A novel bacterial disease of the predatory mite *Phytoseiulus persimilis*:

disease syndrome, disease transmission and pathogen isolation

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CONTENTS

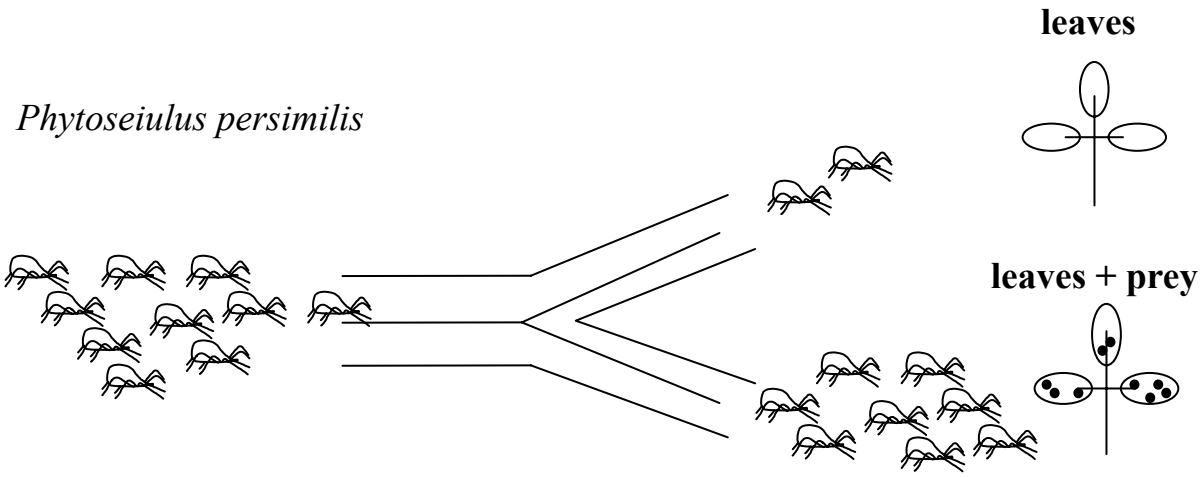
ABSTRACT

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) is a specialist predator of spider mites. Since more than three decades *P. persimilis* has been successfully applied worldwide in biological control of the two-spotted spider mite *Tetranychus urticae* Koch (Acari, Tetranychidae) in several greenhouse and field crops. The importance of *P. persimilis* and other predatory mites in integrated pest control has stimulated research, particularly on predator-prey interactions and foraging behaviour.

During the past two decades studies by different research groups have consistently demonstrated that adult female predatory mites are attracted to volatiles emanating from Lima bean plants infested with their prey *T. urticae*. These so-called herbivore-induced plant volatiles (=HIPV) are produced by the plant after herbivore attack. However, in mid-1992 a sudden and permanent change in behavioural response to HIPV was recorded in our laboratory: adult female *P. persimilis* of our laboratory population, subsequently designated **non-responding (=NR-) population**, showed a lower degree of attraction to HIPV than adult females of other *P. persimilis* populations designated **responding (=R-)** populations.

Moreover, adult female predators of the NR-population show a characteristic set of symptoms, subsequently designated **non-responding (=NR-) syndrome**. Predators shrink after mating, cease oviposition immediately after shrinkage and die several days later. Other characteristics of the syndrome are a tendency to leave a prey patch with ample food, moving fast between places, ceasing predation altogether, having a low excretion rate and carrying excretory crystals in the legs. The NR-syndrome was induced in non-symptomatic adult female predators after exposure to symptomatic predators and faeces and debris released by such predators. Moreover, it was shown that bacteria present in faeces and debris play a role in this process.

Several bacterial species were isolated from symptomatic adult female predators, their faeces and debris. For one of these isolates the Koch's postulates were satisfied, which constitutes the final proof that the isolate in question is the causative agent of the novel disease. The isolate represents a new bacterial species in a new bacterial genus, described as ***Acaricomes phytoseiuli***. This is the first record of a bacterial pathogen in predatory mites. The results presented in this thesis are of great importance to applied and fundamental science, as *P. persimilis* is a cornerstone in biological control and plays a major role in research on predator-prey interactions worldwide.


leaves
Phytoseiulus persimilis
leaves + prey

Chapter 1

General introduction

Phytoseiulus persimilis in biological control

Spider mites (Acari, Tetranychidae) are polyphagous herbivores that often achieve pest status in several protected and field crops. The emergence of pesticide resistance in spider mites has led to early development of biological control by the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae), a specialist predator of spider mites (Helle and van de Vrie, 1974; Helle and Sabelis, 1985). In *P. persimilis* the reproducing female determines the predation capacity of a population because: (1) the developmental time (egg, larva, protonymph, deutonymph, preoviposition female) is short (=less than 1 week under favourable conditions) compared to the oviposition period (=more than 3 weeks); (2) the sex-ratio is female-biased; (3) the predation rate of a female is considerable because the mean daily egg biomass produced is high (=it may be as high as their own body weight) (Sabelis, 1981). As these characteristics may account for high rates of population increase and eradication of local spider mite populations, *P. persimilis* was an early candidate for biological control of spider mites. Small-scale application in greenhouses started in 1968 with the use of *P. persimilis*. Only one commercial company was then producing these natural enemies (van Lenteren and Woets, 1988). The founder population of this commercial stock was very small. Only ten *P. persimilis* reached the German Federal Republic in 1959 on plant material imported from Chile. During the 1980's *P. persimilis* was produced on *Tetranychus urticae* Koch feeding on bean plants in the greenhouse with a maximum weekly production capacity of 20 million predatory mites (van Lenteren and Woets, 1988). High production capacities are needed as periodically large numbers of *P. persimilis* are released to obtain immediate control of spider mites (= inundative release method). The importance of *P. persimilis* and other species of phytoseiid mites in integrated pest control has stimulated numerous research activities all over the world, particularly on predator-prey interactions and foraging behaviour (see for reviews: Dicke *et al*., 1998; Sabelis *et al*., 1999; de Boer and Dicke, 2005). Hence, during the past three decades, *P. persimilis* has been reared by many laboratories and numerous commercial natural enemy producers all over the world.

Quality control of natural enemies

The success of biological control and research programmes is among others depending on the quality of the natural enemy. For biological control programmes the overall quality of an organism can be defined as the ability to function as intended after release into the field; for research programmes the overall quality of an organism can be defined as the ability to show functions that would be shown under field conditions. However, a field population is confronted with numerous changes when introduced into the laboratory, which may affect the quality of the natural enemies. The following changes may occur: (1) stable biotic and abiotic factors may influence which characteristics have the largest effect on fitness, (2) a lack of interspecific competition may change genetic variability, (3) conditions that are made suitable for the average or poorest genotype may decrease genetic variability, (4) laboratory conditions may affect density-dependent characteristics, (5) restricted escape possibilities of unmated or recently mated females may change mate-selection processes, (6) restricted dispersal possibilities may change dispersal behaviour, (7) high rearing densities may cause high rates of cannibalism, (8) rearing on unnatural prey or under unnatural conditions may cause changes in prey preference; (9) rearing on unnatural prey or on prey fed unnatural diets may cause reduced vigour, (10) rearing conditions may enhance infectious and non-infectious diseases (see for further discussion van Lenteren, 2003a). Despite this long list of factors that may reduce quality of mass-reared natural enemies, no proper quality control has been practised during the first two decades of biological control. Poorly performing natural enemies have resulted in failures of biological control and low performance of this pest control method (van Lenteren, 2003a). It was only during the 1980's and 1990's that the issue of quality control gained more interest in Europe and North-America, respectively (van Lenteren, 2003a; Glenister *et al*., 2003). In Europe several workshops of European producers and scientists were organised that resulted in quality control guidelines for 30 species of natural enemies. Parameters that are relatively easy to determine in the laboratory, including emergence, sex ratio, longevity, fecundity, adult size and predation rate are currently measured (van Lenteren, 1998, van Lenteren *et al.*, 2003a). The guidelines have been tested and adapted by commercial producers of Europe and are currently updated and further developed by the International Biocontrol Manufacturers Association (van Lenteren, 2003a). Moreover, it has been suggested that in the future parameters such as flight, walking, searching and/or field performance should be tested in addition to standard laboratory tests or for validation of such tests (Silva *et al*., 2000; Steinberg and Cain, 2003; Lewis *et al*., 2003).

One limiting factor of general quality control programmes today is the structure of the biocontrol industry sector. Natural enemy producers form a rather diverse group including

small and large commercial producers, large companies of associated industries and governmental production units (van Lenteren, 2003a). Numerous companies have appeared and disappeared over the past 30 years and the vast majority of the 85 commercial producers that exist nowadays is small. Only 3 producers have more than 50 employees (van Lenteren, 2003a). Up to now mass-rearing methods are usually developed on an ad hoc basis by the individual producer and means for general research and quality control programmes are rather limited in most of the companies.

Performance of *P. persimilis*

Since its first commercial application in 1968, *P. persimilis* has remained one of the key organisms in augmentative biological control, being reared by the majority of commercial producers. It is the most widely available species of all marketed natural enemies and it is released in large populations mainly against *T. urticae*, but *T. cinnabarinus* can be controlled as well (Cranshaw *et al*., 1996; van Lenteren, 2003b). During the first 20 years of mass production no measurable deterioration in performance of mass-reared *P. persimilis* has been reported. The first reports of low performance in commercial and laboratory populations of *P. persimilis* came from Canada in the early 1990's (Steiner, 1993a, b; Steiner and Bjørnson, 1996). Scientists examined the performance of three mass-produced species from three commercial sources located in Canada, Europe and the United States. In many cases shipments of *P. persimilis* were short on stated contents and surviving mites performed poorly under optimal conditions (Steiner, 1993a, b). During a follow-up study, 6 commercial populations and 3 populations of research facilities from Australia, New Zealand, the United Kingdom and Israel were evaluated. Both longevity and short-term fecundity were rather low for all populations, whereas egg sterilisation led to a consistently higher performance of mites (Steiner and Bjørnson, 1996). These results made the authors pose the provocative question, whether present quality standards and results of scientific investigations of *P. persimilis* are based on unsound results, obtained from *P. persimilis* populations that have not been established as fit and healthy (Steiner and Bjørnson, 1996).

More recent reports suggest that the problem of quality loss in commercial populations of *P. persimilis* did not change essentially since then (Bjørnson *et al*., 2000; Raworth and Bjørnson, 2002; Blümel and Hausdorf, 2002). In 1998 Blümel and Hausdorf (2002) tested among others four batches of commercial *P. persimilis* offered on detached bean leaves (*Phaseolus vulgaris*) according to the guidelines proposed at that time (Steinberg and Dale, 1998). For all batches the percentage of live female predators did not meet the requirement. In addition, one of the four batches did not match the quality criteria with regard to the reproduction per female predator (Blümel and Hausdorf, 2002). During a large scale study of *P. persimilis* from four commercial sources Bjørnson *et al*. (2000) used comparable test methods. The

authors found low fecundity and low survival rates. Raworth and Bjørnson (2002) determined short-term fecundity and survival according to current quality control guidelines (van Lenteren *et al*., 2003a) for predators from six commercial sources directly after delivery and after rearing them 30 days at their laboratory. Surprisingly the overall quality of all populations was low and did not improve by rearing them in the laboratory. Survival rates were even lowest for females that had been reared in the laboratory.

Foraging behaviour of *P. persimilis*

Natural enemy producers traditionally define performance of a natural enemy by using life history parameters such as those integrated in the quality control guidelines (van Lenteren, 2003a). However, a crucial factor influencing the quality of a natural enemy is its foraging behaviour, and this is not measured in quality control procedures (Steinberg and Cain, 2003; Lewis *et al*., 2003). A predator that locates its prey efficiently and will stay in prey patches until they are exploited will be more effective in biological control than individuals that do not show these traits. Moreover, it is important for efficient biological control that the natural enemy is able to locate its host at low host densities (Sabelis and Dicke, 1985; van Lenteren, 1986).

In our laboratory, foraging behaviour of *P. persimilis* has been studied for many years. An important behavioural characteristic of adult females is their attraction to plant odours, currently called "herbivore-induced plant volatiles" (HIPV), that are released in response to feeding damage by their prey *T. urticae*. (Sabelis and van de Baan, 1983). Since 1983 this behavioural response has been reported in numerous laboratories (see for reviews Dicke *et al*., 1998, Sabelis *et al*., 1999) and it has been shown that it plays an important role in successful host location in the field (Zemek and Nachman, 1999; Janssen, 1999).

However, since mid-1992 our laboratory population showed a lower degree of attraction to herbivore-induced plant volatiles than in the first part of 1992 and the previous year. This so-called non-responding (=NR-) population originated from the normally responding population from a Dutch natural enemy producer which had been reared in our laboratory for many years prior to 1992. Several attempts were made to regain a laboratory population of *P. persimilis* showing a "normal" behavioural trait by introducing other commercial and laboratory populations to our laboratory and testing the NR-population at places outside Wageningen (Schütte *et al*. unpublished data). As all attempts failed and behavioural research on *P. persimilis* remained hampered in our laboratory, it was decided to start the present project with the aim of detecting the cause of the behavioural change.

A similar phenomenon has been observed earlier in two other species of phytoseiid mites reared at our laboratory. Between July 1985 and November 1987 the attraction to herbivore-

induced plant volatiles fluctuated widely in three laboratory populations of *Amblyseius potentillae* and one laboratory population of *Typhlodromus pyri* (Dicke *et al*., 1991a). At some times predatory mites preferred the odour of prey-infested leaves to the odour of uninfested leaves whereas at other times their preference was the opposite. Several possible causes for this variation were investigated, but no definite conclusions could be drawn (Dicke *et al*., 1991a).

Working hypothesis and outline of the thesis (Figure 1)

Changes in behavioural response to herbivore-induced plant volatiles in mass-reared natural enemies may be caused by a variety of factors, including changes of (1) the environment, (2) the odour source, (3) the previous experience of the natural enemy (4) the physiological state of the natural enemy (5) the genetic constitution of the natural enemy or (6) by an infectious agent (see also Lewis *et al*., 2003). As preliminary experiments indicated that the latter factor was the most probable explanation for the behavioural change, the following working hypothesis was formulated: **The sudden and permanent behavioural change in adult female *P. persimilis* of the NR-population is a symptom of an infectious disease.**

After a literature study of diseases in phytoseiid mites **(chapter 2)** several mite pathologists were asked to investigate the NR-population for the presence of known and/or novel pathogens. However, no known pathogens or suspicious entities were detected during studies with light microscopy, electron microscopy and molecular methods by Ellen Beerling (Research Station for Floriculture and Glasshouse Vegetables, Aalsmeer, The Netherlands), Marilyn Steiner (Horticultural Research and Advisory Station, NSW Agriculture, Gosford, Australia), Susan Bjørnson (Department of Biology, Saint Mary's University, Halifax, Canada), Hans Breeuwer (Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands) and Regina Kleespies (Institute for Biological Control, Federal Biological Research Centre for Agriculture and Forestry, Darmstadt, Germany) (unpublished data).

Moreover, several authors had earlier applied intensive microscopic investigations on phytoseiid mites that were suspected to suffer from a disease (Hess and Hoy, 1982; Bjørnson, 1998; Bjørnson and Keddie, 2000, Beerling and van der Geest, 1991a, b, Ellen Beerling, personal communication). However, in only one case did microscopic investigations lead to the description of a novel pathogen (Bjørnson *et al.*, 1996; Bjørnson and Keddie, 1999, 2001). Therefore we decided to follow a rather traditional methodology to test the working hypothesis of the present thesis (Figure 1). First, experimental work concentrated on the behavioural change and was aimed at its characterisation and at the eliminating of other possible explanations as listed above **(chapter 3)**.

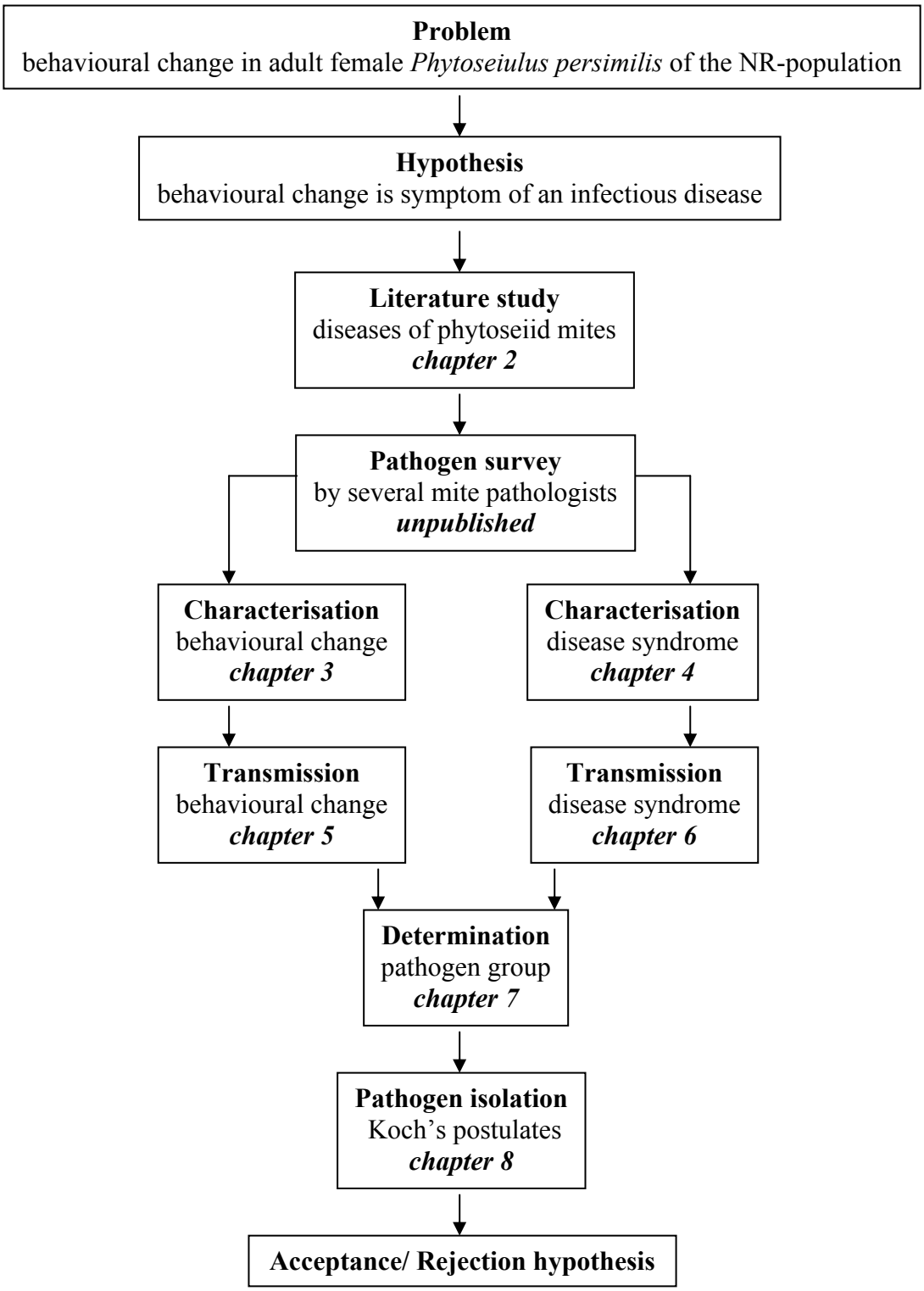


Figure 1: Outline of the research presented in this thesis

A crucial step towards verification of the working hypothesis was evidence of the infectious character of the behavioural change **(chapter 5)**. However, the definite way to make a conclusive disease diagnosis is satisfying Koch's postulates, for which the following steps must be taken (Lacey and Brooks, 1997):

(1) The pathogen must be isolated from all of the diseased individuals examined and the signs and/or symptoms of the disease recorded.
(2) The pathogen must be grown in culture and it must be identified and/or characterised.
(3) The pathogen must be inoculated on/in healthy individuals of the same or a related species and signs and symptoms must be the same.
(4) The pathogen must be isolated in culture again and its characteristics must be exactly like those observed in step 2.

As satisfying the Koch's postulates was the final aim of the present research, it was necessary to determine more characteristics of the NR-population in order to describe a distinct disease syndrome **(chapter 4)**.

Moreover, pathogen numbers were most probably extremely low in infected individuals, as despite intensive investigations (potential) pathogens had not been detected in individuals of the NR-population. In such a case, knowledge about the main route of disease transmission is of utmost importance, as investigations can then be concentrated on the main pathogen reservoir **(chapter 6)**. With this knowledge about the main reservoir of the infectious agent we could target on determination of the pathogen group **(chapter 7)**, isolation of one or more pathogens with the final aim of satisfying the Koch's postulates **(chapter 8)**. Hence the present research outline would lead us to acceptance or rejection of the working hypothesis. In the last part of the thesis the main findings of this project are discussed and summarized **(chapter 9)**.

Acknowledgements

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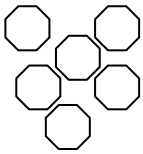
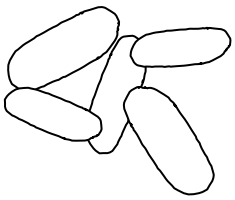
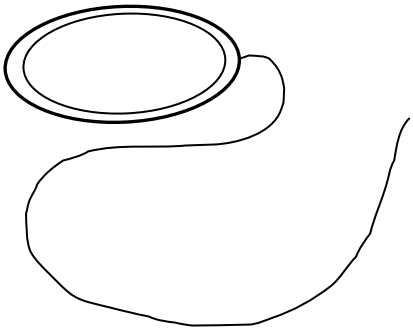
viruses



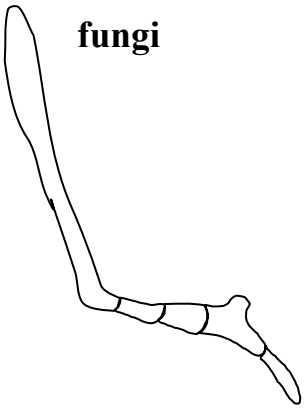
bacteria



microsporidia



fungi



Chapter 2

An overview of diseases of phytoseiid mites

Abstract

Several species of phytoseiid mites (Acari, Phytoseiidae), including species of the genera *Amblyseius*, *Galendromus, Metaseiulus*, *Mesoseiulus, Neoseiulus*, *Phytoseiulus* and *Typhlodromus*, are currently reared for biological control of various crop pests and/or as model organism for the study of predator-prey interactions. Pathogen-free phytoseiid mites are important to obtain high efficacy in biological pest control and to get reliable data in mite research, as pathogens may affect the performance of their host or alter their reproduction and behaviour. Potential pathogens and pathogens in the true sense have been reported for phytoseiid mites during the past 25 years. The present chapter provides an overview of diseases of phytoseiid mites, including potential pathogens with unknown host effects (14 reports), endosymbiotic Wolbachia (6 reports), other endosymbiotic bacteria (*Cardinium*) (2 reports), cases of unidentified diseases (3 reports) and cases of pathogens in the true sense of the word (5 reports). From the latter group 4 reports refer to microsporidia and 1 to a fungus. Only four entities have been studied in detail, including *Wolbachia* infecting 6 predatory mite species, other endosymbiotic bacteria infecting *Metaseiulus occidentalis*, the microsporidium *Microsporidium phytoseiuli* infecting *Phytoseiulus persimilis* and the microsporidium *Oligosporidium occidentalis* infecting *Metaseiulus occidentalis*. In three cases (*Wolbachia*, *M. phytoseiuli* and *O. occidentalis*) infection may be connected with fitness costs of the host. Moreover, infection is not readily visible as no obvious gross symptoms are present. Monitoring of these entities on a routine and continual basis should therefore get more attention, especially in commercial mass-production. Special attention should be paid to field-collected mites before introduction into the laboratory or mass rearing, and to mites that are exchanged among rearing facilities. However, at present general pathogen monitoring is not yet practical as effects of many entities are unknown. More research effort is needed concerning diseases of commercially reared arthropods and those used as model organisms in research.

Introduction

Several species of phytoseiid mites, including species of the genera *Amblyseius*, *Galendromus, Metaseiulus*, *Mesoseiulus, Neoseiulus*, *Phytoseiulus* and *Typhlodromus*, are currently reared for biological control of pests including spider mites (*Tetranychus* spp.) and thrips (*Thrips tabaci* and *Frankliniella occidentalis*) in protected crops, outdoor vegetables, fruit and other horticultural crops (van Lenteren, 2003a, b). Predatory phytoseiid mites include specialists such as *Phytoseiulus persimilis*, which attack spider mites (*Tetranychus* spp.), selective predators such as *Neoseiulus californicus* and generalists such as *Neoseiulus cucumeris,* that prey on microarthropods but can reproduce on a pollen diet and utilise plant exudates, honeydew and fungi as food supplements (McMurtry and Croft, 1997). Among the 30 species that are produced in commercial insectaries on a large scale are 4 phytoseiid species (van Lenteren, 2003a, b). The success of biological control programmes is, among others, dependent on the health of the beneficials that are used. In several cases reports of poor performance in mass-reared phytoseiid mites have raised questions regarding their quality and efficacy in biological control (Steiner, 1993a, b; Steiner and Bjørnson, 1996; Bjørnson *et al*., 2000; Raworth and Bjørnson, 2002; Blümel and Hausdorf, 2002) and have stimulated research in mite pathology (Poinar and Poinar, 1998; van der Geest *et al*., 2000). Moreover, phytoseiid mites are used in several research groups for the study of predator-prey interactions and foraging behaviour (Yao and Chant, 1990; Margolies *et al*., 1997; Dicke *et al*., 1998; Sabelis *et* al., 1999; Zemek and Nachman, 1999; Janssen, 1999; Schausberger and Croft, 2000; Maeda *et al*., 2001; Skirvin and Fenlon, 2003a, b). Pathogens may also alter the behaviour of their host (Horton and Moore, 1993), thereby influencing outcomes of behavioural research. Hence, care should be taken to maintain healthy laboratory stocks.

Pathogens have been reported in phytoseiid mites collected from the field (Furtado *et al.*, 1996), from those currently mass-produced for biological pest control (Beerling and van der Geest, 1991a, b; Bjørnson and Keddie, 2000) and from laboratory populations (Hess and Hoy, 1982; Becnel *et al*., 2002). For the latter two cases it could not be determined whether the pathogens originated from field-collected natural enemies or arose in mass-rearing systems as a result of intense and continuous rearing under laboratory conditions. Mass-reared host populations may be more susceptible to diseases than field populations, as genetic variation is lower and immune responses may be compromised by stress factors including sub-optimal climatic conditions, starvation and overcrowding (Lighthart *et al*., 1988; Sikorowsky and Lawrence, 1994). Moreover, in mass-production of arthropods climatic conditions may be more optimal for pathogens and horizontal pathogen transmission may be more effective than in natural situations (Sikorowsky and Lawrence, 1994). These factors may thus enhance disease incidence, the development of novel diseases and/or virulent

pathotypes in mass-reared populations. The following review of pathogens in phytoseiid mites includes cases with unknown host effects, cases of infection with endosymbiotic bacteria, cases of unidentified diseases and cases of identified diseases, with known pathologies and transmission modes. Diseases are presented according to pathogen group.

Diseases caused by viruses

"Viruses are the ultimate agents provocateurs of biology, for they appear to be welcomed into the trusting arms of the cell…."
Robert Gallo, Virus Hunting, 1991 (cited by Boucias and Pendland, 1998)

General characteristics

Viruses may be defined as biological macromolecules that have the ability to multiply within living cells. They are reported from virtually every insect order and are the smallest of all entomopathogens. These pathogens, comprised of genomic RNA or DNA bound to a protein coat (capsid), are considered the simplest entities capable of replication (Boucias and Pendland, 1998). Viral diseases are one of the most widely investigated infections in insects (Tanada and Kaya, 1993). Some viruses are occluded at random in proteinaceous occlusion bodies that can be detected under the light microscope, whereas most non-occluded viruses can be detected only with the aid of the electron microscope (Lacey, 1997).

In general, infection occurs after viruses have been ingested, but transmission may occur via the host egg (=transovarially), through natural body openings (for example spiracles) or through wounds (Tanada and Kaya, 1993). Diagnostic features considered as general characteristics of viral infection include: coloration (white, yellow, light blue, iridescent blue, green, purple or orange) of the gut, the fat body or the entire body, blackening of the body after death, weakening of the outer skin leading to rupturing and release of liquefied body contents (Evans and Shapiro, 1997). Infected individuals may show reduced feeding, poor breeding performance, extended development, extremely extended longevity, body paralysis or lethal sensitivity to CO_2 (Evans and Shapiro, 1997). Behavioural changes of insects infected by viruses include: changes in level of activity (wandering behaviour) and changes of microhabitat preference, such as elevation seeking behaviour (="tree-top" diseases), movement to exposed locations and diurnal behaviour of nocturnal insects (Horton and Moore, 1993).

Viruses of phytoseiid mites

Only three reports exist on viruses of phytoseiid mites (Table 1). In all cases virus-like particles were detected in electron microscopic studies, but host effects have not been studied. Unidentified, non-occluded virus-like particles were observed in the yolk of developing eggs inside *Neoseiulus cucumeris* (Oudemans) females (Bjørnson *et al.*, 1997). Also gravid *Phytoseiulus persimilis* Athias-Henriot females carried unidentified, non-occluded virus-like particles in the yolk of developing eggs. (Steiner, 1993; Bjørnson *et al.*, 1997).

Phytoseiulus persimilis infected with *Rickettsiella phytoseiuli* contained non-occluded virus-like particles, which both were abundant and visible in the dorsal part of the body, immediately below the cuticle (Šut'áková and Rüttgen, 1978). The authors report an interaction of both entities: viruses were only present in the cytoplasm of cells infected with *R. phytoseiuli* and morphological and structural changes were induced in *R. phytoseiuli* when the host was also carrying the virus-like particles (Šut'áková and Rüttgen, 1978).

Diseases caused by bacteria

"Martians-dead-slain by the putrefactive and disease bacteria against which their systems were unprepared…."
H. G. Wells, War of the Worlds, 1934 (cited by Boucias and Pendland, 1998)

General characteristics

Bacteria are unicellular prokaryotes, their genetic information being contained within a single, double-stranded DNA molecule and small self-replicating DNA molecules termed plasmids or prophages (Boucias and Pendland, 1998). Many bacteria are opportunistic pathogens that may exist in nature as saprophytes and may become pathogenic if conditions are favourable. Others are more fastidious and can grow only in the appropriate host (Boucias and Pendland, 1998).

Bacterial pathogens invade their hosts mostly through the mouth and digestive tract. Less often, they are transmitted through the egg, trachea or wounds in the integument (Tanada and Kaya, 1993). Upon invasion, bacterial pathogens may develop as intracellular pathogens (Rickettsiaceae) or extracellular pathogens (many opportunistic bacteria). Bacterial infections may be classified as (1) bacteremia when bacteria multiply in the hemolymph of the host without producing toxins; (2) septicaemia when bacteria multiply in the hemocoel, produce toxins and kill the host (3) toxaemia when bacteria stay confined to the gut lumen where they produce toxins (Tanada and Kaya, 1993).

Diagnostic features considered as general characteristics of bacterial infection include: distinct colour changes (white, red, amber, black or brown), a lack of appetite, stopping of feeding, excretion of diarrhoea-like faeces, vomiting, weakening of the outer skin, degeneration of internal tissues, cadavers becoming black, odiferous, shrivelled, dry and hard (Tanada and Kaya, 1993; Lacey, 1997).

The vast majority of research on bacterial insect pathogens over the past thirty years has been focused on the toxin-producing *Bacillus* species (Boucias and Pendland, 1998). However, studies on the effects of *ß*-exotoxin from *Bacillus thuringiensis* on phytoseiid mites are not included in the present review as they do not represent a pathogen in the true sense of the word (for a review see van der Geest *et al.,* 2000). Only very little work has been done on other bacterial pathogens. This is mainly due to the fact that bacteria isolated from insects that have been described as opportunistic pathogens belong to genera containing species that may infect plants and vertebrates, which makes them less interesting for the development as microbial control agents (Boucias and Pendland, 1998). Several entomopathogenic species have been identified in the genus *Serratia* including *S. marcescens*. Various entomopathogenic strains of *S. marcescens* are characterised by the production of enzymes and exocellular toxins. However, it is still unclear whether this pathogen is able to actively invade its host. In many cases diseases have been associated with poor sanitation and crowded rearing conditions (Boucias and Pendland, 1998).

Bacteria belonging to the family Rickettsiaceae are obligatory intracellular and multiply in eukaryotic cells. Entomopathogens of this group belong to the genera *Rickettsia*, *Rickettsiella* and *Wolbachia* (Boucias and Pendland, 1998). Members of the genus *Rickettsiella* are common pathogens, whereas those of the genus *Wolbachia* are seldom pathogenic in the true sense but have evolved various means to manipulate their hosts in order to enhance their own transmission (see Stouthamer *et al.*, 1999).

The genus *Rickettsiella* is comprised of a heterogeneous group of bacteria, all members being highly fastidious arthropod pathogens. A lack of homology has been demonstrated for certain members of this genus, suggesting the eventual revision of this group (Boucias and Pendland, 1998). *Rickettsiella* have developmental cycles involving the production of various cell phenotypes. The infectious particle is a small, dense rod or disc-shaped cell. All species are transmitted by feeding or through wounds. Many *Rickettsiella* undergo extensive replication in the fat body following ingestion and penetration of the alimentary tract. At present relatively few species associated to insects have been found (Boucias and Pendland, 1998). Rickettsial infections may induce prominent behavioural changes in the host, including elevation-seeking behaviour and changes in temperature preference (Horton and Moore, 1993).

Wolbachia are common cytoplasmic symbionts of insects, crustaceans, mites and filarial nematodes (see Stouthamer *et al.*, 1999). They are rarely pathogenic but may manipulate the host biology by inducing parthenogenesis (whereby infected females exclusively produce daughters), feminisation (whereby infected genetic males reproduce as females), male-killing (whereby infected male embryos die while female embryos develop into infected females), cytoplasmic incompatibility (unidirectional in its simplest form: whereby the crossing of an uninfected female and infected male result in embryo mortality) or by enhancing host fecundity (Stouthamer *et al.*, 1999).

Wolbachia may be present in various tissues but are predominately present in gonadal tissue (Stouthamer *et al.*, 1999). The symbionts are transmitted vertically through the egg. Therefore, infected mothers give rise to infected offspring. Phylogenetic studies of *Wolbachia* indicate that horizontal transmission must have taken place rather frequently. An intraspecific horizontal transfer of *Wolbachia* has recently been reported (Huigens *et al.,* 2000). Because culturing of *Wolbachia* outside hosts has been successful in only one case, molecular techniques such as the polymerase chain reaction (PCR) are used in detecting *Wolbachia* infections (Stouthamer *et al.,* 1993).

Recently a novel lineage of intracellular bacteria has been shown to be associated with several reproductive disorders, including (1) parthenogenesis in a number of parasitoid wasps in the genus *Encarsia* (Zchori-Fein *et al*., 2001; Zchori-Fein *et al*., 2004), (2) feminization in the mite *Brevipalpus phoenicis* (Weeks *et al*., 2001) and (3) cytoplasmic incompatibility in *Encarsia pergandiella* (Hunter *et al*., 2003). Phylogenetic analysis of the 16S rRNA gene placed this bacterium in the Bacteroidetes group (=*Cytophaga-Flexibacter-Bacteroides* or CFB group). This bacterium has been called the *Encarsia* bacterium (Zchori-Fein *et al*., 2001), the CFB-BP (Weeks and Breeuwer, 2003), and the *Cytophaga*-like organism (CLO) (Hunter *et al*., 2003; Weeks *et al*., 2003; Weeks and Stouthamer, 2004). Recently it has been suggested to classify this symbiont from *Encarsia* as "*Candidatus Cardinium hertigii*" (Zchori-Fein *et al*., 2004). A large screening study has shown that the bacterium is prevalent among arthropods, and that double infection with *Wolbachia* may occur (Weeks *et al*., 2003).

Bacteria of phytoseiid mites

The majority of the identified bacteria recorded in phytoseiid mites belong to the genera *Rickettsiella*, *Wolbachia* and *Cardinium* (Table 1). *Wolbachia* seem to be widespread among phytoseiid mites, as they are found by several authors in numerous populations of six phytoseiid species.

Intracellular, rickettsia-like entities named *Rickettsiella phytoseiuli* have been observed during microscopic studies of *P. persimilis* (see for a review Šut'áková, 1994). Predators originated from a laboratory population of the Ukraine (Šut'áková and Rüttgen, 1978) and did not show developmental abnormalities, morphological changes or increased mortality. However, all investigated mites contained polymorphous entities that were considered to represent six different stages of the reproduction cycle: dense, intermediate, bacterial, giant, crystal-forming and small dark particles (Šut'áková and Rüttgen, 1978). In adult mites, infection was detected in all organs except the nervous tissue, whereas larvae and nymphs and prey spider mites (*Tetranychus urticae* Koch) were never infected with *R. phytoseiuli* (Šut'áková, 1988; Šut'áková, 1991). A *P. persimilis* population from Slovakia exhibited the same infection, whereas a population from the Armenian Republic did not. However, other apparently symbiotic micro-organisms were present in the ovaries of predators from the latter population (Šut'áková and Arutunyan, 1990). *R. phytoseiuli* isolated from *P. persimilis* could be cultivated in adult female *Dermacentor reticulatus* Fabricius ticks, where it formed all six known developmental stages (Šut'áková and Řeháček, 1989). Pathological effects were never recorded, though some individuals carried the microbes in high densities (Šut'áková, 1991).

Hess and Hoy (1982) observed two different pathological manifestations in several laboratory populations of *Metaseiulus occidentalis* (Nesbitt). (1) Some adult females were plumb and had a cream-coloured to pink rectal “plug” that extruded from their posterior end and occasionally caused mites to become glued to the substrate. The rectal plug was associated with motor dysfunction, reduced oviposition and eventually death, and was most common in older females. Immatures and males rarely had rectal plugs. (2) Mites became very pale and so thin that they became translucent. Females failed to oviposit, immatures exhibited high mortality and colonies died out. According to the authors both pathologies were associated with overcrowding. (Hess and Hoy, 1982). The authors described two morphologically distinct unidentified micro organisms in symptomatic and non-symptomatic *M. occidentalis*. Whether these forms represent one or two species was not established. One form (which they called type A) was exclusively intracellular. This type was present in all mites in varying numbers and in all tissues examined, except ovarian and nervous tissues. According to the authors this micro-organism did not appear to be detrimental. The second rickettsia-like form (which they called type B) occurred both intra- and extracellularly. This type was present in two thirds of symptomatic and asymptomatic mites. In some cases it completely dominated the internal organs and the hemocoel and was associated with the rectal plug. Thin and pale mites also contained predominantly the second type, but tissues of these mites appeared more damaged, perhaps accounting for their lucidity. When present in moderate numbers, these micro-organisms were observed in the hemocoel, the Malpighian tubules

and within the ovarian tissue, which may suggest transovarial transmission (Hess and Hoy, 1982). The authors did not determine whether the increase of the second bacterial type was the primary cause of the disease or a secondary effect. Recently it has been suggested by Weeks and Breeuwer (2003) that this second endosymbiont is likely to be *Cardinium*.

By using molecular methods (PCR with *Wolbachia*-specific primers), *Wolbachia* endosymbionts were detected in eight of nine laboratory populations of *M. occidentalis* and in four laboratory populations of *Tetranychus urticae* Koch that served as food for *M. occidentalis* (Johanowicz and Hoy, 1996). In *M. occidentalis*, *Wolbachia* caused non-reciprocal reproductive incompatibilities between infected males and uninfected females. Uninfected females crossed with infected males produced few eggs and no female progeny. Many of the produced eggs were shrivelled (Johanowicz and Hoy, 1998b). The mechanisms by which *Wolbachia* cause reproductive incompatibilities in *M. occidentalis* are unknown. *Wolbachia* infection seems to be associated with fitness costs as the number of female progeny was lower in infected control crosses than in uninfected control crosses. These fitness costs may have prevented the rapid spread of *Wolbachia* in three laboratory populations of *M. occidentalis* (Johanowicz and Hoy, 1999). *Wolbachia* were eliminated from *M. occidentalis* when the predators were reared at an elevated temperature (33ºC) (Johanowicz and Hoy, 1998a, b).

Moreover, Breeuwer and Jacobs (1996) detected *Wolbachia* in a population of *M. occidentalis* from the USA, in a commercial population of *P. persimilis* from the Netherlands, in a population of *Neoseiulus barkeri* (Hughes) collected in the Netherlands and a population of *N. bibens* (Blommers) from Madagascar. The effects of *Wolbachia* on the species other than *M. occidentalis* have not yet been investigated, but it is likely that *Wolbachia* are associated with non-reciprocal reproductive incompatibilities (for a discussion, see Breeuwer and Jacobs, 1996). In a recent study *Wolbachia* infection has also been found in *Galendromus annectens* and *Mesoseiulus longipes* (Weeks *et al*., 2003). Rickettsia-like particles, belonging to the genus *Wolbachia* were also reported by Steiner (1993b) and Bjørnson *et al.* (1997). The latter author detected with molecular methods that *Wolbachia* was present in commercial *P. persimilis* populations from seven sources. However, recently Enigl *et al*. (2005) screened several strains from *Phytoseiulus persimilis* (7 strains obtained from Europe, Africa and the USA and alcohol samples of 10 other strains) for the occurrence of *Wolbachia* and no sample tested positive. They therefore suggested that infection of *P. persimilis* with *Wolbachia* seems to be rare and of minor importance (Enigl *et al*., 2005). It has been suggested that the rickettsia-like organisms reported in the earlier microscopic surveys of phytoseiid mites are probably all members of the genus *Wolbachia* (for a discussion see van der Geest *et al*., 2000).

