33: 341-348.

Chant, D.A. 1959. Phytoseiid mites (Acarina: Phytoseiidae). Part I. Bionomics of seven species in southeastern England. Part II. A taxonomic review of the family Phytoseiidae, with descriptions of thirty-eight new species. Can. Entomol. supplement, 12: 1-166.

Chant, D.A. and Fleschner, C.A. 1960. Some observations on the ecology of phytoseiid mites (Acarina: Phytoseiidae) in California. Entomophaga 5: 131-139.

Chant, D.A. and McMurtry, J.A. 2005. A review of the subfamily Amblyseiinae Muma (Acari : Phytoseiidae): Part VI. The tribe Euseiini n. tribe, subtribes Typhlodromalina n. subtribe, Euseiina n. subtribe, and Ricosciina n. subtribe. Int. J. Acarol., 31: 187-224 (Abstract).

Chat-Locussol, I., Rivenez, M.O., Javoy, M. 1998. Synthese de 7 annees d'experimentations réalisees contre *Frankliniella occidentalis* en serre de concombre. 1er colloque transnational sur les luttes biologique, integree et raisonnee , 21, 22 et 23 janvier 1998, Lille, France.

Chittenden, A.R. and Saito Y. 2001. Why are there feeding and nonfeeding larvae in phytoseiid mites (Acari: Phytoseiidae)? J. Ethol. 19: 55-62.

Clements, D.R. and Harmsen R. 1993. Prey preferences of adult and immature *Zetzellia mali* Ewing (Acari: Stigmaeidae) and *Typhlodromus caudiglans* Schuster (Acari: Phytoseiidae). Can. Entomol. 125: 967-969.

Cock, M.J.W. 1978. The assessment of preference. J. Anim. Ecol. 47: 805-816.

Conti, F., Perrotta, G., Colazza, S., Maltese, U. and Azzaro, F. 2004. Efficacia e selettività di prodotti fitosanitari ecocompatibili per il contenimento di *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) su limone. ATTI Giornate Fitopatologiche 1: 59-64.

Conti, F., Tumminelli, R., Amico, C., Fisicaro, R., Frittitta, C., Perotta, G. and Marullo, R. 2001a. Monitoring *Pezothrips kellyanus* on citrus in eastern Sicily. Thrips and tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera, 207-210.

Conti, F., Tumminelli, R., Amico, C., Fisicaro, R., Frittitta, C., Perotta, G., Marullo, R. and Siscaro, G. 2001b. Il nuova tripide degli agrumi *Pezothrips kellyanus*. L' Informatore Agrario, 57: 43-46.

Conti, F., Tumminelli, R., Fisicaro, R., Perotta, G., Marullo, R. and G. Liotta. 2003. An IPM system for new citrus thrips in Italy. IOBC/WPRS Bulletin 26: 203-208.

Cook, S.M., Bartlet, E., Murray, D.A. and Williams, I.H. 2002. The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. Entomol. Exp. Appl. 104: 43-50.

Croft, B.A., Luh, H.-K., and Schausberger, P. Larval size relative to larval feeding, cannibalism of larvae, egg or adult female size and larval-adult setal patterns among 13 phytoseiid mite species. Exp. Appl. Acarol. 23: 599-610.

De Boer, J.G., Snoeren, T.A.L. and Dicke, M. 2005. Predatory mites learn to discriminate between plant volatiles induced by prey and nonprey herbivores. Anim. Behav. 69: 869-879.

de Courcy Williams, M.E., Kravar-Garde, L. 2002. The interplay between biological characteristics and interactions among predatory mites in biocontrol on protected crops. IOBC/WPRS Bulletin 25: 61-64.

de Courcy Williams, M.E., Kravar-Garde, L., Fenlon, J.S. and Sunderland, K.D. 2004a. Phytoseiid mites in protected crops: the effect of humidity and food availability on egg hatch and adult life span of *Iphiseius degenerans*, *Neoseiulus cucumeris*, N. *californicus* and *Phytoseiulus persimilis* (Acari: Phytoseiidae). Exp. Appl. Acarol. 32: 1-13.

de Courcy Williams, M.E., Kravar-Garde, L., Fenlon, J.S. and Sunderland, K.D. 2004b. The relationship between dietary specialism and availability of food and water on cannibalistic interactions among predatory mites in protected crops. Exp. Appl. Acarol. 33: 31- 44.

de Moraes, G.J., McMurtry, J.A. and Yaninck, J.S. 1989. Some phytoseiid mites (Acari: Phytoseiidae) from tropical Africa with description of a new species. Int. J. Acarol. 15: 95-102.

Degheele, D., Gerin, C., Lebrun, Ph., Van de Veire, M. and Van Impe, G. 1997. De Californische trips, *Frankliniella occidentalis* (Pergande). Ecologie en geïntegreerde bestrijding. Ministère des classes moyennes et de l'agriculture. 69 pp.

Dicke, M. 1988. Prey preference of the phytoseiid mite *Typhlodromus pyri*. 1. Response to volatile kairomones. Exp. Appl. Acarol. 4: 1-13.

Dicke, M. and De Jong, M. 1988. Prey preference of the phytoseiid mite *Typhlodromus pyri*. 2. Electrophoretic diet analysis. Exp. Appl. Acarol. 4: 15-25.

Dicke, M. and Vet, L.M.. 1999. Plant-carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In: Herbivores: Between plants and predators. H. Olff, V.K. Brown and R.H. Drent (eds.), p 483 – 520. Blackwell Science, Oxford, UK.

Dicke, M., Sabelis, M.W., De Jong, M. and Alers, M.P.T. 1990. Do phytoseiid mites select the best prey species in terms of reproductive success? Exp. Appl. Acarol. 6: 161-173.

Dickens, J. 1999. Predator-prey interactions: olfactory adaptations of generalist and specialist predators. Agric. For. Entomol. 1: 47-54.

Dong, H. and Chant, D.A. 1986. The olfactory response of three species of predacious phytoseiid mites (Acarina: Gamasina) to a prey tetranychid species. Int. J. Acarol. 12: 51-55.

Duso, C. and Camporese, P. 1991. Developmental times and oviposition rates of predatory mites *Typhlodromus pyri* and *Amblyseius andersoni* (Acari: Phytoseiidae) reared on different foods. Exp. Appl. Acarol. 13: 117-128.

Düzgüneş, Z. 1963. Türkiyede yeni bulunan akarlar. Bitki Koruma Bülteni 3: 237-246

Elbadry, E. 1970. Taxonomic review of the Phytoseiid mites of Egypt. Bull. Soc. Ent. Egypte LIV: 495-510.

Espino, A., Barroso, J. and Carnero, A. 1988. Resultados preliminaries de la lucha integrada sobre pepino en Canarias. Bol. San. Veg. Plagas 14: 55-66.

Evans, G.O, 1954. The genus *Iphiseius* Berl. (Acarina-Laelaptidae). Proc. Zool. Soc. London 124: 517-526.

Eveleigh, E.S. and Chant, D.A. 1981a. Experimental studies on acarine predator-prey interactions: effects of predator age and feeding history on prey consumption and the functional response (Acarina: Phytoseiidae). Can. J. Zool. 59: 1387-1406.

Eveleigh, E.S. and Chant, D.A. 1981b. Experimental studies on acarine predator-prey interactions: the numerical response of immature and adult predators (Acarina: Phytoseiidae). Can. J. Zool. 59: 1407-1418.

Eveleigh, E.S. and Chant, D.A. 1981c. The feeding and searching behaviour of two species of phytoseiid mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius degenerans* (Berlese), at different prey densities (Acarina: Phytoseiidae). Can. J. Zool. 59: 1419-1430.

Eveleigh, E.S. and Chant, D.A. 1982a. Experimental studies on acarine predator-prey interactions: effects of temporal changes in the environment on searching behaviour, predation rates, and fecundity (Acarina: Phytoseiidae). Can. J. Zool. 60: 2992-3000

Eveleigh, E.S. and Chant, D.A. 1982b. Experimental studies on acarine predator-prey interactions: the distribution of search effort and predation rates of a predator population in a patchy environment. (Acarina: Phytoseiidae). Can. J. Zool. 60: 3001-3009.

Eveleigh, E.S. and Chant, D.A. 1982c. Experimental studies on acarine predator-prey interactions: the distribution of search effort and the functional and numerical responses of predators in a patchy environment. (Acarina: Phytoseiidae). Can. J. Zool. 60: 2979-2991.

Eveleigh, E.S. and Chant, D.A. 1982d. Experimental studies on acarine predator-prey interactions: the effects of predator density on immature survival, adult fecundity and emigration rates, and the numerical response to prey density (Acarina: Phytoseiidae). Can. J. Zool. 60: 630-638

Eveleigh, E.S. and Chant, D.A. 1982e. Experimental studies on acarine predator-prey interactions: the effects of predator density on prey consumption, predator searching efficiency, and the functional response to prey density (Acarina: Phytoseiidae). Can. J. Zool. 60: 611-629.

Eveleigh, E.S. and Chant, D.A. 1982f. Experimental studies on acarine predator-prey interactions: the response of predators to prey distribution in an homogeneous area. (Acarina: Phytoseiidae). Can. J. Zool. 60: 639-647.

Eveleigh, E.S. and Chant, D.A. 1982g. The searching behaviour of two species of phytoseiid mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius degenerans* (Berlese), in relation to the density and distribution of prey in an homogeneous area (Acarina: Phytoseiidae). Can. J. Zool. 60: 648-658.

Everson, P. 1979. The functional response of *Phytoseiulus persimilis* (Acarina: Phytoseiidae) to various densities of *Tetranychus urticae* (Acarina: Tetranychidae). Can. Ent. 111: 7-10.

Fan, Y. and Petit, F.L. 1994. Functional response of *Neoseiulus barkeri* Hughes on two-spotted spider mite (Acari : Tetranychidae). Exp. Appl. Acarol. 18: 613-621.

Faraji, F., Janssen, A. and Sabelis, M.W. 2001. Predatory mites avoid ovipositing near counterattacking prey. Exp. Appl. Acarol. 613-623.

Faraji, F., Janssen, A. and Sabelis, M.W. 2002a. Oviposition patterns in a predatory mite reduce the risk of egg predation caused by prey. Ecol. Entomol. 27: 660-664.

Faraji, F., Janssen, A. and Sabelis, M.W. 2002b. The benefits of clustering eggs: the role of egg predation and larval cannibalism in a predatory mite. Oecologia 131: 20-26.

Faraji, F., Janssen, A., van Rijn, P.C.J. and Sabelis, M.W. 2000. Kin recognition by the predatory mite *Iphiseius degenerans*: discrimination among own, conspecific and heterospecific eggs. Ecol. Entomol. 25: 147-155.

Fernando, M.H.J.P. and Hassell, M.P. 1980. Predator-prey responses in an acarine system. Res. Popul. Ecol. 22:301-322.

Flechtmann, C.H.W. and McMurtry, J.A. 1992a. Studies on how phytoseiid mites feed on spider mites and pollen. Exp. Appl. Acarol. 18: 157-162.

Flechtmann, C.H.W. and McMurtry, J.A. 1992b. Studies of cheliceral and deutosternal morphology of some Phytoseiidae (Acari: Mesostigmata) by scanning electron microscopy. Exp. Appl. Acarol. 18: 163-169.

Gardiner, M.M., Eigenbrode, S.D., Cervantes, D.E. and Barbour, J.D. 2005. Response of *Neoseiulus fallacis* Garmen and *Galendromus occidentalis* Nesbitt (Acari: Phytoseiidae) to *Tetranychus urticae* Koch (Acari: Tetranychidae)-damaged hop *Humulus lupulus* (L.) (Urticales: Cannabaceae). Agricultural and Forest Entomology, 7: 245-251.

Gerling, D. 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Prot. 20: 779-799.

Gillespie, D.R. and Ramey, C.A. 1988. Life history and cold storage of *Amblyseius cucumeris* (Acarina: Phytoseiidae). J. Entomol. Soc. Brit. Columbia 85: 71-76.

Gotelli, N.J. 1995. A primer of Ecology. Sinauer Associates, Inc., Sunderland. 206pp.

Gotoh, T., Nozawa, M. and Yamaguchi, K. 2004. Prey consumption and functional response of three acarophagous species to eggs of the two-spotted spider mite in the laboratory. Appl. Entomol. Zool. 39:97-105.

Grafton-Cardwell, E.E., Ouyang, Y. and Striggow, R.A. 1999. Predacious mites for control of citrus thrips, *Scirtothrips citri* (Thysanoptera: Thripidae) in nursery citrus. Biol. Control 14: 29-36.

Grenier, S., Guillaud, J. Delobel, B. and Bonnot, G. 1989. Nutrition et élévage du predateur polyphage *Macrolophus caliginosus* (Heteroptera : Miridae) sur milieux artificiels. Entomophaga 34: 77-86.

Grostal, P. and Dicke, M. 2000. Recognising one's enemies: a functional approach to risk assessment by prey. Behav. Ecol. Sociobiol., 47: 258-264.

Hansell, R.I.C. and Chant, D.A. 1973. Taxonomic methods: relative weight applied to characters by classical taxonomists in the genus *Iphiseius* Berlese (Acarina: Phytoseiidae). Can. Entomol. , 775-785.

Holling, C. S. 1966. The functional response of invertebrate predators to prey density. Memoirs of the Entomological Society of Canada 48: 1-85.

Holling, C.S. 1961. Principles of insect predation. Annu. Rev. Entomol. 6: 163-182.

Hongo, T. and Obayashi, N. 1997. Use of diapause eggs of brine shrimp, *Artemia salina* (Linné), for artificial diet of coccinellid beetle, *Harmonia axyridis* (Pallas). Jap. J. Appl. Entomol. Zool. 41: 101-105.

Hulting, F.L., Orr, D.B. and Obrycki, J.J. 1990. A computer program for calculation and statistical comparison of intrinsic rates of increase and associated life table parameters. Fla. Entomol. 73: 601-612.

Janssen, A., Pallini, A., Venzon, M. and Sabelis, M. 1998. Behaviour and indirect interactions in food webs of plant-inhabiting arthropods. Exp. Appl. Acarol. 22: 497-521.

Janssen, A., Willemse, E. and Van der Hammen, T. 2003. Poor host quality causes omnivore to consume predator eggs. J. Animal Ecol., 72: 478-483.

Janssens, A., Faraji, F., van der Hammen, T., Magalhães, S. and Sabelis, M. 2002. Interspecific infanticide deters predators. Ecology Letters, 5, 490-494

Jeppson, L.R., McMurty, J.A., Mead, D.W., Jesser, M.J. and Johnson, H.G. 1975. Toxicity of citrus pesticides to some predaceous phytoseiid mites. J. Econ. Entomol. 68: 707- 710.

Jeyaprakash, A. and Hoy, M.A. 2002. Mitochondrial 12S rRNA sequences used to design a molecular ladder assay to identify six commercially available phyoseiids (Acari: Phytoseiidae). Biol. Control 25: 136-142.

Juliano, S.A. 2001. Nonlinear curve fitting: predation and functional response curves. In: Scheiner, M.S., Gurevitch, J. (eds). Design and analysis of ecological experiments. New York, Oxford University Press, 178-196.

Karg, W. 1993. Acari (Acarina), Milben Parasitiformes (Anactinochaeta), Cohors Gamasina Leach, Raubmilben. Die Tierwelt Deutschlands, 59. Gustav Fischer Verlag, Jena. 524 pp.

Kazak, C., Yildiz, S. and Sekeroglu, E. 2002. Biological characteristics and life table of *Neoseiulus umbraticus* Chant (Acari, Phytoseiidae) at three constant temperatures. J. Pest. Science 75: 118-121.

Kennett, C.E. and Hamai, J. 1980. Oviposition and development in predaceous mites fed with artificial and natural diets (Acari: Phytoseiidae). Entomol. Exp. Appl. 28: 116-122.

Kirk, W.D.J. 1985. Pollen-feeding and the host specificity and fecundity of flower thrips (Thysanoptera). Ecol. Entomol. 10: 281-289.

Koppert. 2006. http://www.koppert.nl

Kreiter, S., Tixier, M.-S., Ferragut, L.A. and Lebdi, K.G. 2005. Preliminary observations on the diversity of phytoseiid mites in the Magreb and comparisons to the fauna of Gran Canaria. Phytophaga XIV: 477-484.

Lawton, J.H., Beddington, J.R. and Bonser, R. 1974. Switching in invertebrate predators. In: Ecological stability. M.B. Usheran M.H. Williamson (eds), Chapman and Hall, London, UK, p 141-158.

Lester, P.J. and Harmsen, R. 2002. Functional and numerical responses do not always indicate the most effective predator for biological control: an analysis of two predators in a two-prey system. J. Appl. Ecol. 39: 455-468.

Lo Pinto, M., Wajnberg, E., Colazza, S., Curty, C. and Fauvergue, X. 2004. Olfactory response of two parasitoids, *Lisiphlebus testaceipes* and *Aphidius colemani*, to aphid-infested plants from a distance. Entomol. Exp. Appl. 110: 159 – 164.

Ludwig, S.W. and Oetting, R.D. 2001. Susceptibility of natural enemies to infection by *Beauveria bassiana* and impact of insecticides on *Ipheseius degenerans* (Acari: Phytoseiidae). J. Agric. Urban Entomol. 18: 169-178.

Maeda, T. and Takabayashi, J. 2001. Production of herbivore-induced plant volatiles and their attractiveness to *Phytoseiulus* persimilis (Acari: Phytoseiidae) with changes of *Tetranychus urticae* (Acari: Tetranychidae) density on a plant. Appl. Entomol. Zool. 36: 47-52.

Manly, B.F.J. 1973. A linear model for frequency-dependent selection by predators. Res. Popul. Ecol. 14: 137-150.

Manly, B.F.J. 1974. A model for certain types of selection experiments. Biometrics 30: 281-294.

Manly, B.F.J., Miller, P., Cook, L.M. 1972. Analysis of a selective predation experiment. Am. Nat. 106: 719-736.

McGregor, R.R. and Gillespie, D.R. 2004. Olfactory response of the omnivorous generalist predator *Dicyphus hesperus* to plant and prey odours. Entomol. Exp. Appl. 112: 201-205.

McMurtry, J.A., 1977. Some predaceous mites (Phytoseiidae) on citrus in the Mediterranean region. Entomophaga 22: 19-30.

McMurtry, J.A. and Bounfour, M. 1989. Phytoseiid mites of Morocco, with descriptions of two new species and notes on the genera *Kuzinellus*, *Typhloctonus* and *Typhlodromus* (Acari: Phytoseiidae). Acarologia, XXX, 13-24.

McMurtry, J.A. and Croft, B.A. 1997. Life-styles of phytoseiid mites and their roles in biological control. Annu. Rev. Entomol. 42: 291-312.

McMurtry, J.A. and Scriven, G.T. 1965. Insectary production of phytoseiid mites. J. Econ. Entomol. 58: 282-283.

McMurtry, J.A. and Scriven, G.T. 1966. The influence of pollen and prey density on the number of prey consumed by *Amblyseius hibisci* (Acarina: Phytoseiidae). Ann. Entomol. Soc. Am. 59: 147-149.

McMurtry, J.A., Badii, M.H. and Johnson, H.G. 1984. The broad mite, Polyphagotarsonemus latus, as a potential prey for phytoseiid mites in California. Entomophaga 29: 83-86.

McMurtry, J.A., Johnson, H.G. and Badii, M.H. 1984. Experiments to determine effects of predator releases on populations of *Oligonychus punica* (Acarina: Tetranychidae) on avocado in California. Entomophaga 29: 11-19.

Meyer, J.S., Ingersoll, C.G., McDonald, L.L. and Boyce, M.S. 1986. Estimating uncertainty in population growth rates: Jackknife vs. bootstrap techniques. Ecology 67: 1156–1166.

Meyerdirk, D.E. and Coudriet, D.L. 1985. Predation and developmental studies of *Euseius hibisci* (Chant) (Acarina: Phytoseiidae) feeding on *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Environ. Entomol. 14: 24-27.

Meyerdirk, D.E. and Coudriet, D.L. 1986. Evaluation of two biotypes of *Euseius scutalis* (Acari: Phytoseiidae) as predators of *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 79: 659-663.

Morrison, R.K., 1985. Trichogramma spp. In: P. Singh and R. F. Moore (eds), Handbook of insect rearing. Elsevier, Amsterdam. pp. 413-417.

Munthali, D.C. 1989. The predatory activity of *Iphiseius degenerans* (Berlese) against *Mononychellus progresivus* (Doreste) on Cassava leaves. Discovery and Innovation, 1, 82-84.

Murdoch, W.W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecol. Monogr. 39: 335-354.

Murdoch, W.W. 1973. The functional response of predators. J. Appl. Ecol. 10: 335-342.