Table 1: Overview of entities recorded for phytoseiid mites

Pathogen	Phytoseiid host / origin*	Symptoms	Reference
Viruses			
Non-occluded virus	*Neoseiulus cucumeris* / c	Unknown	Bjørnson *et al*., 1997
Non-occluded virus	*Phytoseiulus persimilis* / c	Unknown	Steiner, 1993b; Bjørnson *et al.*, 1997
Virus-like particles	*Phytoseiulus persimilis* / l	Unknown	Šut'áková & Rüttgen, 1978
Bacteria			
Rickettsiella phytoseiuli	*Phytoseiulus persimilis* / l	Unknown	Šut'áková & Rüttgen, 1978
Wolbachia	*Galendromus annectens*	Unknown	Weeks *et al*., 2003
	Mesoseiulus longipes	Unknown	Weeks *et al*., 2003
	Metaseiulus occidentalis / l	**Known****	Johanowicz & Hoy, 1996; Breeuwer & Jacobs, 1996; Weeks *et al*., 2003
	Neoseiulus barkeri / f	Unknown	Breeuwer & Jacobs, 1996
	Neoseiulus bibens / l	Unknown	Breeuwer & Jacobs, 1996
	Phytoseiulus persimilis / c	Unknown	Steiner, 1993b; Breeuwer & Jacobs, 1996; Bjørnson *et al*., 1997; Weeks *et al*., 2003
Cardinium	*Metaseiulus occidentalis* l/f	**Known****	Weeks *et al*., 2003; Weeks and Stouthamer, 2004; Hoy & Jeyaprakash, 2005
Bacteroidetes & *Enterobacter*	*Metaseiulus occidentalis* l/f	Unknown	Hoy & Jeyaprakash, 2005
Unidentified bacteria	*Metaseiulus occidentalis* / l	**Known**	Hess & Hoy, 1982
	Phytoseiulus persimilis / c	Unknown	Steiner, 1993b; cited in Schütte *et al.*, 2005
	Neoseiulus cucumeris / c	Unknown	cited in Schütte *et al*., 2005
	Neoseiulus barkeri / c	Unknown	cited in Schütte *et al*., 2005
Protozoa			
Microsporidium phytoseiuli	*Phytoseiulus persimilis* /c	**Known****	Bjørnson *et al*., 1996
Oligosporidium occidentalis	*Metaseiulus occidentalis* / l	**Known****	Becnel *et al*., 2002
Nosema steinhausi	*Neoseiulus cucumeris* /c	Unknown	Huger, 1988
	Neoseiulus barkeri /c	Unknown	Huger, 1988
Unidentified microsporidia	*Neoseiulus barkeri* / c	**Known**	Beerling & van der Geest, 1991
	Neoseiulus cucumeris / c	**Known**	Beerling & van der Geest, 1991
	Phytoseiulus persimilis / c	Unknown	Bjørnson & Keddie, 2000
	Phytoseiulus persimilis / c	Unknown	Bjørnson & Keddie, 2000
Fungi			
Neozygites sp.	*Euseius citrifolius* / f	**Known**	Furtado *et al*., 1996
Neozygites acaricida	*Euseius citrifolius* / f	Unknown	Keller, 1997
Neozygites cf. *acaridis*	*Euseius citrifolius* / f	Unknown	Keller, 1997
Unidentified fungi	*Phytoseiulus persimilis* / c	Unknown	cited in Schütte *et al*., 2005
Unidentified disease			
	Neoseiulus hibisci / l	**Known**	Tanigoshi *et al.*, 1981
	Phytoseiulus persimilis / c	**Known**	Bjørnson *et al*. 1997, 2000

*origin: c = commercial population, l = laboratory population, f = field population;
** symptom induction established by experiments

In a large-scale survey of arthropod hosts infection with the endosymbiotic bacterium *Candidatus Cardinium hertigii* was detected by sensitive hemi-nested PCR in *M. occidentalis* (Weeks *et al*., 2003). Test results were negative for *P. persimilis*, *Phytoseiulus macropilis*, *Neoseiulus fallacis*, *Mesoseiulus longipes*, *Galendromus helveolus* and *Galendromus annectens*. Interestingly *M. occidentalis* showed double infection of *Wolbachia* and *Cardinium*. In another study Weeks and Stouthamer (2004) reported that three inbred lines of *M. occidentalis* showed a clear and significant increase in fecundity associated with infection by *Cardinium.* Fecundity advantage of infected females versus non-infected females was approximately 1.6 times over a 6-day oviposition period. As the endosymbiont described by Hess and Hoy (1982) has recently been identified as *Cardinium* (Weeks and Breeuwer, 2003) and as *M. occidentalis* may harbour both *Wolbachia* and *Cardinium* at the same time, the authors suggest that the results of the studies of Johanowicz and Hoy (1998a) on cytoplasmic incompatibility in *M. occidentalis* may have been influenced by the presence of *Cardinium* (Weeks *et al*., 2003). In a molecular screening, using a high-fidelity PCR protocol (allowing the detection of as few as 100 copies of *Wolbachia* DNA; Jeyaprakash and Hoy, 2004), several bacterial species were detected in *M. occidentalis* after the clones were sequenced: one each was closely related to species in the genera *Enterobacter*, *Wolbachia* and *Cardinium*, and one was related to an unnamed micro organism in the phylum Bacteroidetes (Hoy and Jeyaprakash, 2005). PCR tests with newly designed primers for the sequences of the detected bacteria were positive for several laboratory and field-collected populations suggesting that all bacteria are important in the biology of *M. occidentalis* (Hoy and Jeyaprakash, 2005).

In a microscopic study of the digestive tract of *P. persimilis*, bacteria-like entities detected in the gut lumen were thought to have entered the digestive tract during feeding (Arutunyan, 1985). However, these bacteria bear a marked similarity to birefringent dumbbell-shaped crystals that are frequently observed in the Malpighian tubules, the digestive tract and rectum of phytoseiid mites (Steiner, 1993b; Schütte *et al*., 1995; Di Palma, 1996; Bjørnson *et al.*, 1997, 2000; R. G. Kleespies, personal communication).

Bacterial micro-organisms other than rickettsia have been recorded for dead and moribund *P. persimilis* (Steiner, 1993b). However, the author stated that these bacteria are secondary opportunistic invaders rather than a primary infection source. Moreover, unidentified bacteria were reported in microscopic investigations of several diseased mite populations of *P. persimilis*, *Neoseiulus cucumeris* and *Neoseiulus barkeri* (cited in Schütte *et al*., 2005).

Lighthart *et al.* (1988) tested the effect of several stress factors on the susceptibility of *M. occidentalis* to the weak bacterial pathogen *Serratia marcescens*. However, the isolate did not originate from mites. A high pre-inoculation temperature pulse under relatively un-

crowded conditions was most effective in enhancing susceptibility, higher mortality being the only disease symptom. Remarkably, starvation did not have such an effect.

Diseases caused by protozoa

"The pathogen could be transmitted through the egg…eggs from moths showing no corpuscules in their tissues would yield silkworms free of disease."
E.A. Steinhaus, Diseases in a minor Chord, 1975; referring to one of the first descriptions of a microbe as disease agent by Louis Pasteur (cited by Boucias and Pendland, 1998)

General characteristics

All protozoa recorded for phytoseiid mites belong to the phylum Microspora. Microsporidia are small, spore-forming protozoa. However, recent molecular studies indicate that they are related to fungi, which may in part explain the sensitivity of microsporidia to selected antifungal drugs (Boucias and Pendland, 1998). Microsporidia infect a wide range of hosts from all major animal phyla, fish and arthropods being their most common hosts (Tanada and Kaya, 1993). They are obligate intracellular parasites that lack typical mitochondria, a classical Golgi apparatus, centrioles and peroxisomes (Boucias and Pendland, 1998). Many species cause severe and acute infections in insects, but some produce only unapparent and chronic infections, that nonetheless may play an important role in host regulation (Tanada and Kaya, 1993).

The microsporidia have complex biologies that may involve two obligate hosts, vertical or horizontal transmission and/or multiple cell-types (Boucias and Pendland, 1998). The lifecycle consists of two phases, the vegetative phase and the sporulation phase, which results in the production of transmissible spores. In most cases the spore-to-spore cycle takes place in one cell (Tanada and Kaya, 1993). Microsporidia may invade the host tissues when spores are ingested, when the pathogen is transmitted from parent to progeny, or occasionally through wounds in the integument (Tanada and Kaya, 1993). Microsporidian spores are structurally unique and contain a characteristic tube-like polar filament through which an infective stage (sporoplasm) is injected into an adjacent host cell. This begins the infective cycle of the pathogen.

Diagnostic features considered as general characteristics of microsporidian infection are variable and may include: retardation of development and growth, reduced activity, abnormal coloration, diapause alterations, reduction of longevity and reproductive performance (Boucias and Pendland, 1998). Microsporidia-infected insects may also exhibit behavioural changes including changes in temperature preference (Horton and Moore, 1993).

Protozoa of phytoseiid mites

Microsporidia seem to be rather common among phytoseiid mites. Microsporidiosis has been observed in 4 phytoseiid species of varying origins (Table 1). A new microsporidian pathogen has recently been isolated from a laboratory population of *M. occidentalis* (Becnel *et al*., 2002). Immature stages and mature spores were found in the cytoplasm of ceacal cells, lyrate organ cells, ganglia, epithelial cells, muscle, ovary and mature eggs (Becnel *et al*., 2002). Microsporidia were never detected in the spider mite (*Tetranychus urticae*) prey of *M. occidentalis* (Olson and Hoy, 2002). Two classes of uninucleate spores were produced, differing primarily in the length of the polar filaments and the presence of a large posterior vacuole in one spore type (Becnel *et al*., 2002). The authors suspect that spores with long filaments are involved in horizontal disease transmission, which may take place by cannibalism of infected eggs (Olson and Hoy, 2002), whereas spores with the short polar filament may play a role in autoinfection and vertical transovarial transmission, that is highly efficient (99% infected offspring is produced by infected parents) (Olson and Hoy, 2002).

Molecular data (analysis of small subunit ribosomal DNA) indicated that this microsporidium is a new species and that it is most closely related to the *NosemalVariomorpha* clade of microsporidia, whereas developmental and morphological data suggest a placement into the genus *Unikaryon* or *Oligosporidium*. The authors discuss this conflict of morphological and molecular data and assign the new species the name *Oligosporidium occidentalis*. Predators infected by *O. occidentalis* did not exhibit any external or gross signs of infections. However, *O. occidentalis* has clear negative effects on its host. Infected female predators had a shorter life span, a lower oviposition rate and a lower number of female offspring, as infected mites have a male biased sex-ratio (Olson and Hoy, 2002). Heat treatment was effective to cure infected populations of *M. occidentalis* and did induce relatively low mortality (ca. 20%). Predator colonies initiated from mites that were reared from egg to adult at 33ºC showed an initial reduction in infection. However, disease incidence raised to 98% after 10 weeks. Colonies initiated from progeny of the heat-treated mites remained healthy during the observation period of 10 weeks (Olson and Hoy, 2002).

Unidentified microsporidia were reported in commercial mass-rearings of *Neoseiulus* (formerly *Amblyseius*) *cucumeris* and *Neoseiulus* (formerly *Amblyseius*) *barkeri* (Beerling and van der Geest, 1991a, b). This was the first report of microsporidia in mass-reared predatory mites. Predators of the commercial populations showed a low reproduction rate and unsatisfactory predation capacity. Moreover, mites were sluggish and had a swollen and whitish appearance (Beerling and van der Geest, 1991a). Squash preparations of symptomatic mites revealed the presence of numerous microsporidian spores and heavily infected

predators released spores after death (Beerling and van der Geest, 1991a). Microsporidia were also present in the prey mites but the mechanisms of pathogen transmission have not been determined for this system. Three types of microsporidian spores have been found in *N. cucumeris* and *N. barkeri* (Beerling *et al.*, 1993) but it is unclear if these represent one species of microsporidia with three different spore types or three distinct species. Oblong spores were detected in both predator and prey species, small and more oval spores were exclusively found in prey mites. Beerling *et al.* (1993) developed a monoclonal antibody ELISA as a bioassay for the detection of microsporidia in mass-reared *N. cucumeris* and *N. barkeri*. Monoclonal antibodies were produced for one spore type that was present in both predator and prey species. Further work is needed to determine the sensitivity of this test as a suitable screening method for microsporidia in mites. Interestingly, Huger (1988) detected the microsporidium *Nosema steinhausi* in diseased mass-reared populations of the same phytoseiid species (*N. cucumeris* and *N. barkeri*).

Three distinct species of microsporidia have been reported from *P. persimilis* from three commercial sources. The species assigned as *Microsporidium phytoseiuli* was isolated from a European population (Bjørnson *et al*., 1996), one unnamed species (A) was found in a population from North America and another unnamed species (B) in a population from Israel (Bjørnson and Keddie, 2000). Becnel *et al*. (2002) suggested that *Microsporidium phytoseiuli* may also be a member of the genus *Oligosporidium*, because of a number of biological and morphological similarities with *Oligosporidium occidentalis*.

The microsporidia of *P. persimilis* were not restricted to specific tissues and spores were found in muscle fibres, the super- and sub-oesophageal ganglia, ovaries, eggs, cells underlying the cuticle, and cells lining the caecal lumen and Malpighian tubules. Early development of all three microsporidia occurred in cells of the lyrate organ. The lyrate organ occupies a significant portion of the body and is thought to be involved in oogenesis or embryogenesis. Each microsporidium occupied a specific site within these cells. Infection of the lyrate organ may be necessary for the efficient vertical transmission of microsporidia in *P. persimilis* (Bjørnson *et al*., 1996; Bjørnson and Keddie, 2000).

M. phytoseiuli was not present in the prey mites, *Tetranychus urticae*. Therefore, prey mites did not contribute to pathogen transmission among *P. persimilis* mites. Maternal-mediated vertical transmission of *M. phytoseiuli* was 100%. Males did not contribute to infection of the progeny. Horizontal transmission of *M. phytoseiuli* did not occur when uninfected adult predators were kept together with infected *P. persimilis* females or on leaves carrying solutions of microsporidian spores. Horizontal transmission was low (about 15%) when uninfected immatures were kept together with infected adult and immature mites (Bjørnson and Keddie, 2001). At present little is known regarding the mechanisms of transmission.

Microsporidia-infected *P. persimilis* did not exhibit any obvious external symptoms. Therefore, routine monitoring is necessary to detect microsporidia when disease prevalence is low (Bjørnson and Keddie, 1999). *P. persimilis* infected by *M. phytoseiuli* produced fewer eggs, had a shorter longevity and lower prey consumption rate than healthy predators. Moreover, infected females produced fewer female progeny than uninfected females, as the sex ratio of offspring of infected females is male biased (Bjørnson and Keddie, 1999).

Several methods to cure an infection with microsporidia were tested by Bjørnson (1998). The antimicrobial compounds albendazole, fumagillin, metronidazole and nifedipine were ineffective for control of microsporidia in *P. persimilis*, regardless of their dose. The author doubted whether the chemical compounds were able to penetrate the egg corion. Rearing predators at 30ºC did not eliminate microsporidian infections either. The Pasteur method, whereby progeny of healthy mothers is selected for the rearing, was the only effective means to eliminate microsporidia from *P. persimilis* populations (Bjørnson, 1998).

Diseases caused by fungi

"The bewitched were the children and young women who were thought to have the symptoms of diabolical possession but which most likely were those of ergot fungus poisoning."
Kenneth Kipple, 1997 describing witches tried in 1692, (cited by Boucias and Pendland, 1998)

General characteristics

Fungi are eukaryotic heterotrophes that obtain nutrients either from dead organic matter (saprobes) or from living organisms (parasites). Some parasitic fungi are obligate pathogens, but the majority are facultative pathogens capable of growing without their host (Tanada and Kaya, 1993). Entomopathogenic fungi are characterized by their ability to attach to and penetrate host cuticle or spiracles; however, some penetrate through the gut. They replicate inside the host, usually in the hemocoel, where they compete for soluble nutrients and may release mycotoxins, which interfere with normal host development and metamorphosis and in some cases with the immune defense mechanisms (Boucias and Pendland, 1998). Fungi then invade and digest tissues and cause premature death of the host. Thereafter the fungus lives as a saprophyte on the cadaver, producing spores. Under unfavorable conditions resting forms are produced (Tanada and Kaya, 1993). Adhesion and germination of fungal spores on the host cuticle are highly dependent on relative humidity and temperature but light conditions and nutritional requirements are also important factors (Tanada and Kaya, 1993).

Diagnostic features considered as general characteristics of fungi infection in insects may include: blackening surfaces at sites where fungi have penetrated, coloration (white, yellow,

black), loss of appetite, the presence of filamentous hyphae, the presence of characteristically coloured reproductive structures (fruiting structures, spores) on the external surface of the dead host, weakness and partial paralysis, bodies may be hard (Boucias and Pendland, 1998).

In some cases, behavioural changes occur prior to death. Symptoms may include restlessness, loss of coordination and body tremors, loss of reproductive behaviour and changes in microhabitat preference (Horton and Moore, 1993; Boucias and Pendland, 1998). The latter include elevation-seeking behaviour (fungal "summit disease"), movement to exposed locations, change in oviposition or foraging sites and change in temperature preference (Horton and Moore, 1993).

Fungi in phytoseiid mites

Several reports exist on pathogenic fungi of phytoseiid mites up to now (Table 1). Field-collected *Euseius* (formerly *Amblyseius*) *citrifolius* Denmark and Muma were heavily infected by the fungus *Neozygites* sp. (Furtado *et al.*, 1996) and showed a high rate of mortality. Some cadavers carried near-white hyphae that produced pear-shaped conidia. However, *Amblyseius idaeus* Denmark and Muma and *Amblyseius limonicus* Garman and McGregor were not infected by *Neozygites* sp. isolated from the cassava green mite in laboratory tests (De Moraes and Delalibera, 1992). *Euseius citrifolius* collected in Brazil on two subsequent occasions contained viable resting spores and hyphal bodies of two distinct fungal species identified as *Neozygites acaricida* and *Neozygites* cf. *acaridis* (Keller, 1997). During an inventory of pathogens infecting plant inhabiting mites resting spores of Entomophtorales were observed in phytoseiid mites from Brazil (van der Geest *et al*., 2002). Moreover unidentified fungi were reported in microscopic investigations of a diseased population of *P. persimilis* (cited in Schütte *et al*., 2005).

Unidentified diseases

General characteristics

Insect diseases may be broadly categorised as either infectious or non-infectious, based on the respective presence or absence of a transmissible living organism. Diseases classified as non-infectious may be caused by mechanical injury, adverse physical environmental factors, chemical agents, injuries made by predators and parasitoids, genetic factors, nutritional deficiencies and hormonal disruption (Tanada and Kaya, 1993). Traditionally, insect pathologists have focused their research on infectious diseases that might be caused by a variety of pathogens. However, non-infectious diseases may play an important role in insect populations (Tanada and Kaya, 1993).

Unidentified diseases of phytoseiid mites

For phytoseiid mites several reports exist on poor performance, anatomical peculiarities and peculiar colorations (Tanigoshi *et al.*, 1981; Tanigoshi, 1982; Hess and Hoy, 1982; Bjørnson *et al*., 1997, 2000). However, in these cases it was not unambiguously shown that pathogens may have been involved (Table 1).

Tanigoshi *et al.* (1981) observed the formation of a dark-red occlusion within the alimentary tract near the distal end of the opisthosoma for *Neoseiulus (*formerly *Amblyseius*) *hibisci* Chant of both sexes when fed exclusively on *Panonychus citri* McGregor. Newly eclosed *N. hibisci* larvae acquired a red coloration of the gut directly after feeding and became less robust and vigorous after each moult. Complete immature mortality occurred at 32 and 35ºC. Immediately after the last moult female predators became dorso-ventrally flattened, more concave in profile, lethargic, did not lay eggs and exhibited the characteristic dark-red gut occlusion prior to their death. The pigmented mass inside the mite was thought to be associated with the incomplete digestion of the prey mites, as symptoms were not observed in mites fed a diet of pollen from the ice plant, *Malephora crocea* Jacq. (Tanigoshi *et al.*, 1981).

Birefringent, dumbbell-shaped crystals have been observed in *P. persimilis* from several sources (Bjørnson *et al*., 1997, 2000). Excessive crystal formation was associated with white discoloration of the opisthosoma. Discoloration may include (1) a white dorsal spot at the distal end of the opisthosoma, (2) two white stripes along the dorsal lateral sides of the body in the region of the Malpighian tubules or (3) a combination of both forms (Bjørnson *et al*., 2000). Mites carrying discoloration(s) appeared lethargic and provided poor pest control (Steiner, 1993b; Bjørnson *et al*., 1997). Rectal plugs, which were observed when symptoms were more pronounced, often disrupted normal excretion and might cause the affected individual to become stuck to the leaf surface (Bjørnson *et al*., 1997). The frequent occurrence of a prominent white dot in the opisthosoma of *P. persimilis* was correlated with reduced fecundity and predation rate in mites examined following shipment from commercial producers (Bjørnson *et al*., 2000). Crystals were observed in immature and adult *P. persimilis* (Bjørnson *et al*., 1997); therefore, non-excessive crystal formation is likely a normal physiological process (Bjørnson *et al*., 1997). An examination of *P. persimilis* from 14 commercial and academic sources revealed no correlation between the occurrence of crystals and the presence of microsporidia, rickettsia or virus-like particles in *P. persimilis* (Bjørnson *et al*., 1997). In a follow up study Bjørnson and Raworth (2003) found that the expression of white opisthosomal discolorations in *P. persimilis* does not necessarily affect predator performance and concluded that the opisthosomal discolorations are an expression of normal excretory function in *P. persimilis* related to plant nutrition (Bjørnson and Raworth, 2003).

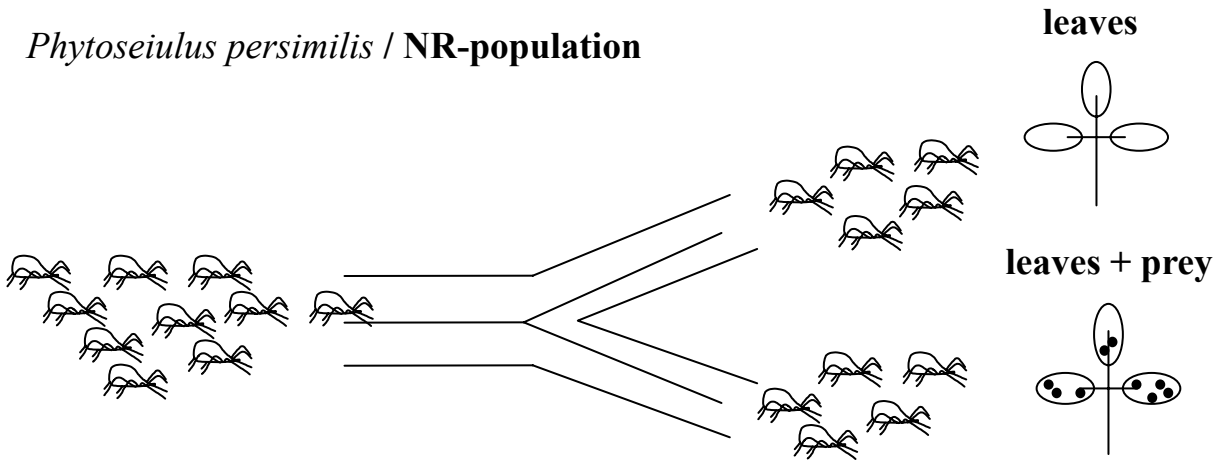
Conclusions

Several potential pathogens and pathogens in the true sense have been reported for phytoseiid mites. However, the status and impact of many described entities on their hosts is not clear. Fourteen reports are descriptive with unknown host effects; three reports mention pathological manifestations without proving the final cause of the symptoms and eight reports describe endosymbiotic bacteria. Only five reports present pathogens in the true sense of the word. From the latter group four reports refer to microsporidia and one to a fungus. Microsporidian infections often appear not to be readily visible as no obvious gross symptoms are present. Such infections may thus be undetected for extended periods meanwhile spreading in the case of exchange of predator populations among producers and laboratories. Screening of these pathogens on a regular base is therefore advisable for maintenance of healthy predator populations over long periods. However, as only few pathogens in the true sense are described up to now it is too early to plead for regular general pathogen screening in phytoseiid mites. The final conclusion of this review may thus be that more research on diseases of beneficial mites that are applied in biological pest control is needed in future.

Acknowledgements

We are grateful to Joop van Lenteren for his helpful comments on an earlier version of this chapter.

Phytoseiulus persimilis / **NR-population**



Chapter 3

Change in behavioural response to herbivore-induced plant volatiles of adult female predators

Abstract

Damage by herbivorous spider mites induces plants to produce volatiles that attract predatory mites that consume the spider mites. A clear attraction to volatiles from Lima bean plants infested with the spider mite *Tetranychus urticae* has been consistently reported during more than 15 years for the predatory mite *Phytoseiulus persimilis*. We have monitored the response to volatiles from spider-mite infested Lima bean plants for a laboratory population of the predatory mite from 1991-1995 on a regular basis. A reduction in the level of attraction in the laboratory population of *P. persimilis* was recorded in mid-1992. The attraction of this so-called non-responding (=NR-) population was weaker than that of a commercial population in the latter part of 1992, but the responses of these two populations were similarly weak in 1994 and 1995. Therefore, a behavioural change has also occurred in this commercial population. Experiments were carried out to address the potential causes of this change in attraction. The attraction of predators from a commercial population with a strong response decreased after being reared in our laboratory. Within a predator population with a low degree of attraction, strongly responding predators were present and they could be isolated on the basis of their behaviour: predators that stayed on spider-mite infested plants in the rearing set-up had a strong attraction, while predators that had dispersed from the rearing set-up were not attracted to prey-infested bean plants. From the NR-population isofemale lines were initiated and maintained for more than 20 generations. All isofemale lines exhibited a consistently strong attraction to spider mite-induced plant volatiles, similar to the attraction recorded for several populations in the past 15 years. Neither in a population with a strong attraction nor in two with a weak attraction was the response of the predators affected by a starvation period of 1-3 hr. Based on these results, possible causes for the observed reduction in predator attraction to herbivore-induced plant volatiles are discussed. The predatory mite *P. persimilis* is a cornerstone of biological control in many crops worldwide. Therefore, the change in foraging behaviour recorded in this predator may have serious consequences for biological control of spider mites.

Introduction

Carnivorous arthropods have well-developed foraging behaviours to find their herbivorous prey or host in complex environments (Vinson, 1976; Nordlund *et al*., 1981; Lewis, 1984; Vet and Dicke, 1992; Turlings *et al*., 1993; Dicke and Vet, 1999). A well-known source of information that is used during foraging consists of chemical cues (infochemicals, *sensu* Dicke and Sabelis, 1988b), produced either by their herbivorous victim or by the food plant of the herbivore. It is generally accepted that the efficient use of these cues is of crucial importance to the carnivore's fitness (Price, 1981; Sabelis and Dicke, 1985; Vet *et al*., 1990; Lewis *et al*., 1990; Vet and Dicke, 1992; Turlings *et al*., 1993; Godfray, 1994; Janssen *et al*., 2002). Variation in the responses to infochemicals that mediate prey/host location is commonly observed among individuals of a carnivore population and may have different causes: differences in experience with herbivore prey/hosts in certain microhabitats, differences in physiological state among carnivores, differences in environmental conditions or stimulus strength used during experiments, a disease, or genetic variation (e.g., Vet *et al*., 1990; Geden *et al*., 1992; Turlings *et al*., 1993; Dicke *et al*., 1998; Gu and Dorn, 2000; Colazza and Rosi, 2001; Maeda *et al*., 2001; Lewis *et al*., 2003).

Predatory mites (Acari, Phytoseiidae) are well known for their ability to use volatile infochemicals during distant prey location. The infochemicals involved are so-called herbivore-induced plant volatiles (=HIPV). They are produced by plants in response to feeding damage of spider mites, which are preyed upon by the predatory mites. These herbivore-induced plant volatiles affect foraging decisions of the predators during long-distance and short-distance prey searching (Sabelis and Dicke, 1985). The behavioural responses of predatory mites towards these HIPV have been reported in many studies for more than 15 years (for reviews see Sabelis and Dicke, 1985; Dicke *et al*., 1990a, 1998; Takabayashi *et al*., 1994; Sabelis *et al*., 1999; de Boer and Dicke, 2005). Most work has been done on the predator species *Phytoseiulus persimilis*, which is a specialist that feeds on spider mites in the genus *Tetranychus*. The ability of the predators to discriminate between volatiles from prey-infested and uninfested plants can be studied in a Y-tube olfactometer. When given a choice between *Tetranychus urticae*-infested bean leaves and uninfested bean leaves in a Y-tube olfactometer 70-95% of the predators prefer the volatiles of the infested leaves (Sabelis and Van de Baan, 1983; Sabelis *et al*., 1984a; Dong and Chant, 1986; Dicke *et al*., 1990b, 1991a, 1993). Such a discrimination between volatiles from *T. urticae*-infested and uninfested plants by *P. persimilis* has also been recorded for many other plant species (Dicke and Sabelis, 1988a; Dicke *et al*., 1990a, 1998; Bruin *et al*., 1992; Takabayashi *et al*., 1994; Krips *et al*., 1999b). Olfactometer data are supported by experiments on predator choices in a multiplant set-up (Janssen, 1999; Zemek and Nachman, 1999). The combined knowledge shows that the responses to HIPV are an impor-

tant determinant of local extermination of prey populations by the predatory mites (Sabelis and Van der Meer, 1986).

The response of the predators is phenotypically plastic. For instance, variation in the predator's response can be caused by experience with spider mites on a certain plant species. This results in an increase of the predator's response to HIPV emitted by that particular plant species (Dicke *et al*., 1990a, b; Takabayashi *et al*., 1994; Krips *et al*., 1999b; Koveos, *et al*., 1999). Another factor that may cause variation in the response is hunger level. An increase in degree of starvation can lead to an increase in the level of attraction to HIPV (e.g., Sabelis and Dicke, 1985). Furthermore, variation among individuals of a *P. persimilis* population with respect to the response to HIPV can also be of genetic nature (Margolies *et al*., 1997; Jia *et al*., 2002).

Here, we identify a change over time in the discrimination of individuals from *P. persimilis* populations between volatiles from *T. urticae*-infested and uninfested Lima bean leaves. This change is characterized by a reduction in the attraction to volatiles emitted by prey-infested plants. Our experiments address the potential causes of this phenomenon.

Materials and Methods

Plants

Lima bean plants (*Phaseolus lunatus* L. cultivar 'Sieva') were reared in a greenhouse at 20-25^{o}C, 50-80% relative humidity, under a light regime of at least 16 hr of light per day. Mercury discharge lamps switched on during the photophase when the light intensity dropped below 150 W/m^2.

Mites

The two-spotted spider mite, *Tetranychus urticae* Koch, was reared on Lima bean plants in a greenhouse under the same conditions as described for the bean plants.

The predatory mite *Phytoseiulus persimilis* Athias-Henriot was reared on detached Lima bean leaves that were infested by two-spotted spider mites. The bean leaves were placed on clay flower pots that were placed upside down in a water basin that served as a barrier to prevent predators from escaping. The water basin was placed in a cage in a greenhouse compartment under similar conditions as described for plant rearing.

Different predator populations were kept in different cages within the same greenhouse compartment. The cages were each surrounded by a water barrier. The non-responding population (=NR) had originally been obtained from Koppert Biological Systems B.V. and has been

reared in our laboratory for many years. In addition, we obtained predator populations A and C from two commercial mass productions in W-Europe.

Olfactometer

The behavioural assays were carried out in a closed-system glass Y-tube olfactometer. An air-streams was generated with pressurized filtered air and the vacuum system of the building. The air speed was 4 litres/min in each olfactometer arm, resulting in a speed of 8 litres/min in the basal tube. For a more detailed description of the olfactometer see Takabayashi and Dicke (1992).

The odour sources used were 9 trifoliate Lima bean leaves infested with ample amounts of two-spotted spider mites versus 9 uninfested trifoliate Lima bean leaves obtained from uninfested plants. Predatory mites were released individually into the olfactometer on the iron wire that was positioned in the centre of the glass tube. They were observed until they reached the end of one of the olfactometer arms. Predators that did not make a choice within 5 min were classified under "no-choice". These predators (typically ca. 5% of all predators tested) were excluded from statistical analysis and from the calculation of the percentage predators that chose the odour of infested leaves.

In experiments where the responses of two predator populations were compared on the same day, the same odour source was used for the two populations. In these cases predators from the two populations were alternatingly introduced into the olfactometer.

Unless stated otherwise, all predators used in the olfactometer were well-fed adult females, a total of 20 predators were used from a population per experimental day and the experiment was performed in our laboratory in Wageningen.

Experiment 1: Monitoring attraction to HIPV of predators from the NR-population and population A

Twenty adult female predators were collected from the mass rearing of the NR-population and their response in the olfactometer was recorded. This was repeated on different days, separated by at least one week, during 1991-1995.

A similar procedure was followed for population A during 1992-1995. Batches of predators from this population were obtained on a weekly basis and reared on spider mites from our laboratory *T. urticae* culture. Two days after their arrival, 20 adult females were collected and their response in the olfactometer was recorded. Subsequently, the predator batch was discarded. In many cases the predators from our laboratory population and from population A were tested on the same day, with the same odour source.

Experiment 2: Effect of rearing predators from populations A and C in our laboratory on their attraction to HIPV

Predators from populations A and C were shipped to Wageningen in the presence of bean leaves infested with *T. urticae*. This was done once every two weeks over 25 weeks in 1995 for population C and over 12 weeks in 1996 for population A. The predators arrived in well-fed condition and were immediately tested in the olfactometer. As a comparison, predators from the same source that arrived in our laboratory in the first shipment and that had been reared in our laboratory since their arrival, were tested on the same day with the same odour source.

Experiment 3: Attraction to HIPV of predators from population A tested in the United States

During a visit to Washington State University, Pullman, Washington, in the first half of 1993, predators were obtained from source A and reared on local spider mites (*T. urticae*) on Lima bean plants (same cultivar as used in Wageningen). This was done several times. A sample of 20 predators was tested in the olfactometer on each of 8 different days spread over 6 months. The predators were from different batches obtained from population A at the commercial mass rearing in western Europe.

Experiment 4: Comparison of predators that left and those that stayed in the rearing set-up

In a Perspex cage [120x120x30 cm (length x width x height)], we placed test tubes with detached Lima bean plants infested with spider mites and introduced predators from source A. The plants contacted the top of the cage, which was not completely closed: openings were present at the top edges through which predators could escape from the Perspex cage. A clay flowerpot was placed upside down on top of the cage, on top of which an Erlenmeyer flask filled with water was positioned. Although the density of spider mites on the plants was high, predatory mites were found on the top of the Erlenmeyer flask every day. These were collected once a day and tested immediately in the olfactometer with the same odour source as predators from the same population but collected on the spider mite-infested plants in the Perspex cage. This experiment was carried out at Washington State University, in the first half of 1993.

Experiment 5: Attraction to HIPV of predators from isofemale lines obtained from the NR-population

In 1994, isofemale lines were initiated with predators collected from the NR-population. Adult mated females were individually isolated in Petri dishes with a Lima bean leaf infested with spider mites. The Petri dishes were sealed with parafilm. Each individual female that survived and had laid sufficient eggs during the next 48 hr (2-9 eggs per female) was used to start an isofemale line. The eggs of one female were placed together in a Petri dish with a piece of bean leaf infested with spider mites. A new infested bean leaf was added after 2 and 4 days. After 7 days the offspring had developed into adults. Mating occurred among brother and sisters. Five adult females per Petri dish were transferred to a new Petri dish to lay eggs for the next generation. After 48 hr the females were discarded and their offspring were reared to adult and so on for subsequent generations. The isofemale lines were maintained during 20 generations. Predator rearing was done in a climate room at 23 ± 2 °C.

Two to three predators per isofemale line were tested in the olfactometer on each experimental day. All isofemale lines were tested on each experimental day with the same odour source. This was done over a period of 20 weeks. For each isofemale line a total of 30-42 individual predators were tested.

Experiment 6: Effect of short-term starvation

The effect of short-term starvation on the attraction to HIPV of *P. persimilis* was tested in the olfactometer in 1995. This was done for three populations: the NR-population, and populations A and C. Population C was shipped on spider-mite infested bean leaves and was tested immediately after arrival in our laboratory. Population A was shipped in the absence of food and the predators were reared on *T. urticae* on Lima bean for two days in our laboratory. For starvation, adult female predators were randomly collected from the populations and individually placed in an Eppendorf vial for 1-3 hr. For each population separately, these females were tested in the olfactometer together with satiated females, by alternately introducing females from the two treatments in the olfactometer. This experiment had two replicates (with 20 predatory mites each) for each population.

Statistics

The χ-square test was used to test for differences from a 50:50 distribution of predators over the two arms of the olfactometer. A contingency table was used to identify homogeneous groups of data within an annual data set (Sokal and Rohlf, 1981). An ANOVA was used to test for differences among years in the average percentage of predators that chose the infested

leaves in the olfactometer. If the ANOVA yielded a significant difference, an LSD multiple comparison test with Bonferroni correction was carried out. To compare the mean responses of two populations that were tested on several experimental days, we used a paired t-test.

Results

Change in degree of attraction in the NR-population (Experiment 1)

Olfactometer experiments with the predators usually result in 70-95% of the predators choosing *T. urticae*-infested leaves. However, in 1992 the NR-population shows a marked decrease in attraction after day 241 (Figure 1). Before day 197 on average 78% of the predators chose the infested leaves, but after day 241,this value is only 50%. The data points in 1992 do not form a homogeneous group (contingency table, $\chi^2 = 68.9$, df = 28, P = 0.001). The data points before day 197 and those after day 241 each comprise a homogeneous group (contingency tables, $\chi^2 = 8.6$, df = 13, P = 0.80 and $\chi^2 = 12.3$, df = 14, P = 0.58, respectively) and the two groups of data are significantly different (Figure 2).

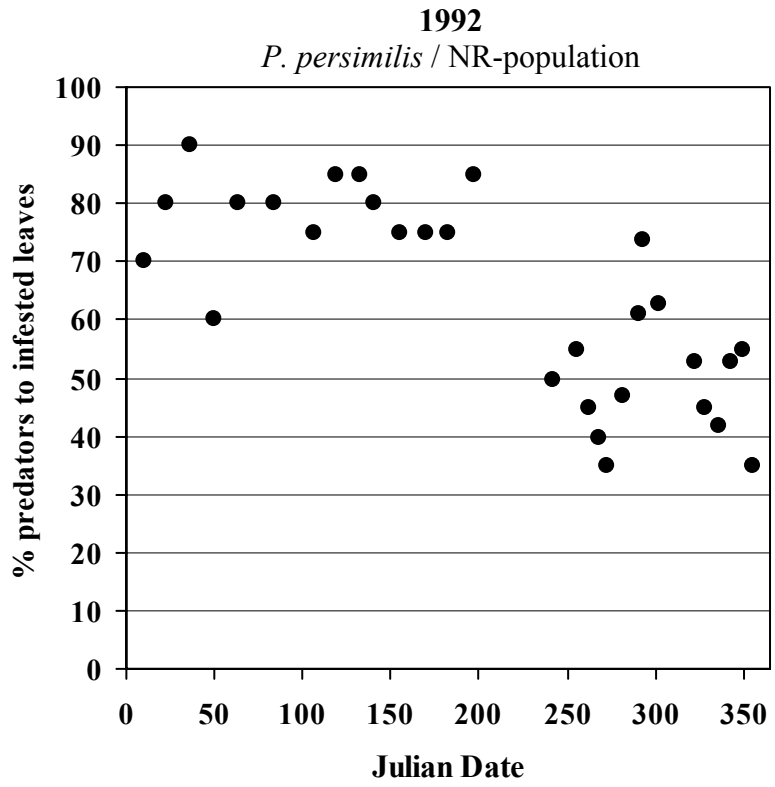


Figure 1: Percentage of adult female *P. persimilis* from the NR-population that chose the volatiles from spider-mite infested Lima bean leaves in the olfactometer. Recordings made at different dates throughout 1992. Each data point represents a test with 20 predators.

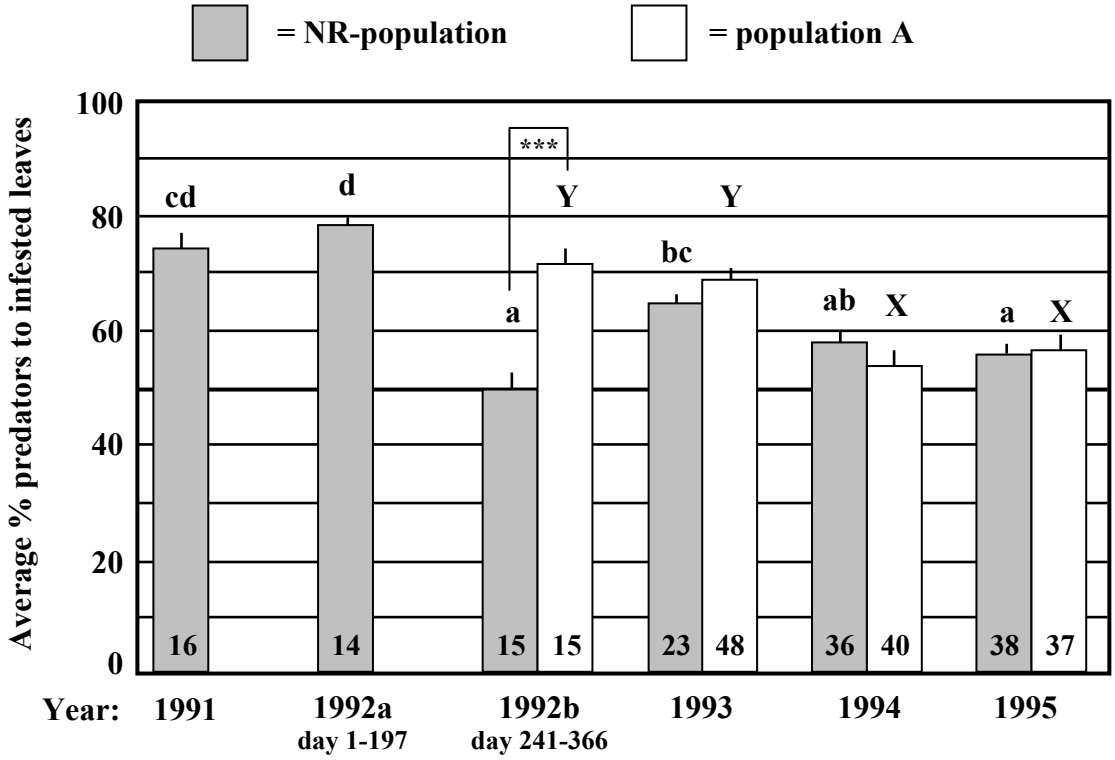


Figure 2: Average (± SE) annual percentage of adult female *P. persimilis* that chose the volatiles from spider-mite infested Lima bean leaves in the olfactometer. Data for NR-population and population A. Averages are significantly different within each population (ANOVA, NR-population: F = 17.3, P << 0.001; Population A: F = 13.8, P << 0.001). Different letters above bars of the same population indicate significant differences (LSD multiple comparisons test with Bonferroni correction). ***= P<0.001 (paired *t* test); numbers in bars represent the number of olfactometer tests with 20 predators each.

This difference between the early and late data for 1992 is not caused by an inferior quality of the odour source in the latter part of 1992: no striking changes were observed in the chemical profiles emitted by infested Lima bean leaves (M.A. Posthumus and M. Dicke, unpublished data). This is also supported by behavioural data: a *P. persimilis* population obtained from source A and tested in the latter part of 1992 had a response that did not differ from the NR-population's response in the first part of 1992 (paired *t* test, $t = 1.75$, df = 29, P = 0.09) (Figure 2). However, the response of population A in the second part of 1992 clearly differed from that of the NR-population in the second part of 1992 (Figure 2) (paired *t* test, $t = 5.2$, df = 28, P<<0.001). It should be noted that most of the data points for the 2 predator populations in the latter part of 1992 were obtained on the same experimental days with the same odour source.

Moreover, the data for the NR-population in the first 197 days of 1992 are not significantly different from data obtained in 1991, while the data in the latter part of 1992 are not significantly different from data obtained in 1994 and 1995 (Figure 2). In conclusion, a change in the attraction of the NR-population of *Phytoseiulus persimilis* occurred in mid-1992. A similar phenomenon has been recorded for population A, which had a significantly lower response in 1994 and 1995 than in 1992 and 1993 (Figure 2, see also below).

Change of degree of attraction in population A (Experiments 2 and 3)

We have investigated the attraction of *P. persimilis* from population A that were reared and tested in Pullman, Washington. The percentage of predators that chose the infested leaves varied between 30 and 92% (65±19%, average ± SD, N = 8). On several occasions the predator population died out within a few weeks after arrival from source A in Pullman, despite abundant food supplies and carefully controlled climatic conditions. In those cases dead females were observed from the day after arrival of the batch and few eggs were found in the culture. This was also observed from time to time in Wageningen with predators from source A since 1993. In some cases it was observed that predators which were obtained from source A would not lay eggs. Such situations were always encountered when the behavioural response of the predators was impaired. Thus, predators from population A that were reared in a laboratory in Pullman had, on average, a low degree of attraction in 1993.

In 1996 we recorded the attraction of newly arrived predators from population A versus predators from population A that were reared in our laboratory. This was done in our laboratory in Wageningen. Predators from population A that were tested immediately upon arrival in

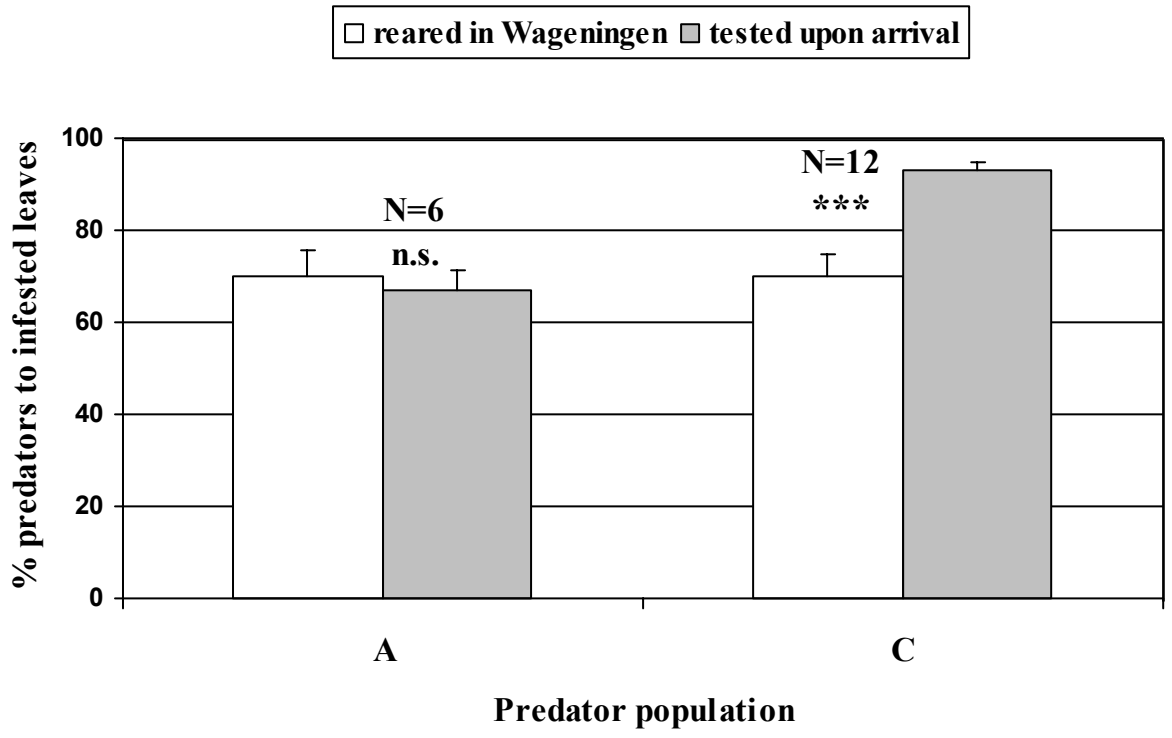


Figure 3: Percentage of predators (average ± SE) from two populations (A, C) that chose the volatiles from spider-mite infested Lima bean leaves in the olfactometer. For each population two groups of predators were tested simultaneously with the same odour source: predators that had been reared in our laboratory and predators that were tested immediately after arrival in our laboratory. n.s.= not significant, P > 0.05; ***= P< 0.001 (paired *t* test). N = number of olfactometer tests with 20 predators each.

Wageningen and predators from population A that had been reared in Wageningen had a similar degree of attraction: the average attraction was 70 vs. 67% (paired *t* test, $t = 0.54$, df = 10, P = 0.60) (Figure 3).

Thus, the population from source A also suffered from an impaired response to HIPV. This is not dependent on rearing the predators in our laboratory in Wageningen. In this context it should be stressed that transfer of predators from Wageningen to source A has never occurred.

Change in degree of attraction after transfer of predators from different populations to our laboratory (Experiment 2)

After recording the change in attraction of the NR-population, we studied the responses of predators from several other populations. In 1995 we compared the response of predators from population C that were tested immediately upon arrival in Wageningen with predators from population C that had been reared in our laboratory in Wageningen. The two groups were tested on the same day with the same odour sources. Predators newly arrived from source C had a very strong attraction (93%), which was significantly stronger (paired *t* test, $t = 4.49$, df = 22, P = 0.001) than the response of predators from the same population that had been reared in our laboratory (70%) (Figure 3). This shows that a reduced attraction can occur after transfer to our laboratory of predators from a population with a strong attraction.

Variation within a population (Experiments 4 and 5)

Careful observation of the data for the NR-population obtained from late 1992 through 1995 shows that there are oscillations in the percentage predators choosing for the infested leaves. At times the response is completely absent (i.e. ca. 50% of the predators choose the infested leaves at several subsequent dates), but at other times the response is around 70% or higher for

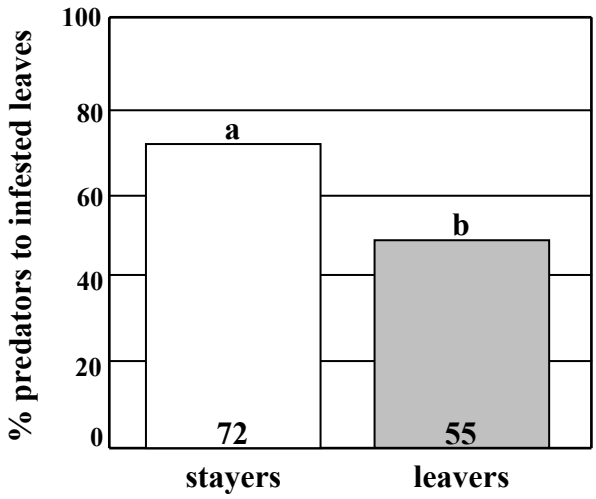


Figure 4: Percentage of adult female *P. persimilis* that chose the volatiles from spider-mite infested Lima bean leaves in the Y-tube olfactometer. Two groups of predators from a population obtained from source A were tested. One group of predators had left the rearing set-up (leavers) and the other had remained in the rearing set-up (stayers) where abundant food was present. For more details see Materials and Methods. Numbers in bars refer to actual number of predators. Different letters above bars indicate significant difference between bars (contingency table, *df* = 1, α = 0.05).

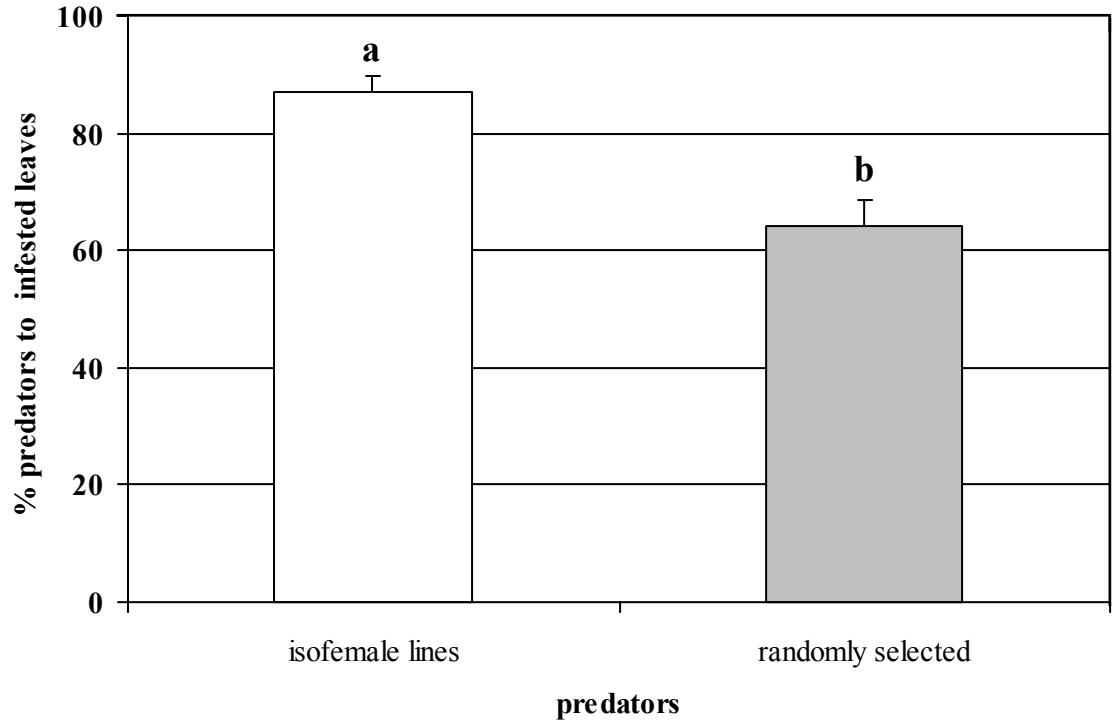


Figure 5: Average (± SE) percentage of adult female *P. persimilis* that chose the volatiles from spider-mite infested Lima bean leaves in the Y-tube olfactometer. Predators were either randomly selected from the NR-population or were taken from isofemale lines that had been obtained from the same population. The two groups of predators were tested 7 times (20 predators each time) on two subsequent days over a 4-month period. Different letters above bars indicate a significant difference between bars (paired *t* test, P<0.001).

several subsequent observations. This might be explained by individual differences among predators where at some times the responding type of individual dominates and at other times the non-responding type dominates in the population.

We compared the behaviour of predators from population A that stayed in the rearing in comparison to that of predators that had left the rearing. This relates to a population, which had a low degree of attraction (65±19%, average ± SD, N = 8; see above). The predators that had left the rearing set-up were not attracted to the odour of infested leaves, while the predators that had stayed in the rearing showed a higher degree of attraction (Figure 4). Thus, within a population that shows a low degree of attraction, there are 2 groups that differ greatly in degree of attraction to HIPV.

During the setting up of isofemale lines from the NR-population, which had a low degree of attraction, 40 females were individually isolated in Petri dishes. Of these, 21 predators survived after 2 days and of these 13 females had produced sufficient eggs (2-9 per female) to start an isofemale line. Two lines were lost due to mortality. The isofemale lines had very similar responses to volatiles from infested plants: 90 ± 3% (average ± SD, N = 11) of preda-

tors chose for the volatiles from infested leaves (30-42 predators tested for each of the 11 isofemale lines). The responses of these isofemale lines were compared to those of randomly selected predators from the NR-population that were tested in the olfactometer in the same weeks as the predators from the isofemale lines. This shows that the predators from the isofemale lines had a significantly stronger response to HIPV than randomly collected predators from the same population (paired *t* test, $t = 4.62$, df = 12, P = 0.001; Figure 5). Thus, isofemale lines with predators showing a high degree of attraction can be selected from a population that has a low degree of attraction on average.

Effect of starvation (Experiment 6)

Starvation for 1-3 hr did not affect the degree of attraction of the predators in any of the three populations tested. The percentages of satiated and starved predators that chose for the infested leaves was 53% (N = 30) and 53% (N = 40) for the NR-population, 38% (N = 40) and 53% (N = 40) for population A and 88% (N = 25) and 92% (N = 39) for population C, respectively. In all cases 40 predators were tested. Deviations of N from 40 indicate the numbers of predators that did not make a final choice within 5 min. Starvation increased the number of predators that made a final choice in the NR-population and population C, but did not affect the distribution of the choices.

Discussion

We have found a striking change in the response of a laboratory population of *P. persimilis* towards volatile infochemicals from *T. urticae*-infested Lima bean plants. Several potential causes for a difference in attraction to HIPV are known (see Introduction). Which of these can explain the observed change in degree of attraction in our laboratory population of *P. persimilis* will be discussed below.

Previous experience and physiological state

Previous experiences of predatory mites can affect the behavioural response towards HIPV. For instance, the attraction of *P. persimilis* to volatiles from cucumber plants or gerbera plants that are infested with *T. urticae* increases after the predators have been feeding for 6-7 days on spider mites on cucumber or gerbera leaves respectively (Dicke *et al*., 1990b; Takabayashi *et al*., 1994; Krips *et al*., 1999b). In addition, physiological state such as starvation level and type of food used for rearing can affect the response of phytoseiid mites to HIPV (Sabelis and Van de Baan, 1983; Dicke *et al*., 1986, 1998; Dicke and Groeneveld, 1986). However, the predators from the NR-population were reared under identical conditions and were all well-fed when tested in the olfactometer experiments carried out before mid-1992 and after mid-1992, and yet their behavioural response to plant odours differed markedly (Figure 1). Short periods

of starvation, which may occur among the predators tested, did not affect the attraction of the predators (Experiment 6). Predators from the isofemale lines were reared on the same food, on the same host plant as predators from the NR-population, and both groups were tested when satiated. Yet, their behavioural responses differed significantly (Figure 5). Thus, experience and physiological state can be excluded as a cause for the observed change in predator behaviour in the NR-population.

Odour quality

The odour sources used in the olfactometer experiments consisted of 9 Lima bean leaves amply infested with *T. urticae* for several days. This is a very strong odour source for the predatory mites (Sabelis and Van de Baan, 1983; Margolies *et al*., 1997). Even 2 Lima bean leaves, each infested with 100 *T. urticae* for 24 hr, result in a strong attraction of *P. persimilis* (Janssen *et al*., 1997). In our study we have recorded various examples of consistent differences among predators from different treatments when offered the same strong odour source (e.g. Figure 4). Furthermore, chemical analyses carried out since 1992 have shown that Lima bean plants infested by *T. urticae* still produce large amounts of the volatiles that were also recorded before 1992 (Dicke *et al*., 1990a, 1999). Thus, there is no evidence to support variation in odour quality as an explanation for the change in attraction of predatory mites.

Environmental variation

Environmental variation such as a decreasing barometric pressure may affect the response of arthropods to infochemicals (Lanier and Burns, 1978; Steinberg *et al*., 1992). However, in the second half of 1992, predators from the NR-population were tested together with predators from population A with the same odour source on the same day. Predators from population A had a significant attraction, while predators from the NR-population were not attracted (Figure 2). This was found for other experiments in this study as well. Therefore, environmental variation cannot explain the recorded change in attraction of *P. persimilis.*

Genetic change

Genetic differences in the strength of the response of the phytoseiid mite *P. persimilis* have been reported (Margolies *et al*., 1997). The data on individual variation among predators in a population (Figures 4 and 5) may suggest that genetic differences can explain our results. However, we have shown several times that the behavioural change invades an entire population at a high speed (Experiment 1 and 2), which is a characteristic of an infectious agent rather than of a genetic change. Therefore, the difference in attraction between females from the isofemale lines and females that were randomly selected from the NR-population (Figure 5) is most probably not a genetic difference, but a difference that can be transferred among

individuals. Another support of the hypothesis that the behavioural change is a symptom of an infectious disease are observations on predator mortality. We observed that the change in response in *P. persimilis* often co-occurred with increased mortality and low reproductive rates. This is also supported by the data on the initiation of the isofemale lines. When starting the isofemale lines with predators from the NR-population, 27 of 40 isolated females died within 48 hr. This is an unusually high mortality. The females that survived after 48 hr were the foundresses of the isofemale lines. They had a strong attraction and a low mortality.

Disease

Pathogens may affect the behaviour of their host, and this has been shown in several insect species (e.g., Horton and Moore, 1993). A few examples exist where micro-organisms affect the foraging decisions of their host (Geden *et al*., 1992; Horton and Moore, 1993). None of our experiments contradict the possibility of disease as the cause of the change in foraging behaviour of the predator *P. persimilis.* It may be expected that this disease is highly contagious as the level of attraction of predators from other populations soon decreased after transfer to our laboratory. We currently have one population with a strong attraction and one with a weak attraction in culture in our laboratory and can only maintain the high degree of attraction in the former by rearing it under very strict hygienic conditions.

Various micro-organisms have been recorded in phytoseiid mites (see chapter 2), including *P. persimilis* (Šut'áková and Rüttgen, 1978; Šut'áková and Arutunyan, 1990; Šut'áková, 1991; Steiner 1993a, b; Bjørnson *et al*., 1996, 1997; Bjørnson and Keddie 2000). There is one report of a microsporidium causing pathological effects, i.e. *Microsporidium phytoseiuli* infecting *P. persimilis* (Bjørnson and Keddie, 1999, 2001). However, there are no reports that micro-organisms cause behavioural changes in phytoseiid mites. Because it is uncommon to report the absence of a behavioural response, it remains obscure how widespread the reduction in attraction observed in the two *P. persimilis* populations is. The finding that the change may invade a population at a high speed suggests that it may be much more common than is currently thought.

The predator *P. persimilis* is an important biological control agent that is a cornerstone in the establishment of biological control in many crops worldwide (Helle and Sabelis, 1985; Van Lenteren and Woets 1988'; Van Lenteren *et al*.,1997). If the performance of this predator should be seriously impaired, biological control of spider mites would be badly hampered. Research into the disease and the possibilities to control it are therefore of great economic importance.

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Phytoseiulus persimilis / **NR-population**

NR-syndrome

Chapter 4

Behavioural and non-behavioural symptoms in adult female predators

Abstract

Adult female *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) of one of our laboratory populations show a lower degree of attraction to herbivore-induced plant volatiles than other laboratory populations. We hypothesized earlier that this consistent change in foraging behaviour is a symptom of a disease. Here we describe more symptoms by comparing adult females of this population (non-responding population) with adult females of other populations that are strongly attracted to herbivore-induced plant volatiles (responding populations). The most apparent characteristic of the non-responding (NR) population was the presence of numerous dorso-ventrally flattened females (76% of all females). These females had a normal size after mating but shrank during adulthood. Independent of their age, shrunken females did not reproduce and died a few days after shrinking. In addition to these profound differences in short term performance, females from the NR-population showed behavioural changes, including a lower degree of attraction to herbivore-induced plant volatiles and a shorter choice time in olfactometertests, a higher tendency to leave a prey-patch and a lower predation rate. Moreover, about half of the live females of the NR-population carried birefringent dumbbell-shaped crystals in the legs whereas live females of a responding population carried crystals only in the lumen of the Malpighian tubules and the rectum. The symptom "crystals in the legs" was correlated with low reproduction. Energy dispersive X-ray diffraction of these crystals revealed that they contain calcium and phosphorus along with carbon and oxygen. Crystals with comparable elemental compositions and the same characteristic concentric layering are well known in insects, where they are thought to play a major role in detoxification of calcium and heavy metals, and in storage of phosphorus. The fraction of predators carrying a white spot in the distal part of the opisthosoma, due to accumulation of excretory material in the rectum, was the same in both populations. Present results are discussed in the context of mite pathology and biological control.

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) has since long been applied in biological control of spider mites and is currently mass-reared for commercial purposes in several countries (Helle and Sabelis, 1985; van Lenteren, 1995, 2000; van Lenteren and Tommasini, 2003). Intensive mass-production together with frequent transport of arthropods may contribute to disease incidence (Sikorowski and Lawrence, 1994). Reports of poor performance of several commercial mite populations (Steiner, 1993a, b; Steiner and Bjørnson, 1996; Raworth and Bjørnson, 2002; Blümel and Hausdorf, 2002) and of consistent changes in foraging behaviour (Schütte *et al*., 1995) have therefore stimulated research in the pathology of this mite (Bjørnson 1998, Bjørnson and Keddie, 1999, 2000, 2001; Bjørnson *et al*., 1996, 1997, 2000; Schütte *et al*., 1995).

In our laboratory foraging behaviour of *P. persimilis* has been studied for many years. An important behavioural characteristic of adult females is their attraction to plant odours, which are released in response to feeding damage caused by their herbivorous prey, the two-spotted spider mite *Tetranychus urticae* Koch. The attraction to these herbivore-induced plant volatiles (=HIPV) has been reported in several other laboratories (see for a review Dicke *et al*., 1998, Sabelis *et al*., 1999). However, since mid-1992 our laboratory population showed a lower degree of attraction to HIPV (= non-responding population). In 1994 the same has happened in a commercially produced population (chapter 3). We demonstrated that neither variation in physiological state of the predators, nor previous predator experience, nor variation in odour blend or environmental parameters explain this consistent behavioural change (chapter 3). Moreover, we have shown several times that the behavioural change invades an entire population at a high speed, which is a characteristic of an infectious agent rather than of a genetic change. We therefore hypothesized that the behavioural change of *P. persimilis* represents a disease symptom.

Insect pathogens generally cause a set of characteristic distinct symptoms (syndrome) in their hosts (Lacey, 1997). Exact knowledge of a characteristic disease syndrome is indispensable for a good diagnosis and helpful for pathogen isolation. Here, we compare several characteristics of adult female predators from the non-responding (= NR-) population with females from a population that is strongly attracted to HIPV (referred to as 'population C' in chapter 3).

We quantified life-history parameters, including 'short-term fecundity' and 'mortality', which are currently used in commercial quality control programs of *P. persimilis* (van Lenteren, 1996, 1998, van Lenteren *et al*., 2003a). Several behavioural parameters including 'response to HIPV', 'tendency to leave a prey patch', 'predation rate' and 'walking speed'

are recorded, as predator behaviour plays a prominent role in foraging success and disease transmission (Andreadis, 1987; Sabelis and Dicke, 1985; Janssen, 1999; Zemek and Nachman, 1999). Moreover, we investigated the anatomical characteristics 'size' and 'opisthosomal discoloration', that have repeatedly been discussed in connection with low quality or (potential) diseases in various mite taxa (Smith & Cressman, 1962; Reed *et* al., 1972; Tanigoshi *et al*., 1981; Hess and Hoy, 1982; Smrž and Čatská, 1987; Zemek, 1993; Steiner 1993b; Bjørnson *et al*., 1997, 2000). In earlier studies we demonstrated that adult female predators of the NR-population may carry dumbbell-shaped crystals in their legs (Schütte *et al*., 1995). As predators belonging to this group showed a higher mortality, a lower short-term fecundity and a lower degree of attraction to HIPV than predators that carried excretory crystals exclusively in the Malpighian tubules and rectum (Schütte *et al*., 1995), we also included the parameter 'crystal location in the mite body' in our study.

Several authors report dumbbell-shaped crystals in phytoseiid mites, but they differ greatly concerning their conception on the role of such crystals in mite biology. Arutunyan (1985) detected such entities in the gut lumen of *P. persimilis* and described them as "bacteria". However, these entities showed a concentric layering, which is typical for excretory crystals of various compositions (Brown, 1982; Dallinger, 1993). Steiner (1993b) reported dumbbell-shaped entities with concentric layering for *P. persimilis* and to a lesser extent for *Neoseiulus cucumeris*. These entities, called "calcium crystals" by the author, were mostly found within the Malpighian tubules, but also in the digestive tract, cytoplasm and legs. The elemental composition of the crystals was not determined in this study. Di Palma (1996) reported similar spherical and dumbbell-shaped entities in the Malpighian tubules of the predatory mite *Typhlodromus rhenanoides*. In phytoseiid mites the Malpighian tubules regulate excretion, i.e. the elimination of non-gaseous by-products of cellular metabolism (Evans, 1992). Di Palma (1996) therefore suspected that the entities are excretory crystals of guanine or uric acid, which are the major nitrogenous excretory products in Acari (Evans, 1992). However, Bjørnson *et al*. (1997) demonstrated that crystals in *P. persimilis* are not common nitrogenous metabolites, as the elements detected by X-ray diffraction in these crystals are not present in guanine or uric acid.

As up to now no clear concept on the role of these crystals in mite biology has been proposed, we determined the elemental composition of the dumbbell-shaped crystals and discuss their possible functions.

Materials and Methods

Cultures

Plants and herbivores

Lima bean plants (*Phaseolus lunatus* L.) were reared in a greenhouse at 20-25 ^{o}C (L16:D8). The herbivorous two-spotted spider mite, *Tetranychus urticae* Koch, was reared on whole bean plants under the same conditions.

Predator populations

The **non-responding** population **(NR)** originated from a commercial mass producer and has been reared in our laboratory for many years in a semi-open rearing system. Detached Lima bean leaves infested with spider mites were placed on a plastic platform in a caged water basin at 20-25 ^{o}C (L16:D8). Fresh leaves were added every 2 to 3 days. Old leaves were removed weekly.

The **responding** population (**R1),** which was used in experiments 1 and 2, originated from another commercial mass producer ('Population C' in chapter 3). The responding population (**R2**), which was used in experiment 3 and for X-ray diffraction of excretory crystals, was obtained from the NR-population by several hygienic measures (see chapter 6). We had to use this population, as we could not get material of satisfying quality in terms of survival and fecundity from the commercial producer of the R1-population during these experiments. The two responding populations did not differ concerning the parameters tested here.

Both responding populations were cultured in a closed rearing system. Detached Lima bean leaves infested with spider mites were placed in Parafilm-sealed plastic Petri dishes (diameter = 9cm) in a climate chamber at 23±1 ^{o}C (L16:D8). In each dish 4 gravid females were kept for egg production during 48 hours after which females were eliminated. New leaves infested with spider mites were added every 2 to 3 days. After one week, when offspring had become mature, gravid females were transferred to new Petri dishes to initiate a new generation or used in experiments. Predators thus were reared in distinct generations. At least 15 dishes were prepared per generation.

Different rearing systems were used for two reasons: (1) a responding population looses its characteristics when reared in a semi-open rearing system at our laboratory (chapter 3); (2) the NR-population dies out when reared for several generations in a closed rearing system (C. Schütte, unpublished data).

Pre-experimental rearing

To minimize the effect of different rearing systems in our experiments all populations were kept in the closed rearing system described above for at least one generation prior to experiments. Twenty dishes were prepared per population. As female predators from the responding populations (R1 and R2) laid more eggs than females from the NR-population, several eggs were eliminated from dishes of the responding populations to obtain similar egg densities for both populations (ca. 10 eggs per dish).

Hygienic measures

All equipment used to handle predators and prey-infested leaves, like brushes and forceps, was sterilized in 0.5% sodium hypochlorite (NaClO) solution prior to use, after which it was rinsed with water several times.

Experimental work

Experiment 1: life-history, anatomy and foraging behaviour parameters

Experimental set-up

We measured several parameters for female predators of three age classes belonging either to the NR- or R1-population. Mated adult female predatory mites (7-9 days old, i.e. ca. 2-4 days since the final moult) were randomly collected from a pre-experimental rearing of the NR- or the R1-population. The response to HIPV and crystal location was determined for one third of the females immediately at the age of 7-9 days, for another third of the females at the age of 9-11 days and for the remaining third at the age of 13-15 days. Between these days the other parameters including size, mortality, fecundity, presence of discoloration and predator position were determined daily. Thus, predators of the second age class were observed during 2 days and predators of the third age class were observed during 6 days. The person measuring the parameters did not know to which population the predators belonged.

During the observation period predators were kept individually on a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm) placed in a climate chamber at 23±1 ^{o}C. Leaf discs were exchanged daily whereby discs cut from the same leaf were evenly distributed over the two populations. In total 120 predators of both the NR- and the R1-population were tested, i.e. 40 predators for each of the three age classes. This was done in two replicates (n=20 per age class). Predators lost during the experiment due to handling were excluded from data analysis.

Predator size

Well-fed mated female *P. persimilis* in the oviposition phase have a swollen pear-shaped body due to the eggs they carry. However, dorso-ventrally flattened female predators, resembling virgin or starved females, may occur in the NR-population. Such female predators can easily be distinguished from the normal-sized predators with the help of a stereomicroscope. As exact numerical size measurement on a daily base would inflict too much stress on live predators, we determined size categorical by classifying predators as either "normal-sized" or "small".

Predator mortality and fecundity

The number of dead predators and of eggs laid by live and dead predators was recorded daily. Egg presence was checked on the leaf disc as well as on the Petri dish.

Attraction to HIPV

The behavioural response of the predatory mites towards odours released by spider mite-infested plants was tested in a two-choice set-up. In a closed-system Y-tube olfactometer the odour from 9 trifoliate Lima bean leaves infested with ample amounts of two-spotted spider mites was offered vs. the odour from 9 uninfested trifoliate Lima bean leaves of comparable size. For a detailed description of the olfactometer see Takabayashi and Dicke (1992). Prior to testing, adult female predators were starved by keeping them individually in plastic Eppendorf vials (volume 1.5 ml) for 1 to 3 hours. Predators were then individually released into the olfactometer and observed until they made a choice for one of the odour sources for a maximum of 5 minutes. The time needed to make the choice and the number of turns during walking was reported for each individual. After testing 5 predators the two odour flows were exchanged, in order to avoid potential influences of asymmetry in the experimental set-up. Predators of the two populations were tested alternately with the same odour source to spread changes in odour emission during an experiment evenly over the two predator groups. The percentage of predators choosing the odour of infested leaves, the mean choice time and the number of turns were calculated from those predators making a choice within 5 minutes. Observations took place at room temperature.

Predator position

When kept on prey-infested leaves in sealed Petri dishes, mated female *P. persimilis* usually reside on the underside of the leaf and are rarely found on the Petri dish as long as ample food is available. The percentage of predators that were found on the leaf as opposed to the Petri dish during more than half of the observations, was calculated.

Opisthosomal discoloration

Opisthosomal discolorations of the predators were recorded daily. Obvious discolorations in *P. persimilis* may include (1) a white dorsal spot at the distal opisthosoma (2) two white stripes along the dorsal sides (3) a combination of both forms (see also Bjørnson *et al*., 1997, 2000).

Crystal location

Dumbbell-shaped crystals light up in polarized light, whereas other objects turn dark. Location of such crystals is therefore possible by observing the mite body under a light microscope equipped with two filters which create polarized light (Schütte *et al*., 1995). A live female predatory mite was placed on a glass slide in a droplet of water. Small pieces of micro- slides were placed next to the mite that was subsequently covered with an intact micro-slide. The micro-slide pieces next to the mite served to prevent damage to the mite. A photograph was taken of each mite. All photographs were pooled and divided into the following groups: (1) crystals present in the rectum and Malpighian tubules (Figure 1, right); (2) crystals present in at least one leg (in front legs crystal invasion must exceed the first segment; Figure 1, left); (3) crystal location not possible due to either bad quality photograph, bad quality of the microscopic preparation or predator damage during handling. The percentage of predators carrying crystals in the legs was calculated from the number of predators for which crystal location was possible.

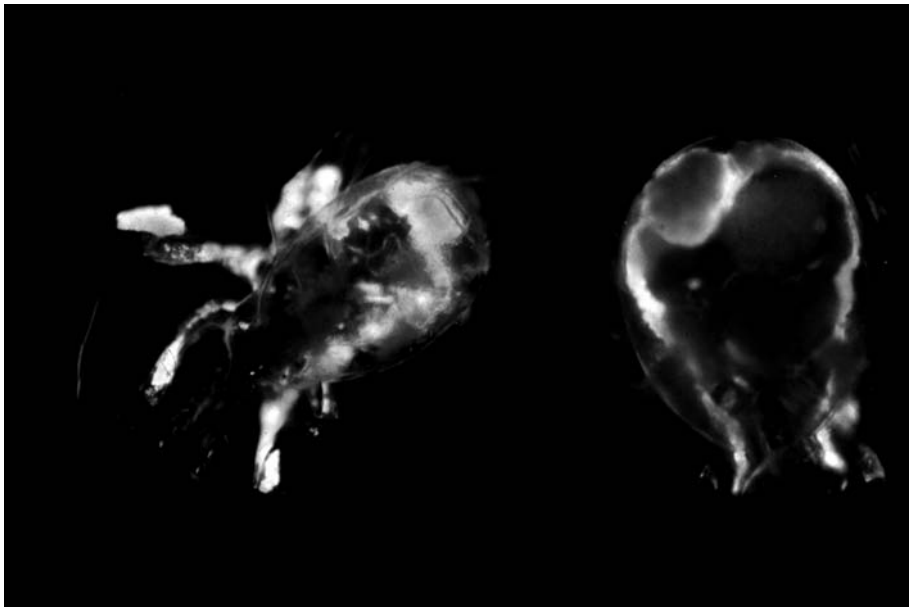

Figure 1: Adult female *P. persimilis* of the NR-population in polarized light with crystals present in their legs (left) and with crystals present in Malpighian tubules and rectum (right). Areas with white colour represent places of crystal accumulation.

Experiment 2: walking speed

The walking speed of adult female predatory mites of the NR- and the R1-population was determined on Lima bean leaf discs (16 cm^2) placed upside down on 1 % (w/v) agar in a Petri dish. The Petri dish was placed under a camera above a ring of fluorescent light. The images from the camera were analysed by the image analysis software Ethovision 1.70 (Noldus Information Technology Inc.).

Prior to testing, 7-9 days old female predators were starved by keeping them individually in plastic Eppendorf vials (volume 1.5 ml) during 1 to 3 hours. Predators were then placed individually on a leaf disc. A new leaf disc was used for each predator tested. The position of the predator on the leaf disc was recorded every 0.96 seconds and the mean walking speed was calculated for each predator until the moment it had left the leaf disc for more than 30 seconds. Observations took place at 23±1 ^oC. Predators of the two populations were tested alternately in order to spread potential fluctuations of environmental factors evenly over the two populations. Per population 20 predators were tested.

Experiment 3: predation and excretion rate

The predation rate of individual adult female predators of the NR- and R2-population was determined on Lima bean leaf discs (4 cm^2) placed upside down on wet cotton wool. Spider mite eggs originating from the general rearing served as prey for the predatory mites. On

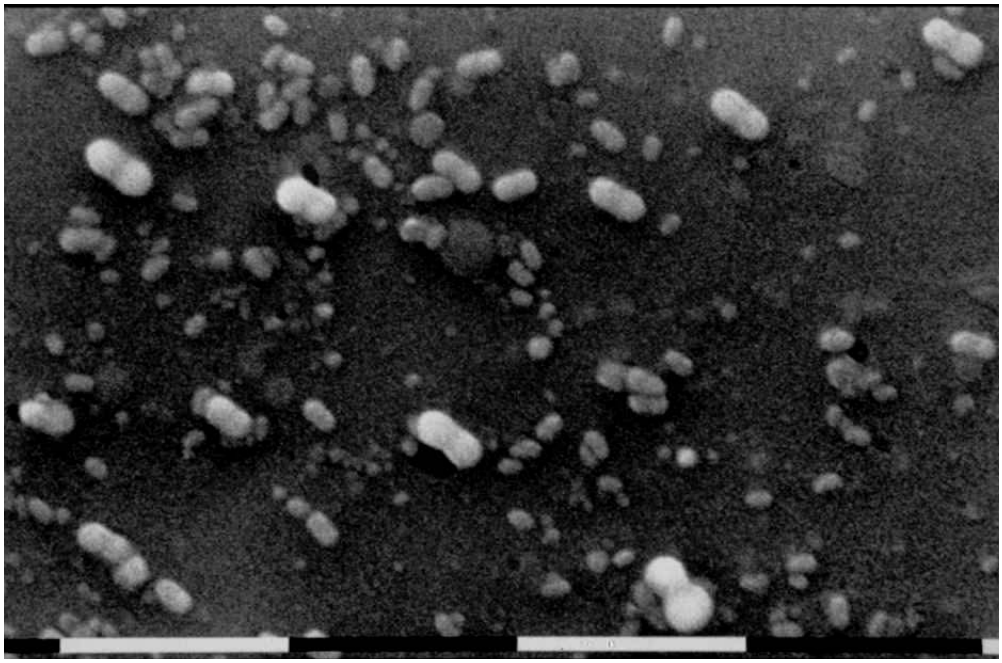

Figure 2: Scanning electron micrograph of dumbbell-shaped crystals of adult female *P. persimilis*. Scale bar = 10 µm

each leaf disc 32 spider mite eggs were placed in 4 rows, thus creating an egg density of 8 eggs/cm^2 (see Krips *et al*., 1999a). One 7-9 days old mated female predator was placed on each leaf disc. During a conditioning period of six hours and an experimental period of six hours, egg densities were checked and consumed eggs were replaced every 2 hours. A time interval of 2 hours in this set-up results in a maximum variation in egg density of 5% between two observations in the case of an average predation rate. The mean number of eggs consumed during 6 hours and the mean number of faeces droplets deposited on the leaf surface during 12 hours was determined per individual predator. For each population 35 predators were tested at the same time.

Elemental composition of dumbbell-shaped crystals

Dumbbell-shaped crystals (Figure 2) were analyzed by energy dispersive X-ray diffraction (EDX) in a Philips 535 scanning electron microscope to determine their composition. Six satiated adult female predators from the NR- or the R2-population were smeared on a scanning stub. The smears were coated with evaporated carbon before analysis. Characteristic dumbbell-shaped particles were then located with the scanning electron microscope. Microanalysis of a single crystal was done by use of an electron beam of 15 keV. Elements with atomic weights equal to carbon and higher can be detected with this method. However, nitrogen is not easily determined, as the nitrogen peak may coincide with the carbon peak.

Statistics

For analysis of numerical data we used the Mann-Whitney U test or the paired t-test. A contingency table test was used for categorical data. The data from the replicates were pooled, as no relevant differences were present between the replicates.

Results

Experiment 1: Life-history, anatomy and foraging behaviour parameters

Size

The most obvious characteristic of the NR-population was that numerous females shrank when becoming adult. Such females occurred infrequently in the R1-population. Shrunken females of the NR-population remained small until death or the end of the experiment. For each age class the fraction of small females was larger in the NR-population than in the R1-population ($P<0.001$ for each age class, Table 1 a-c). Females that did not shrink by the age of 10-12 days remained normal-sized during the whole observation period.

Table 1: Symptoms of adult female *P. persimilis* from the responding population (R1) and the non-responding population (NR). Females were tested at the age of 7-9 days (a), 9-11 days (b) or 13-15 days (c). Numbers in parentheses represent actual predator numbers. For explanation of symptoms see Materials and Methods.

1a) predator age = 7-9 days	**R1** (N = 39)	**NR** (N = 39)	P*
Predator size			
% small ♀♀	**0** (out of 39)	**41** (16 out of 39)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to HIPV	**92** (34 out of 37)	**61** (23 out of 38)	0.004
Choice time (seconds; average ± SD)	**109** ± 47 (37)	**76** ± 22 (38)	< 0.001
Crystal location within predator			
% live ♀♀ with crystals in the legs	**0** (out of 36)	**8** (3 out of 37)	0.25

1b) predator age = 9-11 days	**R1** (N = 40)	**NR** (N = 38)	P*
Predator size			
% small ♀♀	**3** (1 out of 40)	**76** (29 out of 38)	< 0.001
Predator mortality			
% dead ♀♀	**0** (out of 40)	**32** (12 out of 38)	< 0.001
Predator fecundity			
# eggs / ♀ / 2 days (average ± SD)	**8.6** ± 1.4 (40)	**2.5** ± 4.0 (38)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to HIPV	**95** (37 out of 39)	**79** (15 out of 19)	0.16
Choice time (seconds; average ± SD)	**116** ± 39 (39)	**85** ± 25 (19)	0.003
Discoloration on opisthosoma			
% live ♀♀ with white spot	**53** (21 out of 40)	**50** (13 out of 26)	> 0.5
Crystal location within predator			
% live ♀♀ with crystals in the legs	**0** (out of 37)	**56** (14 out of 25)	< 0.001

1c) predator age = 13-15 days	**R1** (N = 39)	**NR** (N = 35)	P*
Predator size			
% small ♀♀	**5** (2 out of 39)	**74** (26 out of 35)	< 0.001
Predator mortality			
% dead ♀♀	**8** (3 out of 39)	**69** (24 out of 35)	< 0.001
Predator fecundity			
# eggs / ♀ / 6 days (average ± SD)	**25.8** ± 4.5 (39)	**7.8** ± 11.9 (35)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to HIPV	**97** (33 out of 34)	**60** (6 out of 10)	0.007
Choice time (seconds, average ± SD)	**113** ± 41 (34)	**86** ± 35 (10)	0.026
Predator position within Petri-dish			
% ♀♀ > half of time on leaf	**95** (37 out of 39)	**46** (16 out of 35)	< 0.001
Discoloration on opisthosoma			
% live ♀♀ with white spot	**39** (14 out of 36)	**36** (4 out of 11)	> 0.5
Crystal location within predator			
% live ♀♀ with crystals in the legs	**0** (out of 34)	**25** (2 out of 8)	0.04

*** Mann -Whitney U test for numerical data, 2 by 2 contingency table test for categorical data.**

Predator mortality and fecundity

Mortality was higher in the NR-population than in the R1-population ($P<0.001$ for each age class, Table 1b & c). Moreover the number of eggs laid by individual females during the experimental period was significantly lower for the NR-population than for the R1-population (Table 1b & c, Mann-Whitney U test, $P<0.001$ for each age class). The NR-population represented two distinct groups of females differing greatly in size and fecundity. One group consisted of shrunken females that laid only 0-5 eggs per 6 days whereas the other group consisted of normal-sized females that deposited as many as 24-30 eggs per 6 days. The average number of eggs laid by normal sized females of the NR-population (9.2±1.1 eggs per 2 days, n=9 females; 27.7±2.3 per 6 days, n= 9 females) is comparable to data of the R1-population (Table 1b & c, first column).

Attraction to HIPV and choice time in the olfactometer

Clear differences in foraging behaviour were found between the two populations. At each age the response to HIPV was lower for the NR-population than for the R1-population. Differences between populations were significant at the age of 7-9 days ($P=0.004$, Table 1a) and at the age of 13-15 days ($P=0.007$, Table 1c). Moreover, predators from the NR-population needed less time to make a choice in the olfactometer than predators from the R1-population. The mean choice time was about 30 seconds shorter for predators from the NR-population, differences between populations being significant at each age (Mann-Whitney U test $P<0.001$ at the age of 7-9 days; $P=0.003$ at the age of 9-11 days and $P=0.026$ at the age of 13-15 days, Table 1a-c). Predators from both populations also differed in turning rate during the olfactometer test: only 8% of the predators of the NR-population made one or more turns (maximum number of turns = 3) whereas as much as 35% of the predators of the R1-population turned around (maximum number of turns = 8).

Predator position within the Petri dish

For 13-15 days old predators, the position within the Petri dish was determined. The percentage predators that were found on the prey-infested leaf as opposed to the Petri dish lid during more than half the number of observations was significantly lower for the NR-population compared to the R1-population ($P<0.001$, Table 1c).

White discoloration on opisthosoma

Live predators showing a prominent white spot at the distal opisthosoma were frequently recorded in both populations, whereas predators with white discoloration along the dorsal sides of the body were never recorded. In both populations the fraction of predators carrying a white spot was about one half for the age group 9-11 days and one third for the age group 13-15 (Table 1 b & c, $P>0.5$ for both age classes). Moreover, the symptom of discoloration

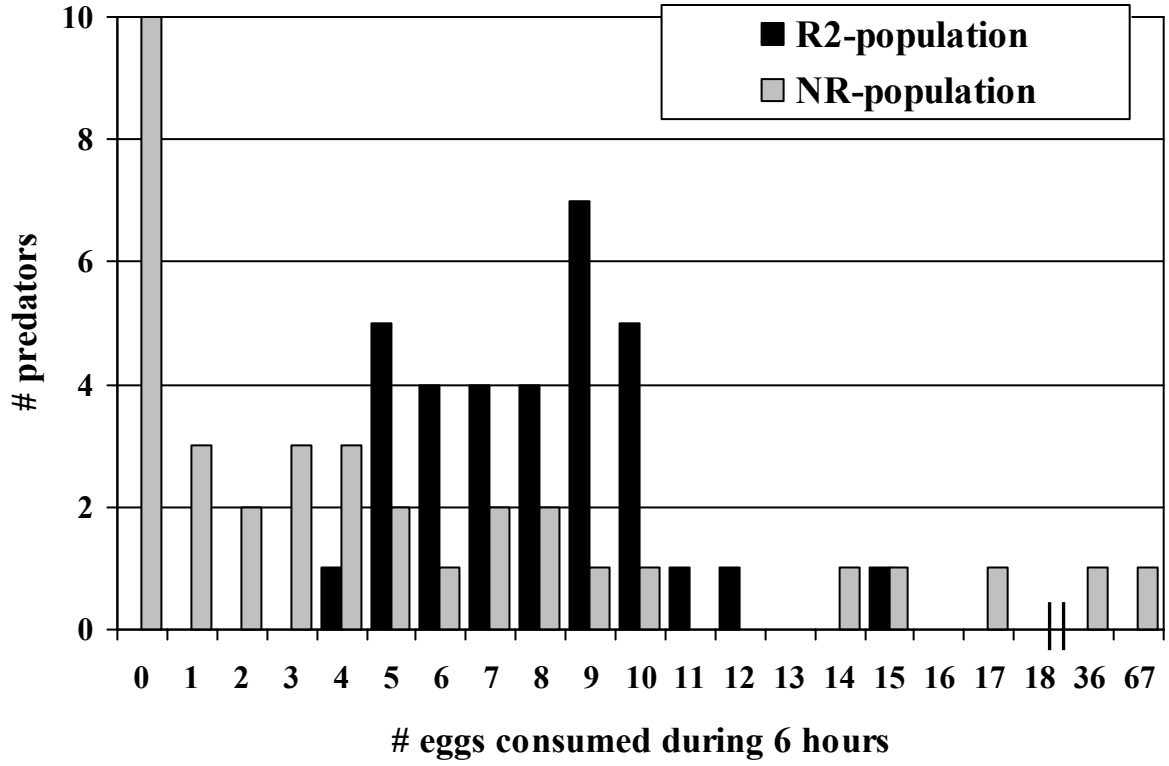

Figure 3: Number of eggs consumed during 6 hours by individual adult female *P. persimilis* collected either from the NR-population or the R2-population.

was reversible, as it changed frequently from one observation day to the next (45% of 226 sequential observations for the R1-population and 42% of 95 sequential observations for the NR-population respectively). In both populations opisthosomal discoloration was not correlated to fecundity at the age of 9-11 days. The 21 predators of the R1-population that showed a white dot laid 3.9±0.4 eggs per day whereas the 19 predators without white dot laid 3.9±0.9 eggs per day (t-test, P=0.9). The 13 predators of the NR-population that showed a white dot laid 1.1±1.9 eggs per day whereas the 13 predators without white dot laid 1.9±2.2 eggs per day (t-test, P=0.4).