Nicoli, G ., Galazzi, D. Mosti, M. and Burgio, G. 1991. Embryonic and larval development of *Chrysoperla carnea* (Steph.) (Neur.: Chrysopidae) at different temperature regimes. IOBC/WPRS Bull. XIV: 43-49.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M.W. 2004. Vulnerability of *Bemisia tabaci* immatures to phytoseiid predators: consequences for oviposition and influence of alternative food. Entomol. Exp. Appl. 110: 95-102.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M.W. 2001. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Exp. Appl. Acarol. 27: 57 - 68.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M.W. 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Exp. Appl. Acarol. 25: 271-291.

Northcraft, P.D. 1987a. First record of three indigenous predacious mites in Zimbabwe. J. Ent. Soc. sth. Afr. 50: 521-523.

Northcraft, P.D. 1987b. Revised survey of Acari in Zimbabwe. Zimbabwe J. Agric. Res. 25: 143-151.

Nunnink, E. 1994. Nieuwe roofmijt loopt op wonderolie. Groenten en Fruit 16: 8-9

Nwilene, F.E. and Nachman, G. 1996a. Functional responses of *Iphiseius degenerans* and *Neoseiulus teke* (Acari: Phytoseiidae) to changes in the density of the cassava green mite, *Mononychellus tanajoa* (Acari: Tetranychidae). Exp. Appl. Acarol. 20: 259-271.

Nwilene, F.E. and Nachman, G. 1996b. Reproductive responses of *Iphiseius degenerans* and *Neoseiulus teke* (Acari: Phytoseiidae) to changes in the density of the cassava green mite, *Mononychellus tanajoa* (Acari: Tetranychidae). Exp. App. Acarol. 20: 273–282.

Opit, G.P. and Peterson, B. 1997. The life cycle and management of *Echinothrips americanus* (Thysanoptera: Thripidae). J. Entomol. Soc. Brit. Columbia 94: 3-6.

Palevsky, E., Argov, Y., Ben David, T. and Gerson, U. 2003. Identification and evaluation of potential predators of the citrus rust mite, *Phyllocoptruta oleivora*, in Israel. Syst. Appl. Acarol. 8: 39-48.

Pande, Y., Carnero, A. and Hernandez, M. 1989. Notes on biological observations on some unrecorded species of phytophagous and predatory mites in Canary Islands. Invest. Agr.: Prod. Prot. Veg. 4: 275-281.

Papaioannou-Souliotis, P., Tsagkarakou, A. and N. Nomikou. 1997. Field observations on some eco-ethological aspects of phytoseiid mites in Greek citrus groves. Acarologia XXXVIII: 29-37.

Porath, A. and Swirski, E. 1965. A survey of Phytoseiid mites (Acarina: Phytoseiidae) on citrus, with a description of one new species. Israel J. Agric. Res. 15: 87-100.

Porres, M.A., McMurtry, J.A. and March, R.B. 1975. Investigations of leaf sap feeding by three species of Phytoseiid mites by labelling with radioactive Phosphoric acid ($H_3{}^{32}PO_4$). Ann. Entomol. Soc. Am. 68: 871-872.

Pritchard, A.E. and Baker, E.W. 1962. Mites of the family Phytoseiidae from Central Africa, with remarks on the genera of the world. Hilgardia 33: 205-309.

Ragusa Di Chiara, S. 1986. A five year study on population fluctuations of phytoseiid mites in a citrus orchard in Sicily. Acarologia XXVII: 193-201.

Ragusa Di Chiara, S. and Tsolakis, H. 1995. The effect of *Phenacoccus madeirensis* Green (Coccoidea: Pseudococcidae) on some biological parameters of four species of phytoseiid mites (Parasitiformes: Phytoseiidae). Israel J. Entomol. XXIX: 301-307.

Ramakers, P.M.J. 1995. Biological control using oligophagous predators. Thrips Biology and Management, B.L. Parker et al. (eds), Plenum Press, New York, 225-229.

Ramakers, P.M.J. and Voet, S.J.P. 1993. Stuifmeel misschien net dat steuntje in de rug. Groenten en Fruit 40: 21.

Ramakers, P.M.J. and Voet, S.J.P. 1995. Use of castor bean, *Ricinus communis*, for the introduction of the thrips predator *Amblyseius degenerans* on glasshouse-grown sweet peppers. Med. Fac. Landbouww. Univ. Gent. 60: 885-891.

Ramakers, P.M.J. and Voet, S.J.P. 1996. Introduction of *Amblyseius degenerans* for thrips control in sweet peppers with potted castor beans as banker plants. IOBC/WPRS Bulletin 19: 127-130.

Rat-Morris, E. 1999. Biological control of *Thrips tabaci* on protected leek seed crops. IOBC/WPRS Bulletin 12: 201-204.

Rogers, D. 1972. Random search and insect population models. J. Anim. Ecol. 41: 369-383.

Rowell, H.J. and Chant, D.A. 1979. Observations on the ontogeny of setae in the family Phytoseiidae (Acarina: Gamasina). Can. J. Zool. 57: 670-682.

Rubin, A. 1969. Observations on the biology of the Israeli predacious mite *Iphiseius degenerans* Berlese in the laboratory, comparing feeding habits in the citrus orchard and on castor bean. Proceedings of the Pacific branch entomology society of America, 53: 29-30 (Abstract).

Rubin, A., Ucko, O., Orr, N. and Offenbach, R. 1996. Efficacy of natural enemies of the western flower thrips *Frankliniella occidentalis* in pepper flowers in the Arava Valley, Israel. IOBC/WPRS Bulletin 19: 139-142.

Sabelis, M. W. and Van de Baan, H. E. 1983. Location of distant spider mite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. Entomol. Exp. Appl. 33: 303-314.

Sabelis, M.W. (1985). Capacity for population increase. In: W. Helle and M.W. Sabelis (Editors), Spider mites. Their biology, natural enemies and control, Vol. B. Elsevier, Amsterdam, 35-43.

Sabelis, M.W. (1985). Predation on spider mites. In: W. Helle and M.W. Sabelis (Editors), Spider mites. Their biology, natural enemies and control, Vol. B. Elsevier, Amsterdam, 103-129.

Sabelis, M.W. 1985. Development. In: Spider Mites. Their Biology, Natural enemies and control. W. S. Helle, M.W. Amsterdam, Elsevier. Volume 1B: 458p.

Sabelis, M.W. 1985. Sex allocation. In: W. Helle and M.W. Sabelis (eds), Spider Mites. Their biology, natural enemies and control. Volume B. Elsevier, Amsterdam. pp 83-94.

Sabelis, M.W. and Bakker, F.M. 1992. How predatory mites cope with the web of their tetranychid prey: a functional view on dorsal chaetotaxy in the Phytoseiidae. Exp. Appl. Acarol. 16: 203-225.

Sabelis, M.W., Van Baalen, M., Bakker, F.M., Bruin, J, Drukker, B., Egas, M., Janssen, A.R.M., Lesna, I.K., Pels, B., van Rijn, P.C.J. and Scutaranu, P. 1999. The evolution of direct and indirect plant defence against herbiviorous arthropods. In: Herbivores: Between plants and predators. H. Olff, V.K. Brown and R.H. Drent (eds.), p 109 – 166. Blackwell Science, Oxford, UK.

Sanderson, J.P. and. McMurtry, J.A 1984. Life history studies of the predacious mite Phytoseius hawaiiensis. Entomol. Exp. Appl. 35: 227-234.

Schanderl, H., Ferran, A. and Garcia, V. 1988. L'élevage de deux coccinelles *Harmonia axyridis* et *Semiadalia undecimnotata* à l'aide d'oeufs d' *Anagasta kuehniella* tués aux rayons ultraviolets. Entomol. Exp. Appl. 49: 235-244.

Schausberger, P. 1999. Predation preference of *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari: Phytoseiidae) when offered con- and hetereospecific immature life stages. Exp. Appl. Acarol. 23: 389-398.

Schulten, G.G.M. 1985. Mating. In: W. Helle and M.W. Sabelis (eds), Spider Mites. Their biology, natural enemies and control. Volume B. Elsevier, Amsterdam. pp 55-66.

Schulten, G.G.M., Van Arendonk, R.C.M., Russell, V.M. and Roorda, F.A. 1978. Copulation, egg production and sex-ratio in *Phytoseiulus persimilis* and *Amblyseius bibens* Acari: Phytoseiidae). Ent. Exp. Appl., 24: 145-153.

Sengonca, C. and Drescher, K. 2001. Laboratory studies on the suitability of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) as prey for the development, longevity, reproduction and predation of four predatory mite species of the genus *Amblyseius* (Acari: Phytoseiidae). Z. Pflanzenk. Pflanzenschutz 108: 66-76.

Shimoda, T. and Dicke, M. 2000. Attraction of a predator to chemical information related to nonprey: when can it be adaptive. Behav. Ecol. 11: 606-613.

Shipp, J.L. and Whitfield, G.H. 1991. Functional response of the predatory mite, *Amblyseius cucumeris* (Acari: Phytoseiidae) on western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Environ. Entomol. 20: 694-699.

Shipp, J.L., Wang, K. and Ferguson, G. 2000. Residual toxicity of avermectin b1 and pyridaben to eight commercially produced beneficial arthropod species used for control of greenhouse pests. Biol. Control 17: 125-131.

Shipp, J.L., Zhang, Y., Hunt, D.W.A. and Ferguson, G. 2003. Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. Environ. Entomol. 32: 1154-1163.

Skirvin, D.J. and Fenlon, J.S. 2001. Plant species modifies the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae): implications for biological control. Bull. Entomol. Res. 91: 61-67.

Skovgård, H., Tomkiewicz, J. Nachman, G. and Munster-Swendsen, M. 1993. The effect of the cassava green mite *Mononychellus tanajoa* on the growth an yield of cassava *Manihot esculenta* in a seasonally dry area in Kenya. Exp. Appl. Acarol. 17: 41-58.

Skovgård, H., Tomkiewicz, J. Nachman, G. and Münster-Swendsen, M. 1993. The dynamics of the cassava green mite *Mononychellus tanajoa* in a seasonally dry area in Kenya. Exp. Appl. Acarol. 17: 59-76.

Smith, L., Cuellar, M. E., Melo, E.L. 1996. Evaluating prey preference by several phytoseiid predators for *Mononychellus tanajoa* (Bondar) and *M. caribbeanae* McGregor (Acari: Phytoseiidae) in cassava. Biol. Control 7: 179-184.

Solomon, M.E. 1949. The natural control of animal populations. J. Anim. Ecol. 18, 1-35.