Location of excretory crystals

None of the live predators of the R1-population carried crystals in the legs (Table 1 a-c). In contrast, some live predators of the NR-population of each age class carried crystals in their legs, differences being significant at the age of 9-11 days and 13-15 days (P<0.001, P=0,04 respectively; Table 1b & c). The presence of crystals in the legs was clearly correlated to lower fecundity. At the age of 9-11 days the 14 predators that carried crystals in their legs laid only 0.1±0.3 eggs per two days whereas the 11 predators without crystals in the legs laid 6.0±4.8 eggs per two days (t-test, P<0.01).

Experiment 2: walking speed

None of the predators tested stood still during the observation period and all predators left the leaf disc within 4 minutes. The walking speed of adult female predators did not differ significantly among the tested populations. The mean walking speed was 0.172±0.07 cm/sec for the NR-population compared to 0.168±0.05 cm/sec for the R1-population (t-test, P=0.68).

Experiment 3: predation and excretion rate

The mean number of eggs consumed during 6 hours was slightly higher for predators of the R2-population (8.0±2.4) than for predators from the NR-population (6.9±12.6, Mann-Whitney U test, P<0.001). Moreover, the data distribution of the NR-population showed a very unusual form (Figure 3). Ten females, which represent about one third of all individuals tested, did not feed at all, whereas two females "consumed" an exaggerate amount of 36 and 67 eggs respectively. Eggs attacked by these two mites appeared shrivelled as a punctured ball. Predators from the NR-population also excreted less faecal material. During the observation period of 12 hours predators from the NR-population deposited on average 0.9±1.9 faeces droplets whereas predators from the R2-population deposited as many as 3.8±2.1 faeces droplets (Mann-Whitney U test, P<0.001). Predators from the NR-population laid fewer eggs (0.2±0.5) than predators from the R2-population (1.7±0.5, Mann-Whitney U test, P<0.001)

Elemental composition of the crystals

Ten dumbbell-shaped crystals of adult female predators of the NR-population were scanned. The main components detected in 8 crystals were carbon, oxygen, calcium and phosphorus (Figure 4a). The amount of carbon was higher than when only the material surrounding the crystals was scanned (Figure 4b). In one of the ten crystals, carbon and oxygen were not detected and in another, calcium and phosphorous were not found.

Crystals originating from the R2-population had a similar composition. In 3 of the 5 scanned crystals carbon, oxygen, calcium and phosphorus were detected, whereas in 2 crystals calcium and phosphorous were not present. Aluminium and copper are background noise, as they are components of the scanning stub. When crystals were scanned on glass plates no aluminium and no copper were detected.

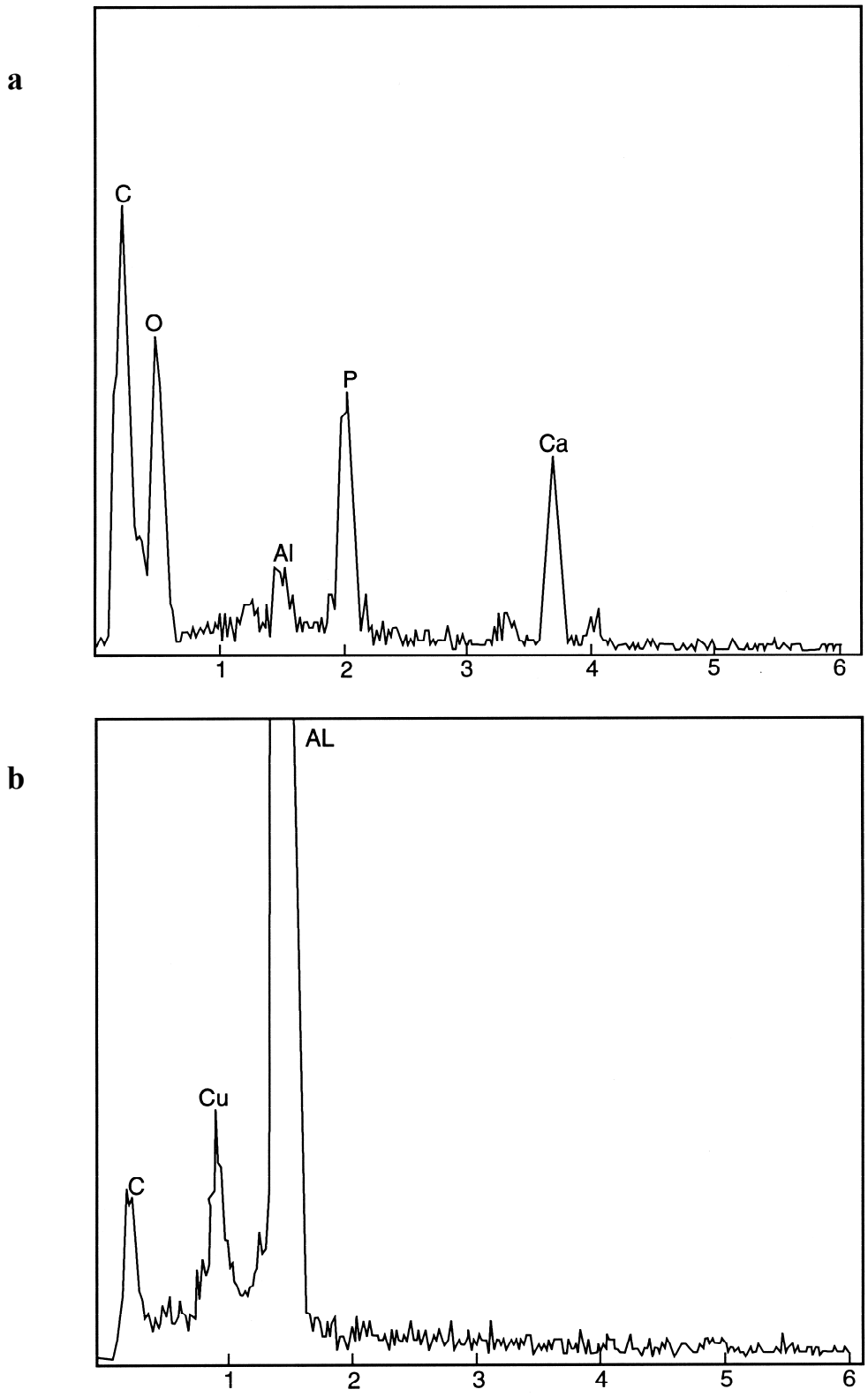

Figure 4: X-ray microanalysis spectra of a dumbbell-shaped crystal of a *P. persimilis* female collected from the NR-population (a) and the surrounding material (b). The horizontal axis represents X-ray energy in keV, the vertical axis indicates number of X-ray counts. C = carbon, O = oxygen, Cu = copper, Al = aluminium, P = phosphorus, Ca = calcium.

Discussion

Size, fecundity and mortality

The most conspicuous characteristic of the NR-population was a rapid and irreversible size change of mated adult females. Seventy-six percent of the adult females shrank, stopped reproduction and died several days later. Similar phenomena have been described for two other phytoseiid species (Tanigoshi *et al*., 1981, Hess and Hoy, 1982). Tanigoshi *et al*. explain shrinkage by nutrient-deficiency. Mature female *Neoseiulus hibisci* were dorso-ventrally flattened and concave, failed to oviposit, were lethargic and carried a dark-red opisthosomal discoloration when fed exclusively on citrus red mites (Tanigoshi *et al*., 1981). These symptoms did not appear when predators were kept on a pollen diet at the same time. Hess and Hoy (1982) describe the size change as part of a pathological manifestation. Female *Metaseiulus occidentalis* failed to oviposit, became very pale and so thin that they were translucent (Hess and Hoy, 1982). Immatures exhibited high mortality, especially at the time of their moults, and dense colonies seemed to dwindle away. Hess and Hoy (1982) observed two morphologically distinct forms of bacteria in these mites, one of them being present in large numbers inside thin and pale mites. However, it was not determined whether high microbial load was the primary cause of the disease or a secondary effect (see chapter 2 for further discussion).

Bjørnson and Keddie (1999) reported only slight size differences between female *P. persimilis* infected with *Microsporidium phytoseiuli* (Microsporidia) and uninfected females on the 5th day of development. Moreover, *M. phytoseiuli* had a less drastic effect on fecundity and survival than the disease of the present study. Microsporidia-infected females laid about 7 eggs less and did not die earlier than uninfected females during a period of 5 days. During the present study females of the NR-population laid about 20 eggs less and died earlier than females of the R1-population during a period of 6 days.

We are aware of one report in the literature of dorso-ventrally flattened non-reproductive female *P. persimilis* (Bjørnson *et al*., 2000). During a large scale study of *P. persimilis* from four commercial sources, 30% of all female predators (216 of 718) were dorso-ventrally flattened or had an intermediate body shape after a 24-h acclimation period during which feeding was possible (Bjørnson *et al*., 2000). Only a third of these predators of smaller body shape (68 of 216) did produce eggs. Known microsporidian diseases did not cause the size differences as no *Microsporidia* were detected in these predators. However, as the mating status was not determined, it could be possible that these females were virgin, as samples contained only 3% males. Nevertheless, these data pose the question whether the novel disease present in our laboratory population may also be present in other populations. We pre-

viously reported that the symptom of a reduced attraction to HIPV is present in several populations of *P. persimilis* (chapter 3).

Foraging behaviour

Adult female predators of the NR-population needed less time to make a final choice in the Y-tube olfactometer than females from the R1-population. The shorter choice time of predators from the NR-population may be caused by (1) a lower turning rate, (2) a higher walking activity, (3) a higher walking speed or a combination of these three components. Predators from the NR-populations made less turns while walking in the olfactometer. Moreover, predators from a responding population tend to stop at the Y-junction of the wire whereas predators from the NR-population tend to pass the junction without stopping (C. Schütte and H. Dijkman, personal observations). A lower turning rate combined with a higher walking activity in predators of the NR-population will lead to a faster movement from one place to the other, i.e. a lower choice time in the olfactometer. As exact determination of walking speed is not possible in the Y-tube olfactometer, we determined it in another set-up, where predators walked on a leaf surface. In this set-up no differences were found concerning walking speed and walking activity between both populations. The walking speed found in the present study (about 0.17 cm/sec) is higher than values found in other studies. In a comparable experimental set-up the highest mean walking speed found was 0.10±0.030 cm/sec on the Gerbera cultivar Bianca (Krips *et al*., 1999a). As leaf hairs have been shown to negatively influence the walking speed of *P. persimilis* (Krips *et al.*, 1999a) a higher walking speed may be expected on Lima bean that does not carry leaf hairs whereas the Gerbera cultivar Bianca carries about 100 hairs per cm^2. However, Sabelis (1981) found a walking speed of 0.12±0.015 cm/sec for starved predators on rose, which is also a plant without leaf hairs. In this study the behavioural observations were used only after a resting period of at least 5 minutes, so that the predator started walking spontaneously. It may be expected that predators walk at a lower speed when being undisturbed for a long time than directly after handling (Sabelis, 1981).

Adult female predators of the NR-population showed a lower degree of attraction to HIPV and a higher tendency of leaving a prey patch than females from the R1-population. This result is in accordance with earlier studies, where we demonstrated that predators that stayed on prey-infested bean plants in a rearing set-up were strongly attracted to HIPV, while predators that had dispersed from the rearing plants were not attracted (chapter 3). Behavioural mechanisms of attraction to, arrestment in and dispersal from prey-patches play an important role in foraging success of *P. persimilis* (Sabelis and Dicke, 1985; Dicke *et al*., 1998; Janssen, 1999; Zemek and Nachman, 1999; Pels and Sabelis, 1999). It may be expected that females from the NR-population will encounter less prey than their conspecifics

from a responding population. In addition they are more likely to disperse and less likely to meet conspecifics, which prefer to remain in prey patches until all prey is consumed (Pels and Sabelis, 1999). Host behaviour and mobility are regarded as key factors in initiating epizootics (Andreadis, 1987), especially in host-pathogen associations where the main transmission mode is horizontal and where infective stages are released by the host throughout the whole life. With the present data we cannot predict whether the behavioural changes recorded here will enhance or reduce pathogen transmission. More information about the timing of the recorded changes, other behavioural characteristics and effects of behavioural changes in (semi-) field situations is required (see for further discussion chapter 9). Besides one report of *Wolbachia*-induced behavioural changes in the spider mite *T. urticae* (Vala *et al*., 2004), we are not aware of other reports of behavioural changes due to pathogen infection in the Acari, whereas behavioural changes are well documented in insect pathology (see for a review Horton and Moore, 1993).

Predation and excretion

The dorso-ventrally flattened females of the NR-population resemble starved females and cannot be distinguished from them with a stereomicroscope. Sabelis (1981) recorded weight loss of well-fed female *P. persimilis* in the initial phase of oviposition from ca. 27µg to ca. 6µg after a 9 day-starvation period. These females were able to recover from starvation and to continue egg laying. Yao and Chant (1990) recorded weight losses from ca. 25µg to ca. 13µg in mated female *P. persimilis* after 21 hours of starvation. Well-fed females had an orange-coloured pear shaped body and were very active. After starvation, predators had a flat translucent body and they were still very active (Yao and Chant, 1990). These drastic changes of the body shape due to starvation are possible, because *P. persimilis* is only lightly sclerotized with a reduced dorsal shield (Yao and Chant, 1990).

It may thus be possible that females of the NR-population stop feeding, a diagnostic symptom in many insect diseases (Lacey, 1997), and that starvation induces some of the diagnostic symptoms recorded here. This hypothesis is supported by the data of the predation rate. Twenty-nine percent of the predators of the NR-population did not feed at all during the observation period of 6 hours, while all female predators of the R2-population did consume at least 4 spider mite eggs during that period. Moreover, predators from the NR-population excreted less faecal material, which is an inevitable result of reduced feeding.

Two females from the NR-population "consumed" as many as 36 and 67 eggs during the 6 hour observation period in addition to 27 and 54 eggs, respectively, during the 6 hour conditioning period. It is most probable that these mites rather attacked the eggs instead of ingesting the whole egg content, as the gut volume (=8.1µg) is not sufficient for such a big prey

turn-over. As the food content of a *T. urticae* egg is about 1µg (Sabelis, 1981), daily food intake would be as high as 126µg and 242µg respectively. These values clearly exceed the total daily food intake of 51µg calculated for adult female *P. persimilis* by Yao and Chant (1990). Moreover, no proportional amount of faeces was deposited by these mites (1 faecal droplet for the female consuming 121 eggs; no faeces for the female consuming 63 eggs). Eggs attacked by these two predators were still visible and resembled a punctured ball. Hence, we assume that the predators attacked the spider mite eggs by puncturing them with their stylets without consuming the (entire) egg content.

The predation rate of the R2-population (8 eggs / 6 hours) is in the range of other literature data. Krips *et al*. (1999a) found predation rates of female *P. persimilis* between 8 and 10 eggs / 8 hours at the same prey densities on three cultivars of *Gerbera jamesonii*. Bjørnson *et al*. (2000) determined the predation rate of female *P. persimilis* on *Phaseolus vulgaris* by offering 20 spider mite eggs to an individual predator. In this set-up a mean predation rate of 4 eggs / 4 hours was found. Sabelis (1981) reported a predation rate of oviposition females of 23 eggs / 24 hours.

Crystal location

Another characteristic of the NR-population is the location of birefringent excretory crystals in the legs of live female predators. Steiner (1993b) also reported the presence of dumbbell-shaped crystals in the legs of *P. persimilis* from commercial populations. During the present study predators with crystals in their legs laid fewer eggs than predators without crystals in the legs. These results are in accordance with an earlier study of the NR-population (Schütte *et al*., 1995). The presence of excretory crystals in the legs of adult female *P. persimilis* could be due either to excessive crystal production as has been described for two viral diseases in mites and insects (Smith and Cressman, 1962; Reed *et* al., 1972; Flipsen *et al*., 1995), or to disintegration of the Malpighian tubules. We favour the second hypothesis, as in individuals carrying crystals in the legs; Malpighian tubules and rectum were not clearly visible (Figure 1, left) while the Malpighian tubules were clearly visible in mites that carried crystals in the Malpighian tubules but not in the legs (Figure 1, right). Premortal tissue disintegration of at least the Malpighian tubules could cause the invasion of excretory material into the legs. Premortal tissue degradation in moribund individuals is common in viral and bacterial diseases of insects (Lacey, 1997).

Opisthosomal discoloration

Live predators showing a prominent white spot at the distal end of the opisthosoma were frequently recorded in both populations. The presence of such a discoloration may therefore not be regarded as a symptom of the disease studied here. In two other studies no relation

was found between discoloration and the presence of pathogens, especially microsporidia, in *P. persimilis* (Bjørnson *et al*., 1997, 2000).

Several studies report a relation between discoloration and performance of *P. persimilis* (Steiner, 1993b; Bjørnson *et al*., 1997, 2000). Mites displaying discoloration(s) appeared lethargic and provided poor pest control (Steiner, 1993b; Bjørnson *et al*., 1997). During a large-scale study of predators from four commercial sources frequent observation of a white spot per individual was correlated with a 45% reduction of fecundity (from 3.8 to 2.1 eggs/ female/day during an observation period of 7 days). Moreover, consumption rate of prey eggs decreased as the number of observations of white discoloration increased (Bjørnson *et al*., 2000).

We did not find a relation between the presence of a white spot in the region of the rectum and fecundity, neither for the NR- nor for the R1-population. This discrepancy may be due to the fact that we only found a discoloration in the form of a white spot whereas the other authors observed another type of discoloration in form of a white coloration along the dorsal sides of the body. This coloration may appear alone or in combination with a white spot (Steiner, 1993b; Bjørnson *et al*., 1997, 2000). However, in a follow-up study Bjørnson and Raworth (2003) also found that the expression of white opisthosomal discolorations in *P. persimilis* does not necessarily affect predator performance. The authors investigated the effect of plant nutrition on the expression of white opisthosomal discoloration in *P. persimilis*. Plants used for the spider mite rearing were treated with distilled water or one of three fertilizer concentrations. The proportion of predators showing white opisthosomal discolorations increased as fertilizer concentrations increased, but no significant differences were found between treatments concerning several life-history traits (total fecundity, mean daily oviposition, oviposition period, post-oviposition period and mortality). The authors concluded that the opisthosomal discolorations are an expression of normal excretory function in *P. persimilis* and that they are related to plant nutrition (Bjørnson and Raworth, 2003).

During the present study the white spot frequently vanished and reappeared between observations (45% of 226 sequential observations for the R1-population and 42% of 95 sequential observations for the NR-population respectively). This is in accordance with earlier data of Bjørnson *et al*. (2000), who recorded that opisthosomal discolorations of adult female *P. persimilis* changed in 53% of 2111 sequential observations. These frequent transformations are most probably caused by excretion of white material. Bjørnson *et al*. (2000) observed 198 egestions of *P. persimilis* during 2692 4-min observation periods. During 92.4% of these egestion events white material was excreted and most cases (79.1%) involved a simultaneous colour change from a prominent white spot to no coloration. Production and excretion of large amounts of excretory products may be expected in female *P. persimilis* as adult

female mites are characterized by a high rate of ingestion and a highly efficient food turnover. A mated female may ingest twice her own body weight during 24 hours (Yao and Chant, 1990), 70% of which is converted to eggs, under ample prey supply and optimal temperature (Sabelis, 1981). The white spot may thus be a normal phenomenon related to temporary accumulation of excretory products in the rectum, whereas a more intensive discoloration, caused by accumulation of excretory products in the Malpighian tubules, and the presence of rectal plugs are caused by problems in the excretory process, which may have negative effects on mite performance. The high relative humidity present in closed Petri dishes, used in the present study, may facilitate regular excretion and minimize extreme accumulations of excretory products and the development of rectal plugs.

Possible function of dumbbell-shaped crystals

In the present study X-ray diffraction of dumbbell-shaped crystals of the NR- as well as the R2-population revealed the presence of large amounts of calcium and phosphorus along with carbon and oxygen. Amounts of the different elements varied. Moreover, in one crystal carbon and oxygen were not detectable whereas in three crystals calcium and phosphorous were not. Crystals containing mainly calcium together with organic material and other elements such as phosphorus, potassium, magnesium and chlorine have been reported in all major phyla of invertebrates (Brown, 1982). The composition of most calcium-containing crystals is generally variable. Variation may be found even in crystals from different tissues of the same animal (Brown, 1982). In insects spherical crystals, showing a prominent concentric layering, are often found in the Malpighian tubules (Brown, 1982). Such crystals are thought to play a major role in detoxification of calcium and heavy metals, and in storage of phosphorus (Dallinger, 1993). Dumbbell-shaped crystals of *P. persimilis* most probably belong to this group of excretory products.

X-ray diffraction of dumbbell-shaped crystals of *P. persimilis* from a different source revealed a different elemental composition. Bjørnson *et al*. (1997) found high levels of potassium, low levels of phosphorus and traces of chlorine. In this case a carbon tape was used for adherence of the smears to the scanning stubs and smears were coated with gold. Such a coating makes detection of phosphorous and carbon difficult, as their peaks may coincide with the peak of gold (Anke Clerkx, personal communication). Differences in crystal composition may also be caused by different rearing conditions of the predatory mites. Wessing and Zierold (1992) found that diet affected composition of excretory crystals located in the Malpighian tubules of larval *Drosophila melanogaster*. Calcium, which was added to the diet of *D. melanogaster,* was mainly stored in the metal-containing crystals situated in the Malpighian tubules. Supply of calcium salts to bean plants induced the formation of a high amount of calcium oxalate crystals in the bean leaves (Zindler-Frank, 1995). As the prey of

P. persimilis is a leaf-parenchyma feeder, it is possible that prey mites take up calcium from plants, which in turn has to be effectively detoxified and stored in herbivorous prey mites and their predators. This hypothesis is in accordance with the findings of Raworth and Bjørnson (2002) that the occurrence of white discolorations in *P. persimilis* increases with increasing fertilisation of the plants, the food source of their prey. It could be possible that in this case higher amounts of phosphorous were ingested by *P. persimilis*, which were subsequently stored in dumbbell-shaped crystals. An elemental analysis was done only for the leaf-tissue, where elevated levels of several elements, including phosphorus, were detected.

Implications for biological control

Female *P. persimilis* of the NR-population show the following set of symptoms termed "non-responding syndrome" (=NR-): predators (1) shrink several days after mating, (2) cease egg production immediately after shrinking, (3) die several days after shrinking, (4) show the tendency to leave a prey patch with ample food, (5) show a lower degree of attraction to HIPV (6) move faster in the olfactometer (7) may cease prey consumption (8) carry excretory crystals in the legs and (9) have a lower excretion rate. It may be expected that this syndrome, which resembles a disease syndrome, has a negative impact on the effectiveness of *P. persimilis* in the biological control of spider mites. However, the present experiments have been executed in closed Petri dishes. Studies in a more realistic set-up in the open field or in glasshouses are needed to assess the effect of the NR-syndrome on predator performance in biological control.

Knowledge on pathogens of mass produced natural enemies is rather scarce (Bjørnson and Schütte, 2003; Schütte *et al*., 2005), whereas quality control has received more attention. Over the past decade, quality control guidelines have been established for more than 20 species of natural enemies, in order to assure and standardize an acceptable quality level of natural enemies on the market (van Lenteren, 1996, 1998, van Lenteren *et* al., 2003a). According to these quality control guidelines female *P. persimilis* should produce at least 10 eggs/female/5days and at least 80% of the tested females should be alive at the end of the test period of 5 days. Data from females that do not survive must be excluded from fecundity analysis (van Lenteren *et al*., 2003a). The NR-population of the present study would not meet the requirements for survival, as the survival rate was only 30%. However, the population did meet the requirements for fecundity, as the surviving females laid as many as 21.5 eggs/female/5days.

The results concerning crystal location in live female *P. persimilis* may be of value for the biological control industry. The demand for new, commercially feasible quality control tests is still growing, as maintenance of good quality standards is a key factor in augmentative

types of biological control (Bigler, 1989; van Lenteren, 1996, 2003a). Steinberg *et al*. (1999) suggested that the presence of discolorations and crystals in the legs should be included in quality control tests of *P. persimilis,* if the parameters prove to be correlated to mite performance. We here demonstrated that predator fecundity is negatively correlated with the parameter "presence of crystals in the legs".

We hypothesized earlier that the behavioural change of *P. persimilis* from our NR-population is a symptom of an infectious disease (see chapter 3). The present study supports this hypothesis as several characteristic symptoms were found in mated adult female predators that resemble a disease syndrome.

Acknowledgements

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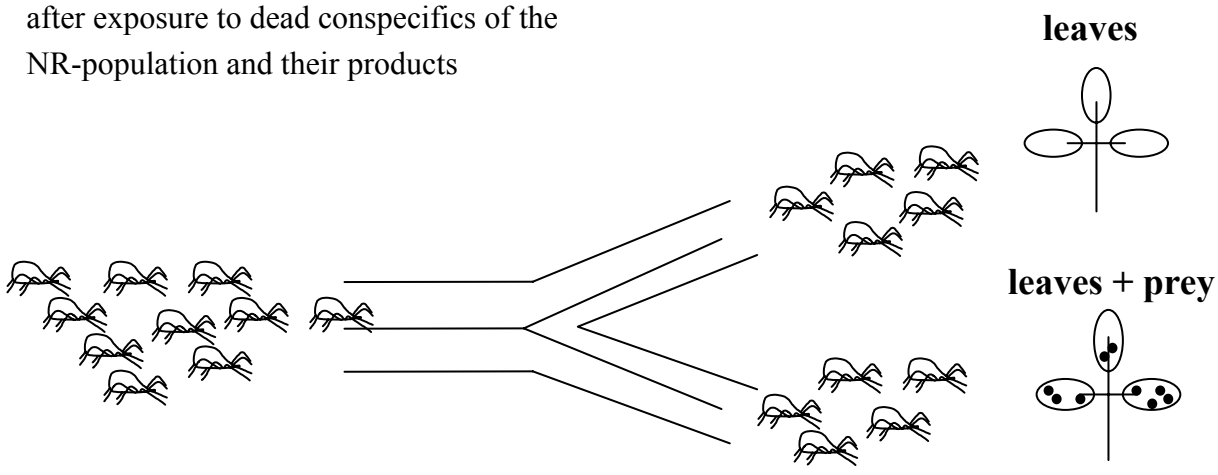
Phytoseiulus persimilis / **R-population**

after exposure to dead conspecifics of the NR-population and their products



Chapter 5

Change in foraging behaviour of adult female predators after exposure to dead conspecifics and their products

Abstract

Adult females of the predatory mite *Phytoseiulus persimilis* Athias-Henriot are strongly attracted to herbivore-induced plant volatiles (=HIPV) released by plants infested with their prey, the two-spotted spider mite *(Tetranychus urticae* Koch), thereby effectively locating their prey. However, we found a consistently lower degree of attraction to HIPV for a population of *P. persimilis*, which is called non-responding (=NR-) population. Here we demonstrate that this low degree of attraction is a contagious phenomenon and that it cannot be explained by differences in abiotic conditions, physiological state and experience of predators or by genetic differences between predator populations. Female predators exposed to dead conspecifics of the NR- population and their products showed a lower degree of attraction to HIPV and a higher mortality, than predators exposed to products of a living conspecific of the NR-population. This was true 6-7 days after contact with dead conspecifics and their products whereas 2 days after contact no effects were detected. The present results are discussed in view of our hypothesis that the change in foraging behaviour as well as the high mortality rate are symptoms of a contagious disease affecting the NR-population.

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot is a common biological control agent of the two-spotted spider mite (*Tetranychus urticae* Koch) being commercially mass-reared in many countries (Helle & Sabelis, 1985; van Lenteren, 1995; van Lenteren 2003b). Numerous studies have shown that adult female predatory mites are attracted to herbivore-induced plant volatiles (=HIPV) released by spider mite-infested plants (for reviews see Dicke *et al*., 1998; Sabelis *et al*., 1999; de Boer and Dicke, 2005). However, we demonstrated a lower degree of attraction to HIPV for a population of *P. persimilis* (Schütte *et al*., 1995). This population will be referred to as non-responding (=NR-) population. The transformation from a responding population to a non-responding population appeared suddenly and was persistent (chapter 3). Such a change in foraging behaviour was a new phenomenon in *P. persimilis* but has been found earlier in the predatory mites *Amblyseius potentillae* and *Typhlodromus pyri* (Dicke *et al*., 1991a).

Variation in foraging behaviour of predatory arthropods may be due to factors, such as varying abiotic conditions (Mikulecký and Zemek, 1992), genetic variation (Margolies *et al*., 1997), experience with prey (Krips *et al.*, 1999b; Papaj and Lewis, 1993;) and changes in physiological state (Dicke *et al*., 1986). A less often considered hypothesis is that infection with a pathogen may lead to altered foraging behaviour in carnivorous arthropods (Geden *et al*., 1992; Horton and Moore, 1993). Here we present a first experiment to test this hypothesis for the predatory mite *P. persimilis*.

If a disease causes a sudden change in foraging behaviour, it might be possible to induce the change in mites of a responding (=R-) population by bringing them in contact with individuals or residues of mites of a NR-population. In a diseased population early-dying individuals are likely candidates to carry and release pathogens. Common routes of horizontal disease transmission consist of pathogen release prior to death or after death (Andreadis, 1987) and cannibalism on dead, diseased conspecifics (Dhandapani *et al*., 1993). Cannibalism on living conspecifics has repeatedly been reported for *P. persimilis* its extent being variable and depending on satiation level of the predator (Walzer and Schausberger, 2002; Schausberger, 2003; De Courcy Williams *et al*., 2004b). Here we tested whether the presence of dead individuals of a NR-population of *P. persimilis* and their products released prior to death induce behavioural changes in mites of a R-population.

Materials and Methods

Cultures

Plants and herbivores

Lima bean plants (*Phaseolus lunatus* L.) were reared in a greenhouse at 20-25 ^{o}C (L16:D8). The two-spotted spider mite, *Tetranychus urticae* Koch, was reared on bean plants under the same conditions.

Predator populations

The **non-responding** population **(NR)** of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (originally obtained from a commercial mass producer in Western Europe) was cultured on detached bean leaves infested with spider mites on a plastic platform in a caged water basin at 20-25 ^{o}C (L16:D8). The **responding** population **(R)** was reared on detached bean leaves infested with spider mites in plastic Petri dishes (diameter= 9cm) sealed with parafilm. The Petri dishes were placed in a climate chamber at 23±1 ^{o}C (L16:D8). This rearing method was used because since the presence of the NR-population in our department, a responding population loses its response to HIPV when reared in an open rearing unit as described above (C. Schütte, unpubl.). The R-population consisted of several isofemale lines selected from the NR-population (see also chapter 3).

Bioassay

The behavioural response of the predatory mites towards HIPV was tested in a two-choice set-up. In a closed-system Y-tube olfactometer the odour of 9 trifoliate Lima bean leaves heavily infested with two-spotted spider mites was offered versus the odour of 9 uninfested trifoliate Lima bean leaves of comparable size. For a detailed description of the olfactometer see Takabayashi and Dicke (1992). Satiated adult female predators were tested individually. In this bioassay three classes of predators can be distinguished: (1) those making no choice during the observation period of five minutes, (2) those choosing the odour of uninfested leaves and (3) those choosing the odour of infested leaves.

Experimental treatment

The experiment was replicated 3 times, using the following treatment. To obtain dead and live predators from the NR-population to serve as "infection source" and control respectively, sixty egg-laying female predatory mites were randomly collected from the NR-population for each replicate. They were placed individually on a spider mite-infested bean leaf in a sealed plastic Petri dish (diameter = 9 cm). The Petri dishes were kept in a climate

chamber at 23±1 °C (L16:D8). The following day the number of dead individuals was recorded. Mortality was 22%, 25% and 33% for each replicate respectively. Dishes carrying a dead individual (for treatment) and a comparable number of dishes carrying an individual still alive (for control) were selected. The remaining Petri dishes were discarded from the experiment. For the treatment, the dead predatory mite from the NR-population was left in the dish; for the control the living predatory mite from the NR- population was eliminated from the dish. One mated female predator of the R-population (age 7-9 days) was introduced per dish. Thus, the introduced responding predator encountered a dead conspecific from the NR-population plus its products released prior to death (eggs, faeces and debris) in the treatment dishes, whereas in the control dishes the introduced responding predator only encountered products (eggs, faeces and debris) released by a live conspecific from the NR-population. After an incubation period of two days, each predator still alive was tested in the Y-tube olfactometer for its response to HIPV. Predators were subsequently transferred to new Petri dishes containing a spider mite-infested leaf. After another 4-5 days all predators still alive were tested again in the olfactometer. In the first and third replicate, treatment and control predators were tested alternately in the olfactometer with the same odour sources. Total numbers of predators from the three replicates were 56 for the control and 48 for the treatment. One control predator was lost due to escape on the third day.

Statistics

The pooled data of three replicates were tested in a contingency table test. Numbers per replicate were too small for separate analysis as well as for testing for homogeneity.

Results

None of the predators had died 2 days after the start of the experiment (Figure 1). In contrast, 6-7 days after the start of the experiment mortality of predators being exposed to dead conspecifics and their products was about three times greater than mortality of predators exposed to products of a living conspecific (Figure 1, contingency table test, P=0.033). Mortality was 16% (n=19), 0% (n=16) and 10% (n=20) for each control replicate respectively, whereas it was 39% (n=13), 13% (n=15) and 30% (n=20) for the corresponding treatment replicates respectively.

The percentage of predators choosing the odour of spider mite-infested leaves in the Y-tube olfactometer was more than 80% for both predator groups 2 days after the start of the experiment (Figure 2, contingency table test, P=0.94). A response of more than 80% to the odour of prey-infested leaves is comparable to data of responding predator populations (Sabelis and Van de Baan, 1983; Dicke *et al*., 1993; chapter 3). However, 6-7 days after the start of the experiment, only 67% of the predators exposed to dead conspecifics and their

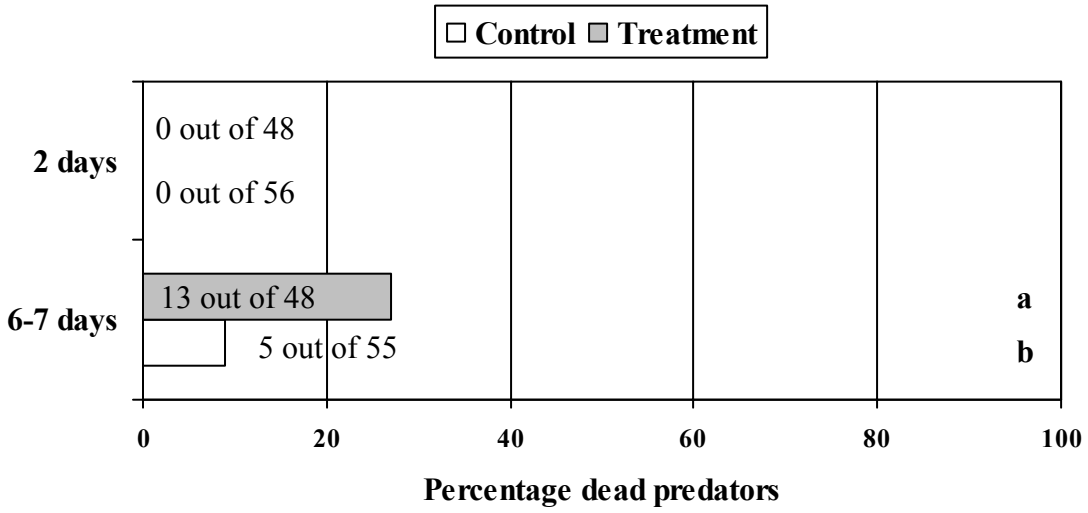


Figure 1: Percentage dead *P. persimilis* females 2 and 6-7 days after the start of the experiment. Females were previously exposed individually to a dead conspecific and its products (treatment), or to products of a living conspecific (control). Numbers in bars refer to actual numbers of dead predators. Different letters next to bars indicate significant differences (contingency table test per experimental day, α = 0.05).

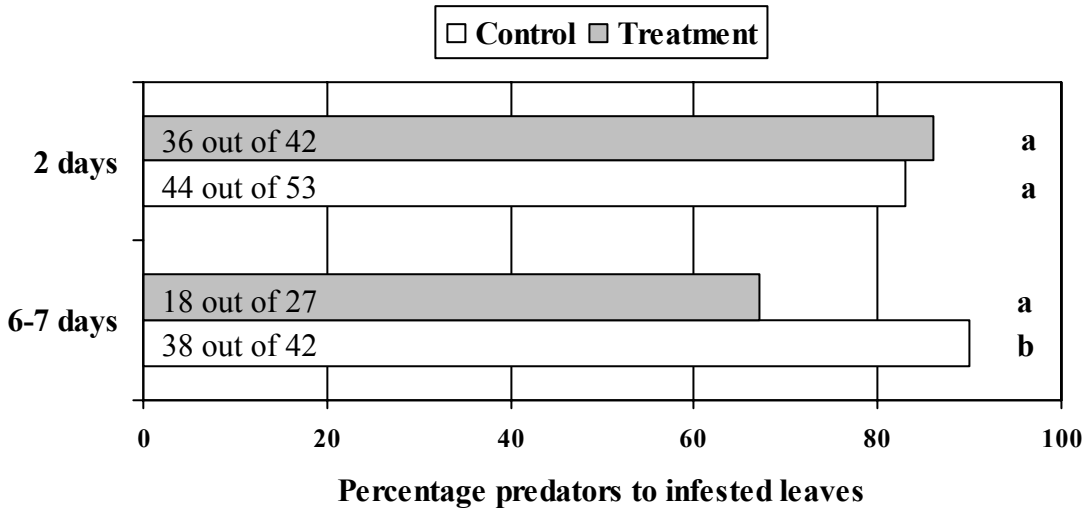


Figure 2: Percentage *P. persimilis* females that chose the odour of spider mite-infested leaves in a Y-tube olfactometer 2 and 6-7 days after start of the experiment. Percentages were calculated from numbers of predators that make a choice. Females were previously exposed individually to a dead conspecific and its products (treatment), or to products of a living conspecific (control). Numbers in bars refer to actual numbers of predators choosing the odour of prey-infested leaves. Different letters next to bars indicate significant differences (contingency table test per experimental day, α = 0.05).

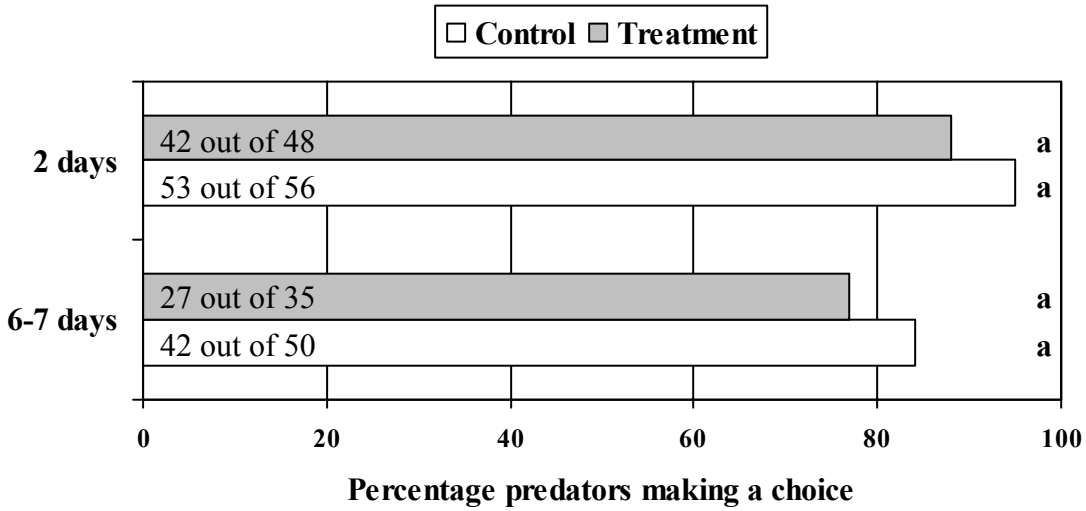


Figure 3: Percentage *P. persimilis* females making a choice for either of the two odour sources in a Y-tube olfactometer 2 and 6-7 days after start of the experiment. Females were previously exposed individually to a dead conspecific and its products (treatment), or to products of a living conspecific (control). Numbers in bars refer to actual numbers of predators making a choice. Different letters next to bars indicate significant differences (contingency table test per experimental day, $\alpha = 0.05$).

products chose the odour of spider mite-infested leaves, whereas 91% of predators exposed to products of a living conspecific did so (Figure 2, contingency table test, P=0.031). The response to the odour of prey-infested leaves was 100% (n=14), 75% (n=12) and 94% (n=16) for each control replicate respectively whereas it was only 50% (n=4) 75% (n=12) and 64% (n=11) for the corresponding treatment replicates respectively. A response of less than 70% to the odour of infested leaves is comparable to data of the NR-population (Schütte *et al*., 1995; chapter 3).

The percentage of predators that did not make a choice in the Y-tube olfactometer was not significantly different for the two predator groups on each test day (Figure 3). However, 6-7 days after the start of the experiment the percentage of non-choosers was slightly higher than for other data reported on responding populations. Usually less than 10% of predators from a responding population do not make a choice in the olfactometer during an observation period of 5 minutes (Dicke *et al*., 1993).

Discussion

The presence of dead conspecifics of a NR-population and their products induced behavioural as well as non-behavioural changes in predatory mites of the R-population in only about a week. Predators exposed to dead conspecifics and their products showed a lower degree of attraction to HIPV and a higher mortality than control predators. The fast changes

in mortality and response to HIPV cannot be explained by varying abiotic conditions, physiological state and experience of predators, or by genetic changes in the predators, as these factors should have the same impact on treatment and control predators. The results therefore support our hypothesis that a pathogen is inducing the change in foraging behaviour of *P. persimilis*. The disease-causing agent may have been released prior to, or after death.

There is a possibility that the described changes would also be induced in the presence of dead conspecifics of a responding population. We did not expose control predators to dead conspecifics of the R-population. Such an experiment was not feasible in an acceptable time, because it is impossible to obtain enough predators of the R-population dying a natural death while being of a comparable age (i.e. that were still carrying eggs) as predators of the NR-population (C. Schütte, unpubl.). Moreover, even no predator of the R-population may die during 24 hours when a batch of predators more than one month old was observed (C. Schütte, unpubl.). Furthermore, we may expect that predatory mites of the R-population regularly encounter dead conspecifics and their products in the standard rearing used for *P. persimilis*, where densities are high. On the other hand, this did not lead to induction of the described changes in numerous other studies (Sabelis and van de Baan, 1983; Dicke *et al*., 1993). Therefore, it is unlikely that dead conspecifics of the R-population induce the behavioural changes in conspecifics. Sterilisation of dead predators of the NR-population, which would be another possible control, was not done because the sterilizing agent may also affect the predator's behaviour.

Despite the intensive use of phytoseiid mites in biological control and reports of poor quality and performance (Steiner, 1993a, b; Steiner and Bjørnson, 1996; Bjørnson *et al*., 2000; Raworth and Bjørnson, 2002; Blümel and Hausdorf, 2002) information on diseases of these predators is rather scarce (van der Geest *et al*., 2000; Bjørnson and Schütte, 2003; Schütte *et al*., 2005; chapter 2). Only three micro-organisms have been studied in detail: *Rickettsiella phytoseiuli* (Šut'áková and Rüttgen, 1978; Šut'áková, 1988, 1991, 1994), *Wolbachia* (Steiner, 1993b; Breeuwer and Jacobs, 1996; Bjørnson *et al*., 1997; Weeks *et al*., 2003) and *Microsporidium phytoseiuli* (Bjørnson *et al*., 1996; Bjørnson and Keddie, 1999, 2001). Clear pathological effects have been reported only for *M. phytoseiuli* (see for a detailed discussion chapter 2 and chapter 9). At the time of this study light- and electron microscopic investigations did not reveal any obvious infections of microsporidia or other pathogens in predatory mites of our NR-population (S. Bjørnson, personal communication; C. Schütte, unpubl.). Future investigations will therefore aim at gaining more insight into the pathology and main transmission routes in order to identify the disease agent responsible for the behavioural change in *P. persimilis*.