SPSS for Windows. Release 12.0, 2001. SPSS Inc., 1989-2003.

Stark, J.D., Tanigoshi, L., Bounfour, M. and Antonelli, A. 1997. Reproductive potential: its influence on the susceptibility of a species to pesticides. Ecotoxicology and Environmental Safety, 37, 273-279.

Sterk, G., Bolckmans, K., Van de Veire, M., Sels, B. and Stepman, W. 1995. Side-effects of the microbial insecticide PreFeRal (*Paecilomyces fumosoroseus*, Strain Apopka 97) on different species of beneficial arthropods. Med. Fac. Landbouww. Univ. Gent. 60: 719-724.

Swirski, E. and Amitai, S. 1961. Some phytoseiid mites (Acarina: Phytoseiidae) of Israel, with a description of two new species. Israel J. Agric. Res. 11: 193-202.

Swirski, E. and Amitai, S. 1984. Notes on phytoseiid mites (Mesostigmata: Phytoseiidae) from the Mediterranean littoral zone of Israel, with a description of a new species of *Typhloctonus*. Israel J. Entomol. XVIII: 71-85.

Swirski, E. and Amitai, S. 1990. Notes on phytoseiid mites (Mesostigmata: Phytoseiidae) from the Sea of Galilee region of Israel, with a description of a new species of *Amblyseius*. Israel J. Entomol. XXIV: 115-124.

Swirski, E. and Ragusa, S. 1978. Three new species of phytoseiid mites from Kenya (Mesostigmata: Phytoseiidae). Zoological Journal of the Linnean Society, 63, 397-409.

Swirski, E. and Shechter, R. 1961. Some Phytoseiid mites (Acarina: Phytoseiidae) of Hong Kong, with a description of a new genus and seven new species. Israel J. Agric. Res. 97-117.

Swirski, E., Blumberg, D., Wysoki, M. and Izhar, Y. 1987. Biological control of the Japanese bayberry whitefly, *Parabemisia myricae* (Kuwana) (Homoptera: Aleyrodidae), in Israel. Isr. J. Entomol. XXI: 11-18.

Takafuji, A. and Chant, D.A. 1976. Comparative studies of two species of predacious phytoseiid mites (Acarina: Phytoseiidae), with special reference to their responses to the density of their prey. Res. Popul. Ecol. 17: 255-310.

Toyoshima, S. and Amano, H. 1998. Effect of prey density on sex ratio of two predacious mites, *Phytoseiulus persimilis* and *Amblyseius womersleyi* (Acari: Phytoseiidae). Exp. Appl. Acarol. 22: 709-723.

Tsoukanas, V.I., Papadopoulos, C.D., Fantinou, A.A. and Papadoulis, G.T. 2006. Temperature-dependent development and life table of *Iphiseius degenerans* (Acari : Phytoseiidae). Environ. Entomol. 35: 212-218.

Ueckermann, E.A. 1992. Some Phytoseiidae of the Cape Verde Islands (Acari: Mesostigmata). Phytophylactica 24: 145-155.

Vacante, V., Cocuzza, G.E. De Clercq, P. Van de Veire, M. and Tirry, L. 1997. Development and survival of *Orius albidipennis* and *O. laevigatus* (Het.: Anthocoridae) on various diets. Entomophaga 42: 493-498.

Valentin, R. 1997. Amerikaanse trips speelt verstoppertje. Groenten en Fruit 30: 18.

Van den Berg, M.A., Deacon, V.E. Fourie, C.J. and Anderson, S.H. 1987. Predators of the citrus psylla, *Trioza erytreae* (Hemoptera: Triozidae), in the Lowveld and Rustenburg areas of Transvaal. Phytophylactica 19: 285-289.

Van den Meiracker, R.A.F. and Ramaker, P.M.J. 1991. Biological control of the western flower thrips Frankliniella occidentalis, in sweet pepper, with the anthocorid predator Orius insidiosus. Med. Fac. Landbouww. Univ. Gent. 56: 241 – 249.

van der Merwe, G.G. 1968. A taxonomic study of the family Phytoseiidae (Acari) in South Africa with contributions to the biology of two species. Entomol. Mem. 18, pp 198.

van der Merwe, G.G. and Ryke, P.A.J. 1963. Key characteristics of the predacious family Phytoseiidae with special reference to the genus *Amblyseius* Berlese (Acarina). J. Entomol. Soc. S. Africa, 26, 82-93.

Van Driesche R.G. and Bellows, T.S. 1996. Biological Control. Chapman and Hall, New York. 539 pp.

van Houten, Y.M. and van der Staay, M. 1993. Beter af met *Amblyseius degenerans*. Groenten + Fruit, 45, 14-15.

van Houten, Y.M. and van Stratum, P. 1993. Biological control of western flower thrips in greenhouse sweet peppers using non-diapausing predatory mites. IOBC/WPRS Bull. 16: 77-80.

van Houten, Y.M. and van Stratum, P. 1995. Control of Western flower thrips on sweet pepper in winter with *Amblyseius cucumeris* (Oudemans) and *A. degenerans* Berlese. In: Thrips Biology and Management. B.L. Parker et. (eds), p 245-248, New York

van Houten, Y.M., van Rijn, P.C.J., Tanigoshi, L.K., van Stratum, P. and Bruin, J. 1993. Potential of phytoseiid predators to control Western flower thrips in greenhouse crops, in particular during the winter period. IOBC/WPRS Bull. 16: 98-101.

van Houten, Y.M., van Rijn, P.C.J., Tanigoshi, L.K., van Stratum, P. and Bruin, J. 1995a. Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. Entomol. Exp. Appl. 74: 225-234.

Van Lenteren, J.C. and Bakker, K. 1976. Functional responses in invertebrates. Neth. J. Zool. 26:567-572.

van Lenteren, J.C. and Loomans, A.J.M. 1998. Is there a natural enemy good enough for biological control of thrips? The 1998 Brighton conference – Pests and Diseases, 5B-3, 401-408.

van Lenteren, J.C. and Woets, J. 1988. Biological and integrated pest control in greenhouses. Ann. Rev. Entomol. 33: 239-269.

van Rijn, P.C.J. and Sabelis, M.W. 1990. Pollen availability and its effect on the maintenance of populations of *Amblyseius cucumeris*, a predator of thrips. Med. Fac. Landbouww. Univ. Gent 55: 335-341.

van Rijn, P.C.J. and Tanigoshi, L.K. 1999a. Pollen as food for the predatory mites Iphiseius degenerans and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Exp. Appl. Acarol. 23: 785-802.

van Rijn, P.C.J. and Tanigoshi, L.K. 1999b. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. Exp. Appl. Acarol. 23: 281-296.

van Rijn, P.C.J., van Houten, Y.M. and Sabelis, M.W. 2002. How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology 83: 2664-2679.

van Rijn, P.C.J., van Houten, Y.M. and Sabelis, M.W. 1999. Pollen improves thrips control with predatory mites. IOBC/WPRS Bull. 22: 209-212.

van Schelt, J. 1999. Biological control of sweet pepper pests in The Netherlands. IOBC/WPRS Bull. 22: 217-220.

van Schelt, J. 2000. Improved methods of testing and release of *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) for aphid control in glasshouses. Eur. J. Entomol. 97, 511-515.

Van Stappen, G., 1996. Use of cysts. In: P. Lavens and P. Sorgeloos (eds.), Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper no.361, Rome. pp. 107-136.

Vet, L.E.M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37:141-172.

Walzer, A. and Schausberger, P. 1999. Predation preferences and discrimination between con- and hetereospecific prey by the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. BioControl 43: 469-478.

Wei, Q. and Walde, S.J. 1997. The functional response of *Typhlodromus pyri* to its prey, *Panonychus ulmi*: the effect of pollen. Exp. Appl. Acarol. 21: 677-684.

Willemse, E. 2002. Een ei hoort erbij! Ent. Ber. Amst. 62, 27-29.

Wittmann, E.J. and Leather, S.R. 1997. Compatibility of *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) with *Neoseiulus* (*Amblyseius*) *cucumeris* Oudemans (Acari: Phytoseiidae) and *Iphiseius* (*Amblyseius*) degenerans Berlese (Acari: Phytoseiidae) in the biocontrol of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Exp. Appl. Acarol. 21: 523-538.

Wysoki, M. and Bolland, H.R. 1983. Chromosome studies of phytoseiid mites (Acari: Gamasida). Int. J. Acarol. 9: 91-94.

Wysoki, M. and Swirski E. 1971. Studies on overwintering of predacious mites of the genera *Amblyseius* Berlese, *Typhlodromus* Scheuten and *Iphiseius* Berlese (Acarina: Phytoseiidae) in Israel. Entomological essays to commemorate the retirement of Prof. K. Yasumatsu.: 265-292.

Wysoki, M. and Swirski, E. 1968. Karyotypes and sex determination of ten species of phytoseiid mites (Acarina: Mesostigmata). Genetica 39: 220-228.

Yao, D.S. and Chant, D.A. 1989. Population growth and predation interference between two species of predatory mites (Acarina: Phytoseiidae) in interactive systems. Oecologia 80: 443-455.

Yao, D.S. and Chant, D.A. 1990. Changes in body weight of two species of predatory mites (Acarina: Phytoseiidae) as a consequence of feeding in an interactive system. Exp. Appl. Acarol. 8: 195-220.

Yue, B. and Tsai, H.J. 1996. Development, survivorship and reproduction of *Amblyseius largoensis* (Acari: Phytoseiidae) on selected plant pollens and temperatures. Biol. Control 25: 488-494.

Zannou, I.D., Hanna, R., de Moraes, G.J., Kreiter, S., Phiri, G. and Jone, A. 2005. Mites of cassava (*Manihot esculenta* crantz) habitats in southern Africa. Int. J. Acarol. 31: 149-164

Zemek, R. and Prenerova, E. 1997. Powdery mildew (Ascomycotina: Erysiphales) – an alternative food for the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae). Exp. Appl. Acarol. 21: 405-414.

APPENDICES

APPENDIX I

VBA MACRO "JACKKNIFE METHOD TO CALCULATE LIFE TABLE PARAMETERS"

1. INTRODUCTION

In this Appendix a VBA Macro (Visual Basic for Applications version 6.3) is described. The macro was compiled in order to calculate Jackknife estimates of r_m.

The Jackknife is a general nonparametric procedure for obtaining estimated standard errors for statistics which are complex functions of the data (Tukey, 1958). This technique has been used to obtain standard errors for intrinsic rates of increase (Meyer *et al.*, 1986, Hulting *et al.*, 1990)

A brief description of the technique used for the calculations of the intrinsic rate of increase follows.