The fact that parasites and pathogens may alter the behaviour of their hosts in a distinct way has been an interesting phenomenon to functional ecologists (Moore & Gotelli, 1990; Kuris, 1997; Thomas *et al*., 2005). Behavioural changes may result simply from direct physiological effects of the infection (Müller *et al*., 1997; Chow and Mackauer, 1999). However, behavioural changes may benefit the pathogen by enhancing disease transmission, and it has been argued that they are controlled by the pathogen (Stamp, 1981; Brodeur and McNeil, 1989, 1990, 1992; Schmid-Hempel and Müller, 1991; Müller and Schmid-Hempel, 1992; Krasnoff *et al*., 1995; Goulson, 1997). In other cases it was found that parasite-induced behavioural changes were advantageous to the host and it was argued that they may be controlled by the host (Shapiro, 1976; Smith Trail, 1980; Müller and Schmid-Hempel, 1993; McAllister and Roitberg, 1987; McAllister *et al*., 1990; Poulin and Brodeur, 1994). The pathogen inducing the behavioural changes in *P. persimilis* would offer interesting possibilities for studies on adaptive forces of behavioural changes induced by pathogens (see also chapter 9). A lower degree of attraction to HIPV results most probably in an early death from starvation of diseased predators, but it would on the same hand reduce disease transmission to conspecifics. More insight into the pathology and other behavioural changes induced by the pathogen is needed to enable a functional analysis of the behavioural changes in the interaction between *P. persimilis* and its pathogen.

Acknowledgements

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Phytoseiulus persimilis / **R-population**

after exposure to faeces and debris released
by predators from the NR-population

NR-syndrome

Chapter 6

Vertical and horizontal syndrome transmission by adult female predators

Abstract

Adult female *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) of one of our laboratory populations (=NR-population), show the following set of symptoms: predators shrink several days after mating, cease egg production and die several days after shrinking. They show a lower degree of attraction to herbivore-induced plant volatiles and a shorter choice time in olfactometertests, have the tendency to leave a prey patch with ample food, may carry excretory crystals in the legs, may cease prey consumption, and have a lower excretion rate. We hypothesized earlier that this characteristic syndrome, called non-responding (=NR-) syndrome, is caused by a pathogen infecting *P. persimilis*. To further support this hypothesis we here study several transmission modes of the NR-syndrome. In all tests we measured size, short-term fecundity, mortality, predator position, response to plant odours and crystal location, thus including 6 of the 9 symptoms known yet. No evidence was found for vertical syndrome transmission from parent to offspring. Eggs from symptomatic females of the NR-population mated by males of the NR-population gave rise to normal-sized, well-performing predators, when they had been surface sterilized or transferred to a new leaf. However, such eggs gave rise to shrunken females (17%) when left on the leaf where they had been laid. In the latter case transmission via products deposited on the leaf by the mothers was possible. We therefore tested several modes of horizontal transmission by exposing females of a commercial population that never showed the NR-syndrome (=R1-population) to products related to the symptomatic NR-population. No evidence was found for syndrome transmission via food or via squashed adult females. However, symptoms were induced in adult females of the R1-population after a 3-day exposure to a live adult female of the NR-population (incubation period = 3-7 days, fraction shrunken females = 53%) and after a 1-day exposure to faeces and debris collected from such females (incubation period = 2-4 days, fraction shrunken females = 65%). Contact with live females and faeces of the R1-population did not induce the syndrome. These results clearly indicate that the NR-syndrome is a contagious phenomenon and that the factor inducing the syndrome is transmitted horizontally among and between generations via faeces and debris deposited by symptomatic females. The results are discussed in the context of mite pathology and biological control.

Introduction

Adult female *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) of one of our laboratory populations (=NR-population), show the following set of symptoms: predators shrink several days after mating, cease egg production immediately after shrinking, die several days after shrinking, show a lower degree of attraction to herbivore-induced plant volatiles (=HIPV) and a shorter choice time in olfactometertests, have the tendency to leave a prey patch with ample food, may carry excretory crystals in the legs, may cease prey consumption and have a lower excretion rate (Schütte *et al*., 1995; chapter 3 and 4). The syndrome is called non-responding (=NR-) syndrome because the first symptom recorded was the reduced attraction to HIPV (Schütte *et al*, 1995; chapter 3). It is expected that predators showing this NR-syndrome will be less successful predators of spider mites than non-symptomatic predators. *P. persimilis* has become a biological control agent of large economical importance (van Lenteren *et al*., 1997; Garthwaite, 2000) and is used in research on predator-prey relations in several research groups (see for reviews Sabelis and Dicke, 1985; Dicke *et al*., 1998; Sabelis *et al*., 1999; de Boer and Dicke, 2005). Therefore, research aimed at curing of populations that show the NR-syndrome and sustaining of populations with a normal performance in laboratories and industrial rearings is important from a fundamental and applied perspective.

We have previously demonstrated that the lower degree of attraction to HIPV and the higher mortality are contagious phenomena (chapter 5) and that the lower degree of attraction to HIPV is present in at least one commercial population of *P. persimilis* (chapter 3). These findings led to the hypothesis that the behavioural change may represent a symptom of an infectious disease.

Several pathogens and potential pathogens have been described for phytoseiid mites (see for reviews: van der Geest *et al*., 2000; Bjørnson and Schütte, 2003; Schütte *et al*., 2005; Hoy and Jeyaprakash, 2005; chapter 2). Until now only five micro-organisms detected in *P. persimilis* have been studied in more detail: the rickettsia *Rickettsiella phytoseiuli* (see for a review Šut'áková, 1994) and *Wolbachia* sp. (Breeuwer and Jacobs, 1996), the microsporidium *Microsporidium phytoseiuli* (Bjørnson 1998, Bjørnson *et al*., 1996, Bjørnson and Keddie, 1999, 2001) and two non-identified species (Bjørnson and Keddie, 2000). Only for *M. phytoseiuli* clear pathological effects have been reported including negative effects on fecundity, longevity, predation rate and progeny sex ratio (Bjørnson and Keddie, 1999). However, it is unlikely that *M. phytoseiuli* or other microsporidia cause the characteristics of the NR-population, as microsporidia have never been detected in individuals of this population (S. Bjørnson and E. Beerling, personal communication; C. Schütte, unpublished data). It is thus

most likely that a novel disease is manifested in our laboratory population. In the present case, where no potential pathogens could be detected in microscopic studies, knowledge about the main transmission mode of the NR-syndrome may be an important step towards pathogen isolation. Moreover, information on the mode(s) of transmission is indispensable for the development of techniques for disease cure and prevention.

Pathogen transmission may be vertical or horizontal. Vertical transmission is defined as direct pathogen transfer from a parent organism to its offspring (Andreadis, 1987). Transmission from father to offspring (paternal-mediated) is not very common. Pathogens may be transferred to offspring via infected sperm or via veneral transfer during mating to the female with subsequent transfer to the eggs (Andreadis, 1987). Transmission from mother to offspring via the egg (maternal-mediated) is an important mode of transmission of many viruses and protozoa and may be the principal mean of transmission (Andreadis, 1987; Bjørnson and Keddie, 2001). Pathogens may be present on the egg surface (transovum transmission) or inside the egg (transovarial transmission).

Horizontal disease transmission is defined as pathogen transfer from individual to individual but not directly from parent to offspring. This can occur among and between generations and between different host species (Andreadis, 1987). In horizontal transmission pathogens may gain entrance into the host by passing the integument (many fungi and nematode species) or by entering body openings (all pathogen groups). Infection through the mouth (per os) is the most common way of entrance by insect pathogens under natural conditions (Andreadis, 1987). Consumption of contaminated food, faeces or conspecifics may cause infections and thus horizontal transmission.

We have investigated whether parent predators from the NR-population of *P. persimilis* may transfer the NR-syndrome directly to their offspring (vertical transmission), and whether the NR-syndrome is induced in non-symptomatic female predators of the R1-population after exposure to products related to the NR-population (horizontal transmission). To include as many symptoms as possible we have applied the earlier developed bioassay (chapter 4), in which 6 of the 9 symptoms of the NR-syndrome known can be measured simultaneously.

Materials and Methods

Cultures

Plants and herbivores

Lima bean plants (*Phaseolus lunatus* L.) were reared in a greenhouse at 20-25 ^{o}C (L16:D8). The herbivorous two-spotted spider mite, *Tetranychus urticae* Koch, was reared on whole bean plants under the same conditions.

Predator populations

The **non-responding** population **(NR)** originated from a commercial mass producer and has been reared in our laboratory for many years in a semi-open rearing system. Detached Lima bean leaves infested with spider mites were placed on a plastic platform in a caged water basin at 20-25 ^{o}C (L16:D8). Fresh leaves were added every 2 to 3 days. Old leaves were removed weekly.

The **responding** population **(R1)** originated from another commercial mass producer and was cultured in a closed rearing system. Detached Lima bean leaves infested with spider mites were placed in Parafilm-sealed plastic Petri dishes (diameter = 9cm) in a climate chamber at 23±1 ^{o}C (L16:D8). In each dish 4 gravid females were kept for egg production during 48 hours after which the females were removed. New leaves infested with spider mites were added every 2 to 3 days. After one week, when offspring had become mature, gravid females were transferred to new Petri dishes to initiate a new generation or they were used in experiments. Predators thus were reared in distinct generations. At least 15 dishes were prepared per generation.

Different rearing systems were used for two reasons: (1) the responding population looses its characteristics when reared in a semi-open rearing system in our laboratory (chapter 3); (2) the non-responding population dies out when reared for several generations in a closed rearing system (C. Schütte, unpublished data).

Pre-experimental rearing

To eliminate the effect of different rearing systems in our experiments both predator populations were kept in the closed rearing system described above for at least one generation prior to experiments. Twenty dishes were prepared per population. As female predators from the R1-population laid more eggs than females from the NR-population (Schütte *et al*., 1995, chapter 4), several eggs were eliminated from dishes of the R1-population to obtain similar egg densities for both populations (ca. 10 eggs per dish).

Pre-infection rearing of the R1-population

To minimize genetic variation and variation due to accidental contamination of rearing dishes we reared sisters of comparable age, mated by a brother, which then were equally distributed over control and treatment in experiment 2.3 and 2.4. Twenty-five mated female predators of the R1-population were placed individually on a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm). After 24 hours the females were removed. Dishes with a dead predator or less than 3 eggs were eliminated. The eggs of the remaining dishes were transferred to the underside of a prey-infested leaf in a new dish, as female predators prefer the underside for egg deposition. After 4 days new food was

added to each dish. After 8 days each Petri dish contained the adult offspring of one mother. Each Petri dish contained at least one male, which had inseminated its sisters, because in a batch of 4-5 eggs, which is the daily egg production of a healthy female, the first egg produced is usually a male (Amano and Chant, 1978).

Hygienic measures

All equipment used to handle predators and prey-infested leaves, like brushes and forceps, was sterilized in 0.5% sodium hypochlorite (NaClO) solution prior to use, after which they were rinsed with water several times.

Predator eggs were surface-sterilized by dipping them in a 0.5% sodium hypochlorite solution for 30 seconds. Subsequently they were dipped 3 times in sterilized water for 30 seconds each. Eggs were shortly dried on tissue paper and transferred to the underside of prey-infested bean leaves.

General bioassay set-up and symptom assessment

The following method was applied in each experiment. Adult female predators were kept individually on a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm) placed in a climate chamber at 23±1 ^{o}C. Leaf discs cut from one leaf were evenly distributed over treatments. Size, mortality, fecundity and predator position, were assessed daily. The response to HIPV and the location of excretory crystals were determined once on the last experimental day. The person measuring the parameters did not know to which population or treatment group the predators belonged. For a detailed description of symptom assessment see chapter 4.

Experimental treatments

Experiment 1: Vertical syndrome transmission (see Figure 1)

Here we tested whether parent predators from the NR-population can transmit the NR-syndrome to their offspring. Normal-sized mated females from the NR-population were collected for egg production from the semi-open predator rearing. Egg collection was done in the same way as for the pre-infection rearing. To eliminate effects of secondary micro-organisms, which may colonize dead individuals, eggs were only collected from dishes carrying a live predator. To estimate the infection of the mothers, we checked whether they became symptomatic. This experiment consisted of two replicates, with 71 and 79 eggs respectively.

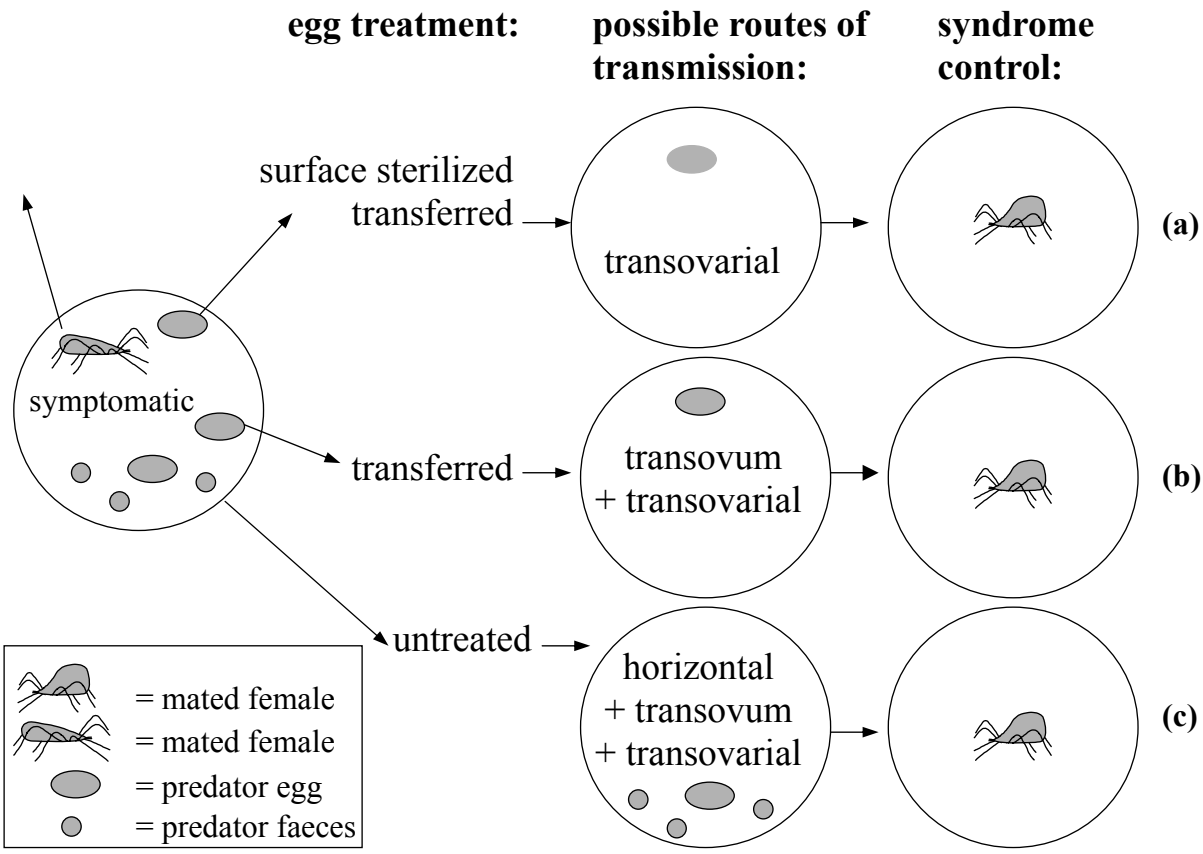

Figure 1: Schematic representation of experimental set-up to investigate the involvement of vertical transmission (Experiment 1)

Eggs were evenly distributed over the following three treatments:

(a) Eggs were surface-sterilized with sodium hypochlorite solution and transferred to the underside of a prey-infested leaf disc (viable microbes may only be present inside the egg = **transovarial transmission**).

(b) Eggs were transferred to the underside of a new prey-infested leaf disc that had not been in contact with a predatory mite (viable microbes may be present inside the egg and/or on the egg surface = **transovum transmission** + transovarial transmission).

(c) Eggs were left at the place where they had been deposited (viable microbes may be present inside the egg, on the egg surface and/or in products left on the leaf by the mother = **horizontal transmission** + transovum transmission + transovarial transmission).

The number of individuals not recovered for treatment a, b and c, was 4, 2 and 1 respectively during the egg stage and 3, 0 and 3 respectively during juvenile development. Juveniles were transferred to a fresh prey-infested leaf after 3 days. After 6 days all offspring had become adult and females and males belonging to the same treatment group were allowed to mate. A single copulation is sufficient for a female to reach maximum egg production (Schulten, 1985). Because the sex ratio of *P. persimilis* is female biased, males had to mate more than once. The duration of mating was observed to be sure that it was not interrupted early. A complete mating takes about 150 minutes (Schulten, 1985). After mating, size, mortality, fecundity and position of the female predators were recorded daily. The number of females lost during handling was 2, 0, and 1 for treatments a, b and c, respectively. When the post-oviposition period had lasted for at least 5 days for all predators, surviving predators were tested in the Y-tube olfactometer and crystal position was determined (see chapter 4 for methodology). During a period of 30 days, symptoms were measured for 27, 28 and 30 females for treatments a, b and c, respectively.

To test if the NR-syndrome appeared in the second generation, eggs were collected near the end of the oviposition period (= day 17 after mating). The number of eggs collected was 75, 75 and 61 for treatments a, b and c, respectively. These eggs were reared individually in a Petri dish rearing and from the age of 8 days the size of mated females was checked daily. When the females were 14 days old the following parameters were determined: number of eggs/female alive during 2 days, response to HIPV and crystal location (see chapter 4 for methodology). This experiment consisted of two replicates, with 15-18 mated females per treatment. In this way we tested 34, 31 and 33 second generation females for treatments a , b and c, respectively.

Experiment 2: Horizontal syndrome transmission (see Figure 2)

Experiment 2.1: Horizontal transmission via food

We tested whether consumption of prey mites from our laboratory rearing may induce the syndrome in predators from the R1-population. Mated adult female predators of unknown age were randomly taken from a batch of the R1-population directly after shipment from the producer and distributed over two groups. Predators were kept either on spider mite-infested leaves originating from the same commercial producer who delivered the R1-population (= control) or on spider mite-infested leaves originating from our laboratory (=treatment). As the quantities of food delivered by the commercial producer were a limiting factor we transferred predators to new leaf discs every second day. Eggs laid during the first day were eliminated. Parameters were measured for a period of 6 days. Three replicates were run, each with 10 predators per treatment.

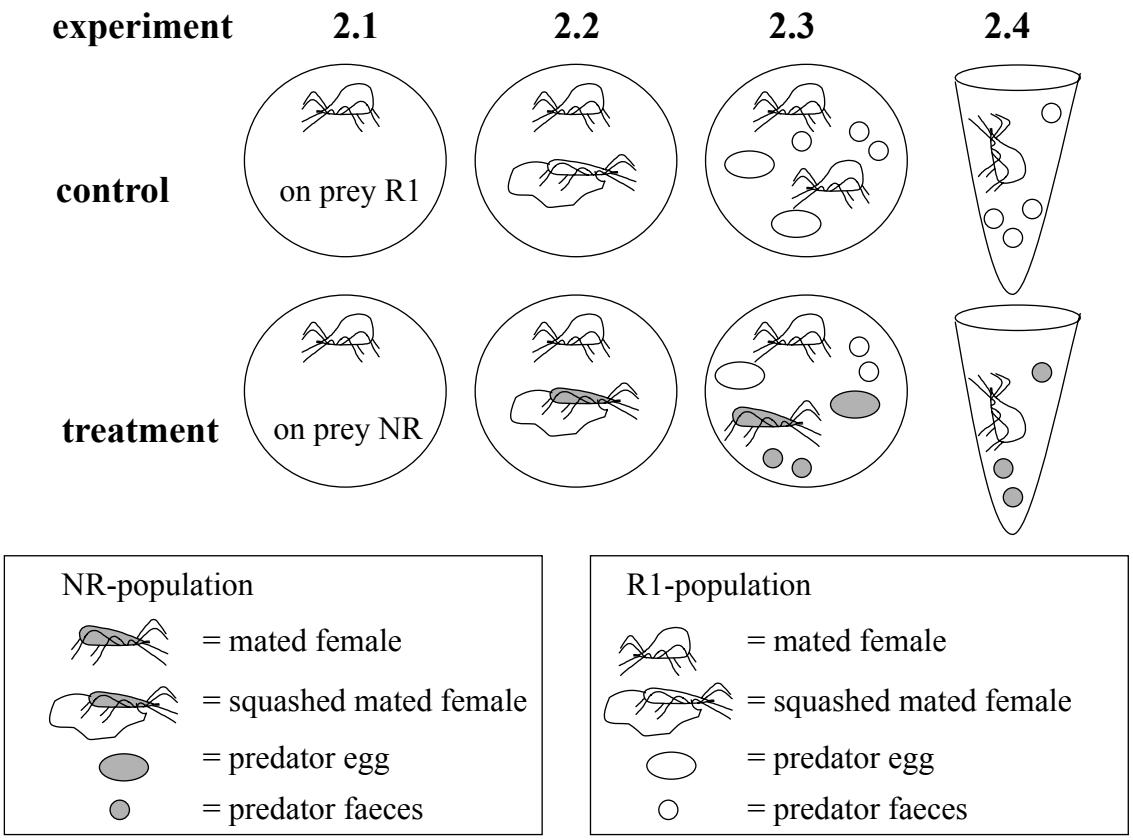

Figure 2: Schematic representation of experimental set-up to investigate the involvement of horizontal transmission (Experiment 2)

Experiment 2.2: Horizontal transmission via squashed female predators

Next, we tested whether the presence of squashed female predators from the NR-population can induce the syndrome in predators of the R1-population. Experimental set-up and replicate number were the same as in experiment 2.1. Mated adult female predators of the R1-population were distributed over two groups. Predators of both groups were kept on spider mite-infested leaf discs originating from the commercial producer who also delivered the R1-population**.** Leaf discs either carried two squashed female predators of the R1-population (=control) or two squashed female predators of the NR-population (=treatment). The squashed females were collected from newly delivered material of the R1-population and from the open rearing of the NR-population.

Experiment 2.3: Horizontal transmission via live female predators

Tested was whether the presence of a live adult female of the NR-population may induce the NR-syndrome in predators of the R1-population. Mated adult female predators (age = 7

days) from the pre-infection rearing of the R1-population were distributed over two groups. Predators of both groups were kept on spider mite-infested leaf discs originating from the spider mite rearing of our laboratory. A leaf disc carried either an additional live female (age = 9-11 days) from the R1-population (=control) or an additional live female (age = 9-11 days) from the NR-population (treatment). In order to distinguish these females from the other female present in the dish, they were marked with a small spot of water-soluble ink. The marked females from both populations originated from a pre-experimental rearing. After three days the marked females were removed. The unmarked females were transferred individually to new Petri dishes and NR-syndrome parameters were measured for a period of 6 days. Two replicates with 20 predators each were run.

Experiment 2.4: Horizontal transmission via faeces and debris of live female predators

Finally, we tested whether faeces and debris excreted by predators from the NR-population can induce the NR-syndrome in predators from the R1-population. Mated adult female *P. persimilis* are of minute size (length of the body ca. 0.45 mm; Gaede, 1992) and they excrete only liquid faeces, which strongly adheres to the surface after evaporation. Therefore it was not possible to collect faeces without debris left on the surface by the excreting predator.

Faeces and debris were collected by keeping four adult female predators (age = 9-11 days) originating from a pre-experimental rearing in a plastic Eppendorf vial (volume = 1.5 ml) together with a small piece of wet cotton wool (ambient temperature 23±1 ^{o}C). Per replicate fifteen vials were prepared for the NR- and the R1-population. After 24 hours the predators and their eggs were removed. Vials carrying dead predators were excluded from the experiment.

Adult female predators (age = 7 days) from the pre-infection rearing of the R1-population were distributed over two groups. Four predators were either kept in an Eppendorf vial carrying faeces and debris from the R1-population (=control) or in a vial carrying faeces and debris from the NR-population (=treatment) (ambient temperature 23±1 ^{o}C). After 24 hours the predators were transferred individually to new Petri dishes. In this experiment the NR-syndrome parameters were measured for a period of only 3 days in order to prevent an excess loss of predators through death. The number of replicates and predators was the same as in experiment 2.3.

Statistics

We used the Mann-Whitney U test or the paired t-test to test numerical data. A contingency table test was used for categorical data. The data from the replicates were pooled, because no relevant differences were present between the replicates.

Results

Vertical disease transmission

Characteristics of mothers

The 73 female predators from the NR-population whose eggs were used for this experiment clearly showed the NR-syndrome that has previously been described in chapter 4. Ninety-three percent of the mothers shrank and only 48% were present on the leaf at the moment of egg collection. Moreover, they laid only 2.1±1.1 eggs during one day. Seventy-four percent of the females chose the odour of prey-infested plants and 15% carried crystals in their legs.

Characteristics of juvenile and adult offspring prior to mating

Less than 10% of the offspring was lost during development due to non-hatching of eggs and juvenile mortality (Table 1). This was true for all three treatments. The overall female sex ratio was 78%. As only 5 males developed from eggs that had been transferred to a new leaf, these males had to mate more times than those of the other two treatments (Table 1).

Characteristics of mated females

The syndrome was not transmitted vertically via the egg. Only normal-sized, well-performing females developed from eggs that were surface-sterilized or transferred to another leaf whereas untreated eggs gave rise to dorso-ventrally flattened females (17%). These females shrank 2-6 days after mating and died 4-8 days later. Females that did not shrink during that period remained normal-sized until the end of the experiment. Even when they entered the post-oviposition period, i.e. when they stopped carrying eggs, they could be

Table 1: Pre-mating characteristics of predators reared from eggs collected from the NR-population. Eggs were transferred to another leaf after surface sterilization (= eggs sterilized), transferred to another leaf (= eggs transferred) or left at the place where the mother had laid them (= eggs untreated). Numbers in parentheses represent actual predator numbers.

	Eggs sterilized (51)	Eggs transferred (47)	Eggs untreated (52)
% eggs not hatched	**2** (1 out of 47)	**0** (out of 45)	**0** (out of 51)
% dead juveniles	**5** (2 out of 43)	**2** (1 out of 45)	**8** (4 out of 48)
% females	**71** (29 out of 41)	**89** (39 out of 44)	**73** (32 out of 44)
# matings / ♂ (average)	**2.4**	**5.6**	**2.6**
	29 mated females	**28** mated females *	**31** mated females **

*impossible to accomplish mating of all females during 24 hours, because of a limited number of males
**1 female escaped prior to mating

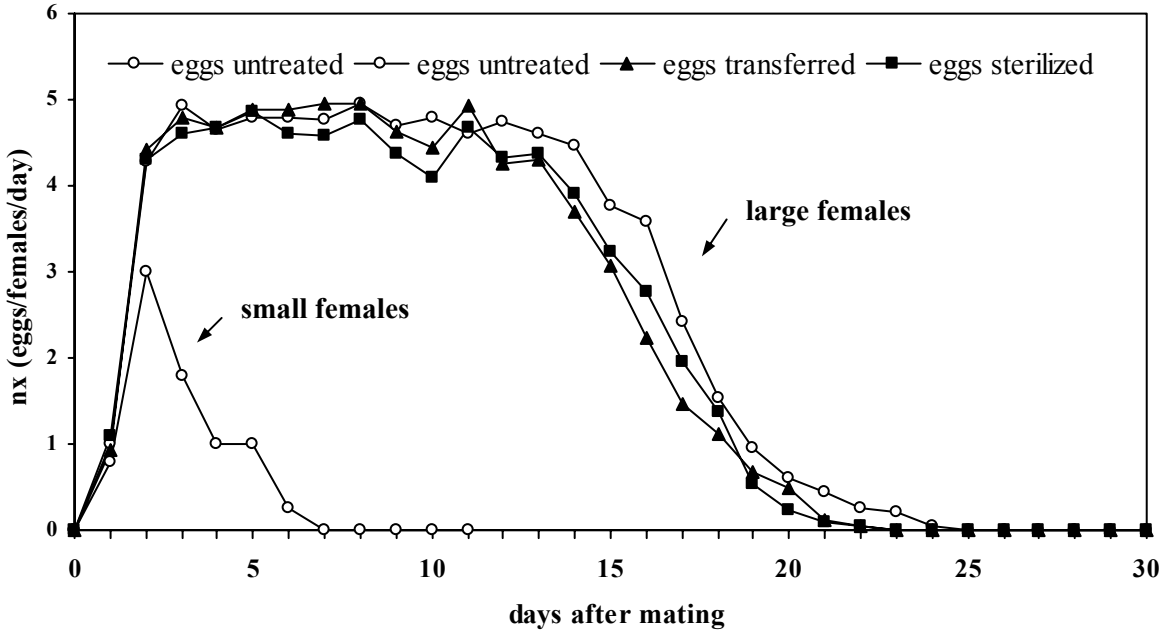

Figure 3: Age-specific oviposition n(x) (number of eggs per living female of age class x) of normal-sized and shrunken *Phytoseiulus persimilis* reared from eggs collected from the NR-population. Eggs were transferred to another leaf after surface sterilization (= eggs sterilized), transferred to another leaf (= eggs transferred) or left at the place where the mother had laid them (= eggs untreated). Day 0 is the 7th day of development and mating took place on this day.

distinguished from dorso-ventrally flattened individuals. In the following part, data recorded from females originating from untreated eggs are presented separately for small and normal-sized females.

The age-specific oviposition curves (Figure 3) of the normal-sized females of the three treatments were very similar and resembled the form of a trapezoid. After mating the age-specific oviposition rate rapidly increased. On the 3rd day after mating it reached a peak of 4.6, 4.8 and 4.9 eggs per female per day for predators reared from sterilized eggs, transferred eggs and untreated eggs respectively (defined as peak rate of oviposition by Sabelis and Janssen, 1994). The end of the plateau phase was reached on the 13th to 14th day where after it decreased to zero at the end of the reproductive life on the 24th to 25th day. However, the curve of the shrunken females originating from untreated eggs is totally different, having a triangular shape (Figure 3). The oviposition rate of these females steeply rose to a top of 3 eggs per female at the 2nd day after mating. Subsequently, it steeply decreased to zero at the end of the reproductive life, which was reached on the 7th day already. The last shrunken female already died on the 11th day after mating. During their short life, shrunken females laid an average of only 7.8±7.4 eggs and only 2 of the 5 females were found on the leaf during more than half of the time. As all shrunken females died early, crystal location and the attraction to HIPV were not determined for this group.

Table 2: Characteristics of mated **normal-sized** female *P. persimilis* reared from eggs collected from the non-responding population. Eggs were surface-sterilized and transferred to another leaf (= eggs sterilized), transferred to another leaf (= eggs transferred) or left at the place where the mother had laid them (= eggs untreated). Numbers in parentheses represent actual predator numbers. Values in the same row carrying different letters are significantly different (paired t-test for numerical data, 2 by 2 contingency table test for categorical data, 2 comparisons: column 1 with 2, column 2 with 3, a = 0.025).

	Eggs sterilized (N =27)	Eggs transferred (N=28)	Eggs untreated (N=25)
% dead ♀♀	**22** (6 out of 27) **a**	**11** (3 out of 28) **a X**	**12** (3 out of 25) **X**
# eggs / ♀ / 30 days (mean ± SD)	**60.0** ± 24.7 (27) **a**	**67.9** ± 13.7 (28) **a X**	**71.9** ± 15 (25) **X**
% ♀♀ > half of time on leaf	**100** (27)	**100** (28)	**100** (25)
% ♀♀ to plant odours (HIPV)	**88** (14 out of 16) **a**	**76** (16 out of 21) **a X**	**67** (10 out of 15) **X**
% ♀♀ with crystals in legs	**0** (out of 21)	**0** (out of 24)	**0** (out of 20)

The normal-sized females of all three treatments performed better than their shrunken mothers and sisters. Mortality of normal-sized females up to day 30 after mating was low and fecundity was high. The mean number of deposited eggs was 60, 67.9 and 71.9 for normal-sized females of the three treatments, respectively (Table 2) compared to only 8 eggs of the shrunken females. Mortality was highest and fecundity was lowest in normal-sized females reared from sterilized eggs, but not significantly different from females reared from transferred eggs (mortality: P=0.3; fecundity: P= 0.65, paired t-test). No differences were found between normal-sized females of the three treatments concerning foraging behaviour. All normal-sized females were recorded on the leaf during more than half of the observations (Table 2). Although the females tested in the olfactometer for attraction to HIPV were 37 days old and had laid no eggs for at least 5 days, they were still attracted to the odour of prey-infested plants (Table 2). The attraction to HIPV was lowest for females reared from untreated eggs, but not significantly different from females reared from transferred eggs (P = 0.71). None of these old normal-sized females carried crystals in the legs (Table 2).

Characteristics of second generation females

Eggs were collected from normal-sized females from all 3 treatments on the 17th day after mating and reared until adulthood. We did not find any evidence of syndrome occurrence in the second generation, as none of the females grown from these eggs turned small up to the age of 14 days after mating and none of these females carried crystals in the legs at that age. The mean number of eggs laid on day 13 plus 14 was 8.6±2.7, 8.9±2.3 and 8.0±2.7 for treatments a, b and c, respectively ($P \geq 0.16$, paired t-tests). Moreover, females of all three treatments showed a strong attraction to HIPV. The percentage of females choosing the odour of

prey-infested plants was 90% (N=30), 93% (N=28) and 85% (N=26) for treatments a, b and c, respectively ($P \geq 0.5$).

Horizontal disease transmission

Transmission via food and squashed females

No symptoms were induced in female predators from the R1-population when fed with prey-mites reared in our laboratory (Table 3a). The same is true for predators from the R1-population when exposed to squashed female predators from the NR-population (Table 3b). The data for all NR-syndrome parameters of the control predators as well as treated predators were very similar to the data obtained for predators from the R1-population in earlier experiments (chapter 4).

Transmission via live females

All but one of the symptoms tested were induced in female predators from the R1-population after a three day-exposure to female predators from the NR-population (Table 3 c). The data for size, mortality, fecundity and predator position of treated predators were very similar to data obtained for predators of the NR-population during earlier studies (chapter 4). None of the control predators shrank or died during the experimental time, whereas about half of the treated predators shrank 3-7 days after the first contact with a symptomatic female and died ($P<0.001$, for both parameters). Females, which did not shrink during this period, remained normal-sized and alive until the end of the experiment. Treated predators laid significantly fewer eggs than control predators ($P<0.001$, Mann-Whitney U test) and significantly fewer treated females were found on the leaf in more than half of the observations ($P<0.001$). No significant difference was found between treated and control predators concerning their response to HIPV ($P=0.83$). However, due to high mortality in the treated predators, only 1 shrunken individual was present among the 15 treatment females making a choice. None of the live control predators carried crystals in the legs whereas a quarter of the live treatment predators did so ($P = 0.015$).

Transmission via faeces and debris

All but one of the symptoms tested were induced in predators from the R1-population after a 24h contact with faeces and debris released by female predators from the NR-population (Table 3d). The first shrunken females appeared 2 days after contact. Two days later 65% of the treated predators had shrunk, whereas all control predators were still normal-sized ($P<0.001$). Treated predators laid significantly fewer eggs during this period than control predators ($P<0.001$, Mann-Whitney U test). The fraction of females that resided on the leaf at the last parameter-assessment day was 85% for the control predators versus only 58% for

Table 3: Symptoms of adult female *P. persimilis* of the R1-population. Females were incubated **a)** on prey-infested leaves originating either from the commercial producer of the R1-population (=control) or from our laboratory (=treatment), **b)** together with 2 squashed adult female predators of the R1-population (=control) or the NR-population (=treatment), **c)** together with 1 live adult female of the R1-population (=control) or the NR-population (=treatment) during 3 days and **d)** on faeces and debris of adult female predators of the R1-population (=control) or of the NR-population (=treatment) during 1 day. Numbers in parentheses represent actual predator numbers. For explanation of symptoms see chapter 4.

3a) FOOD	**Control** (N = 27)	**Treatment** (N = 29)	P*
Predator size			
% small ♀♀	**0** (out of 27)	**0** (out of 29)	
Predator mortality			
% dead ♀♀	**26** (7 out of 27)	**7** (2 out of 29)	0.11
Predator fecundity			
# eggs / ♀ / 6 days (average ± SD)	**21.8** ± 6.1 (27)	**21.9** ± 3.8 (29)	0.99
Predator behaviour in olfactometer			
% ♀♀ to plant odours (HIPV)	**94** (15 out of 16)	**85** (23 out of 27)	0.70
Predator position within dish			
% ♀♀ > half of time on leaf	**96** (26 out of 27)	**83** (24 out of 29)	0.23
Crystal location within predator			
% ♀♀ with crystals in legs	**0** (out of 19)	**0** (out of 26)	

3b) SQUASH	**Control** (N = 25)	**Treatment** (N = 24)	P*
Predator size			
% small ♀♀	**0** (out of 25)	**4.2** (1 out of 24)	0.98
Predator mortality			
% dead ♀♀	**16** (4 out of 25)	**8.3** (2 out of 24))	0.70
Predator fecundity			
# eggs / ♀ / 6 days (average ± SD)	**21.6** ± 4.7 (25)	**29.9** ± 5.7 (24)	0.27
Predator behaviour in olfactometer			
% ♀♀ to plant odours (HIPV)	**88** (14 out of 16)	**82** (14 out of 17)	1.0
Predator position within dish			
% ♀♀ > half of time on leaf	**92** (23 out of 25)	**92** (22 out of 24)	1.0
Crystal location within predator			
% ♀♀ with crystals in legs	**5** (1 out of 20)	**0** (out of 18)	1.0

3c) ALIVE	**Control** (N = 37)	**Treatment** (N = 38)	P**
Predator size			
% small ♀♀	**0** (out of 37)	**53** (20 out of 38)	< 0.001
Predator mortality			
% dead ♀♀	**0** (out of 37)	**47** (18 out of 38)	< 0.001
Predator fecundity			
# eggs / ♀ / 6 days (average ± SD)	**25.9** ± 1.5 (37)	**11.8** ± 10.8 (38)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to plant odours (HIPV)	**81** (25 out of 31)	**73** (11 out of 15)	0.83
Predator position within dish			
% ♀♀ > half of time on leaf	**95** (35 out of 37)	**50** (19 out of 38)	< 0.001
Crystal location within predator			
% ♀♀ with crystals in legs	**0** (out of 27)	**25** (4 out of 16)	0.015

Table 3 continued:

3d) FAECES	Control (N = 39)	Treatment (N = 43)	P**
Predator size			
% small ♀♀	**0** (out of 39)	**65** (28 out of 43)	< 0.001
Predator mortality			
% dead ♀♀	**0** (out of 39)	**0** (out of 43)	
Predator fecundity			
# eggs / ♀ / 3 days (average ± SD)	**11.9** ± 1.9 (39)	**7.3** ± 3.4 (43)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to plant odours (HIPV)	**88** (22 out of 25)	**68** (25 out of 37)	0.06
Crystal location within predator			
% ♀♀ with crystals in legs	**0** (out of 35)	**35** (14 out of 40)	< 0.001

* Paired t-test for numerical data, 2 by 2 contingency table test for categorical data
**Mann-Whitney U test for numerical data, 2 by 2 contingency table test for categorical data

treated predators (P = 0.017). Treated predators also showed a lower degree of attraction to HIPV than the control predators, differences being marginally insignificant (P = 0.06). None of the control predators carried crystals in the legs compared to 35% of the treatment predators (P<0.001).

Discussion

Vertical transmission

No evidence was found for maternal- or paternal-mediated vertical transmission of the NR-syndrome. Eggs laid by symptomatic females of the NR-population mated by males of the NR-population gave rise to normal-sized well-performing females, which produced non-symptomatic offspring themselves, when eggs were surface sterilized or transferred to an uncontaminated substrate. This is in accordance with earlier studies, where 11 isofemale lines could be started from the NR-population, by transferring eggs to an uncontaminated bean leaf. Predators from these lines showed a stronger attraction to HIPV than predators from the NR-population during the same period (chapter 3).

In contrast, Bjørnson and Keddie (2001) found 100% vertical transmission of the microsporidian pathogen *M. phytoseiuli* in *P. persimilis*. All progeny that hatched from surface-sterilized eggs were infected. Male predators did not contribute to microsporidian infection of their progeny. In another study, surface sterilization of eggs collected from a poorly performing commercial population of *P. persimilis* had a positive effect on short-term survival and oviposition (Steiner and Bjørnson, 1996). Short-term performance was best in predators originating from eggs rinsed in water, compared to eggs washed with formaldehyde or tetracycline hydrochloride.

Horizontal transmission via residues of the mother

In the present study syndrome transmission from mother to offspring was only achieved horizontally via products left on the leaf by the mother. Five females shrank 2-6 days after mating and died 4-8 days later. Infection of these females must have taken place during the larval or protonymphal stage, as contact with products left by the mother was only possible during the first 3 days of the experiment. This is remarkable, because larvae of *P. persimilis* do not feed at all and only move small distances (Nagelkerke, 1993; Schausberger and Croft, 1999b). Such a phenomenon, i.e. when larvae or nymphs acquire benign or sublethal infections and survive to adulthood wherein they may transmit the pathogen and/or become symptomatic, is called transstadial transmission. This may allow pathogens to multiply to higher numbers in older individuals (Andreadis, 1987).

Horizontal transmission via food and squashed females

Consumption of *T. urticae* spider mites that had been reared in our department did not induce the NR-syndrome in females of the R1-population. Hence, the syndrome-inducing factor is not present in the food source of the NR-population. These results are in accordance with earlier studies where non-symptomatic predators could be reared with spider mite prey from the mass rearing in our laboratory (chapter 3). Spores of the microsporidian pathogen *M. phytoseiuli* have also never been detected in *T. urticae*, not even in colonies that were fed to a *P. persimilis* population that was 100% infected with the microsporidian. In contrast, *Wolbachia* may be present in *T. urticae* as well as in *P. persimilis* (Breeuwer and Jacobs, 1996).

No evidence was found for syndrome transmission via females that were squashed on the leaf surface, although transmission of the behavioural change via females that had died has been reported earlier (chapter 5). In the latter case, however, the leaf did not carry only an individual that had died, but also products such as faeces that had been left on the leaf prior to death. It is possible that body fluid of diseased predators is not infectious, or that it does not stay infectious for a long time. Another possible explanation is predator avoidance behaviour: female *P. persimilis* may avoid dead conspecifics and body fluids of conspecifics, whereas they do not avoid faeces of conspecifics. Avoidance of dead conspecifics has been reported for the American cockroach *Periplaneta americana*, which is repelled by intact and ruptured corpses of conspecifics (Rollo *et al*., 1995). Moreover, female *T. urticae* avoid places with artificially damaged conspecifics (eggs or dead adults) (Grostal and Dicke, 1999).

Horizontal transmission via live females and faeces

Female predators from the R1-population showed the NR-syndrome following a 3-day exposure to a live conspecific of the NR-population and after a 1-day exposure to faeces and debris of such females; transmission rates were 53% and 65%, respectively. These results support our hypothesis that the NR-syndrome is caused by a disease. Excretion of infective pathogen stages in faeces is characteristic of many bacterial, viral and protozoan pathogens that infect the digestive tract of insects (Andreadis, 1987). Bjørnson and Keddie (2001) report that horizontal transmission of *M. phytoseiuli* in *P. persimilis* is rather low (14%). It only occurred when immature *P. persimilis* developed while in contact with infected immature and adult predators during at least 5 days. Horizontal transmission did neither occur when uninfected adult female predators were placed on leaf surfaces previously contaminated by infected predators or by application of microsporidian spores, nor when those predators were exposed to infected female predators during 48 hours (Bjørnson and Keddie, 2001). We are aware of one other report of horizontal disease transmission via faeces in mites. Pathogen transfer via defecation has been reported for the herbivorous mite *P. citri* infected with a non-occluded virus (Reed et al., 1975).