1. Compute r_m based upon the complete sample (e.g., n = 30 females) by solving the equation $\sum e^{-r_m x} l_x m_x = 1$

2. Compute the corresponding statistics r_{mi} (i = 1, 2, …, n) based upon the sample data with each of the i^{th} element (e.g., i^{th} female) ignored in turn

3. Compute the Jackknife pseudo-values $\tilde{r}_i$ as follows:

$$\tilde{r}_i = (nr_m) - ((n-1)r_{mi})$$

4. Compute the Jackknife estimate of the intrinsic rate of increase $\hat{r}_m$:

$$\hat{r}_m = \frac{1}{n}\sum_{i=1}^{n} \tilde{r}_i$$

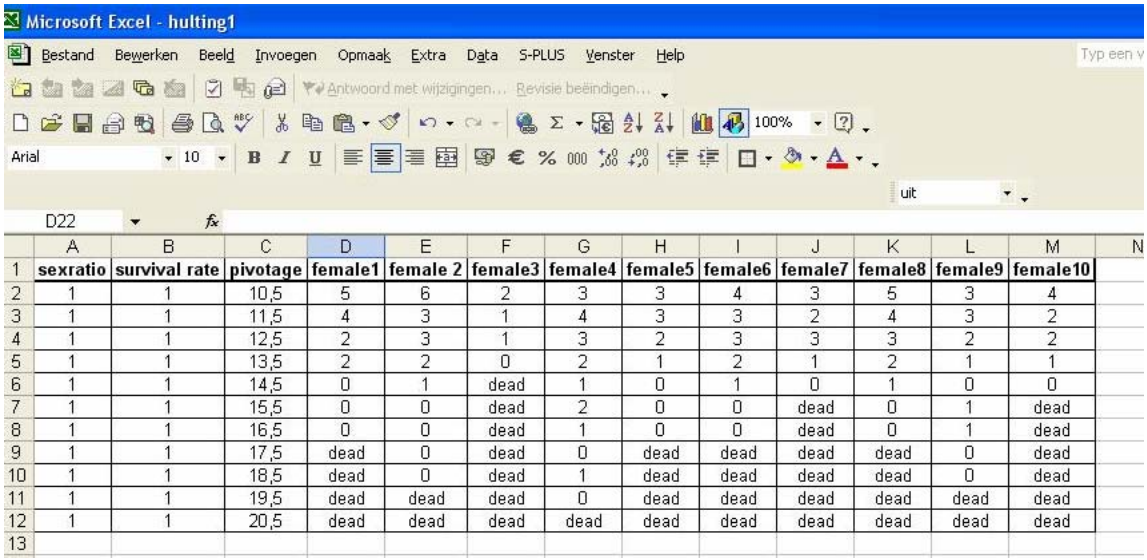
5. The standard error of $\hat{r}_m$ is calculated as follows:

$$\hat{\sigma}_m = \sqrt{\frac{1}{n(n-1)}\sum_{i=1}^{n} (\tilde{r}_i - \hat{r}_m)^2}$$

2. PROGRAM DESCRIPTION

2.1 Input file

Input in the Excel worksheet consists of data from one replicate for a group of females. The first line of the worksheet contains titles: sex ratio, survival rate, pivotal age and a female number. The following lines of input consist each of: the overall sex ratio of the progeny on pivotal age *x*, the overall survival ratio of the progeny on pivotal age *x*, the pivotal age *x* and the number of eggs of each female at age x (Fig. I.1).



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	sexratio	survival rate	pivotage	female1	female 2	female3	female4	female5	female6	female7	female8	female9	female10
2	1	1	10,5	5	6	2	3	3	4	3	5	3	4
3	1	1	11,5	4	3	1	4	3	3	2	4	3	2
4	1	1	12,5	2	3	1	3	2	3	3	3	2	2
5	1	1	13,5	2	2	0	2	1	2	1	2	1	1
6	1	1	14,5	0	1	dead	1	0	1	0	1	0	0
7	1	1	15,5	0	0	dead	2	0	0	dead	0	1	dead
8	1	1	16,5	0	0	dead	1	0	0	dead	0	1	dead
9	1	1	17,5	dead	0	dead	0	dead	dead	dead	dead	0	dead
10	1	1	18,5	dead	0	dead	1	dead	dead	dead	dead	0	dead
11	1	1	19,5	dead	dead	dead	0	dead	dead	dead	dead	dead	dead
12	1	1	20,5	dead	dead	dead	dead	dead	dead	dead	dead	dead	dead
13													

Figure I.1. Input file for the VBA-Macro.

2.2 Program code

```
Sub Jacknife_rm

' This macro has been created by ir. Isabelle Vantornhout and ir. Hilde Minnaert (2003)

‘ STEP A
‘ Create a new dataset containing the number of daughters per females per pivotal age ‘
by multiplying the number of eggs per female by the sex ratio and the survival rate
```

' **Step A1**: Count the number of rows and columns in order to know the number of
' females (*i.e.*, mothers) and the duration of the experiment (*i.e.*, number of observations)

```
Dim numbercolumn As Integer, numberrow As Integer, numberfemales As Integer
Dim numberobservations As Integer
Sheets("blad1").Select
ActiveSheet.Name = "eggfemaleday"
Range("A1").Select
Selection.CurrentRegion.Select
numbercolumn = Selection.Columns.Count
numberrow = Selection.Rows.Count
numberfemales = numbercolumn - 3
numberobservations = numberrow - 1
```

' **Step A2**: Add a column, insert a title (sex*surv) and multiply the sexratio with the
‘ survival rate for each pivotal age

```
Columns("C:C").Select
Selection.Insert shift:=xlToRight
Range("C1").Select
ActiveCell.FormulaR1C1 = "sex*surv"
Range("C2").Select
ActiveCell.FormulaR1C1 = "=RC[-2]*RC[-1]"
Selection.Copy
Columns("C:C").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
```

' **Step A3**: Make a dataset containing the number of daughters per female on a new
‘ sheet

```
Dim j As Integer
Sheets("blad2").Select
For j = 1 To numberfemales
counter = j
Cells(1, j).Select
ActiveCell.FormulaR1C1 = counter
Next j

Dim i As Integer
For i = 2 To numberrow
Cells(i, 1).Select
ActiveCell.FormulaR1C1 = "=eggfemaleday!RC[4]*eggfemaleday!RC3"
Selection.Copy
Rows(i).Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Next i

Range("a1").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets("blad3").Select
ActiveSheet.Name = "daughtersfemaleday"
Range("a1").Select
Selection.PasteSpecial Paste:=xlValues
Selection.Columns.AutoFit

For i = 2 To numberrow
For j = 1 To numberfemales
Cells(i, j).Select
If ActiveCell.Text = "#WAARDE!" Then ActiveCell.FormulaR1C1 =
"=eggfemaleday!RC[4]"
```

```
Next j
Next i

Sheets("blad2").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete

' STEP B:
‘ Create an MxLx table

‘ Step B1: Calculate the survival of the females

Sheets("daughtersfemaleday").Select
Range("A1").Select
Selection.CurrentRegion.Select
numberrow = Selection.Rows.Count
numberfemales = Selection.Columns.Count
numberobservations = numberrow - 1

Sheets.Add
ActiveSheet.Name = "numberfemales"
 Range("a1") = numberfemales

Dim counterescaped As Integer, counterdead As Integer
Sheets("daughtersfemaleday").Select
For i = 2 To numberrow
Rows(i).Select
counterescaped = 0
counterdead = 0
For j = 1 To numberfemales
For Each word In Worksheets("daughtersfemaleday").Cells(i, j)
If word.Value = "escaped" Then counterescaped = counterescaped + 1
```

```
For Each word2 In Worksheets("daughtersfemaleday").Cells(i, j)
If word2.Value = "dead" Then counterdead = counterdead + 1
Next
Next
Next j
Cells(i, numberfemales + 2) = counterescaped
Cells(i, numberfemales + 3) = counterdead
Next i
Cells(1, numberfemales + 1).Select
ActiveCell.FormulaR1C1 = "number"
Cells(1, numberfemales + 2).Select
ActiveCell.FormulaR1C1 = "number escaped"
Cells(1, numberfemales + 3).Select
ActiveCell.FormulaR1C1 = "number dead"
Cells(2, numberfemales + 1).Select
ActiveCell.FormulaR1C1 = "='numberfemales'!R1C1-'daughtersfemaleday'!RC[1]-
'daughtersfemaleday'!RC[2]"
Selection.Copy
Columns(numberfemales + 1).Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Columns(numberfemales + 1).Select
Selection.Copy
Sheets.Add
ActiveSheet.Name = "survivalratio"
Selection.PasteSpecial Paste:=xlValues
Sheets("daughtersfemaleday").Select
Columns(numberfemales + 2).Select
Selection.Copy
Sheets("survivalratio").Select
Range("B1").Select
Selection.PasteSpecial Paste:=xlValues
```

```
Sheets("daughtersfemaleday").Select
Columns(numberfemales + 3).Select
Selection.Copy
Sheets("survivalratio").Select
Range("C1").Select
Selection.PasteSpecial Paste:=xlValues
Range("D1").Select
ActiveCell.FormulaR1C1 = "Survivalratio"
Range("D2").Select
ActiveCell.FormulaR1C1 = "=(RC[-3]-RC[-1])/RC[-3]"
Range("D3").Select
ActiveCell.FormulaR1C1 = "=(R[-1]C[-3]-(RC[-1]-R[-1]C[-1]))/R[-1]C[-3]"
Selection.Copy
Columns("D:D").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

Range("E1").Select
ActiveCell.FormulaR1C1 = "Lx"
Range("E2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]* 1"
Range("E3").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*R[-1]C"
Selection.Copy
Columns("E:E").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

Sheets("daughtersfemaleday").Select
Columns(numberfemales + 1).Select
Selection.Delete shift:=xlLeft
Columns(numberfemales + 1).Select
```

```
Selection.Delete shift:=xlLeft
Columns(numberfemales + 1).Select
Selection.Delete shift:=xlLeft

‘ Step B2: Create a table to be used in the Jackknife technique (removal of one female ‘
on each turn

Sheets.Add
ActiveSheet.Name = "database"
Range("a1").Select

ActiveCell.FormulaR1C1 = "adult age"
For i = 1 To numberrow
counter2 = i
Cells(i + 1, 1).Select
ActiveCell.Value = counter2
Next i

Sheets("eggfemaleday").Select
Columns("D:D").Select
Selection.SpecialCells(xlCellTypeConstants, 3).Select
Selection.Copy
Sheets("database").Select
Range("B1").Select
Selection.PasteSpecial Paste:=xlValues
Sheets("daughtersfemaleday").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets("database").Select
Range("c1").Select
Selection.PasteSpecial Paste:=xlValues
Sheets.Add
```

```
ActiveSheet.Name = "original database"
Sheets("database").Select
Range("a1").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets("original database").Select
Selection.PasteSpecial Paste:=xlValues
Sheets("database").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete

Sheets("original database").Select

Range("a1").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets.Add
ActiveSheet.Name = "copy database"
Range("a1").Select
ActiveSheet.Paste

Sheets("daughtersfemaleday").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete
Sheets.Add
ActiveSheet.Name = "daughtersfemaleday"
Sheets("copy database").Select
Range("A1").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets("daughtersfemaleday").Select
Range("a1").Select
```

```
Selection.PasteSpecial Paste:=xlValues
Columns("a:b").Select
Selection.Delete shift:=xlLeft

' STEP B3 : Create a MxLx table

Sheets("copy database").Select
Columns("b:b").Select
Selection.SpecialCells(xlCellTypeConstants, 23).Select
Selection.Copy
Sheets.Add
ActiveSheet.Name = "xLxMx table"
Range("A1").Select
ActiveSheet.Paste
Range("b1").Select
ActiveCell.FormulaR1C1 = "Mx"
Range("b2").Select
ActiveCell.FormulaR1C1=
"=SUM(daughtersfemaleday!R)/COUNT(daughtersfemaleday!R)"
Range("b2").Select
Selection.Copy
Columns("b:b").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

For j = 2 To numberrow
Cells(j, 2).Select
If ActiveCell.Text = "#DEEL/0!" Then ActiveCell.FormulaR1C1 = "0"
Next j

Sheets("survivalratio").Select
Columns("E:E").Select
```

```
Selection.SpecialCells(xlCellTypeFormulas, 3).Select
Selection.Copy
Sheets("xLxMx table").Select
Range("C2").Select
Selection.PasteSpecial Paste:=xlValues
Range("C1").Select
ActiveCell.FormulaR1C1 = "Lx"

Sheets("xLxMx table").Select
Range("D1").Select
ActiveCell.FormulaR1C1 = "MxLx"
Range("D2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*RC[-2]"
Range("D2").Select
Selection.Copy
Columns("D:D").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("E1").Select
ActiveCell.FormulaR1C1 = "xMxLx"
Range("E2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*RC[-4]"
Range("E2").Select
Selection.Copy
Columns("E:E").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
```

' **STEP C**

' Solve the equation $\sum e^{-r_m x} l_x m_x = 1$ and copy the life table parameters for the complete dataset on a new worksheet ‘ named “final parameters”

```
Sheets.Add
ActiveSheet.Name = "parameters"
Sheets("copy database").Select
Sheets("copy database").Move Before:=Sheets(2)
Sheets("xLxMx table").Select
Sheets("xLxMx table").Move Before:=Sheets(4)

Sheets("parameters").Select
ActiveCell.FormulaR1C1 = "iterationnumber"
Range("B1").Select
ActiveCell.FormulaR1C1 = "som MxLx"
Range("C1").Select
ActiveCell.FormulaR1C1 = "som xMxLx"
Range("D1").Select
ActiveCell.FormulaR1C1 = "Tc"
Range("E1").Select
ActiveCell.FormulaR1C1 = "Ro"
Range("F1").Select
ActiveCell.FormulaR1C1 = "rc"
Range("G1").Select
ActiveCell.FormulaR1C1 = "r"
Range("H1").Select
ActiveCell.FormulaR1C1 = "T"
Range("I1").Select
ActiveCell.FormulaR1C1 = "iterationsum"
Range("B2").Select
ActiveCell.FormulaR1C1 = "=SUM('xLxMx table'!C[2])"
Range("C2").Select
ActiveCell.FormulaR1C1 = "=SUM('xLxMx table'!C[2])"
Range("D2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]/RC[-2]"
Range("E2").Select
```

```
ActiveCell.FormulaR1C1 = "=RC[-3]"
Range("F2").Select
ActiveCell.FormulaR1C1 = "=LN(RC[-1])/RC[-2]"
Range("F2").Select
Selection.Copy
Range("G2").Select
Selection.PasteSpecial Paste:=xlValues
Range("H2").Select
ActiveCell.FormulaR1C1 = "=LN(RC[-3])/RC[-1]"

Sheets("xLxMx table").Select
Range("F1").Select
ActiveCell.FormulaR1C1 = "minrx"
Range("F2").Select
ActiveCell.FormulaR1C1 = "=-parameters!R2C7*'xLxMx table'!RC[-5]"
Range("F2").Select
Selection.Copy
Columns("F:F").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("G1").Select
ActiveCell.FormulaR1C1 = "exp(minrx)"
Range("G2").Select
ActiveCell.FormulaR1C1 = "=EXP(RC[-1])"
Range("G2").Select
Selection.Copy
Columns("G:G").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("H1").Select
ActiveCell.FormulaR1C1 = "som =1"
Range("H2").Select
```

```
ActiveCell.FormulaR1C1 = "=RC[-1]*RC[-4]"
Range("H2").Select
Selection.Copy
Columns("H:H").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Sheets("parameters").Select
Range("I2").Select
ActiveCell.FormulaR1C1 = "=SUM('xLxMx table'!C[-1])"

SolverOk SetCell:="$I$2", MaxMinVal:=3, ValueOf:="1", ByChange:="$G$2"
SolverSolve Userfinish:=True

Sheets.Add
ActiveSheet.Name = "final parameters"
Sheets("parameters").Select
Rows("1:2").Select
Selection.Copy
Sheets("final parameters").Select
Selection.PasteSpecial Paste:=xlValues, Transpose:=True

Sheets("final parameters").Select
Sheets("final parameters").Move After:=Sheets(6)

‘ STEP D:
' In the following loop Jackknife estimates of r m are calculated

‘ Step D1: one female at the time is removed from the dataset

For x = 1 To numberfemales

Sheets("daughtersfemaleday").Select
```

```
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete

Sheets.Add
ActiveSheet.Name = "daughtersfemaleday"

Sheets("original database").Select
Range("a1").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets("copy database").Select
Range("a1").Select
ActiveSheet.Paste
Columns(x + 2).Select
Selection.Delete shift:=xlLeft

Range("a1").Select
Selection.CurrentRegion.Select
Selection.Copy
Sheets("daughtersfemaleday").Select
Range("a1").Select
Selection.PasteSpecial Paste:=xlValues
Columns("a:b").Select
Selection.Delete shift:=xlLeft

‘ Step D2: Calculate the survival of the females

Sheets("daughtersfemaleday").Select
Range("A1").Select
Selection.CurrentRegion.Select
numberrow = Selection.Rows.Count
numberfemales = Selection.Columns.Count
```

```
numberobservations = numberrow - 1
Sheets("numberfemales").Select
Range("a1") = numberfemales

Sheets("daughtersfemaleday").Select
For i = 2 To numberrow
Rows(i).Select
counterescaped = 0
counterdead = 0
For j = 1 To numberfemales
For Each word In Worksheets("daughtersfemaleday").Cells(i, j)
If word.Value = "escaped" Then counterescaped = counterescaped + 1
For Each word2 In Worksheets("daughtersfemaleday").Cells(i, j)
If word2.Value = "dead" Then counterdead = counterdead + 1
Next
Next
Next j
Cells(i, numberfemales + 2) = counterescaped
Cells(i, numberfemales + 3) = counterdead
Next i

Cells(1, numberfemales + 1).Select
ActiveCell.FormulaR1C1 = "number"
Cells(1, numberfemales + 2).Select
ActiveCell.FormulaR1C1 = "number escaped"
Cells(1, numberfemales + 3).Select
ActiveCell.FormulaR1C1 = "number dead"
Cells(2, numberfemales + 1).Select
ActiveCell.FormulaR1C1 = "='numberfemales'!R1C1-'daughtersfemaleday'!RC[1]-
'daughtersfemaleday'!RC[2]"
Selection.Copy
Columns(numberfemales + 1).Select
```

```
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

Columns(numberfemales + 1).Select
Selection.Copy
Sheets("survivalratio").Select
Range("a1").Select
Selection.PasteSpecial Paste:=xlValues
Sheets("daughtersfemaleday").Select
Columns(numberfemales + 2).Select
Selection.Copy
Sheets("survivalratio").Select
Range("B1").Select
Selection.PasteSpecial Paste:=xlValues
Sheets("daughtersfemaleday").Select
Columns(numberfemales + 3).Select
Selection.Copy
Sheets("survivalratio").Select
Range("C1").Select
Selection.PasteSpecial Paste:=xlValues
Columns("D:e").Select
Selection.Delete shift:=xlLeft
Range("D1").Select
ActiveCell.FormulaR1C1 = "Survivalratio"
Range("D2").Select
ActiveCell.FormulaR1C1 = "=(RC[-3]-RC[-1])/RC[-3]"
Range("D3").Select
ActiveCell.FormulaR1C1 = "=(R[-1]C[-3]-(RC[-1]-R[-1]C[-1]))/R[-1]C[-3]"
Selection.Copy
Columns("D:D").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
```

```
Range("E1").Select
ActiveCell.FormulaR1C1 = "Lx"
Range("E2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]* 1"
Range("E3").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*R[-1]C"
Selection.Copy
Columns("E:E").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

Sheets("daughtersfemaleday").Select
Columns(numberfemales + 1).Select
Selection.Delete shift:=xlLeft
Columns(numberfemales + 1).Select
Selection.Delete shift:=xlLeft
Columns(numberfemales + 1).Select
Selection.Delete shift:=xlLeft

‘ Step D3: Create an MxLx table

Sheets("xLxMx table").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete

Sheets("copy database").Select
Columns("b:b").Select
Selection.SpecialCells(xlCellTypeConstants, 23).Select
Selection.Copy
Sheets.Add
ActiveSheet.Name = "xLxMx table"
Range("A1").Select
```

```
ActiveSheet.Paste
Range("b1").Select
ActiveCell.FormulaR1C1 = "Mx"
Range("b2").Select
ActiveCell.FormulaR1C1 =
"=SUM(daughtersfemaleday!R)/COUNT(daughtersfemaleday!R)"
Range("b2").Select
Selection.Copy
Columns("b:b").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

For j = 2 To numberrow
Cells(j, 2).Select
If ActiveCell.Text = "#DEEL/0!" Then ActiveCell.FormulaR1C1 = "0"
Next j

Sheets("survivalratio").Select
Columns("E:E").Select
Selection.SpecialCells(xlCellTypeFormulas, 3).Select
Selection.Copy
Sheets("xLxMx table").Select
Range("C2").Select
Selection.PasteSpecial Paste:=xlValues
Range("C1").Select
ActiveCell.FormulaR1C1 = "Lx"

Sheets("xLxMx table").Select
Range("D1").Select
ActiveCell.FormulaR1C1 = "MxLx"
Range("D2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*RC[-2]"
```

```
Range("D2").Select
Selection.Copy
Columns("D:D").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("E1").Select
ActiveCell.FormulaR1C1 = "xMxLx"
Range("E2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*RC[-4]"
Range("E2").Select
Selection.Copy
Columns("E:E").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste

Sheets("parameters").Select
Range("B2").Select
ActiveCell.FormulaR1C1 = "=SUM('xLxMx table'!C[2])"
Range("C2").Select
ActiveCell.FormulaR1C1 = "=SUM('xLxMx table'!C[2])"
Range("D2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]/RC[-2]"
Range("E2").Select
ActiveCell.FormulaR1C1 = "=RC[-3]"
Range("F2").Select
ActiveCell.FormulaR1C1 = "=LN(RC[-1])/RC[-2]"
Range("F2").Select
Selection.Copy
Range("G2").Select
Selection.PasteSpecial Paste:=xlValues
Range("H2").Select
ActiveCell.FormulaR1C1 = "=LN(RC[-3])/RC[-1]"
```

```
Sheets("xLxMx table").Select
Range("F1").Select
ActiveCell.FormulaR1C1 = "minrx"
Range("F2").Select
ActiveCell.FormulaR1C1 = "=-parameters!R2C7*'xLxMx table'!RC[-5]"
Range("F2").Select
Selection.Copy
Columns("F:F").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("G1").Select
ActiveCell.FormulaR1C1 = "exp(minrx)"
Range("G2").Select
ActiveCell.FormulaR1C1 = "=EXP(RC[-1])"
Range("G2").Select
Selection.Copy
Columns("G:G").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("H1").Select
ActiveCell.FormulaR1C1 = "som =1"
Range("H2").Select
ActiveCell.FormulaR1C1 = "=RC[-1]*RC[-4]"
Range("H2").Select
Selection.Copy
Columns("H:H").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Sheets("parameters").Select
Range("I2").Select
ActiveCell.FormulaR1C1 = "=SUM('xLxMx table'!C[-1])"
```