Pathogen uptake

Horizontally transmitted insect pathogens may gain entrance to their hosts through natural body openings or through the integument (Andreadis, 1987). The majority of horizontally transmitted insect pathogens enter their host through the mouth (per os). It has been argued earlier that infection of predatory mites through the oral route is unlikely unless the prey is infected, as their mouthparts consist of several sharp stylets that puncture the prey (Bjørnson and Keddie, 2001). We think that per os infection might be possible even if prey is not infected:

(1) In situations where prey is scarce **cannibalism** of infected conspecifics could be a source of infection, as *P. persimilis* is known to feed on conspecifics (see for a review Schausberger, 2003). Interestingly Schausberger and Croft (2001) have demonstrated the presence of kin discrimination for cannibalistic females of *P. persimilis*. In dual choice tests female predators discriminate between related and unrelated conspecific larvae and preferentially prey upon unrelated larvae. This was true for laboratory-reared, commercially mass-reared and field-collected females. A possible reason for kin recognition may be disease avoidance, as genetic similarity between predator and prey poses a greater risk to acquire deleterious pathogens, because of selection for host specificity among pathogens (Pfennig *et al*., 1998). However, *P. persimilis* does not discriminate between con- and heterospecific predatory mites as prey (Schausberger and Croft, 1999a), which contradicts the hypothesis of disease

avoidance. It would thus be interesting to study cannibalistic behaviour of predators from our NR-population.

(2) As *P. persimilis* may **drink from water** droplets (Gaede *et al.*, 1992) contaminated water droplets may as well be a source of oral infection.

(3) Moreover, female *P. persimilis* may **share food with other individuals** when predator density is high (Yao and Chant, 1990), which creates possibilities for pathogen transfer via shared food.

(4) Another possible mechanism of pathogen uptake that might be important in the natural situation is the **grooming of body parts**, which had been previously in contact with faeces and debris deposited by diseased predators. Adult female *P. persimilis* use their chemoreceptor-carrying pedipalps, to drum individual prey or the walking substrate (Dicke *et al.*, 1991b). This behaviour may lead to contamination with the infectious agent, which could gain oral entrance through subsequent pedipalp cleaning.

In the closed Petri dishes and Eppendorf vials used for the present transmission experiments, condense water was always present due to the high relative humidity in the closed system. It is thus possible that predators drank from faeces-contaminated condense water. Horizontal transmission in Eppendorf vials was not recorded when no water was added (C. Schütte, unpublished data). Grooming of contaminated parts of the body may also be a valid explanation in the present experiments. However, cannibalism and food sharing does not hold as possible explanations in the set-up in which non-symptomatic predators were exposed to faeces and debris of infected predators.

In this context reports should be mentioned of insects and mites that are attracted to conspecific faeces (Lorenzo and Lazzari, 1996; Carlson *et al*., 2000, Grenacher, *et al*., 2001). Adult female *P. persimilis* are attracted to conspecifics (Janssen *et* a*l*., 1997), but we are not aware of any study where the role of predator faeces in attraction has been studied. It could be possible that *P. persimilis* uses faeces as information source for the presence of conspecific individuals. Under natural conditions *P. persimilis* shows a clustered distribution and lives in aggregations of conspecifics. As each individual deposits large amounts of faecal material (chapter 4), faeces would be an excellent cue for the presence of conspecifics in a prey patch shared by conspecifics.

Pathogen release into the environment

Bjørnson and Keddie (2001) observed numerous microsporidian spores of *M. phytoseiuli* in smear preparations of faecal pellets of infected predators examined by light microscopy, whereas no spores were detected on leaf surfaces or predator faeces when examined by

SEM. Predator faeces appeared as intact aggregates of dumbbell-shaped crystals (Bjørnson and Keddie, 2001). Hence the authors thought it unlikely that spores are liberated from intact faecal pellets onto leaf surfaces. However, in that study dissected bean leaves were air-dried prior to SEM observation. In contrast, humidity may be near saturation in the region close to the leaf surface (=boundary layer) when a leaf is attached to the plant (Gaede, 1992). It may thus be possible that faeces are diluted and faecal components are liberated onto the leaf surface in such conditions and that they may be picked up by predatory mites in the way described above. In such a case ambient humidity and temperature, air velocity, light regime, plant condition and plant characteristics (including leaf size, leaf shape, leaf position in plant, leaf thickness, leaf surface) could influence disease transmission, as these factors have a direct impact on the humidity within the boundary layer of the leaf (Gaede, 1992).

Virulence and transmission

Insect pathogens may be divided into two groups according to their virulence and transmission (Myers and Rothman, 1995): (1) highly virulent pathogens kill their hosts and are transmitted horizontally via the release of environmentally resistant, infectious particles (nuclear polyhedral viruses, bacteria, fungi). Epizootics can destabilize host populations. (2) Benign pathogens reduce the vigour of infected hosts and can be transmitted vertically between generations without destabilizing host populations (many protozoa, some viruses).

The present case has the characteristics of the first group: symptomatic female predators stop egg laying shortly after mating and die several days after reproduction ceases (chapter 4). They release faeces carrying the infectious agent that stays viable when released into the environment. Moreover, epizootics may in certain cases destabilize host populations and this may lead to eradication of the population (chapter 3; C. Schütte, unpublished data). In such systems predator behaviour is crucial for pathogen transmission (Andreadis, 1987). It may therefore be expected that behavioural changes cause changes in pathogen transmission. Whether the reported behavioural changes benefit the pathogen by ensuring high transmission rates or the host by minimizing disease transmission to conspecifics, will be an interesting topic for further study (see for further discussion chapter 9).

In contrast to the disease reported here, the disease caused by *M. phytoseiuli* has the characteristics of the second group: this pathogen causes less severe reductions in fecundity, longevity, prey consumption and female offspring (Bjørnson and Keddie, 1999). Pathogen transmission is mainly vertical and disease prevalence may stay at a low level in infected colonies over a long period (Bjørnson and Keddie, 2001). In this system, where maternal

vertical transmission is 100% and horizontal transmission is low, predator behaviour will have less effect on disease transmission.

Biological control

The results of the present study clearly demonstrate that the NR-syndrome is a contagious phenomenon. It may be expected that the syndrome will be efficiently transferred among and between generations as soon as it has entered a population. Care should be taken to avoid contact of such a population with other populations of *P. persimilis*. The best diagnostic symptoms for the presence of the NR-syndrome are size of adult females and the presence of crystals within the legs. A population is most probably carrying the NR-syndrome when the following requirements are met: (1) numerous dorso-ventrally flattened females are present, (2) these flattened females do not become normal sized after offering ample food and a male conspecific and die several days after shrinking (3) live adult females carrying birefringent crystals in the legs are present (for further discussion see chapter 9). It remains to be detected whether commercial populations exhibit the novel disease described in this paper.

Only normal-sized well-performing females showing normal foraging behaviour were obtained from surface-sterilized eggs and eggs transferred to an uncontaminated place. Moreover, these females produced non-symptomatic offspring themselves. Egg sterilization did not have great negative effects on the progeny in terms of egg hatchability, fecundity and mortality. Net fecundity was 60±25 eggs for females grown from sterilized eggs and 68±14 eggs for females grown from transferred eggs respectively. These numbers lay within the range of data reported for *P. persimilis* (40 – 80 eggs/female, reviewed by Sabelis and Janssen, 1994). Transfer and/or surface sterilization of eggs are thus promising methods of curing the present disease. However, after sterilization eggs have to be transferred to an uncontaminated environment. This may be a bottleneck for many commercial producers of *P. persimilis*, as rearing space and facilities are often scarce. Sterilization methods that may lead to non-contaminated rearing facilities are therefore needed in the future.

Conclusions

The NR-syndrome is transmitted horizontally from mother to offspring and between females by faeces and debris. These findings strongly support our hypothesis that a pathogen induces the syndrome. The ultimate proof of this hypothesis will be to satisfy Koch's postulates (Lacey and Brooks, 1997). Elucidation of faeces as the main transmission mode offers new perspectives for pathogen isolation, which is a prerequisite for addressing Koch's postulates.

Isolation and identification of the putative pathogen is of great interest for commercial producers of biological control agents and for laboratories working with phytoseiid mites, because of its negative effects on both life history and behaviour.

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aqueous suspension of faeces and debris
collected from predators of the NR-population

bacteria present

P. persimilis / **R-population**

NR-syndrome

bacteria eliminated

P. persimilis / **R-population**

no NR-syndrome

Chapter 7

Evidence of the involvement of bacteria

Abstract

Adult female *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) of a laboratory population show drastic changes in foraging behaviour, anatomy and life history compared to typical laboratory populations. We demonstrated earlier that the set of characteristic symptoms, called non-responding (=NR-) syndrome, is transmitted horizontally between and among predator generations via faeces and debris deposited by symptomatic females. Here, we prove that bacteria present in faeces and debris deposited by symptomatic females are involved in the induction of the NR-syndrome. The potential of predator products to induce the NR-syndrome was assayed by keeping healthy adult female predators during a period of 3 days on prey-infested bean leaves, which had previously been sprayed with an aqueous suspension of faeces and debris. The NR-syndrome was clearly induced in those predators that had been exposed to a suspension collected from symptomatic females (incubation time 4-6 days, 93% shrunken females), whereas predators exposed to a suspension collected from non-symptomatic females did not show the NR-syndrome. Moreover, predators from the first group transmitted infectious products themselves already 5 days after the initial exposure, whereas this was not the case for the second predator group. The bioassay used in the present study is important for laboratories and companies as it can be applied for testing the presence of the novel disease in populations of *P. persimilis*. To investigate the involvement of bacteria in syndrome induction we (1) eliminated bacteria from a faeces-and-debris suspension of symptomatic females by passing the suspension through a bacterial micro-filter and (2) added the antibiotic tetracycline to a suspension of faeces and debris from symptomatic females. A suspension of faeces and debris collected from symptomatic females did not induce the NR-symptom after bacteria had been eliminated, whereas an untreated portion of the same suspension did so. Moreover, the NR-syndrome was induced in predators exposed to an aqueous suspension of the residues that had not passed the bacterial filter. A suspension of faeces and debris collected from symptomatic females, to which the antibiotic tetracycline had been added, did not induce the NR-syndrome whereas the same suspension did induce all symptoms when no tetracycline was added. These findings prove that bacteria are involved in the induction of the NR-syndrome. The results are discussed in the context of mite pathology and biological control.

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) feeds exclusively on herbivorous spider mites (Acari, Tetranychidae), including the two-spotted spider mite *Tetranychus urticae* Koch and has since long been successfully used as a biological control agent in several field and glasshouse crops (Helle and Sabelis, 1985; van Lenteren, 1995; van Lenteren *et al*., 1997; Garthwaite, 2000).

We have previously reported drastic changes in foraging behaviour, anatomy and life history for adult female *P. persimilis* of one of our laboratory populations, designated non-responding (=NR-) population (Schütte *et al*., 1995; chapter 3 and 4). Adult female predators of this population show a lower degree of attraction to herbivore-induced plant volatiles (=HIPV) and a shorter choice time in olfactometertests and have a stronger tendency of leaving patches carrying ample prey (chapter 3 and 4). Attraction to and arrestment in prey patches are two important mechanisms leading to extinction of prey populations, resulting in successful biological control of the prey (Zemek and Nachman, 1999; Janssen, 1999; Pels and Sabelis, 1999). Besides behavioural changes, we detected drastic changes in life history parameters. Female predators shrink when mature, cease oviposition and die several days after shrinkage. In an earlier study symptomatic females laid an average of only 8 eggs during their life and died 11 days after mating, whereas non-symptomatic females laid an average of 72 eggs and were still alive 30 days after mating (chapter 6). Another diagnostic symptom of the NR-population is the appearance of excretory crystals in the legs (Schütte *et al*., 1995; chapter 4).

The characteristic set of symptoms (=NR-syndrome) is not transmitted vertically from parent to offspring via the egg, but horizontally from female to offspring as well as between females (chapter 5 and 6). In both cases the transmission takes place via faeces and debris from symptomatic females (chapter 6). These data indicate that infection with a disease agent causes the NR-syndrome.

Several pathogens and potential pathogens have been described for phytoseiid mites (see for reviews van der Geest *et al.*, 2000; Bjørnson and Schütte, 2003; Schütte *et al*., 2005; chapter 2). In microscopic studies of commercial and laboratory populations of *P. persimilis* several potential pathogens, including protozoa, viruses and bacteria, have been detected (Šut'áková, 1988, Steiner, 1993b, Bjørnson, 1998; Bjørnson *et al*., 1997). However, the status and the impact of most described entities on *P. persimilis* are not yet clear. Detailed studies have been made for one microsporidian pathogen (Bjørnson, 1998; Bjørnson *et al.,* 1996; Bjørnson and Keddie, 1999, 2001). *Microsporidium phytoseiuli* (Microsporidia), which has been isolated from a commercial predator population from Europe, had clear negative effects on

sex ratio, predation capacity, fecundity and longevity of *P. persimilis* (Bjørnson, 1998; Bjørnson and Keddie, 1999). The main transmission route of *M. phytoseiuli* is vertical from mother to offspring via the interior of the egg (Bjørnson and Keddie, 2001). However, neither *M. phytoseiuli* nor any other microsporidia have ever been detected in predators from the NR-population (C. Schütte unpublished data, S. Bjørnson and E. Beerling, personal communication).

The only peculiarity, incidentally found in Giemsa-stained smears of moribund or dead predators of the NR-population, was the presence of numerous bacteria (C. Schütte, unpublished data, S. Bjørnson, personal communication). However, such bacteria were never detected in smears of living symptomatic predators whereas symptomatic females deposit the infectious agent in faeces and debris before death (chapter 6). Yet, bacteria detected in moribund and dead predators might represent secondary infections of opportunistic pathogens invading predators that are weakened by the disease. Many bacterial pathogens of insects are considered to be opportunistic and may exist in nature as saprophytes (Boucias and Pendland, 1998). Therefore, in the present study we preferred to use faeces and debris deposited by symptomatic females as pathogen source for experimental work.

First, we designed a valid bioassay to test the potential of an aqueous suspension to induce the NR-syndrome in *P. persimilis.* The bioassay was used to study the following questions: (1) does an aqueous suspension of faeces and debris collected from symptomatic females induce the NR-syndrome in healthy predators? (2) Can predators that have been exposed to an infectious suspension of predator products, pass the syndrome to other predators after exposure? (3) Are bacteria involved in syndrome induction?

Materials and Methods

Cultures

Plants and herbivores

Lima bean plants (*Phaseolus lunatus* L.) were reared in a greenhouse at 20-25 °C (L16:D8). The herbivorous two-spotted spider mite, *Tetranychus urticae* Koch, was reared on whole bean plants under the same conditions.

Predator populations

The **non-responding** population **(NR)** originated from a commercial mass producer and has been reared in our laboratory for many years in a semi-open rearing system. Detached Lima bean leaves infested with spider mites were placed on a plastic platform in a caged water basin at 20-25 °C (L16:D8). Fresh leaves were added every 2 to 3 days. Old leaves were re-

moved weekly. The NR-population consists of symptomatic and non-symptomatic predators (chapter 3 and 4).

The **responding** population **(R)** originated from the NR-population. It was started with surface sterilized eggs from the NR-population and was cultured in a closed rearing system. Detached Lima bean leaves infested with spider mites were placed in Parafilm-sealed plastic Petri dishes (diameter = 9cm) in a climate chamber at 23±1 ^{o}C (L16:D8). In each dish 4 gravid females were kept for egg production during 48 hours after which the females were eliminated. New leaves infested with spider mites were added every 2 to 3 days. After one week, when the offspring had become mature, gravid females were transferred to new Petri dishes to initiate a new generation or they were used in experiments. Thus, predators were reared in distinct generations. At least 15 dishes were prepared per generation. The R-population consisted of non-symptomatic predators only (chapter 4).

Different rearing systems were used for two reasons: (1) a responding population looses its characteristics when reared in a semi-open rearing system at our laboratory (chapter 3); (2) the NR-population dies out when reared for several generations in a closed rearing system (C. Schütte, unpublished data).

Pre-experimental rearing

To eliminate the effect of different rearing systems in the experiments, both populations were kept in the closed rearing system described above for at least one generation prior to experiments. Twenty dishes were prepared per population. As female predators from the R-population laid more eggs than females from the NR-population (chapter 4), eggs were eliminated from dishes of the R-population, to obtain similar egg densities of ca. 10 eggs per Petri dish for both populations.

Pre-infection rearing of the R-population

To minimize variation due to accidental contamination of rearing dishes we reared sisters of comparable age, mated by a brother, which were then equally distributed over the treatments of an experiment. Twenty-five mated female predators of the R-population were placed individually on a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm). The females were removed after 24 hours in experiment 1 and after 48 hours in experiments 2 and 3. Dishes that contained a dead predator or few eggs after these time periods were discarded. The eggs of the remaining dishes were transferred to the underside of a prey-infested leaf in a new dish, as female predators prefer the underside for egg deposition. After 4 days new food was added to each dish. After 8 days each Petri dish contained the adult offspring of one mother. As in a batch of 4-5 eggs, which is the daily

egg production of a healthy female, the first egg produced is usually a male (Amano and Chant, 1978) each Petri dish contained at least one male which had inseminated its sisters.

Hygienic measures

All equipment used to handle predators and prey-infested leaves, like brushes and forceps, was sterilized in 0.5% sodium hypochlorite (NaClO) solution prior to use, after which it was rinsed with water several times.

For the start of the R-population, predator eggs from the NR-population were surface- sterilized by dipping them in a 0.5% sodium hypochlorite solution for 30 seconds. Subsequently they were dipped 3 times in sterilized water for 30 seconds each. Eggs were shortly dried on tissue paper and transferred to the underside of prey-infested bean leaves. Ca. 500 eggs were used to initiate the R-population.

Experimental work

Bioassay to test the effects of an aqueous suspension of predator products on healthy *P. persimilis* females

Preparation of an aqueous suspension of faeces and debris

Adult female *P. persimilis* are of minute size (length of the body ca. 0.45 mm; Gaede, 1992) and they excrete liquid faeces, which strongly adhere to the surface after desiccation. Therefore, it was not possible to collect faeces exclusively, without including debris left on the surface by the excreting predator. Faeces and debris were collected by keeping four adult female predators of a pre-experimental rearing (age = 9-11 days, i.e. ca. 4 days after the final moult) in plastic Eppendorf vials (volume = 1.5 ml) together with a small piece of wet cotton wool (ambient temperature 23±1 ^{o}C). After 24 hours the predators and their eggs were removed and the number of faecal droplets deposited was counted for each vial. Vials carrying dead predators were not used, to exclude micro-organisms that are associated with dead mites. Faecal droplets deposited by *P. persimilis* were then dissolved in distilled water. For each replicate faeces and debris deposited by 36-40 predators (i.e. 9-10 vials) were dissolved in 8 ml water.

Application of the suspension on spider mite–infested leaves

Twenty leaf pieces heavily infested with all stages of spider mites were placed upside down on a 58 x 47cm filter paper in a fume hood. Pieces of comparable size (ca. 12 cm^2) cut from one leaf were evenly distributed over treatments. Four ml of the faecal solution was sprayed evenly over the whole surface of the filter paper with the help of a hand-held atomizer (Preval sprayer, 61ml, 59.5g Precision Valve Corporation, New York), in such a way that

each leaf piece was entirely covered with small droplets. The fume hood was disinfected after each spraying and for each treatment a new sprayer was used.

General bioassay set-up

Adult female predators (age = 7 days) from the pre-infection rearing of the R-population were kept individually on sprayed leaf pieces in sealed Petri dishes (diameter = 9 cm) during 3 days (ambient temperature 23±1 ^{o}C). They were then transferred individually to a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm) placed in a climate chamber at 23±1 ^{o}C. Leaf discs cut from one leaf were evenly distributed over treatments.

During a period of 3 days (2 days in experiment 1a) predator size, mortality and fecundity were measured each day. We determined **predator size** by classifying predators as either "normal- sized" or "small". Dorso-ventrally flattened female predators can easily be distinguished from the normal-sized predators with the help of a stereomicroscope. **Mortality** and **fecundity** were assessed by counting the number of dead predators and of eggs deposited on the leaf disc as well as on the Petri dish.

The response to herbivore-induced plant volatiles, the predator position within the Petri dish and the location of excretory crystals within the predator were determined once on the last experimental day. **The attraction to HIPV** was tested in a two-choice set-up. In a closed-system Y-tube olfactometer the odour from 9 trifoliate Lima bean leaves infested with ample amounts of two-spotted spider mites was offered vs. the odour from 9 uninfested trifoliate Lima bean leaves of comparable size. Predators were individually released into the olfactometer and observed until they made a choice for one of the odour sources. The percentage of predators choosing the odour of infested leaves was calculated from those predators making a choice within 5 minutes. To determine **predator position** we noted their position within the Petri dish directly after selecting a dish for symptom assessment and calculated the fraction of females staying on the leaf. **Crystal location** within a predatory mite was assessed by investigating live mites under a light microscope, equipped with two filters that create polarized light. Dumbbell-shaped crystals light up in polarized light, whereas other objects turn dark. A photograph was taken of each mite. All photographs were pooled and divided into the following groups: (1) crystals present in the rectum and Malpighian tubules; (2) crystals present in at least one leg; (3) crystal location not possible The percentage of predators carrying crystals in the legs was calculated from the number of predators for which crystal location was possible. For a detailed description of the symptom assessment, see chapter 4. The person measuring the parameters did not know to which treatment group the predator belonged.

Experimental treatments

Experiment 1: Syndrome transmission via faeces-and-debris-suspension

a) Faeces-and-debris-suspension of predators from the R- and NR-population

Here we tested whether an aqueous suspension of faeces and debris collected from the NR-population can induce the NR-syndrome in predators from the R-population. Predators from the R-population were exposed to a suspension which had been derived either from females from the R-population (faeces R = control) or from females from the NR-population (faeces NR = treatment). Two replicates with 19 predators each were run. In the control two predators were lost due to handling. On the 4th day after the start of the exposure all live predators from the control and treatment were transferred to Eppendorf vials for collection of faeces. After collection of faeces all live predators were again transferred to leaf discs for symptom assessment for one more day.

b) Faeces-and-debris-suspension of predators from the R-population, previously exposed to faeces suspension

Next, we examined whether females from the R-population that had been exposed to faeces-and-debris suspension of the NR-population deposit the infectious agent themselves. Predators from the R-population were exposed to a suspension that had been derived from predators that had been exposed to either faeces of the R-population (faeces R on R = control) or to faeces of the NR-population (faeces R on NR = treatment). Twenty predators were tested for the treatment as well as the control.

Experiment 2: Syndrome transmission via faeces suspension / effect of filtration

Further, we studied whether the infectious agent can pass through a bacterial filter. Four ml of faeces-and–debris-suspension from the NR-population were passed through a bacterial filter (Millipore, Millex, pore size 0.22 μm). Four ml of sterilized water were then passed through the filter in order to wash out all particles that can pass the filter. This procedure was repeated twice, after which the filter was washed with 4 ml of sterilized water, in order to recover the residues that did not pass the filter.

Predators from the R-population were distributed over four groups. They were exposed to: (1) faeces-and-debris-suspension from the R-population (= faeces R) (2) faeces-and-debris-suspension from the NR-population (= faeces NR) (3) filtered faeces-and-debris-suspension from the NR-population (faeces NR / filtrate) (4) aqueous suspension of the filter residues (faeces NR / residues). Two replicates with 16 predators each were run. The effects of treatment 2-4 were compared to effects of treatment 1.

Experiment 3: Syndrome transmission via faeces suspension / effect of tetracycline

Finally, we tested whether the broad-spectrum antibiotic tetracycline can prevent syndrome prevalence, by treating the aqueous faeces-and-debris-suspension with 0.5% w/v tetracycline and an aqueous tetracycline suspension of the same concentration.

Predators from the R-population were distributed over three groups. They were exposed to (1) faeces-and-debris-suspension of the NR-population (faeces NR), (2) faeces-and-debris-suspension of the NR-population to which tetracycline had been added (faeces NR / antibiotic) (3) water plus tetracycline (water / antibiotic). Effects of treatment 2 were compared to effects of treatments 1 and 3.

Statistics

The Mann-Whitney U test was used to test numerical data. A contingency table test was applied for categorical data. The data from the replicates were pooled, because no differences were found between the replicates. The Bonferroni Inequality Rule was used to correct for multiple comparisons.

Table 1 Faeces and debris collection in Eppendorf vials during 24 hours. The number of females used for collection, the number of eggs and faeces droplets deposited by these females during collection and the faeces concentration of the sprayed aqueous suspension per replicate (replicate1/ replicate2) for experiment 1a (**a**), 1b (**b**), 2 (**c**) and 3 (**d**) are presented. For a detailed explanation of the experiments see Materials and Methods.

a) EXPERIMENT 1a	# ♀♀	**#** eggs	**#** faecal droplets	**# faecal droplets / ml**
R-population	36 / 36	65 / 66	167 / 196	**21 / 25**
NR-population	36 / 36	6 / 14	57 / 78	**7 / 10**

b) EXPERIMENT 1b	# ♀♀	# eggs	# faecal droplets	**# faecal droplets / ml**
R-population (on faeces R)	20	33	89	**11**
R-population (on faeces NR)	20	8	34	**4**

c) EXPERIMENT 2	# ♀♀	# eggs	# faecal droplets	**# faecal droplets / ml**
R-population	36 / 40	70 / 79	287 / 391	**36 / 49**
NR-population	36 / 40	5 / 5	76 / 119	**10 / 15**

d) EXPERIMENT 3	# ♀♀	# eggs	# faecal droplets	**# faecal droplets / ml**
NR-population	40 / 40	6 / 19	40 / 133	**5 / 17**

Results

Collection of faeces

Egg and faeces deposition by adult female predators varied among experiments and replicates (Table 1a-d). In all replicates predators from the R-population laid more eggs and had a higher excretion rate than predators from the NR-population. Females from the R-population laid 4-15 times more eggs and deposited 2-3 times more faeces droplets. The concentration of the faecal suspension was therefore higher for the R-population than for the NR-population for all replicates of experiment 1 and 2.

Experiment 1: Syndrome transmission via aqueous faeces suspension

a) Effect of faeces-and-debris-suspension collected from predators from the R- and NR-population

Symptoms of the NR-syndrome were induced in female predators from the R-population after a 3 day-exposure to faeces-and-debris-suspension from the NR-population, whereas the NR-syndrome was not induced by faeces-and-debris-suspension from the R-population, despite being of a higher faecal concentration (Table 2a; Table 1a). None of the control predators (= faeces R in Table 2a) shrank during the experimental time, whereas 90% of the treated predators exposed to faeces suspension of the NR-population (= faeces NR, in Table 2a) shrank 4-6 days after start of the exposure ($P<0.001$). Mortality was higher for treated predators than for control predators ($P= 0.003$), whereas oviposition rate was lower ($P<0.001$). Behavioural symptoms were induced as well: significantly fewer treated predators were found on the leaf on the last experimental day ($P<0.001$). No significant differences were found for the response to HIPV ($P=1.0$). Due to a high mortality among treated predators, the number of treated predators tested in the olfactometer was too low ($N=11$) to reach adequate replicate numbers in this test. Significantly more treated predators carried crystals in the legs ($P<0.001$).

b) Effect of faeces-and-debris-suspension collected from predators from the R-population, that had previously been exposed to faeces suspension

Two symptoms of the NR-syndrome were clearly induced in females of the R-population after a 3 day-exposure to faeces-and-debris-suspension of predators of the R-population that had previously stayed on faeces and debris of the NR-population (Table 2b). As many as 65% of the treated predators (= faeces R on NR in Table 2b) shrank within 4-6 days after exposure start, whereas none of the control predators (= faeces R on R in Table 2b) shrank during the experimental time ($P<0.001$). Mortality was somewhat higher for treated predators than for control predators, but this difference was not significant ($P=0.5$). Oviposition

Table 2: Symptoms of adult female *P. persimilis* from the R-population after a 3 day-exposure to prey-infested leaf pieces that were sprayed with an aqueous faeces-and-debris-suspension. (**a**) Faeces and debris were collected either from the R-population (=faeces R) or from the NR-population (=faeces NR). (**b**) Faeces and debris were collected from females from the R-population that had previously stayed on leaf pieces sprayed with a suspension collected from either the R-population (faeces R on R) or the NR-population (faeces R on NR). Numbers in parentheses represent actual predator numbers. For explanation of symptoms see chapter 4.

a) EXPERIMENT 1a	**Faeces R** (N = 36)	**Faeces NR** (N = 38)	P*
Predator size			
% small ♀♀	**0** (out of 36)	**90** (34 out of 38)	< 0.001
Predator mortality			
% dead ♀♀	**6** (2 out of 36)	**37** (14 out of 38)	0.003
Predator fecundity			
# eggs / ♀ / 2 days (average ± SD)	**7.5** ± 1.9 (36)	**1.2** ± 1.5 (38)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to HIPV	**82** (22 out of 27)	**64** (7 out of 11)	1.0
Predator position within dish			
% ♀♀ on leaf	**97** (33 out of 34)	**58** (14 out of 24)	< 0.001
Crystal location within predator			
% ♀♀ with crystals in legs	**3** (1 out of 31)	**82** (18 out of 22)	< 0.001

b) EXPERIMENT 1b	**Faeces R on R** (N = 20)	**Faeces R on NR** (N = 20)	P*
Predator size			
% small ♀♀	**0** (out of 20)	**65** (13 out of 20)	< 0.001
Predator mortality			
% dead ♀♀	**0** (out of 20)	**10** (2out of 20)	0.5
Predator fecundity			
# eggs / ♀ / 3 days (average ± SD)	**12.5** ± 3.9 (20)	**2.5** ± 2.0 (20)	< 0.001
Predator behaviour in olfactometer			
% ♀♀ to HIPV	**79** (15 out of 19)	**83** (10 out of 12)	1.0
Predator position within dish			
% ♀♀ on leaf	**80** (16 out of 20)	**72** (13 out of 18)	0.7
Crystal location within predator			
% ♀♀ with crystals in legs	**11** (2 out of 19)	**29** (4 out of 14)	0.4

*Mann-Whitney U test for numerical data, 2 by 2 contingency table test for categorical data

rate of treated predators was much lower than of control predators (P<0.001). No significant differences were found for the two behavioural symptoms, i.e. attraction to HIPV and predator position within the Petri dish (P=1.0 and P=0.7 respectively), and the presence of crystals in the legs (P=0.4).

Experiment 2: Syndrome transmission via faeces-and-debris-suspension / effect of filtration

Again, faeces-and-debris-suspension from the R-population did not induce the NR-syndrome in predators from the R-population (Table 3). The data for size, mortality, oviposition rate, attraction to HIPV, predator position and crystal location of control predators (= faeces R in Table 3), were similar to the data obtained in experiment 1a (compare first column in Table 2a with first column in Table 3). The oviposition rate was lower in experiment 1a than in experiment 2, because symptom assessment was done during only 2 days in experiment 1a compared to 3 days in experiment 2.

Faeces-and-debris-suspension of the NR-population induced all but one symptoms (i.e. mortality) in predators of the R-population (Table 3). Treated predators (=faeces NR in Table 3) differed significantly from control predators (=faeces R in Table 3) for five symptoms: size ($P<0.001$), oviposition rate ($P<0.001$), attraction to HIPV ($P=0.011$), predator position within dish ($P=0.006$) and crystal location within predator ($P<0.001$). The faeces-and-debris-suspension of the NR-population induced no mortality and a lower fraction of predators with crystals in the legs during the experimental time (compare second columns in Table 2a and Table 3). In experiment 1a predators were kept during 24 hours without food for faeces collection. This stress may have induced a higher mortality and a greater fraction of predators with crystals in their legs.

Table 3: Symptoms of adult female *P. persimilis* from the R-population after a 3 day-exposure to prey-infested leaf pieces that were sprayed with an aqueous faeces-and-debris-suspension collected either from the R-population (=faeces R), or from the NR-population (=faeces NR). Half of the suspension from the NR-population was sprayed after passage through a microbial sieve (faeces NR /filtrate). The residues not passing the filter were re-dissolved in water and sprayed (faeces NR/ residues). Numbers in parentheses represent actual predator numbers. For explanation of symptoms see chapter 4. Column 2, 3 and 4 are compared to column 1. Data carrying asterisks are significantly different from data in the first column (Mann-Whitney U test for numerical data and 2 by 2 contingency table test for categorical data, $\alpha=0.05/3= 0.0167$).

EXPERIMENT 2	**Faeces R** (N = 30)	**Faeces NR** (N = 32)	**Faeces NR /filtrate** (N = 32)	**Faeces NR/residues** (N = 32)
Predator size				
% small ♀♀	**0** (out of 30)	**88** (28 out of 32) *	**0** (out of 32)	**69** (22 out of 32) *
Predator mortality				
% dead ♀♀	**7** (2 out of 30)	**0** (out of 32)	**6** (2 out of 32)	**0** (out of 32)
Predator fecundity				
# eggs / ♀ / 3 days (average±SD)	**13.0** ± 2.3 (30)	**1.8** ± 3.2 (32) *	**12.8** ± 2.1 (32)	**7.7** ± 5.2 (32) *
Predator behaviour				
% ♀♀ to HIPV	**83** (20 out of 24)	**52** (16 out of 31) *	**72** (18 out of 25)	**50** (14 out of 28)
Predator position within dish				
% ♀♀ on leaf	**93** (26 out of 28)	**59** (19 out of 32) *	**80** (24 out of 30)	**69** (22 out of 32)
Crystal location within predator				
% ♀♀ with crystals in legs	**4** (1 out of 26)	**48** (15 out of 31) *	**0** (out of 28)	**60** (18 out of 30) *

In contrast, the same suspension of the NR-population did not induce any of the symptoms in predators from the R-population when bacteria had been eliminated by filtration (Table 3). For none of the measured parameters significant differences were found between predators from the filtrate treatment (=faeces NR / filtrate in Table 3) and predators exposed to faeces-and-debris-suspension of the R-population: mortality (P=1.0), oviposition rate (P=0.1), attraction to HIPV (P = 0.5), predator position (P=0.3) and crystal location (P=0.5).

Moreover, the aqueous suspension of the filtration residues did induce symptoms of the NR-syndrome in predators from the R-population (Table 3). For 3 symptoms significant differences were found between predators exposed to an aqueous suspension of filter residues (=faeces NR/residues in Table 3) and predators exposed to the faeces suspension of the R-population: predator size (P<0.001), oviposition rate (P<0.001) and crystal location (P<0.001). For the two behavioural symptoms the differences were marginally insignificant: attraction to HIPV (P=0.018) and predator position (P=0.025).

In Figure 1 the percentage of small females, which is the most obvious symptom of the NR-syndrome, is plotted against time. For predators exposed to unfiltered faeces suspension of the NR-population the percentage of small females showed a rapid increase 3-5 days after

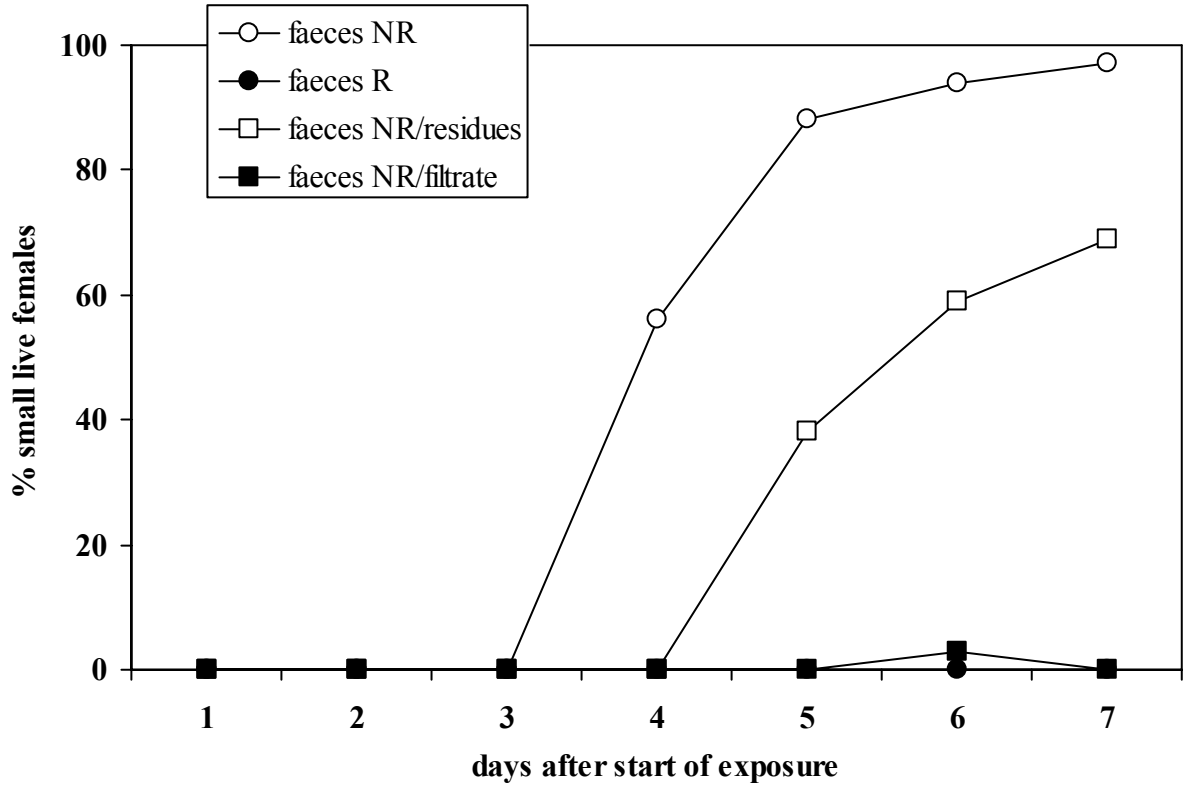

Figure 1: Percentage of live small female *P. persimilis* of the R-population after a 3 day-exposure to faeces suspension from the R-population (faeces R) faeces suspension from the NR-population (faeces NR), filtered faeces suspension from the NR-population (faeces NR / filtrate) aqueous suspension of the filter residues (faeces NR / residues).

start of the exposure after which it reached a plateau near 100%. The curve for the predators exposed to the filtrate residues of the NR-faeces is similar to the curve for the predators exposed to unfiltered faeces. However, the increase was less steep and started one day later.

Experiment 3: Syndrome transmission via-faces-and-debris-suspension / effect of tetracycline

Again, the NR-syndrome was induced in predators from the R-population after exposure to faeces and debris of the NR-population (=faeces NR in Table 4). The data were very similar to the data obtained in experiment 2 (compare second column Table 3 and Table 4). However, when tetracycline was added to a fraction of the same faeces-and-debris-suspension the symptoms were not induced (= faeces NR/antibiotic in Table 4). For 3 symptoms the differences between predators exposed to a faeces suspension of the NR-population (faeces NR in Table 4) and the same suspension plus tetracycline (faeces NR/antibiotic in Table 4) were significant: predator size ($P<0.001$), oviposition rate ($P<0.001$) and crystal location ($P<0.001$). Differences found for the behavioural symptoms and mortality were not significant: attraction to HIPV ($P=0.3$), predator position ($P=0.045$) and mortality ($P= 0.2$).

Moreover, for none of the symptoms significant differences were found between predators exposed to faeces-and-debris-suspension plus tetracycline (=faeces NR/antibiotic in Table 4) and predators exposed to an aqueous tetracycline suspension of the same concentration

Table 4: Symptoms of adult female *P. persimilis* from the R-population after a 3 day-exposure to prey-infested leaf pieces sprayed either with an aqueous faeces-and-debris-suspension collected from the NR-population (=faeces NR), or with the same suspension plus tetracycline (=faeces NR/ tetracycline) or with water plus tetracycline (=water/tetracycline). Numbers in parentheses represent actual predator numbers. For explanation of symptoms see chapter 4. Column 1 and 3 are compared to column 2. Data carrying different letters are significantly different from data of the second column (Mann-Whitney U test for numerical data and 2 by 2 contingency table test for categorical data, $\alpha=0.05/2= 0.025$).

EXPERIMENT 3	**Faeces NR** (N = 38)	**Faeces NR/antibiotic** (N = 38)	**Water/antibiotic** (N = 38)
Predator size			
% small ♀♀	**100** (38 out of 38) **a**	**8** (3 out of 38) **bX**	**3** (1 out of 38) **X**
Predator mortality			
% dead ♀♀	**3** (1 out of 38) **a**	**13** (5 out of 38) **aX**	**8** (3 out of 38) **X**
Predator fecundity			
# eggs /♀ / 3 days (average ± SD)	**2.0** ± 2.6 (38) **a**	**11.6** ± 4.7 (38) **bX**	**11.9** ± 4.0 (38) **X**
Predator behaviour in olfactometer			
% ♀♀ to HIPV	**60** (12 out of 20) **a**	**78** (18 out of 23) **aX**	**62** (13 out of 21) **X**
Predator position within dish			
% ♀♀ on leaf	**50** (18 out of 36) **a**	**76** (25 out of 33) **aX**	**86** (30 out of 35) **X**
Crystal location within predator			
% ♀♀ with crystals in legs	**68** (21 out of 31) **a**	**0** (out of 31) **bX**	**7** (2 out of 29) **X**

(=water/antibiotics in Table 4): size (P=0.6), mortality (P=0.7), oviposition rate (P=0.8), attraction to HIPV (P=0.3), predator position (P=0.4) and crystal location (P=0.2).

Discussion

Effects of aqueous faeces-and-debris-suspension

The NR-syndrome was induced in adult females from the R-population after a 3-day exposure to an aqueous suspension of faeces and debris collected from the NR-population that was sprayed onto the leaf surface. In contrast, in a study by Bjørnson and Keddie (2001) no disease transmission was observed when uninfected female *P. persimilis* stayed on leaf surfaces contaminated with spores of the microsporidian pathogen *M. phytoseiuli*. In this case the spores had been obtained from crushed infected predator eggs or as a suspension of spores in distilled water (Bjørnson and Keddie, 2001). These results are in accordance with other data, as *M. phytoseiuli* did neither infect female *P. persimilis* predators after exposure to infected live females nor after exposure to leftovers of infected females (Bjørnson and Keddie, 2001), whereas the NR-syndrome was induced in adult female *P. persimilis* both after exposure to symptomatic live females and after exposure to faeces collected from symptomatic females (chapter 6).

The effects recorded in the present study are comparable to data of experiments mimicking the natural transmission route more exactly, i.e. exposure to faeces and debris and exposure to a live mite (chapter 6). However, transmission rates were higher: as much as 93% (N=108) of exposed predators became symptomatic during the present experiments compared to 53% (N=38) of predators exposed to live mites and 65% (N=43) of predators exposed to faeces and debris (chapter 6). These higher rates of transmission are most probably caused by higher pathogen encounter rates and better pathogen entrance. In the present bioassay faecal components were distributed over the entire prey patch whereas their distribution was patchy in the other set-ups (chapter 6). Moreover, faecal components were diluted in water. Both factors facilitate pathogen entrance via body openings, which is the most likely entrance mode, as at present no cuticle-penetrating bacteria are known to exist (Boucias and Pendland, 1998). Possible ways of pathogen entrance via the mouth (per os) in *P. persimilis* have been discussed previously (chapter 6). In the present bioassay the pathogen may gain entrance into its host per os, by drinking aqueous faeces-and-debris-suspension present on the leaf surface, as *P. persimilis* is known to drink from water droplets (Gaede *et al.*, 1992). Another possible mechanism of pathogen entrance per os is the grooming of body parts, which had previously been in contact with faeces-and-debris-suspension.

Despite the differences in faeces concentrations between replicates and experiments the effects were very stable. Thus, spraying leaves with an aqueous faeces-and-debris-suspension is a valid bioassay for testing the infectiousness of faeces and debris deposited by adult female *P. persimilis* showing the NR-syndrome. We conclude that predator size, oviposition rate, predator position and crystal location are the most consistent and reliable symptoms in the present bioassay set-up. For mortality rates to be consistently recorded, the observation period has not been long enough. However, we may expect that all females that had shrunk during the observation period will die several days after shrinkage, as has been observed in experiments with a longer time span (chapter 4 and 6). For a satisfactory statistical analysis of the behavioural response to HIPV replicate number was too low in the present set-up. However, combined data from all experiments clearly demonstrate that aqueous faeces-and-debris-suspension of the NR-population induces the behavioural change in the response to HIPV. As much as 82% (N =51) of the females exposed to faeces of the R-population chose the odour of herbivore-infested plants compared to only 57% (N=62) of females exposed to faeces of the NR-population (P=0.006). This agrees with previous data on this NR-symptom (chapter 3 and 5).