' **STEP D4**

'Solve $\sum e^{-r_m x} l_x m_x = 1$ and copy the Jackknife pseudovalues of the life table ‘parameters for the complete dataset on the worksheet named “final parameters”

```
SolverOk SetCell:="$I$2", MaxMinVal:=3, ValueOf:="1", ByChange:="$G$2"
SolverSolve Userfinish:=True

Sheets("parameters").Select
Rows("2:2").Select
Selection.Copy
Sheets("final parameters").Select
Columns(x + 2).Select
Selection.PasteSpecial Paste:=xlValues, Transpose:=True
ActiveCell.FormulaR1C1 = "=column(RC[-2])"

Next x
```

‘STEP E:

‘Calculate the Jackknife estimate of the intrinsic rate of increase and its standard error

```
Sheets("parameters").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete
Sheets("copy database").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete
Sheets("numberfemales").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete
Sheets("daughtersfemaleday").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete
```

```
Sheets("xLxMx table").Select
Application.DisplayAlerts = False
ActiveWindow.SelectedSheets.Delete
Sheets("final parameters").Select
Range("b1").Select
ActiveCell.FormulaR1C1 = "alle"
Selection.SpecialCells(xlCellTypeConstants, 1).Select
Selection.NumberFormat = "0.000"
Range("a10").Select
ActiveCell.FormulaR1C1 = "numberfemales"

Sheets("eggfemaleday").Select
Range("A1").Select
Selection.CurrentRegion.Select
numbercolumn = Selection.Columns.Count
numberfemales = numbercolumn - 4
Sheets("final parameters").Select
Range("b10") = numberfemales

Range("a11").Select
ActiveCell.FormulaR1C1 = "jacknife pseudovalues"
Range("C11").Select
ActiveCell.FormulaR1C1 = "=R10C2*R7C2-(R10C2-1)*R[-4]C"
Range("C11").Select
Selection.Copy
Rows("11:11").Select
Selection.SpecialCells(xlCellTypeBlanks).Select
ActiveSheet.Paste
Range("B11").Select
Selection.ClearContents

Range("A12").Select
```

```
ActiveCell.FormulaR1C1 = "Jacknife rm"
Range("C12").Select
ActiveCell.FormulaR1C1 = "=AVERAGE(R[-1])"
Range("A13").Select
  ActiveCell.FormulaR1C1 = "standard deviation"
  Range("C13").Select
  ActiveCell.FormulaR1C1 = "=STDEV(R[-2])"
  Range("A14").Select
  ActiveCell.FormulaR1C1 = "standard error"
  Range("C14").Select
  ActiveCell.FormulaR1C1 = "=R[-1]C/SQRT(R[-4]C[-1])"
  Range("A15").Select

  Sheets("final parameters").Select
  Range("A1").Select
  Selection.CurrentRegion.Select
  Selection.Columns.AutoFit

End Sub
```

APPENDIX II
ANALYSIS OF FUNCTIONAL RESPONSE EXPERIMENTS: SAS PROGRAM CODE

1. INTRODUCTION

In this Appendix SAS code (SAS LE) for data input and regression analysis of functional response data is described. The SAS code presented here is an extension of the code available on http://www.oup-usa.com/sc/0195131878/chapter10.html.

2. PROGRAM CODE

2.1 Analysis of one functional response experiment with prey depletion

2.1.1 Data input lines

```
DATA FUNCRESP;
INPUT N0 REP FATE NE; /* N0 = initial number of prey, REP =replicate number,
FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each FATE */
N02=N0**2; /* initial number of prey squared */
N03=N0**3; /*initial number of prey cubed */
cards; /*below this line data is inserted NO REP FATE NE */
```

2.1.2 Procedure LOGISTIC for logistic regression

```
/* cubic model, if the parameters of this model are non-significant, next step is to
eliminate the cubic term N03*/
proc logistic data=FUNCRESP;
MODEL FATE = N0 N02 N03;
```

```
WEIGHT NE;
/* quadratic model, if the parameters of this model are non-significant, next step is to eliminate the cubic term N02*/
proc logistic data=FUNCRESP;
MODEL FATE = N0 N02;
WEIGHT NE;

/* simple logistic regression, lowest order model that can be fitted*/
proc logistic data=FUNCRESP;
MODEL FATE = N0;
WEIGHT NE;

DATA FUNCRES2; /* obtaining means and SE's for observed proportions eaten */
SET FUNCRESP;
PROC MEANS DATA=FUNCRES2;
BY N0 NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=FUNCMEAN MEAN=FUNCPROP;
DATA FUNCRES3; /* generating predicted proportions eaten */
SET FUNCMEAN;
K=EXP(-0.6831+(-0.0570*N0)+(0*N0**2)+(0*N0**3)); /* insert parameter estimates of logistic regression in this expression */
PRED=K/(1+K);

PROC PLOT DATA=FUNCRES3; /* plotting observed means and predicted values */
PLOT PRED*N0='o';
```

2.1.3 Procedure NLIN for non-linear regression

The choice for one of the program codes given below depends on the type of functional response (Type II or Type III) determined by the logistic regression.

A. TYPE II functional response

```
proc nlin data=FUNCRES2
method=dud;
PARMS A = 0.0001 0.001 0.01 0.1 /* initial parameter estimates */
THHAT=8.0;
BOUNDS A>0, THHAT>0; /* parameter bounds */
T=24; /* experimental period in H */
X=NE; /* initial predicted value */
/* define the implicit function */
C1=EXP(-A*T); /* components of the implicit function */
C2=A*THHAT;
H=N0*C1*EXP(C2*X)+X-N0; /* the implicit function */
ITER=0; /* iterations for Newton's method */
/* Newton's method employed to find predicted number eaten */
DO WHILE(ABS(H)>0.0001 AND ITER<50); /* stop criteria for Newton's method */
X=X-H/(N0*C1*C2*EXP(C2*X)+1); /* new predicted value */
H=N0*C1*EXP(C2*X)+X-N0; /* new value of implicit function */
ITER=ITER+1; /* iteration counter */
END;
MODEL NE=X; /* model for nonlinear least squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */
```

B. TYPE III functional response

```
/* full model */
proc nlin data=FUNCRES2
method=dud;
PARMS BHAT= 0.001 0.01 0.1 /* initial parameter estimates */
CHAT= 0.001 0.01 0.1
DHAT= 0 THHAT=3.0;
BOUNDS BHAT>0,CHAT>=0,
THHAT>0; /* parameter bounds */
```

```
T=24; /* experimental period in H */
X=NE; /* initial predicted value */
A=(DHAT+BHAT*N0)/(1+CHAT*N0); /* expression for A */
/* define the implicit function */
C1=EXP(-A*T); /* components of the implicit function */
C2=A*THHAT;
H=N0*C1*EXP(C2*X)+X-N0; /* the implicit function */
ITER=0; /* iterations for Newton's method */
/* Newton's method employed to find predicted number eaten */
DO WHILE(ABS(H)>0.0001 AND ITER<50); /* stop criteria for Newton's method */
X=X-H/(N0*C1*C2*EXP(C2*X)+1); /* new predicted value */
H=N0*C1*EXP(C2*X)+X-N0; /* new value of implicit function */
ITER=ITER+1; /* iteration counter */
END;
MODEL NE=X; /* model for nonlinear least squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */

/* reduced model: chat omitted */
PROC NLIN DATA=FUNCRES2
method =dud;
PARMS BHAT=0.001 0.01 0.1 /* initial parameter estimates */
DHAT=0
THHAT=3.0;
BOUNDS BHAT>0,THHAT>0; /* parameter bounds */
T=24; /* experimental period in h */
X=NE; /* initial predicted value */
A=(DHAT+BHAT*N0); /* expression for A */
/* define the implicit function */
C1=EXP(-A*T); /* components of the implicit function */
C2=A*THHAT;
H=N0*C1*EXP(C2*X)+X-N0; /* the implicit function */
ITER=0; /* iterations for Newton's method */
```

```
/* Newton's method employed to find predicted number eaten */
DO WHILE(ABS(H)>0.0001 AND ITER<50); /* stop criteria for Newton's method */
X=X-H/(N0*C1*C2*EXP(C2*X)+1); /* new predicted value */
H=N0*C1*EXP(C2*X)+X-N0; /* new value of implicit function */
ITER=ITER+1; /* iteration counter */
END;
MODEL NE=X; /* model for nonlinear least squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */

/* reduced model: chat and dhat omitted */
PROC NLIN DATA=FUNCRES2
method = dud;
PARMS BHAT= 0.001 0.01 0.1 /* initial parameter estimates */
THHAT= 3.0;
BOUNDS BHAT>0,THHAT>0; /* parameter bounds */
T=24; /* experimental period in H */
X=NE; /* initial predicted value */
A=BHAT*N0; /* expression for A */
/* define the implicit function */
C1=EXP(-A*T); /* components of the implicit function */
C2=A*THHAT;
H=N0*C1*EXP(C2*X)+X-N0; /* the implicit function */
ITER=0; /* iterations for Newton's method */
/* Newton's method employed to find predicted number eaten */
DO WHILE(ABS(H)>0.0001 AND ITER<50); /* stop criteria for Newton's method */
X=X-H/(N0*C1*C2*EXP(C2*X)+1); /* new predicted value */
H= N0*C1*EXP(C2*X)+X-N0; /* new value of implicit function */
ITER=ITER+1; /* iteration counter */
END;
MODEL NE=X; /* model for nonlinear least squares */
OUTPUT OUT=PLOTFUNC P=PRED R=RES; /* output data set for plotting */
```

2.2 Program code for experiments without prey depletion

2.2.1 Data input lines

```
DATA FUNCRESP;
INPUT N0 REP FATE NE; /* N0 = initial number of prey, REP =replicate number,
FATE: 0 = prey eaten 1 = prey alive, NE = count of prey in each FATE */
N02=N0**2; /* initial number of prey squared */
N03=N0**3; /*initial number of prey cubed */
cards; /*below this line data are inserted NO REP FATE NE */
```

2.2.2 Procedure LOGISTIC for logistic regression

```
/* cubic model, if the parameters of this model are non-significant, next step is to
eliminate the cubic term N03*/
proc logistic data=FUNCRESP;
MODEL FATE = N0 N02 N03;
WEIGHT NE;

/* quadratic model, if the parameters of this model are non-significant, next step is to
eliminate the cubic term N02*/
proc logistic data=FUNCRESP;
MODEL FATE = N0 N02;
WEIGHT NE;

/* simple logistic regression, lowest order model that can be fitted*/
proc logistic data=FUNCRESP;
MODEL FATE = N0;
WEIGHT NE;

DATA FUNCRES2; /* obtaining means and SE's for observed proportions eaten */
```

```
SET FUNCRESP;
PROC MEANS DATA=FUNCRES2;
BY N0 NOTSORTED;
VAR PROPEAT;
OUTPUT OUT=FUNCMEAN MEAN=FUNCPROP;
DATA FUNCRES3; /* generating predicted proportions eaten */
SET FUNCMEAN;
K=EXP(-0.6831+(-0.0570*N0)+(0*N0**2)+(0*N0**3)); /* insert parameter estimates of logistic regression in this expression */
PRED=K/(1+K);

PROC PLOT DATA=FUNCRES3; /* plotting observed means and predicted values */
PLOT PRED*N0='o';
```

2.2.3 Procedure NLIN for non-linear regression

The choice for one of the program codes given below depends on the type of functional response (Type II or Type III) determined by the logistic regression.

A. TYPE II functional response

```
proc nlin data=FUNCRES2
method=dud;
PARMS A = 0.001 0.01 0.1 /* initial parameter estimates */
THHAT=3.0;
BOUNDS A>0, THHAT>0; /* parameter bounds */
MODEL NE=A*N0*24/(1+A*N0*THHAT); /* model for nonlinear least squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */
```

B. TYPE III functional response

```
/* full model */
```

```
proc nlin data=FUNCRES2
method=dud;
PARMS BHAT= 0.001 0.01 0.1 /* initial parameter estimates */
CHAT= 0.001 0.01 0.1
DHAT= 0 THHAT=3.0;
BOUNDS BHAT>0,CHAT>=0,
THHAT>0; /* parameter bounds */
T=24; /* experimental period in H */
MODEL NE=(DHAT*N0*T + BHAT*N02*T)/(1 +
CHAT*N0+DHAT*N0*THHAT+BHAT*N02*THHAT); /* model for nonlinear least
squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */

/* reduced model: chat omitted */
proc nlin data=FUNCRES2
method=dud;
PARMS BHAT= 0.001 0.01 0.1 /* initial parameter estimates */
DHAT= 0 THHAT=3.0;
BOUNDS BHAT>0,CHAT>=0,
THHAT>0; /* parameter bounds */
T=24; /* experimental period in H */
MODEL NE=(DHAT*N0*T + BHAT*N02*T)/(1 +
DHAT*N0*THHAT+BHAT*N02*THHAT); /* model for nonlinear least squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */

/* reduced model: chat omitted */
proc nlin data=FUNCRES2
method=dud;
PARMS BHAT= 0.001 0.01 0.1 /* initial parameter estimates */
THHAT=3.0;
BOUNDS BHAT>0,
THHAT>0; /* parameter bounds */
```

```
T=24; /* experimental period in H */
MODEL NE= (BHAT*N02*T)/(1 + BHAT*N02*THHAT); /* model for nonlinear least squares */
OUTPUT OUT=PLOTNOTO P=PRED R=RES; /* output data set for plotting */
```

CURRICULUM VITAE

CURRICULUM VITAE

Isabelle VANTORNHOUT

1. PERSONAL DATA

- Name: Isabelle A.S. Vantornhout
- Place and date of birth: Kortrijk (Belgium), 7 January 1975
- Nationality: Belgian
- Address: Roeselarestraat 144
  8560 Wevelgem (Belgium)
- Mobile: 0479/74 95 32

2. DIPLOMAS AND CERTIFICATES

- Bio-Engineer in Agricultural Science (1998)
  - Option: Crop Protection
  - Ghent University, Faculty of Bioscience Engineering
- Qualified Teacher's Degree for Secondary Education - Section 2 in Applied Biological Sciences (2004)
  - Ghent University, Faculty of Psychology and Educational Sciences
- Doctoral Training in Applied Biological Sciences (2004)
  - Ghent University, Faculty of Bioscience Engineering

3. WORK EXPERIENCE

- 1 February 1999 – 30 November 2004:
  - Academic assistent at the Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University.
- From 1 December 2004:

- Study counsellor ("Studietrajectbegeleider") at the Faculty of Bioscience Engineering, Ghent University.

4. PUBLICATIONS

- Vantornhout, I., Van de Veire, M. and Tirry, L. 1999. Toxicity of imidacloprid to *Myzus persicae* and the predatory bugs *Orius laevigatus* and *Macrolophus caliginosus*. Med. Fac. Landbouww. Univ. Gent. 64: 49-57.
- Van de Veire, M., Vantornhout, I. and Tirry, L. 1999. Integrated control of the green peach aphid *Myzus persicae* in sweet peppers using the nicotinyl insecticide imidacloprid. IOBC/WPRS Bull. 22: 263-266.
- Vantornhout, I., Minnaert, H.L., Tirry, L. and De Clercq, P. 2001. Development of *Iphiseius degenerans* Berlese (Acari: Phytoseiidae) on four different kinds of food sources. Med. Fac. Landbouww. Univ. Gent. 66: 321-325.
- Vantornhout, I., Minnaert, H.L., Tirry, L. and De Clercq, P. 2004. Effect of pollen, natural prey and factitious prey on the development of *Iphiseius degenerans*. BioControl 49: 627-644.
- Vantornhout, I., Minnaert, H.L., Tirry, L. and De Clercq, P. 2005. Influence of diet on life table parameters of *Iphiseius degenerans.* Exp. Appl. Acarol. 35: 183-195.
- Mahdian, K., Vantornhout, I., Tirry, L. and De Clercq, P. Effects of temperature on predation by the stinkbugs *Picromerus bidens* and *Podisus maculiventris* (Heteroptera: Pentatomidae) on noctuid caterpillars. Bull. Entomol. Res. In press.

5. ATTENDED SYMPOSIA AND CONFERENCES

- 51st International Symposium on Crop Protection. 4 May 1999, Ghent, Belgium. (presentation: I. Vantornhout, M. Van de Veire and L. Tirry. Toxicity of imidacloprid to *Myzus persicae* and the predatory bugs *Orius laevigatus* and *Macrolophus caliginosus*).

- Studie- en vervolmakingsdag: Gewasbescherming in de toekomst. Coda, 29 maart 2000, Tervuren, Belgium.
- Splus cursus, 20 April 2000, Ghent, Belgium.
- 52nd International Symposium on Crop Protection. 9 May 2000, Ghent, Belgium.
- 53rd International Symposium on Crop Protection. 8 May 2001, Ghent, Belgium.
- 7th FLTBW PhD Symposium. 10 Octobre 2001, Ghent, Belgium. (Poster presentation: I. Vantornhout, H. Minnaert, L. Tirry and P. De Clercq. Development of *Iphiseius degenerans* Berlese (Acari: Phytoseiidae) on four different kinds of food sources.). Poster award 7th FLTBW PhD Symposium 2001.
- 54th International Symposium on Crop Protection. 7 May 2002, Ghent, Belgium. (Poster presentation: I. Vantornhout, H. Minnaert, L. Tirry and P. De Clercq. Development of *Iphiseius degenerans* Berlese (Acari: Phytoseiidae) on four different kinds of food sources.)
- 55th International Symposium on Crop Protection. 6 May 2003, Ghent, Belgium (secretary Section Agricultural entomology and IPM)
- Studie- en vervolmakingsdag: Gewasbescherming: wat blijft ervan over? Coda, 31 March 2004, Tervuren, Belgium.
- 56th International Symposium on Crop Protection. 4 May 2004, Ghent, Belgium (secretary Section Agricultural entomology and IPM)
- 5th Symposium of the European Association of Acarologists, 26-30 July 2004, Berlin, Duitsland. (Presentation: I. Vantornhout, H. Minnaert, L. Tirry and P. De Clercq. Biological parameters of *Iphiseius degenerans* fed on twospotted spider mites and western flower thrips)
- 57th International Symposium on Crop Protection. 10 May 2005, Ghent, Belgium (secretary Section Agricultural entomology and IPM)
- 58th International Symposium on Crop Protection. 23 May 2006, Ghent, Belgium (Vice-chair Section Biological control of pests)