Involvement of bacteria

A faeces-and-debris-suspension of the NR-population that has been filtered through a microbial filter does not induce the NR-syndrome. In contrast, the aqueous suspension of the filter residues containing all particles of the suspension larger than 0.22 μm, does induce the NR-syndrome in predators of the R-population, syndrome expression being less drastic than when crude faeces-and-debris-suspension of the NR-population was sprayed. It may be expected that the applied procedures (filtration, washing and resuspending) inflicted stress onto the pathogen and that a part of the pathogen population was destroyed or left in the filter. This may explain the somewhat smaller effects. When the broad-spectrum antibiotic tetracycline was dissolved in a faeces-and-debris-suspension of the NR-population, the NR-syndrome was not induced, whereas the same suspension did induce all symptoms when no antibiotic was added.

In another case of behavioural changes reported in phytoseiid mites, no effects of antibiotics were found. Adult female predators of three populations of *Amblyseius potentillae* and of one population of *Typhlodromus pyri* were repelled by HIPV whereas attraction had been reported in earlier cases (Dicke *et al*., 1991a). To test the involvement of micro-organisms the antibiotics rifampicin or oxy-tetracycline (1% w/w) were added to the food of *A. potentillae*, by either mixing the crystals with the pollen food or by suspending it in honey. The response of predators reared on such food was not different from the response of predators that had fed on pollen only (Dicke *et al*., 1991a).

Steiner and Bjørnson (1996) treated the surface of *P. persimilis* eggs originating from a population with poor performance, with different concentrations of tetracycline hydrochlorite. Mites originating from such eggs showed a much higher fecundity and longevity than in all previous tests done with this population. However, rinsing the eggs with water only gave the best results, suggesting that in this case no positive additive effect from antibiotics was present (Steiner and Bjørnson, 1996).

The findings of the present study demonstrate that neither viruses nor toxic substances alone can induce the NR-syndrome and that tetracycline-sensitive bacteria of a size larger than 0.22 µm, are clearly involved in this process. These results strongly support our hypothesis that the NR-syndrome is caused by a novel pathogen. The ultimate proof of this hypothesis will be to meet Koch's postulates (Lacey and Brooks, 1997; Boucias and Pendland, 1998). One requirement of these postulates is that the pathogen must be isolated from experimentally infected hosts. In the present study predators of the R-population transmitted the NR-syndrome to non-symptomatic females 5 days after they have been exposed to faeces-and-debris-suspension of the NR-population. Hence, infected predators produce infective bacteria already 5 days after infection, or the bacteria contaminating the mites stay viable during more than five days. In the present case we cannot exclude one of theses mechanisms as faeces cannot be separated from other mite products in a reliable way. Moreover mites were not surface-sterilized after infection as this may lead to elimination of pathogens inside small organisms such as *P. persimilis* (Lacey and Brooks, 1997). The fact that experimentally infected female predators can transmit the disease shortly after infection is an important characteristic of an epizootic. The effects of faeces and debris collected from predators that had previously been exposed to leftovers of the NR-population were milder than the effects of faeces-and-debris-suspension from the NR-population. This could be due to the lower concentration of the faeces suspension (see Table 1) or to a lower concentration of the infectious agent.

Pathogen virulence

None of the female predators that were exposed to the faeces-and-debris-suspension collected from the R-population shrank during the experimental period. Thus, the suspension from non-symptomatic female predators was not detrimental to adult conspecifics in the present experimental set-up. This may be expected, as *P. persimilis* naturally lives in colonies of conspecifics (Helle and Sabelis, 1985), a situation in which encounters with faeces and debris of conspecifics are inevitable. At the same time, the effects of faeces-and-debris-suspension collected from female predators of the NR-population were very drastic. As much as 100 out of 108 healthy predators shrank during only 6 days after start of the exposure to the faeces-and-debris-suspension. The shrunken females may be regarded as repro-

ductively dead as they do not produce any eggs until their death. These data together with previous data (chapter 3 , 4, 5 and 6) demonstrate that the pathogen causing the NR-syndrome in *P. persimilis* is rather virulent. It has been argued that in mainly horizontally transmitted pathogens the evolution of high virulence is favoured in situations that allow for a high rate of horizontal transmission (Ewald, 1994; Myers and Rothman, 1995). The method applied to rear *P. persimilis* from the NR-population in our laboratory represents such a situation, as high numbers of predators are kept together in a relatively small place.

Epizootics of highly virulent pathogens may destabilize host populations leading to eradication of local populations in case the pathogen is too efficient in its spread among hosts or too virulent. However, despite consistent fluctuations in predator densities and syndrome incidence, the NR-population has never been eradicated when reared in an open rearing system (chapter 3; Schütte, unpublished data). This could be due to a combined effect of 1) rapid evolution of disease resistance in *P. persimilis*, due to its short generation time, 2) behavioural mechanisms that minimize contact between infectious material and healthy conspecifics and/or 3) low pathogen viability and pathogen entrance under ambient climatic conditions. In contrast, the same NR-population is eradicated by the pathogen after only several generations, when kept in a closed Petri-dish rearing (C. Schütte, unpublished data). In the latter case infected predators cannot escape from the rearing unit and the relative humidity is higher, and both may enhance pathogen transmission (see for further discussion chapter 9).

Bacteria in *P. persimilis*

Whereas numerous insect-specific and tick-specific bacterial pathogens are known to date (Tanada and Kaya, 1993; Schabereiter-Gurtner *et al*., 2003), knowledge on bacterial pathogens of *Acari* other than ticks is rather scarce. This may be partly due to the small size of many species and to the low economical and human-health importance of the majority of mite species (Poinar and Poinar, 1998; van der Geest *et al.*, 2000). The majority of all bacteria reported for *P. persimilis* belong to the rickettsia. Rickettsia consist of mainly intracellular micro-organisms, that exist in mutualistic, parasitic and pathogenic associations with a wide range of organisms (Boucias and Pendland, 1998). In transmission electron studies Šut'áková and Rüttgen (1978) were the first to report a rickettsia, *Rickettsiella phytoseiuli,* in *P. persimilis*. Rickettsia belonging to the genus *Rickettsiella* are common intracellular pathogens, but the authors did not detect any detrimental effect of this bacterium on development and morphology of infected predators (see for a review Šut'áková, 1994).

In addition, rickettsia-like organisms have been reported by several authors (see chapter 2). It has been suggested that the rickettsia-like organisms reported in the earlier microscopic

surveys of *P. persimilis* are probably members of the genus *Wolbachia* (for a discussion see van der Geest *et al*., 2000). *Wolbachia* are common cytoplasmic symbionts of insects, crustaceans, mites and filarial nematodes (see Stouthamer *et al.*, 1999). They are rarely pathogenic but may manipulate the host biology by inducing parthenogenesis (whereby infected females exclusively produce daughters), feminisation (whereby infected genetic males reproduce as females), male-killing (whereby infected male embryos die while female embryos develop into infected females), cytoplasmic incompatibility (unidirectional in its simplest form: whereby the crossing of an uninfected female and infected male result in embryo mortality) or by enhancing host fecundity (Stouthamer *et al.*, 1999). *Wolbachia* were reported from *P. persimilis* by Steiner (1993b) and Bjørnson *et al.* (1997). Through molecular methods *Wolbachia* was found to be present in commercial *P. persimilis* populations from seven sources (Bjørnson *et al.,* 1997). Breeuwer and Jacobs (1996) used the polymerase chain reaction (PCR) assay to carry out a survey for *Wolbachia* in various spider mite and predatory mite species and *Wolbachia* was detected in a laboratory population of *P. persimilis*. In addition Weeks *et al*. (2003) detected *Wolbachia* in one of two tested *P. persimilis* populations. However, *Wolbachia*-specific PCR products have never been detected in predators of the NR-population (J. A. J. Breeuwer, personal communication).

Bacterial micro-organisms other than rickettsia have only been described in dead and moribund predators by Steiner (1993b) and in diseased predators from several commercial populations (Schütte *et al.*, 2005). However, Steiner (1993b) stated that bacteria were secondary opportunistic invaders rather than a primary infection source.

Biological control

The findings of the present study prove that bacteria are involved in the induction of the detrimental NR-syndrome of *P. persimilis*. The bioassay used in the present study is important for laboratories and companies as it can be applied for testing the presence of the novel pathogen in populations of *P. persimilis.* It is very likely that a *P. persimilis* population is infected with the novel bacterial pathogen when an aqueous suspension of predator faeces collected from this population induces the NR-syndrome in non-symptomatic *P. persimilis*. Care should be taken to avoid contact of such a population with other populations of *P. persimilis*.

The fact that the bacteria are tetracycline sensitive may offer possibilities for the cure of infected populations. However, in the present study we only tested the prophylactic effects of tetracycline, as it was applied together with the infectious agent. The effects of tetracycline on symptomatic predators after infection are not yet known. Moreover application of tetracycline on a large scale in predator populations should be avoided for the following reasons:

1) Emergence of antibiotic resistance is likely as it has been reported earlier for other bacterial invertebrate pathogens; a well-known example being the recently widespread oxytetracycline resistance in the honey bee bacterial pathogen *Paenibacillus larvae*, the causative agent of the important honey bee larval disease American foulbrood (Evans, 2003). 2) Bacterial endosymbionts (for example *Wolbachia*) may be eradicated in the prey as well as the predators (Breeuwer, 1997), what may have negative effects on predatory mite performance. 3) It is unlikely that all predators would take up the antibiotic in large-scale mass productions. 4) In the present study some small predators were found after tetracycline treatment, which may be an indication of negative effects of the antibiotic on the predatory mites.

Application of tetracycline on a small scale, for example if a healthy population is needed to start a new rearing is much safer. However it is not necessary, as surface sterilization of predator eggs is a curative method that is more reliable without any negative effects on *P. persimilis* and its prey (chapter 6). During all the studies of the NR-syndrome done so far, symptomatic female *P. persimilis* did at least produce some eggs before death (chapter 4 and 6). Thus it may be expected that one can find enough eggs in most diseased populations. In our laboratory this method has been successfully applied for several years now and it has repeatedly been recommended to commercial insectaries. However, after sterilization eggs have to be transferred to an uncontaminated environment. This may be a bottleneck for many commercial producers of *P. persimilis*, as rearing space and facilities are often scarce. Sterilization methods that may lead to non-contaminated rearing facilities are therefore needed in the future.

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Phytoseiulus persimilis / **R-population**

after exposure to ***Acaricomes phytoseiuli***

NR-syndrome

Chapter 8

Effects of the bacterium *Acaricomes phytoseiuli* on adult female predators

Abstract

Adult female *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) of a laboratory population show a set of characteristic symptoms, designated as non-responding (NR-) syndrome. Mature predators shrink, cease oviposition and die. They show a lower degree of attraction to herbivore-induced plant volatiles, a shorter choice time in olfactometertests, and a stronger tendency of leaving prey patches carrying ample prey. Moreover, predators may carry excretory crystals in the legs, may cease prey consumption and have a low excretion rate. Here we satisfy the Koch's postulates for a strain of *Acaricomes phytoseiuli* (DSM 14247) that was isolated from symptomatic female *P. persimilis* of the NR-population. Adult female *P. persimilis* were either exposed to a bacterial inoculum suspension (treatment) or to sterile distilled water (control) during a period of 3 days. Control and treated predators were examined for the occurrence of six symptoms characteristic for the NR-syndrome and the presence of *A. phytoseiuli* after inoculation. The latter was done by re-isolation of *A. phytoseiuli* from individual predators and predator faeces on nutrient agar and by histopathological studies of individual predators.

The NR-syndrome was clearly induced in those predators that had been exposed to the bacterial inoculum (incubation time = 2-5 days, fraction shrunken females = 80%), whereas predators exposed to water did not show the NR-syndrome. *A. phytoseiuli* was never isolated from control predators whereas it could be re-isolated from 60% of the treated predators (N=37) and from faeces of 41% of treated predators (N=17). Only one day after exposure *A. phytoseiuli* could not be re-isolated from treated predators and their faeces. Light and electron microscope studies of predators exposed to *A. phytoseiuli* revealed striking bacterial accumulations in the lumen of the alimentary tract together with extreme degeneration of its epithelium. In addition, bacterial foci also occurred in the fat body. These phenomena were not observed in control predators that were exposed to sterile water. The present data prove that *A. phytoseiuli* may infect the predatory mite *P. persimilis* and induce the occurrence of the NR-syndrome in adult female *P. persimilis*. This is the first record of a bacterial pathogen in phytoseiid mites and the first description of pathogenic effects of a bacterial species belonging to the genus *Acaricomes*.

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) feeds exclusively on herbivorous spider mites (Acari, Tetranychidae), including the two-spotted spider mite *Tetranychus urticae* Koch and has since long been used as a biological control agent in several field and glasshouse crops (Helle and Sabelis, 1985; van Lenteren *et al*., 1997). Moreover *P. persimilis* has become a key species in research on multitrophic interactions and predator-prey relationships (for a review see Dicke *et al*., 1998; Sabelis *et al*., 1999; de Boer and Dicke, 2005). Hence during the past three decades *P. persimilis* has been reared in numerous laboratories and insectaries all over the world.

We have previously reported drastic changes in foraging behaviour, anatomy and life history for one of our laboratory populations, designated as the non-responding (=NR-) population (Schütte *et al*., 1995; chapter 3 and 4). Adult female *P. persimilis* of this NR-population show the following set of symptoms: predators shrink when mature, cease oviposition immediately after shrinking and die several days after shrinking. They show a low degree of attraction to herbivore-induced plant volatiles (=HIPV), a short choice time in olfactometertests and have the tendency to leave a prey patch with ample food. In addition they may carry excretory crystals in the legs, may cease prey consumption altogether and have a low excretion rate.

The disease with this characteristic non-responding (=NR-) syndrome is transmitted horizontally from female to offspring, as well as between females via faeces and debris deposited by symptomatic females (chapter 5, 6). We demonstrated earlier that bacteria from predator faeces are involved in syndrome induction (chapter 7). The most definite way to make a conclusive diagnosis is satisfying the Koch's postulates, for which the following steps must be taken (Lacey and Brooks, 1997):

1) The pathogen must be isolated from all of the diseased individuals examined and the signs and/or symptoms of the disease recorded.
2) The pathogen must be grown in culture and it must be identified and/or characterised.
3) The pathogen must be inoculated on/in healthy individuals of the same or a related species and signs and symptoms must be the same.
4) The pathogen must be isolated in culture again and its characteristics must be exactly like those observed in step 2.

We repeatedly isolated a novel bacterial species from surface-sterilized adult female *P. persimilis* of the NR-population and their faeces, whereas this species was never isolated from females of populations that did not show the NR-syndrome or from its prey (postulate 1).

This isolate has been grown in culture and it has been described as *Acaricomes phytoseiuli* gen. nov., sp. nov. by Pukall *et al.* (2006) (postulate 2). In the present study we satisfy the last steps of the Koch's postulates, by testing whether the NR-syndrome is induced in adult female *P. persimilis* after inoculation with *A. phytoseiuli* (postulate 3) and whether *A. phytoseiuli* may be isolated from predators and their faeces after inoculation with *A. phytoseiuli* (postulate 4). Moreover, we did light and electron microscope studies of infected and uninfected predators to examine histopathological changes in adult female predators after inoculation with *A. phytoseiuli*.

Materials and Methods

Cultures

Microbial cultures

A culture of the bacterium *Acaricomes phytoseiuli* (DSM 14247) was maintained on Luria Bertani (LB) agar at 23±1 °C (L16:D8). It was obtained from material stored at –80 ºC that was isolated from surface-sterilized adult female *P. persimilis* of the NR-population.

A small amount of bacterial material grown on LB agar for a period of 5-7 days was diluted in 10 ml sterile distilled water. The suspension of cells had an optical density of 0.13-0.16 on a spectrophotometer (Bio Rad Smart Spec^{TM}) set at 600 µm (corresponding to an *A. phytoseiuli* concentration of approximately 1-1.2 * 10^9 cells/ml). The concentration of *A. phytoseiuli*, expressed as colony forming units (CFU)/ml, could not be determined in faeces and bacterial suspensions, as isolated colonies of *A. phytoseiuli* were never found on LB medium. *A. phytoseiuli* did not grow at all or aggregated over big parts of the nutrient plate.

Plants and herbivores

Lima bean plants (*Phaseolus lunatus* L.) were reared in a greenhouse at 20-25 °C (L16:D8). The herbivorous two-spotted spider mite, *Tetranychus urticae* Koch, was reared on intact bean plants under the same conditions in a separate greenhouse compartment.

Predators

General rearing

The predator population designated responding (=R) population originated from the NR-population. It was started with surface-sterilized eggs from the NR-population and was cultured in a closed rearing system. Detached Lima bean leaves infested with spider mites were placed in Parafilm-sealed plastic Petri dishes (diameter = 9 cm) in a climate chamber at 23±1 °C (L16:D8). In each dish 4 gravid females were kept for egg production during 48 hours after which females were eliminated. New leaves infested with spider mites were

added every 2 to 3 days. After one week, when the eggs had developed into adults, gravid females were transferred to new Petri dishes to initiate a new generation or they were used in experiments. Thus, predators were reared in distinct generations. At least 15 dishes were prepared per generation. The R-population consists of non-symptomatic predators only (chapter 4).

Pre-infection rearing of the R-population

To minimize variation due to accidental contamination of rearing dishes we reared sisters of comparable age, mated by a brother, which were then equally distributed over the different treatments of an experiment. Twenty-five mated female predators of the R-population were placed individually on a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm). Females were removed after 24 hours and dishes with a dead predator or few eggs were eliminated. The eggs of the remaining dishes were transferred to the underside of a prey-infested leaf in a new dish, as female predators prefer the leaf underside for egg deposition. After 4 days new food was added to each dish. After 8 days each Petri dish contained the adult offspring of one mother. As in a batch of 4-5 eggs, which is the daily egg production of a healthy female, the first egg produced is usually a male (Amano and Chant, 1978) each Petri dish contained at least one male which had inseminated its sisters.

Experiments

Infection of predatory mites

Leaf pieces heavily infested with all stages of spider mites were placed upside down on a 58 x 47 cm filter paper in a fume hood. The number of leaf pieces varied between experiments and replicates ($18<N<26$). Pieces of comparable size (ca. 12 cm^2) cut from one leaf were evenly distributed over treatment and control. Four ml of sterilized distilled water (control) or 4 ml of the bacterial inoculum (treatment) was sprayed evenly over the whole surface of the filter paper and leaf pieces with the help of a hand-held atomizer (Preval sprayer, 61 ml, 59.5 g Precision Valve Corporation, New York) in such a way that each leaf piece was entirely covered with small droplets. The fume hood was disinfected after each spray and for each treatment a new atomizer was used.

Adult female predators (age = 7 days) from the pre-infection rearing of the R-population were kept individually on these sprayed leaf pieces in sealed Petri dishes (diameter = 9 cm) during 3 days (ambient temperature 23±1 ^oC). They were then transferred individually to a spider mite-infested leaf disc (diameter = 2.5 cm) in a plastic Petri dish (diameter = 5.5 cm) placed in a climate chamber at 23±1 ^oC. Leaf discs cut from one leaf were evenly distributed

over treatment and control. Symptoms of the NR-syndrome were assessed for individual predators.

Pathogen isolation and detection from predators and predator faeces

Surface-sterilized predators were transferred to a droplet of 50 µl sterile water in a sterile plastic dish and squashed with a flamed needle until the body contents protruded. LB agar plates were streaked with these 50 µl and incubated for 5-7 days in a climate chamber at 23±1 ^{o}C (L16:D8).

Predator faeces and debris were collected by keeping surface-sterilized predators individually in sterile plastic Eppendorf vials (volume = 1.5 ml) carrying a small piece of sterilized wet cotton wool (ambient temperature 23±1 ^{o}C). After 24 hours the cotton wool, predators and predator eggs were removed. Deposited faeces droplets were then dissolved in 0.4 ml sterile distilled water. LB agar plates were streaked with 50 µl of the suspension and incubated for 5-7 days in a climate chamber at 23±1 ^{o}C (L16:D8).

When growing on LB agar under this incubation temperature *A. phytoseiuli* becomes visible after 5-7 days as a yellowish smear, which is easy to distinguish from other bacterial and fungal species regularly present on LB plates inoculated with faeces or predators. Bacterial material showing these characteristics was tested at a later date by a molecular test using a Polymerase Chain Reaction (PCR) with *A. phytoseiuli*-specific primers developed by Gols *et al.* (in prep.). In experiment 3, predators and predator faeces were tested with the same PCR test according to the methods described in Gols *et al.* (in prep.).

Experimental treatments

Experiment 1: The Koch's postulates

The effects of *A. phytoseiuli* on adult female *P. persimilis* were investigated using the bioassay described above. The symptoms predator size, predator mortality, predator fecundity, predator position and crystal location were assessed.

We determined **predator size** by classifying predators as either "normal-sized" or "small". Dorso-ventrally flattened female predators can easily be distinguished from the normal-sized predators with the help of a stereo microscope. **Predator mortality** and **fecundity** were assessed by counting the number of dead predators and of eggs deposited on the leaf disc as well as on the Petri dish. We noted the **position of the predator** within the Petri dish directly after selecting a dish for symptom assessment and calculated the fraction of females present on the leaf. **Crystal location within a predatory mite** was assessed by investigating live mites under a light microscope, equipped with two filters that create polarized light. Dumbbell-shaped crystals light up in polarized light, whereas other objects turn dark. As intact predators were needed for pathogen isolation after this measurement, no micro-slide

was placed on the predator. Predators were categorized into the following groups: (1) crystals present in the rectum and Malpighian tubules; (2) crystals present in at least one leg; (3) crystal location not possible. The percentage of predators carrying crystals in the legs was calculated from the number of predators for which crystal location was possible. The person measuring the parameters did not know to which treatment group the predator belonged. For a more detailed description of the symptom assessment, see chapter 4.

Because the symptoms predator size, predator mortality, and predator position can be observed without opening the Petri dishes, they were assessed daily during the exposure period as well as during the observation period (= 5 days). Fecundity was measured only at day 3-5 following exposure. The location of excretory crystals was determined in predators once, just before pathogen isolation. Two replicate experiments were run with 25 predators in each control and treatment group. Predators were collected at random for re-isolation of *A. phytoseiuli* at day 1-6 following exposure. These predators were surface-sterilized, squashed and streaked on agar plates, or surface-sterilized and used for faeces collection, which were subsequently streaked on agar plates. In some cases both faeces and the predator that deposited the faeces were used. A PCR test with *A. phytoseiuli*-specific primers **(**Gols *et al.,* in prep.) was conducted for14 predators.

Experiment 2: Behavioural response to HIPV

The effect of infection with *A. phytoseiuli* on the attraction of adult female *P. persimilis* to HIPV was also investigated. The **response to HIPV** was tested at 4 days after the start of exposure in a two-choice set-up. In a closed-system Y-tube olfactometer the odour from 9 trifoliate Lima bean leaves infested with ample amounts of two-spotted spider mites was offered vs. the odour from 9 uninfested trifoliate Lima bean leaves of comparable size. Predators were individually released into the olfactometer and observed until they made a choice for one of the odour sources. The percentage of predators choosing the odour of infested leaves was calculated from those predators making a choice within 5 minutes. For a detailed description of the symptom assessment, see chapter 4.

To confirm the occurrence of the NR-syndrome in the predators exposed to *A. phytoseiuli* 3 other symptoms i.e. predator size, predator position and fecundity were determined prior to the olfactometertest. To confirm infection with *A. phytoseiuli*, several predators of each treatment group were streaked on agar plates directly after the olfactometertest. Bacterial material suspected to represent *A. phytoseiuli* was then tested with the *A. phytoseiuli*-specific PCR test (Gols *et al*., in prep).

Three replicates with 19, 21 and 16 predators respectively were run and 15 predators were used for pathogen isolation. During the experiment 4 treated predators were lost and 10 treated predators died prior to symptom measurement. Symptoms could therefore be measured for 56 control and 42 treated predators.

Experiment 3: Histopathology

The effect of *A. phytoseiuli* on the internal structure of adult female *P. persimilis* was investigated using the bioassay described above. Sixty predators were inoculated per treatment group. Three days after inoculation, 20 control and 20 treated predators were randomly selected and shipped together with ample food to Germany where they were prepared one day later for examination with light and electron microscopy.

To confirm the occurrence of the NR-syndrome in exposed predators, 3 symptoms including predator size, predator position and fecundity were measured at 4 days after inoculation for the remaining predators. To confirm infection with *A. phytoseiuli* four different methods were applied: (1) predators were tested directly with the *A. phytoseiuli*-specific PCR test as described by Gols *et al*. (in prep.); (2) predators were streaked on agar plates and bacterial material suspected to represent *A. phytoseiuli* was then tested with the PCR test; (3) predators were used for faeces collection which was subsequently tested with the PCR test; (4) predators were used for faeces collection which was subsequently streaked on agar plates and tested with the PCR test.

During symptom assessment only 1 control and 2 treated predators were lost. Moreover, 3 control predators and 4 treated predators died prior to symptom assessment. Thus symptoms could be determined for 36 control and 34 treated predators.

Light and electron microscopy of adult female *P. persimilis*

For histopathological studies, series of treated predators (exposed to bacterial inoculum) and control predators (exposed to sterile water) were prepared. After cutting off the legs, the body of the mites was fixed for light microscope investigations in Dubosq-Brazil's alcoholic Bouin's and embedded in Histosec (Merck, Darmstadt, Germany). Sections were cut at 4-6 µm, stained with Heidenhain's iron hematoxylin, and counterstained with erythrosin (Merck, Darmstadt, Germany) or stained with 5% Giemsa solution in 0.02M phosphate buffer (pH 6.9) (Romeis, 1989) for 30 min. A Leica DMRB photomicroscope (Leica, Bensheim, Germany) with bright field equipment was used to examine the sections.

For transmission electron microscopy, mites were fixed overnight at 4°C in 3% glutaraldehyde in Veronal buffer (pH 7.2), and post fixed in 2.0% osmium tetroxyde in the same buffer for 5h. Subsequently, they were stained *en bloc* in 2% aqueous uranyl acetate for 5h and then dehydrated through increasing concentrations of ethanol. Finally, the mites were

embedded in a n-butyl-methyl-methacrylate mixture (7:3) (Merck, Darmstadt, Germany). Thin sections were obtained with a Leica Ultracut S microtome and stained with 6% lead citrate, followed by 2% aqueous uranyl acetate. Sections were investigated with a Zeiss 902 transmission electron microscope.

Hygienic measures

All equipment used to handle predators and prey-infested leaves, like brushes and forceps, was sterilized in 0.5% sodium hypochlorite (NaClO) solution prior to use, after which it was rinsed with water several times. Other equipment, distilled water and nutrient media were autoclaved before usage.

For the start of the R-population, predator eggs were surface-sterilized by placing them in a 0.5% sodium hypochlorite solution for 30 seconds. Subsequently, they were rinsed 3 times in sterile water during 30 seconds each. Eggs were shortly dried on tissue paper and transferred to the underside of prey-infested bean leaves. Ca. 500 eggs were used to initiate the R-population.

Adult female predators were surface-sterilized by placing them subsequently in 70% alcohol for a few seconds, briefly in sterile water and in a 0.5% sodium hypochlorite solution for 30 seconds after which they were rinsed briefly in 3 changes of sterile water (procedure according to Lacey and Brooks, 1997).

Statistics

The Mann-Whitney U test was used to test numerical data. A contingency table test was applied for categorical data. The data from the replicates were pooled, as no differences were found between the replicates.

Results

Experiment 1: The Koch's postulates

Symptoms of the NR-syndrome were induced in female predators from the R-population after a 3 day-exposure to an inoculum of *A. phytoseiuli*, whereas the NR-syndrome was not induced in predators from the R-population exposed to sterile water (Figure 1). Only two of the control predators (4%) shrank during the experimental time, whereas 80% of the treated predators were dorso-ventrally flattened 5 days after start of exposure. Differences between control predators and treated predators were significant 3, 4 and 5 days after exposure start ($P<0.01$ at day 3, $P<0.001$ at day 4 and 5, Figure 1A). Significantly fewer treated predators were found on the leaf at 4 days after exposure start ($P<0.001$, Figure 1B). Oviposition rate

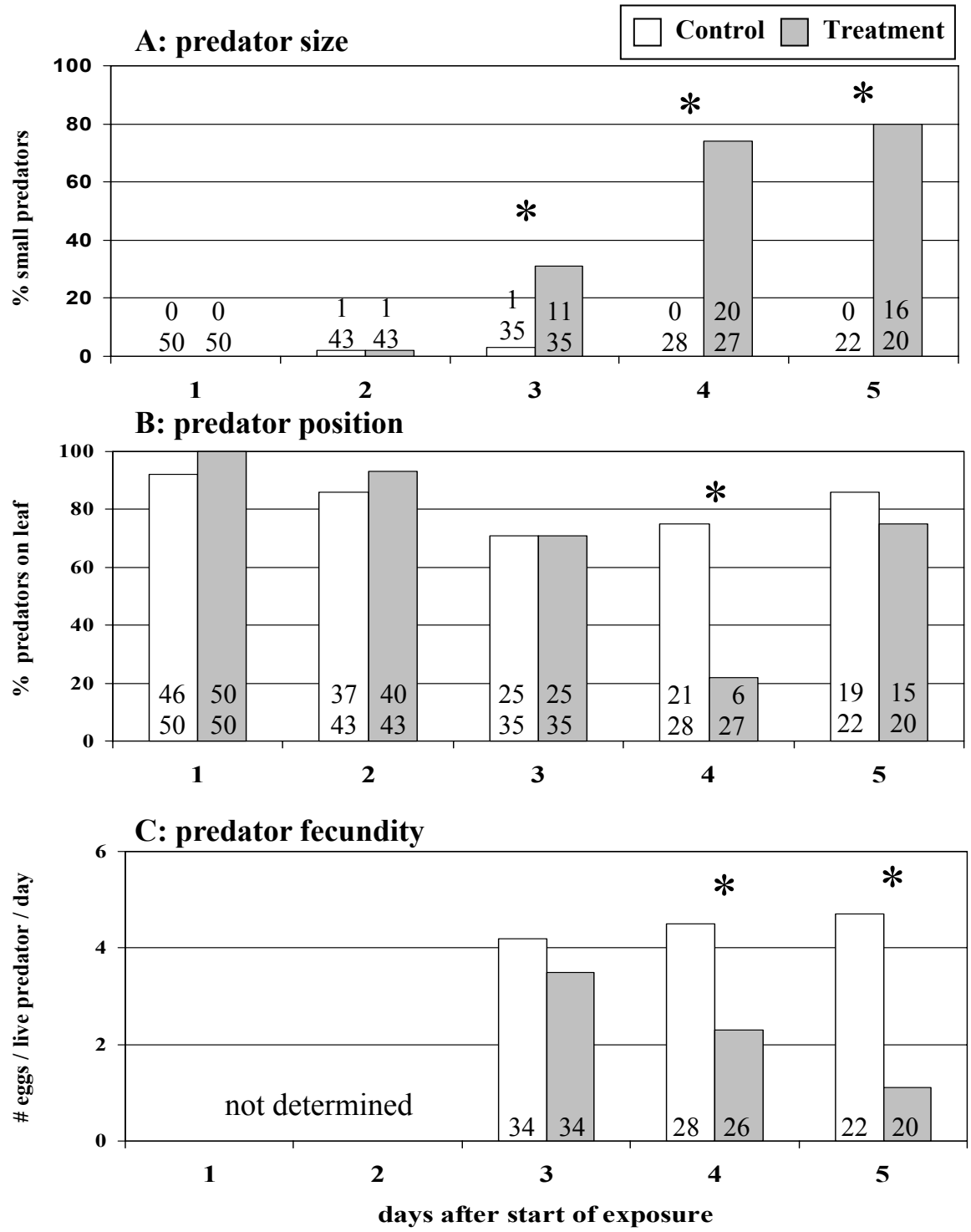

Figure 1: Symptoms of adult female *P. persimilis* from the R-population after a 3 day-exposure to prey-infested leaf pieces that were either sprayed with sterile distilled water (=control) or with an aqueous inoculum of *A. phytoseiuli* (=treatment), A) percentage small predators B) percentage predators residing on prey-infested leaves, C) mean number of eggs/ live predator /day. In graph A and B the first number in the bars represent actual predator numbers being small (A) or residing on the leaf (B), whereas the second number represents the total number of tested predators. In graph C the numbers in bars represent the number of predators for which oviposition was determined. Asterisks indicate significant difference between control and treatment (2 by 2 contingency table test for categorical data (A, B) and Mann-Whitney U test for numerical data (C), α=0.05).

of the treated predators was lower than oviposition rate of the control predators at each observation day, differences being significant 4 and 5 days after exposure start (P<0.001, Figure 1C). The presence of excretory crystals in the legs was also induced by *A. phytoseiuli*. As much as 20% of 40 treated predators analyzed carried crystals in the legs whereas all 40 control predators analyzed carried excretory crystals only in the rectum and Malpighian tubules (P=0.005). Mortality was somewhat higher for treated predators than for control predators. None of the control predators died whereas 10% of the treated predators were dead at the end of the experimental period (P=0.06).

A. phytoseiuli was never isolated from control predators and their faeces, whereas it was isolated directly from the predator in 60% of 37 treated predators and from faeces in 41% of 17 treated predators (Figure 2). For both control and treated predators respectively, more bacteria were isolated from squashed predators than from faeces (P<0.001 for control, P=0.003 for treatment). Only during the first day after exposure *A. phytoseiuli* could not be isolated from treated predators and their faeces, whereas it could be isolated daily from at least some individuals 2 to 6 days after exposure start (Figure 3). *A. phytoseiuli* was not isolated from

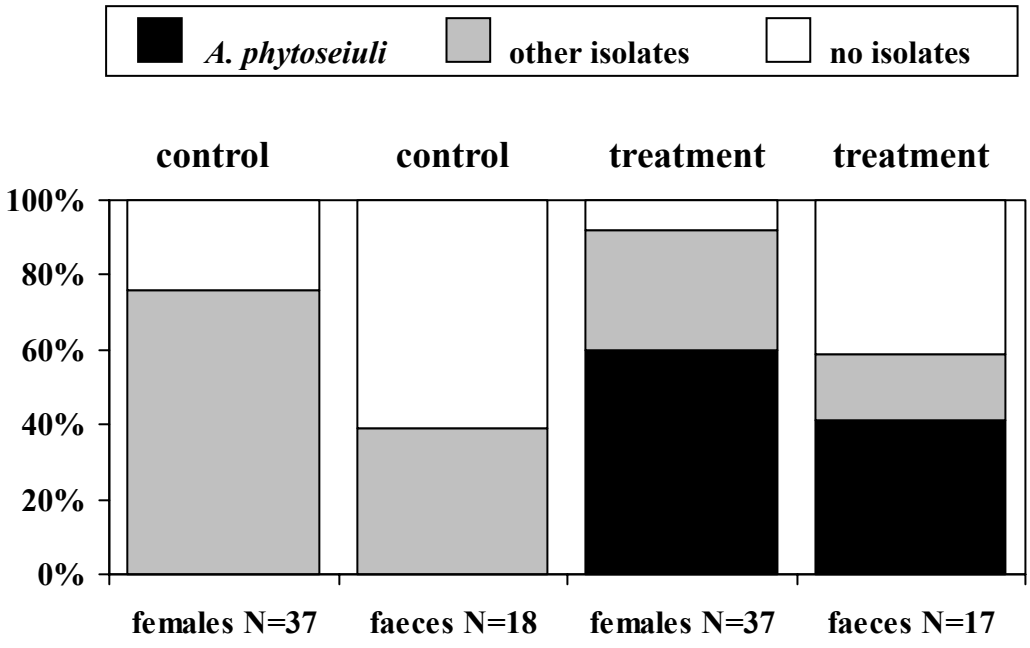

Figure 2: Percentage of adult female *P. persimilis* from the R-population from which we isolated *A. phytoseiuli* (black), other bacterial and fungal isolates (grey) or no isolates (white) on LB agar (summarized data). Bacterial isolates originated from the female predator (=females) and/or from faeces deposited by female predators (=faeces). Predators were previously exposed to prey-infested leaf pieces that were either sprayed with sterile distilled water (=control) or with an aqueous inoculum of *A. phytoseiuli* (=treatment).

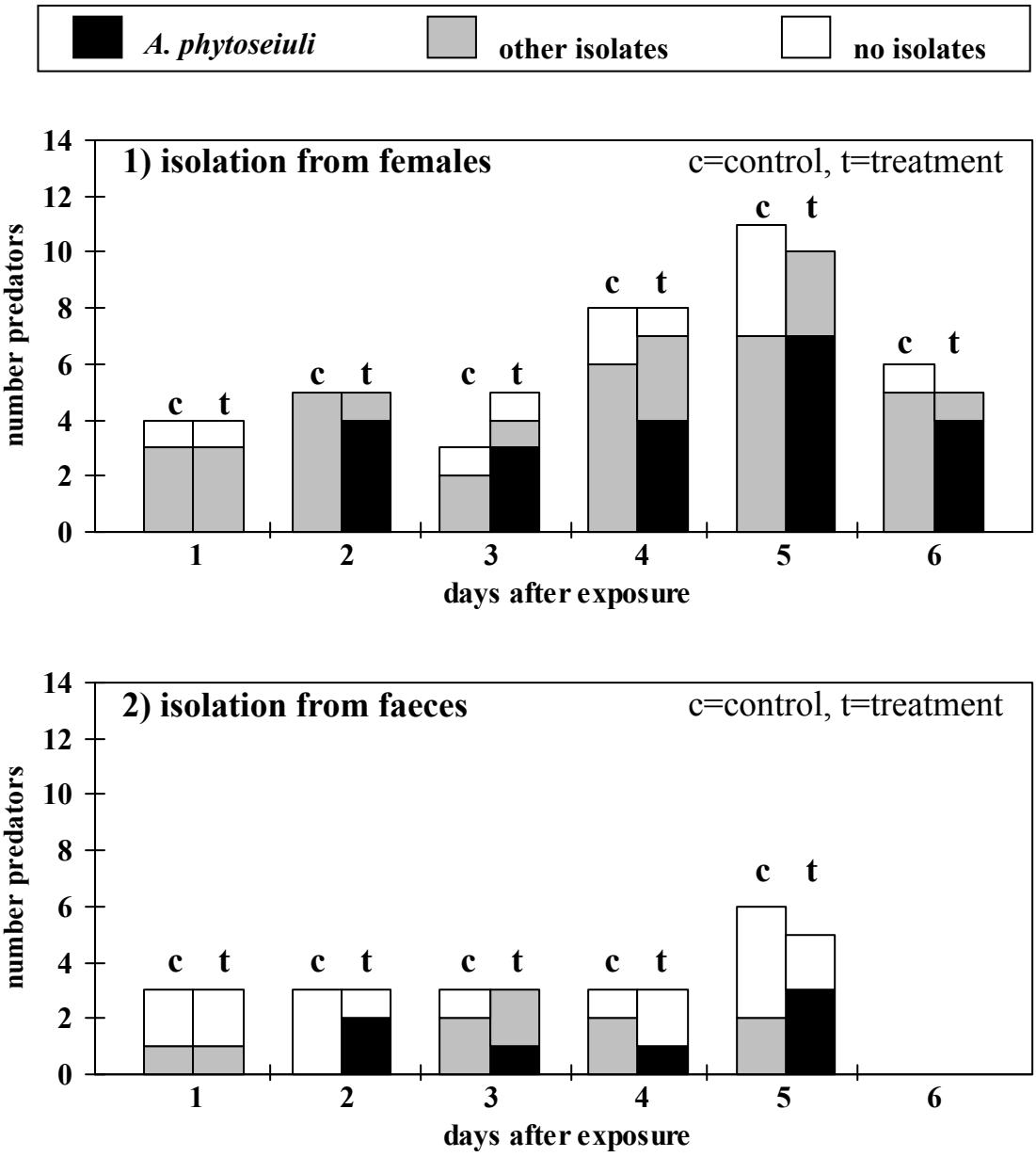

Figure 3: Percentage of adult female *P. persimilis* from the R-population from which we isolated *A. phytoseiuli* (black), other bacterial and fungal isolates (grey) or no isolates (white) on LB agar (daily data). Bacterial isolates originated from the female predator (=1) and/or from faeces deposited by female predators (=2). Predators were previously exposed to prey-infested leaf pieces that were either sprayed with sterile distilled water (=control) or with an aqueous inoculum of *A. phytoseiuli* (=treatment).

all the predators that showed the NR-syndrome, and sometimes it was also isolated from non-symptomatic predators treated with the bacterium: isolation was successful for 15 out of 25 symptomatic predators and for 10 out of 25 non-symptomatic predators of the bacteria-treated predators. In 14 cases material of bacterial colonies was tested with the *A. phytoseiuli*-specific PCR test (Gols *et al*., in prep.) and in all cases the test was positive.

Experiment 2: Response to prey-infested leaves

The vast majority of the adult female predators made a choice for one of the two odour sources when tested in the Y-tube olfactometer, differences between control and treatment being not significant (P=0.44, Figure 4a). In contrast, significant differences were found between treatment and control predators concerning their preference for HIPV. As much as 82% of 44 control predators preferred the odour of spider mite-infested leaves in the Y-tube olfactometer, whereas only 58% of 36 treated predators showed this preference (P=0.027, Figure 4b) A preference of 80% and more for the odour of prey-infested leaves is comparable to data for responding populations (chapter 3 and 4), whereas a preference of less than 70% for the odour of infested leaves is comparable to data for the NR-population (chapter 3 and 4).

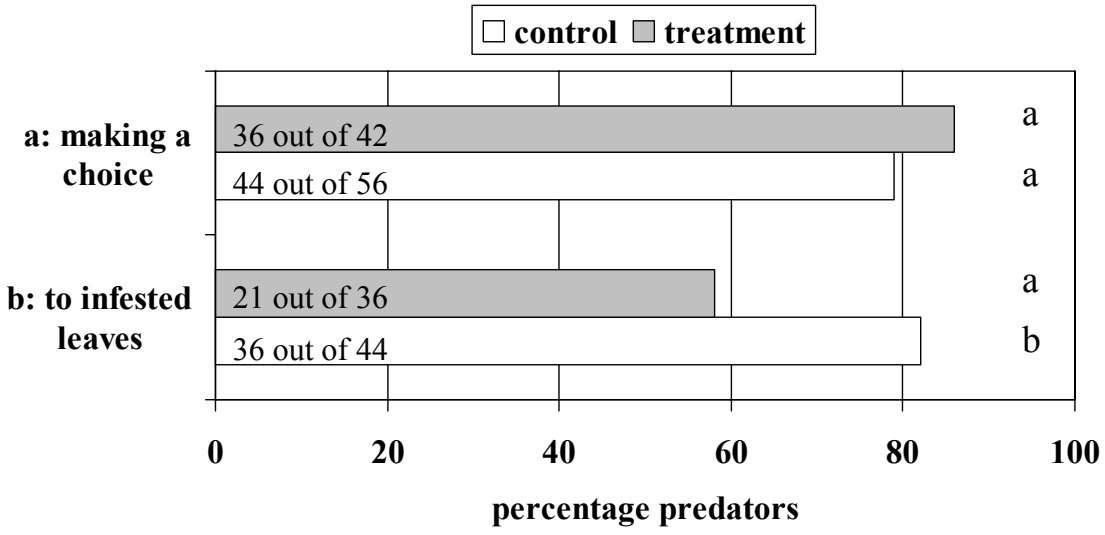

Figure 4: Percentage adult female *P. persimilis* that made a choice in the Y-tube olfactometer (=a) and those that preferred the odour of spider mite-infested leaves (=b). Predators were previously exposed to prey-infested leaf pieces that were either sprayed with sterile distilled water (=control) or with an aqueous inoculum of *A. phytoseiuli* (=treatment). Numbers in bars refer to actual predator numbers that performed the indicated behaviour. Different letters next to bars indicate significant difference between treatment and control (2 by 2 contingency table test, P< 0.05).

Table 1: Symptoms of live adult female *P. persimilis* of the R-population after a 3 day-exposure to prey-infested leaf pieces that were either sprayed with sterile distilled water (=control) or with an aqueous inoculum of *A. phytoseiuli*: (**a**) experiment 2 (**b**) experiment 3. Numbers in parentheses represent actual predator numbers. For explanation of symptoms see chapter 4.

1a) Experiment 2	**Control** (N = 56)	**Treatment** (N = 42)	P*
Predator size			
% small ♀♀	**0** (out of 56)	**88** (37 out of 42)	< 0.001
Predator fecundity			
# eggs / live ♀ / day (average ± SD)	**4.1** ± 0.6 (56)	**0.5** ± 1.0 (42)	< 0.001
Predator position within dish			
% ♀♀ on leaf	**84** (47 out of 56)	**36** (15 out of 42)	< 0.001

1b) Experiment 3	**Control** (N = 36)	**Treatment** (N = 34)	P*
Predator size			
% small ♀♀	**0** (out of 36)	**68** (23 out of 34)	< 0.001
Predator fecundity			
# eggs / live ♀ / day (average ± SD)	**4.4** ± 1.0 (36)	**2.4** ± 1.9 (34)	< 0.001
Predator position within dish			
% ♀♀ on leaf	**72** (26 out of 36)	**41** (14 out of 34)	0.009

* Mann-Whitney U test for numerical data, 2 by 2 contingency table test for categorical data

Exposure to an inoculum of *A. phytoseiuli* also induced other characteristic symptoms of the NR-syndrome in experiment 2 (Table 1a). None of the control predators shrank permanently during the experimental time, whereas as much as 88% of the treated predators were dorso-ventrally flattened 4 days after start of exposure (P<0.001, Table 1a). Oviposition rate of the treated predators was lower than of the control predators (P<0.001 Table 1a) and fewer treated predators than control predators were found on the prey-infested leaf (P=0.001, Table 1a). Infection of treated predators with *A. phytoseiuli* was confirmed in 13 out of 15 cases (87%).

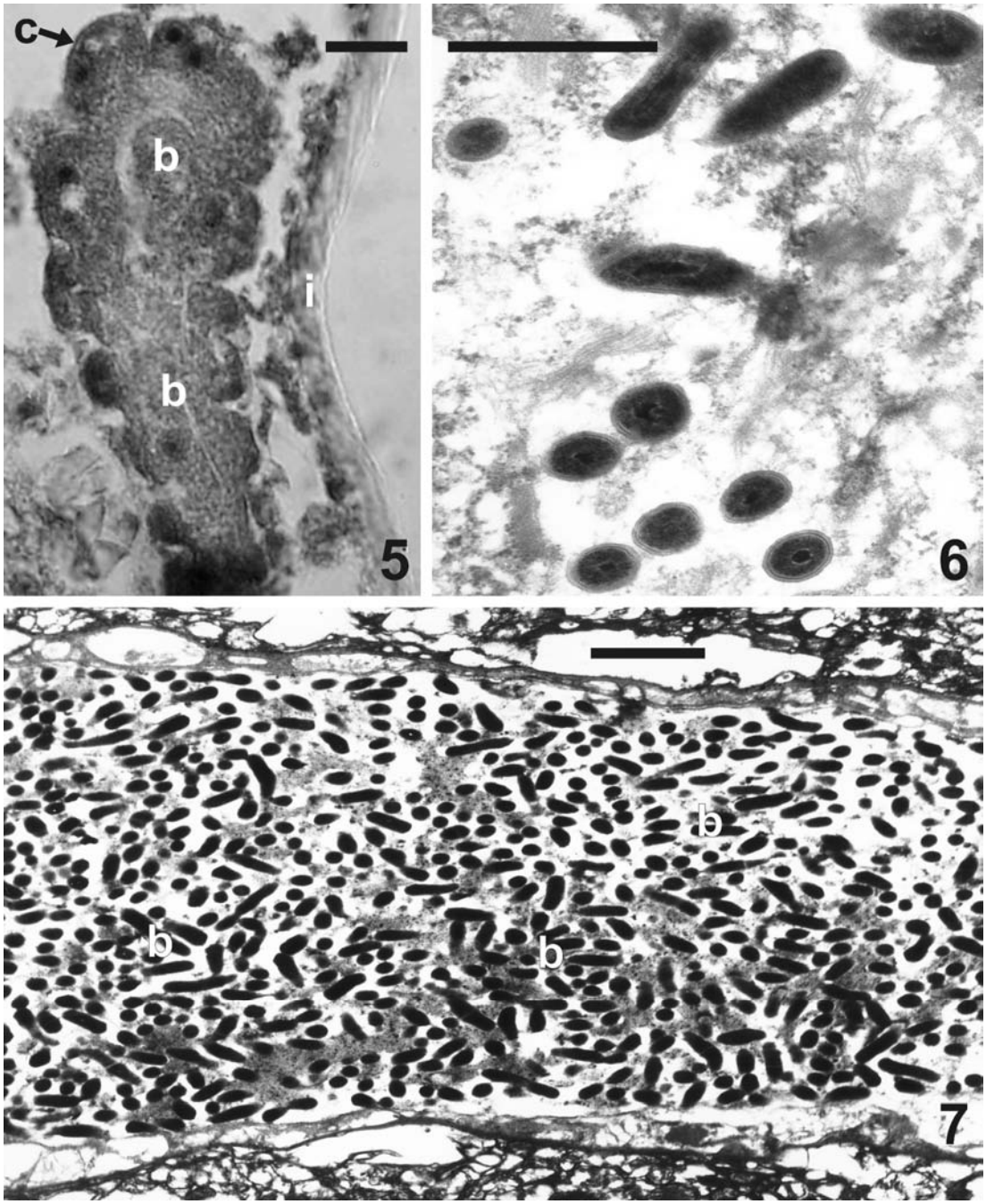

Figure 5: Light micrograph of a section of a caecum (c) of the alimentary tract of an adult female *P. persimilis* that was previously exposed to *A. phytoseiuli* inoculum. Note the high accumulation of bacteria (b) in the lumen, (i) integument; bar = 10µm.

Figure 6: Electron micrograph of a thin section of the fat body of an adult female *P. persimilis* that was previously exposed to *A. phytoseiuli* inoculum infected with rod-shaped bacteria; bar = 1µm.

Figure 7: Electron micrograph of a thin longitudinal section of the digestive tract of an adult female *P. persimilis* that was previously exposed to *A. phytoseiuli* inoculum. The lumen is densely packed with bacteria (b); bar = 2µm.

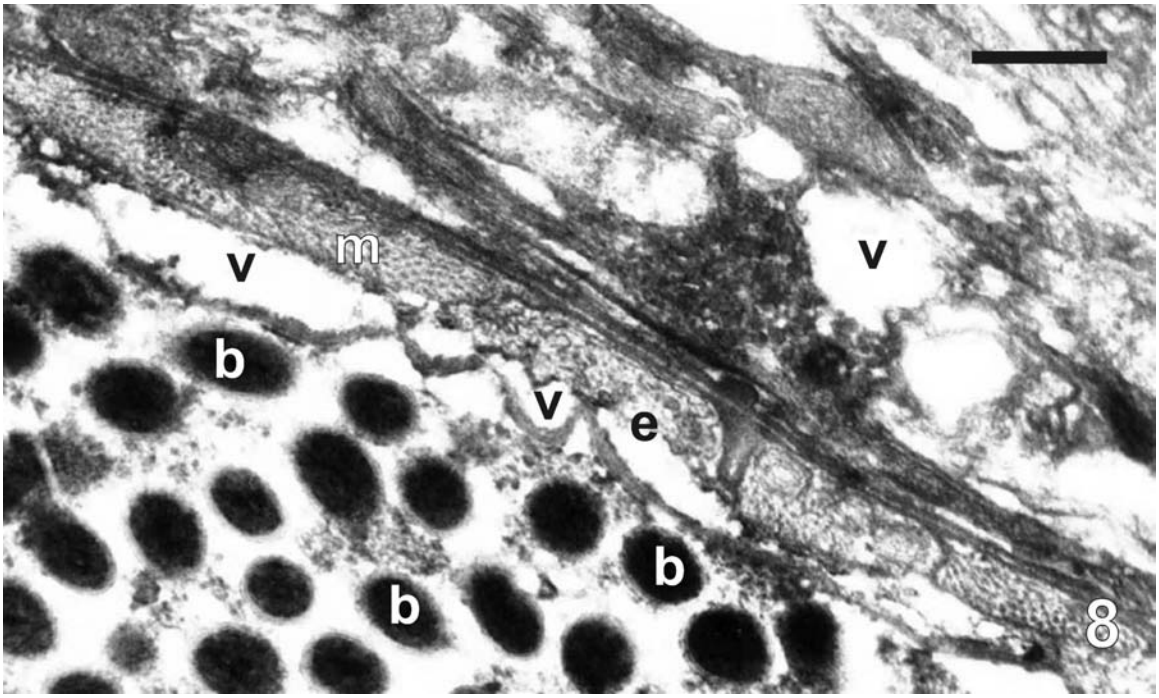

Figure 8: Electron micrograph of a peripheral part of the digestive tract of an adult female *P. persimilis* that was previously exposed to *A. phytoseiuli* inoculum in higher magnification. (b) Bacteria filling the gut lumen; the adjacent epithelium (e) displays drastic degeneration; only large vacuoles (v) and cell borders are left; (m) muscularis; bar = 1µm.

Experiment 3: Histopathology

Analysis of both light and electron microscope sections revealed that in 6 of 11 investigated treated predators unusual amounts of bacteria accumulated in the lumen of the digestive tract. Frequently, the whole gut lumen was densely packed with bacteria (Figures 5, 7 and 8). Together with progressive accumulation of bacteria, the adjacent epithelium of the digestive tract displayed drastic degeneration, finally only leaving large vacuoles and cell borders (Figures 7 and 8). Often, the bacteria had entered the fat body (Figure 6). In contrast, in all of the 9 control mites investigated only relatively low numbers of bacteria were found in the digestive tract and no bacteria could be observed in the fat body.

Exposure to an inoculum of *A. phytoseiuli* induced the characteristic symptoms of the NR-syndrome in experiment 3, too (Table 1b). None of the control predators shrank permanently during the experimental time, whereas as much as 68% of the treated predators were dorso-ventrally flattened 4 days after start of exposure ($P<0.001$, Table 1b). Oviposition rate of the treated predators was lower than of the control predators ($P<0.001$ Table 1b). Moreover, significantly fewer treated predators were found on the leaf ($P=0.009$, Table 1b).

A. phytoseiuli was not detected in any of the control predators, whereas it was found in some treated predators, irrespective of the method used: the PCR test was positive (1) in 2 out of 10 cases (20%) when predators were tested directly with the PCR, (2) in 5 out of 12 cases (42%) when predators were streaked on agar plates, (3) in 2 out of 9 cases (22%) when faeces was used for the PCR, and (4) in 1 out of 5 cases (20%) when faeces were streaked on agar plates.

Discussion

Effects of *A. phytoseiuli*

Exposure to an inoculum of *A. phytoseiuli* induced characteristic symptoms of the NR-syndrome in adult female *P. persimilis* of the R-population in all three experiments within a period of 2-5days after the start of exposure. In contrast, none of these symptoms was recorded in control predators that were exposed to sterile distilled water. The data for the control predators are comparable to previous data of non-symptomatic female predators originating from healthy populations (chapter 4, 6, and 7), whereas the data for treated predators are comparable to previous data of predators from the NR-population (chapter 4) and for predators that were exposed to faeces and debris of symptomatic predators from the NR-population (chapter 6 and 7). Moreover, *A. phytoseiuli* could be isolated only from treated predators and their faeces and debris 2-6 days after the start of exposure, which corresponds well in time with the occurrence of the NR-syndrome. Thus, in the present study the Koch's postulates are satisfied, which constitutes the final proof for our hypothesis that the bacterial pathogen *A. phytoseiuli* is the causative agent of the NR-syndrome in adult female *P. persimilis*.

Exposure to an inoculum of *A. phytoseiuli* induced, next to the characteristic NR-syndrome, histopathological changes in adult female *P. persimilis*. Our histological investigations of females exposed to the bacterial inoculum disclosed striking accumulations of bacteria occupying the lumen of the alimentary tract that may even be blocked totally. In addition a drastic degeneration of the epithelium of the alimentary tract took place. Moreover, the bacteria did also colonize the fat body. As no other possibly pathogenic agents could be found in all our light and electron microscope investigations of the treated predators and as no bacterial accumulations were recorded in control predators, it is very likely that the bacterial accumulations consist of *A. phytoseiuli*. Degeneration of the alimentary tract as observed here could explain the presence of birefringent excretory crystals in the legs of symptomatic predators. Such crystals are normally present in the Malpighian tubules and the rectum. Epithelium degeneration could lead to the invasion of the crystals into other regions of the body.

Effects of *A. phytoseiuli* on host behaviour

Host behaviour and mobility are regarded as key factors initiating epizootics (Andreadis, 1987), especially in host-pathogen associations where the main transmission mode is horizontal as in the present system (chapter 5, 6 and 7) where infective stages are released by the host throughout its life.

Exposure to an inoculum of *A. phytoseiuli* induced a lower degree of attraction to HIPV and a higher tendency of leaving a prey patch in adult female *P. persimilis.* These results are in accordance with earlier studies on the NR-syndrome (chapter 3, 4, 6 and 7). *A. phytoseiuli*-infected females are potentially less likely to encounter prey patches and are more likely to disperse from prey patches than uninfected conspecifics. These two behavioural traits could result in reduced encounters between infected and uninfected predators and thus reduce disease transmission, which would be beneficial to the host. To verify this hypothesis more information about the timing of the recorded changes, as well as other behavioural characteristics and effects of behavioural changes in (semi-) field situations is required (see for further discussion chapter 9).

Besides one report of *Wolbachia*-induced behavioural changes in the spider mite *T. urticae* (Vala *et al*., 2004), we are not aware of reports of behavioural changes due to pathogen infection in the Acari, whereas such behavioural changes including changes in microhabitat preference, are well documented for pathogen infection in insects (see for a review Horton and Moore, 1993). However, information on bacterial entomopathogens is very scarce. Horton and Moore (1993) cite only two cases: (1) a *Rickettsiella*-species induces elevation-seeking behaviour in its Coleopteran host and (2) another *Rickettsiella*-species induces changes in temperature preference in its Orthopteran host.

Pathogens in *P. persimilis*

Several entities including non-occluded viruses, unidentified bacteria, *Rickettsiella*, *Wolbachia* and three species of *Microsporidia* have been reported for *P. persimilis* (see for reviews: van der Geest *et al*., 2000; Bjørnson and Schütte, 2003; Schütte *et al*., 2005; chapter 2). However, for only one microsporidium species, isolated from a European population of *P. persimilis* and assigned as *Microsporidium phytoseiuli*, clear pathological effects on its host have been found, including reduced fecundity, longevity and predation rate. Moreover, infected female mites produced fewer female progeny than uninfected females, as the sex ratio of offspring of infected females was male biased (Bjørnson and Keddie, 1999). Maternal-mediated vertical transmission of *M. phytoseiuli* is 100% and evidence for horizontal transmission of *M. phytoseiuli* was only found when uninfected immatures were kept together with infected adult and immature mites (Bjørnson and Keddie, 2001). Thus *M. phyto-*

seiuli has less obvious and severe effects on *P. persimilis* than *A. phytoseiuli* and has a different transmission strategy (predominantly vertical transmission) than *A. phytoseiuli* (entirely horizontally transmitted) (see for further discussion chapter 6 and 9).

Bacterial pathogens in Acari other than ticks

Whereas numerous insect-specific and tick-specific bacterial pathogens are known (Tanada and Kaya, 1993; Schabereiter-Gurtner *et al*., 2003), knowledge on bacterial pathogens of *Acari* other than ticks is rather scarce. In 1971 Lipa stated that no pathogenic bacteria have ever been isolated from mites and 27 years later Poinar and Poinar (1998) concluded in their review on mite diseases that no mite-specific bacteria have been isolated to date. This may be partly due to the small size of many species and to the low economical and human-health importance of the majority of mite species (Poinar and Poinar, 1998; van der Geest *et al*., 2000). During the past decade research has concentrated on intracellular bacteria such as *Rickettsiella*, *Wolbachia* and *Cardinium*-like organisms that may manipulate host biology in various ways (Šut'áková, 1991; Breeuwer and Jacobs, 1996; Johanowicz and Hoy, 1998a, b; Weeks *et al*., 2003; Weeks and Stouthamer, 2004; Hoy and Jeyaprakash, 2005). In a recent study micro-organisms associated with the predatory mite *Metaseiulus occidentalis* and its prey *Tetranychus urticae* were assessed using a high-fidelity polymerase chain reaction (PCR) protocol (Hoy and Jeyaprakash, 2005). Sequences from four bacterial species related to *Wolbachia*, *Cardinium*, *Bacteroidetes* and *Enterobacter* were obtained from *M. occidentalis*, and three sequences related to *Wolbachia*, *Rickettsia* and *Caulobacter* were obtained from *T. urticae*. Both mites were negative for Archaebacteria (for a further discussion see chapter 2). Only for two bacterial species clear host-effects have been demonstrated for *M. occidentalis*: (1) *Wolbachia* caused non-reciprocal reproductive incompatibilities between infected males and uninfected females. *Wolbachia* infection seems to be associated with fitness costs as the number of female progeny was lower in infected control crosses than in uninfected control crosses (Johanowicz and Hoy, 1998a). (2) A clear and significant increase in fecundity was associated with infection by *Cardinium* (Weeks and Stouthamer, 2004).

Several authors investigated the effects of bacterial entomopathogens on mites with pathogen isolates of non-mite origin. In most studies, effects of commercial formulations of *Bacillus thuringiensis* or its ß-exotoxin have been evaluated (see for a review van der Geest *et al*., 2000). In one study the opportunistic pathogen *Serratia marcescens* was investigated (Lighthart *et al.*, 1988). The authors demonstrated that several stress factors enhanced the susceptibility of the predatory mite *Metaseiulus occidentalis* to *S. marcescens,* elevated mortality being the sole disease symptom. These studies show that mites are potentially susceptible to bacterial pathogens.

Only two reports exist on the isolation of bacteria from diseased mites. (1) Unidentified bacteria were isolated from a diseased specimen of *Dendrolaelaps* sp. from Poland (Thomas and Poinar, unpublished data cited in Poinar and Poinar, 1998). (2) A specimen of *T. urticae* that had been reared at 30 ^{o}C under continuous light was diagnosed as carrying *Pseudomonas aeruginosa*, a well-known insect bacterium with stress-induced pathogenicity (Thomas and Poinar, 1973). However, the Koch's postulates were not satisfied in these cases.

Thus, *A. phytoseiuli* represents the first report of a bacterial pathogen in the true sense for mites other than ticks. This finding may be of utmost importance for the biological control industry, as *P. persimilis* is one of the key organisms of biological control. It is striking, that some symptoms resembling the symptoms of the NR-syndrome of *P. persimilis* have been reported for other populations of *P. persimilis* and for other phytoseiid species. A reduced attraction to HIPV has been reported for a commercial population of *P. persimilis* (chapter 3) and for three laboratory populations of *Amblyseius potentillae* and one laboratory population of *Typhlodromus pyri* (Dicke *et al*., 1991a). Dorso-ventrally flattened females that show less vigour have been reported previously for 4 commercial populations of *P. persimilis* (Bjørnson *et al*., 2000; Steinberg and Cain, 2003), and for two other predatory mite species *Amblyseius hibisci* (Tanigoshi *et al*., 1981) and *Metaseiulus occidentalis* (Hess and Hoy, 1982). In the latter case the authors observed two morphologically distinct forms of bacteria in symptomatic mites, one of them being present in large numbers inside thin and pale mites. However, it was not determined whether high microbial load was the primary cause of the disease or a secondary effect. Later it has been suggested that the rickettsia-like organisms reported in the earlier microscopic surveys of phytoseiid mites are probably members of the genus *Wolbachia* (for a discussion see van der Geest *et al*., 2000). Moreover Weeks and Breeuwer (2003) have recently identified the endosymbiont described by Hess and Hoy (1982) as *Cardinium*. Nevertheless, these data pose the question whether *A. phytoseiuli* may also be present in other predatory mite populations. Future studies will therefore be aimed at addressing this question.

Acaricomes phytoseiuli

A. phytoseiuli is a Gram-positive, rod-shaped, none-spore forming bacterium. Comparative analysis of the 16S rDNA sequence revealed that the strain was a new member of the family of the Micrococcaceae. Nearest phylogenetic neighbours were determined as *Renibacterium salmoninarum* (94.0%), *Arthrobacter globiformis* (94.8%) and *Arthrobacter russicus* (94.6%) (Pukall *et al*., 2006). It appears that the new genus *Acaricomes* is closely related to the genus *Arthrobacter*. The genus *Arthrobacter* consists of a group of catalase-positive, strictly aerobic rod shaped micro-organisms that exhibit a coryneform morphology. Phylogenetically, species of this genus belong to the Actinomyces branch of the Gram-positive

bacteria and are among others closely related to members of the genus Micrococcus (Stackebrandt *et al*., 1997). The genus *Arthrobacter* is phenotypically heterogeneous. A large number of studies have shown that soil is the usual habitat of arthrobacters and that they are a numerically important fraction of the indigenous soil flora from various parts of the world (Keddie *et al*., 1986).

Interestingly one of the species closely related to *A. phytoseiuli* is a well-known pathogen. *R. salmoninarum* is the causative agent of bacterial kidney disease (BKD) in salmonid fishes (Sanders and Fryer, 1980). BKD is one of the most important diseases of wild and cultured salmonid fish and has been reported from many countries (see for a review Evenden, *et al*., 1993). Unlike some other fish pathogens it is not an opportunistic pathogen but an obligate pathogen, causing systematic chronic infections. *R. salmoninarum* can be transmitted vertically and horizontally and the expansion of salmonid fish culture has assisted in its spread.

The present results finally raise the question about the origin of *A. phytoseiuli*. It is not very probable that the bacterium originates from prey populations of *P. persimilis*, as up to now we have never been able to isolate *A. phytoseiuli* or detect it with molecular tests from laboratory populations of *T. urticae* (Gols *et al*., in prep.). Whether *A. phytoseiuli* is a native pathogen of wild *P. persimilis* populations, whether it was introduced in mass-rearing systems or whether it evolved rather recently in mass-rearing systems by horizontal gene transfer remains to be studied (see for further discussion chapter 9). We do not have reliable information about introductions of *P. persimilis* from South-America after its first shipment of 1957, as the biological control industry wants to keep that information confidential. Moreover, we did not test wild populations up to now. With the reliable PCR-test it is now possible to screen *P. persimilis* populations from different origins, including natural populations, which may shed light on the origin of the pathogen.

Acknowledgements

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Acaricomes phytoseiuli

ACARICOMES PHYTOSEIULI

Acaricomes phytoseiuli

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Acaricomes phytoseiuli

ACARICOMES PHYTOSEIULI

Chapter 9

General Discussion

Disease syndrome

The studies presented in this thesis led to the **isolation and description of *Acaricomes phytoseiuli*, a novel bacterial pathogen of the predatory mite *Phytoseiulus persimilis*** (chapter 8, Pukall *et al.*, 2006). *A. phytoseiuli* induces numerous diagnostic symptoms in adult female *P. persimilis* that form together a characteristic disease syndrome, designated NR-syndrome (Table 1). As a behavioural change was the first observed symptom and the starting point of the present thesis (chapter 3), I investigated behavioural symptoms in all chapters of this thesis. This is a rather unusual approach. Most invertebrate pathologists concentrate on the determination of life history parameters (fecundity, mortality, LD50) and anatomical symptoms. However, host behaviour may be a striking diagnostic disease symptom (Horton and Moore, 1993) and it may play a predominant role in disease transmission. (Andreadis,1987).

Several symptoms of the NR-syndrome including reduced fecundity, high mortality, low predation rate, low degree of attraction to herbivore-induced plant volatiles and early dispersal from prey patches negatively affect the performance of *P. persimilis* and thereby its efficacy in biological control programmes and/or its applicability in research programmes. It should be stressed here, that **the NR-syndrome may remain undetected for extended periods**. Dorso-ventrally flattened females can easily be regarded as juveniles (deutonymphs), virgin or starved females. As only mated females are tested in current quality control standards (van Lenteren *et al*., 2003a), dorso-ventrally flattened females could be excluded from testing. According to current quality control guidelines female *P. persimilis* should produce at least 10 eggs/female/5days and at least 80% of the tested females should be alive at the end of the test period of 5 days. Data on females that do not lay eggs during the test period of 5 days and those that do not survive are excluded from fecundity analysis (van Lenteren *et al*., 2003a). The NR-population of our laboratory did not meet the requirements for survival but did meet the requirements for fecundity (chapter 4). It could thus be possible that current quality control tests would not detect an infection with *A. phytoseiuli*.

Table 1: Diagnostic symptoms of the NR-syndrome, present in adult female *P. persimilis*

Symptom 
Behavioural
(1) Low degree of attraction to herbivore-induced plant volatiles in Y-tube olfactometer **(2) Short choice time** in Y-tube olfactometer **(3) High dispersal rate** from prey patches still carrying ample food
Non-behavioural
(4) Size change by shrinkage to dorso-ventrally flattened form shortly after mating **(5) Reduced fecundity** caused by oviposition stop after shrinkage **(6) High mortality** caused by death several days after shrinkage **(7) Low predation rate** caused by feeding stop **(8) Low excretion rate** after feeding stop
Anatomical
(9) Presence of excretory crystals in the legs and not only in excretory organs **(10) Bacterial accumulations inside the alimentary tract** that may be blocked totally **(11)Bacterial accumulations in the fat body** **(12)Degeneration epithelium of the digestive tract** leaving large vacuoles and cell borders

 **Does the NR-syndrome only occur in the NR-population?**

Currently some data suggest that **the NR-syndrome may be widespread among *P. persimilis* populations**. Recent molecular studies have shown that *A. phytoseiuli* is widespread among European commercial populations of *P. persimilis* (Gols *et al*., in prep.). Moreover, we have *A. phytoseiuli*-isolates from three different commercial populations, and faeces and debris collected from these *A. phytoseiuli*-infected populations did induce the NR-syndrome in healthy female *P. persimilis* (Gols *et al*., in prep.). Moreover, I am aware of several publications in which remarkable peculiarities of *P. persimilis* have been stated, that could, among others, be explained by an infection with *A. phytoseiuli*:

(1) Several authors reported poor performance in terms of **fecundity and survival** for several commercial populations of *P. persimilis* (Steiner, 1993 a, b; Steiner and Bjørnson, 1996; Raworth and Bjørnson, 2002; Blümel and Hausdorf, 2002). Raworth and Bjørnson (2002) determined short-term fecundity and survival according to current quality control guidelines (van Lenteren *et al*., 2003a) for predators from six commercial sources directly after delivery and after rearing them 30 days at their laboratory. Surprisingly the overall quality of all populations was low and did not improve by rearing them in the laboratory and

eliminating procedures of mass-production and transport that might affect fecundity and survival. Survival rates were even lowest for females that had been reared in the laboratory. The authors suggest that these unexpected results may have been caused by a temporary starvation period, as colonies were once subjected to limited prey. They hypothesize that cannibalism of young stages has affected the age distribution in such a way, that only older females with lower daily fecundity rates were harvested. However, it could also be possible that the poor performance has been caused by *A. phytoseiuli*.

(2) During a study on the effects of humidity on adult life span of several phytoseiid species a mean **life span** of only 19 days was found for *P. persimilis* (de Courcy Williams *et al*., 2004a). These results were in accordance with only one other study (23 days at 26°C, Takahashi and Chant, 1994), whereas in earlier studies generally much longer mean life spans were reported (33-51 days, various authors cited in Sabelis, 1981; 94-122 days at 20°C and 96% air humidity, Gaede, 1992). The authors suggest that differences may be attributed to likely differences between studies based on field-collected or mass-reared predators. However, it cannot be excluded that the early death of *P. persimilis* has been caused by *A. phytoseiuli*.

(3) During a large scale study of *P. persimilis* from four commercial sources, 30% of all female predators (216 of 718) were **dorso-ventrally flattened** or had an intermediate body shape after a 24-h acclimation period during which feeding was possible (Bjørnson *et al*., 2000). Only one third of these predators of smaller body shape (68 of 216) did produce eggs. As mating status and age of the non-ovipositing females was unknown, it might be possible that these individuals were dorso-ventrally flattened due to an infection with *A. phytoseiuli*.

(4) Surface sterilization of eggs collected from a poorly performing commercial population of *P. persimilis* had a positive effect on short-term survival and oviposition. Remarkably short-term performance was best in predators originating from eggs rinsed in water, compared to eggs washed with formaldehyde or tetracycline hydrochloride (Steiner and Bjørnson, 1996). As the cause of the poor performance of this predator population was not determined, it might be explained by an infection with *A. phytoseiuli*.

(5) Maeda *et al*. (2000) found remarkable effects of rearing conditions on the **olfactory response** of adult female *P. persimilis*. Mites that had been reared on an artificial arena (plastic board onto which periodically prey-infested bean leaves were deposited) were attracted to herbivore-induced plant volatiles in a Y-tube olfactometer whereas predators reared on a detached prey-infested leaf were not attracted. The authors state that disease infection cannot explain these results because of the following two arguments: (1) The *P. persimilis* population used in their study showed a significant positive odour response during 4

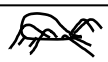
years rearing on the arena. However, also in my studies populations showed high responses during several years before the first occurrence of the behavioural change. Thus, this argument does not hold. It would be more important to give information on the response level of these predator populations at the time of the reported study. (2) The behavioural change was reversible, as predators were attracted to plant odours after being transferred from the detached-leaf culture to the artificial arenas. This argument only holds when in both cases the same individuals were tested. The presented data, however, strongly suggest that this is not the case. Therefore, the data presented by Maeda *et al.* (2000) do not exclude the effect of an infection with *A. phytoseiuli*. In this study rearing condition could have affected disease transmission and disease incidence, which would have effects on predator behaviour. Effects of rearing conditions will be discussed in more detail in the paragraph on transmission.

(6) Remarkable differences in dispersal behaviour between two populations of *P. persimilis* were found during studies on the **location and dispersal** of *P. persimilis* in strawberry fields (van de Vrie and Price, 1997). Commercially produced predators from the Netherlands released from containers had a higher tendency to disperse when released in a strawberry field than predators that were collected from a field population. Dispersal was most pronounced during the first day after release. The authors state that more studies are needed to understand the stimuli for this pronounced dispersal. They suggest that overcrowding in the transport containers and starvation may trigger the enhanced dispersal. However, I suggest that also in this case an infection with *A. phytoseiuli* cannot be excluded.

(7) During a study on **movement** of adult female *P. persimilis* on plants, an unusually high rate of disappeared predators was recorded, ranging from 70-99%. These high losses even made it difficult to draw any firm conclusions regarding the results (Skirvin and Fenlon, 2003a). The authors explain these high losses with high temperatures during the experiments, often being above 30°C sometimes reaching 40°C. However, the authors report that examination of the substrate did not lead to the recovery of any dead mites. The predators apparently left the experimental set-up without leaving any trace. Also this phenomenon could be explained by an infection with *A. phytoseiuli*. In yet another study the same authors found unusual results concerning the **predation rate** of *P. persimilis* (Skirvin and Fenlon, 2003b). In contrast to earlier findings they report a decline of the predation rate from 25°C to 30°C. The authors explained these contradictory results by the different experimental set-up and concluded that other studies may have overestimated predation rates at 30°C. In this context it is interesting to mention, that some data were caused by outlying data, where very low prey consumption was observed. These findings may be an indication of an infection with *A. phytoseiuli,* as infected predators often stop feeding (chapter 4).

(8) Steinberg and Cain (2003) executed intriguing experiments on the **dispersal** of *P. persimilis* in a plant set-up and compared these results with the results of simultaneous laboratory quality control tests (van Lenteren *et al*., 2003a) for predators sampled from the same batch. First of all, also in this study losses of predators in the plant were variable among replicates and could be as high as 100%. Moreover, results were variable among the three tested batches. One batch contained predators that had very high fecundity values (22 eggs/ predator/5days!) a high survival rate (85%) and a response of 73% in the searching ability test (= predators found at the prey patch after 24 hours and predator population established at prey patch after 5 days). Batches that performed less well in terms of fecundity and survival also had lower response levels in the searching ability test (ranging from 37.5% to 57%). The authors also mention the presence of "flat" predators in one of their batches and mention their concern about possible infectious diseases in predatory mites.

Up to now *A. phytoseiuli* has not been detected in predatory mite species other than *P. persimilis* (Gols *et al*., in prep.). Pathogen transfer from one predatory mite species to another could be mediated by intraguild predation, a common phenomenon among phytoseiid mites. Especially adult females of generalist phytoseiid mites are highly aggressive against heterospecific predatory mites (Schausberger and Croft, 2000). To our knowledge two studies mention symptoms similar to symptoms of the NR-population (see chapter 2 for a detailed discussion): (1) Immediately after the last moult female *Neoseiulus (*formerly *Amblyseius*) *hibisci* (Chant) became dorso-ventrally flattened, more concave in profile, lethargic, did not lay eggs and exhibited a characteristic dark-red gut occlusion prior to death (Tanigoshi, *et al.,* 1981); (2) Individuals of *Metaseiulus occidentalis* (Nesbitt) became very pale and so thin that they became translucent. Females failed to oviposit, immatures exhibited high mortality and colonies died out (Hess and Hoy, 1982). In both cases the authors could not unambiguously show the cause of these symptoms. Moreover, another symptom of the NR-syndrome, the lower degree of attraction to herbivore-induced plant volatiles, has been reported for three other predatory mite species: *Amblyseius potentillae*, *Typhlodromus pyri* (Dicke *et al*., 1991a) and *Amblyseius womersleyi* (Maeda *et al*., 2001). It may be clear from the points mentioned above that good tools for disease detection, prevention and cure are of utmost importance for industry and research.



What are current tools for disease diagnosis?

The most secure means of diagnosis are pathogen isolation (chapter 8) and/or the PCR test (Gols *et al*., in prep.). Both tests should preferably be done with surface sterilized adult predators, as the chance for pathogen detection is highest with this method (chapter 8). But these tools are not available for every laboratory and commercial producer. Moreover, nega-

Table 2: Easily observable diagnostic symptoms of the NR-syndrome, present in adult female *P. persimilis*.

Symptom 
Behavioural
(1) High dispersal rate from prey-infested plants when prey is still ample
Non-behavioural
(2) Presence of many dorso-ventrally flattened non-ovipositing individuals These individuals should not regain size after offering ample amounts of prey These individuals should not start egg production after mating These individuals should show high mortality rates
(3) Excretion of faeces and debris that may induce the NR-syndrome in *P. persimilis*
Anatomical
(4) Presence of excretory crystals in the legs

tive test results will not guarantee in all cases that the disease is not present. In many cases both methods have resulted in negative test results for symptomatic predators (chapter 8, Gols *et al*., in prep.). These problems can be overcome by keeping the sample size big. To avoid (expensive) repeated testing of numerous samples, knowledge on diagnosis in the field situation is of great importance. The demand for new, commercially feasible quality control tests is still growing, as maintenance of good quality standards is a key factor in augmentative types of biological control (Bigler, 1989; van Lenteren, 2003a ; Bolckmans, 2003). **Some of the diagnostic symptoms of the NR-symptom are easily observed with no or relatively cheap equipment** (Table 2). Attention should be paid to predator dispersal, predator size and the position of excretory crystals in the mite body.

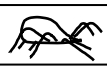
Method 1 (see also symptoms (1) and (2) in Table 2): Healthy adult female *P. persimilis* remain in prey patches until all prey is consumed (Pels and Sabelis, 1999; Mayland *et al*., 2000). A high dispersal rate before extinction of the prey is therefore an important suspicious symptom. In such a case the size of adult females should be analyzed. If numerous dorso-ventrally flattened individuals, resembling starved females, virgin females or deutonymphs are present, it should be tested whether these individuals are indeed starved or virgin. If they do not regain their normal pear-shaped form after feeding and/or mating, the population should be tested with an *Acaricomes*-specific PCR test (Gols *et al*., in prep.).

Method 2 (see also symptom (3) in Table 2): A more time consuming method is the use of the bioassay described in chapter 7. It is very likely that a population is infected with *A. phy-*

toseiuli when an aqueous suspension of predator faeces collected from this population induces the NR-syndrome in non-symptomatic *P. persimilis*.

Method 3 (see also symptom (4) in Table 2): When a microscope with polarized light is available crystal location in the body of living mated female predators may be observed. If crystals regularly appear in the legs of live predators the population should be tested with an *Acaricomes*-specific PCR test (Gols *et al*., in prep.).

In all cases care should be taken to avoid contact of a suspicious population with other populations of *P. persimilis*.



What are current tools for disease cure and prevention?

Bacterial insect pathogens are usually opportunists, i.e. they may only infect individuals that are stressed in one or the other way (Tanada and Kaya, 1993). Therefore, a general advice for prevention of bacterial diseases in arthropod rearings is avoiding stress and contaminations of the rearing facilities (R. G. Kleespies, personal communication; Tanada and Kaya, 1993). However, this is not true for the present disease. *P. persimilis* populations became infected with *A. phytoseiuli*, even when they were kept under optimal conditions with ample food. In the present system **disease outbreak can only be prevented by avoiding pathogen invasion of a population**. Therefore special attention should be paid to populations transferred from the field or those received from other rearing facilities. Such populations should be checked for suspicious symptoms as stated above. They should also be kept separately from other populations as long as their infection status is unclear.

The fact that *A. phytoseiuli* is tetracycline-sensitive may offer possibilities for the curing of infected populations. However, up to now only the prophylactic effects of tetracycline have been shown (chapter 7), whereas the effects of tetracycline on symptomatic predators after infection are not yet known. At the present time I do not recommend long-term **application of tetracycline** on a large scale in predator populations because of the following:

(1) Under such application regimes emergence of antibiotic resistance is likely, as it has been reported earlier for bacterial invertebrate pathogens. Recently, widespread oxytetracycline resistance has been reported for the honey bee bacterial pathogen *Paenibacillus larvae*, the causative agent of the important honey bee larval disease American foulbrood (Evans, 2003). This pathogen has been treated in bee colonies with oxytetracycline for fifty years. Antibiotic resistance was uncorrelated with haplotype, suggesting either that resistance has evolved multiple times in *P. larvae* or that resistance involves recent horizontal transfer via a non-genomic (e.g. plasmid or conjugal transposon) route. Evans (2003) proclaims that, as new antibiotics become available for the treatment of this disease, strict guidelines should be

established for the rotation of different antibiotic treatments, to prolong their effectiveness as controls.

(2) Treatment with tetracycline may lead to the elimination of bacterial endosymbionts (e.g. *Wolbachia*) in predatory mites as well as in the prey mites (Breeuwer, 1997). As (long term) effects of such eliminations are not known currently, attention should be paid to possible negative effects of the elimination of endosymbionts.

(3) In our studies some small predators were found after tetracycline treatment (chapter 7), which may be an indication of direct negative effects of the antibiotic on *P. persimilis*. Possible negative effects on predatory mites should therefore be checked before tetracycline treatment is applied on a regular and large scale.

Periodic application of tetracycline on a small scale, for example when a healthy population is needed to start a new rearing, is not necessary. **Surface sterilization of predator eggs** is a simple curing method that is more reliable and without any negative effects on *P. persimilis* and its prey (chapter 6). During all our studies, female *P. persimilis* infected with *A. phytoseiuli* did at least produce some eggs before death (chapter 4, 6, 7 and 8). Thus it may be expected that one can find enough eggs in most infected populations. In our laboratory this method has been successfully applied for several years now.

How can the two diseases of *P. persimilis* be distinguished?

The most characteristic diagnostic symptom that distinguishes the bacterial disease from the microsporidian disease in *P. persimilis* is shrinkage of mated females (Table 3). This phenomenon has never been reported for the microsporidian disease caused by *Microsporidium phytoseiuli*. Bjørnson and Keddie (1999) reported only slight size differences between female *P. persimilis* infected with *M. phytoseiuli* (Microsporidia) and uninfected females on the 5th day of development. The dorso-ventrally flattened females of *Acaricomes*-infected populations resemble starved females and cannot be distinguished from them with a stereomicroscope. Several authors record heavy weight losses of female *P. persimilis* due to starvation (Sabelis, 1981; Yao and Chant, 1990). Starved females were able to recover from starvation and to continue egg laying. Well-fed females had an orange-coloured pear shaped body and were very active. After starvation, predators had a flat translucent body and they were still very active (Yao and Chant, 1990). It may thus be possible that females from *Acaricomes*-infected populations stop feeding, a diagnostic symptom in many insect diseases (Lacey, 1997), and that starvation induces some of the other diagnostic symptoms recorded here, including a low excretion rate, a high dispersal rate, oviposition stop and a low degree of attraction to herbivore-induced plant volatiles.

Table 3: Diagnostic symptoms of the disease caused by *Acaricomes phytoseiuli* and of the disease caused by *Microsporidium phytoseiuli* (Bjørnson and Keddie,1999).

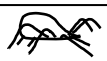
Symptom 	***Acaricomes phytoseiuli***	***Microsporidium phytoseiuli***
Behavioural		
Low degree of attraction to HIPV	+	**?**
Short choice time in Y-tube olfactometer	+	**?**
High dispersal rate	+	**?**
Non-behavioural		
Size change to dorso-ventrally flattened shape	+	**-**
Slightly smaller size on 5th day of development	**?**	+
Reduced fecundity	+	+
Reduced longevity	+	+
Reduced predation rate	+	+
Low excretion rate	+	+
Distorted sex ratio	**?**	+
Anatomical		
Presence of excretory crystals in the legs	+	**?**
Pathogen present in alimentary tract and fat body	+	+
Degeneration epithelium of the digestive tract	+	**-**
Pathogen not restricted to specific tissues	**-**	+
Pathogen present inside eggs	**-**	+

Disease transmission

In this thesis no evidence is reported for vertical disease transmission from parent to offspring. However, we found **high rates of horizontal disease transmission via faeces and debris** of female *P. persimilis* that were infected with *A. phytoseiuli*. As many as 100 out of 108 healthy predators shrank only 6 days after start of the exposure to faeces and debris suspension collected from symptomatic females (chapter 7). The shrunken females may be regarded as reproductively dead as they do not produce any eggs until death. These data together with data from chapters 4, 6 and 8 demonstrate that *A. phytoseiuli* is rather virulent. It has been argued that in mainly horizontally transmitted pathogens the evolution of high

virulence is favoured in situations that allow for a high rate of horizontal transmission (Ewald, 1994; Myers and Rothman, 1995). The method applied to rear *P. persimilis* in our laboratory represents such a situation, as high numbers of predators are kept together in a relatively small place.

Host behaviour and mobility are regarded as key factors in initiating epizootics (Andreadis, 1987), especially in host-pathogen associations where the main transmission mode is horizontal and where infective stages are released by the host throughout the whole life. This is most probably the case for the disease described here (chapters 4, 7, 8). Thus, another intriguing question to be discussed here is the role of the behavioural changes in disease transmission.



How can behaviour change due to disease?

Behavioural changes caused by parasites and pathogens are rather common in invertebrates. According to Horton and Moore (1993) they may be classified in four broad categories: (1) changes in microhabitat preferences, including diurnal activity by normally nocturnal hosts; (2) changes in activity level; (3) reproductive activity expressed under inappropriate conditions, that is, by castrated hosts (4) modifications in feeding behaviour of vectors. The most intriguing question in this field, that has stimulated a vast number of studies, is whether a given behavioural change is: (1) a "by-product" of infection (i. e. a consequence of pathology or stress) or an ancestral legacy that may or may not benefit either party of the host-parasite complex (Müller *et al*., 1997; Chow and Mackauer, 1999); (2) an adaptive change mediated by the pathogen that is beneficial to the pathogen (Stamp, 1981; Brodeur and McNeil, 1989, 1990, 1992; Schmid-Hempel and Müller, 1991; Müller and Schmid-Hempel, 1992) ; (3) an adaptive change mediated by the host that is beneficial to the host (Shapiro, 1976; Smith Trail, 1980; Müller and Schmid-Hempel, 1993; McAllister and Roitberg, 1987; McAllister *et al*., 1990). Behavioural changes may benefit the pathogen by inducing an increased pathogen dissemination and/or increased pathogen survival in the host, whereas they may benefit the host by inducing decreased pathogen survival in the host and/or decreased infection of kin (Figure 1; based on Horton and Moore, 1993).

Especially the idea that parasites can manipulate the phenotype of their host and thus enhance their own transmission became rapidly popular and resulted in an impressive number of studies performed during the past three decades. Parasite-induced alterations are now documented for a wide range of parasites (see Thomas *et al*., 2005 for a recent review). Examples for pathogen-induced behavioural changes that may enhance pathogen transmission include: Viruses that cause elevation-seeking behaviour in caterpillars which is likely to re-

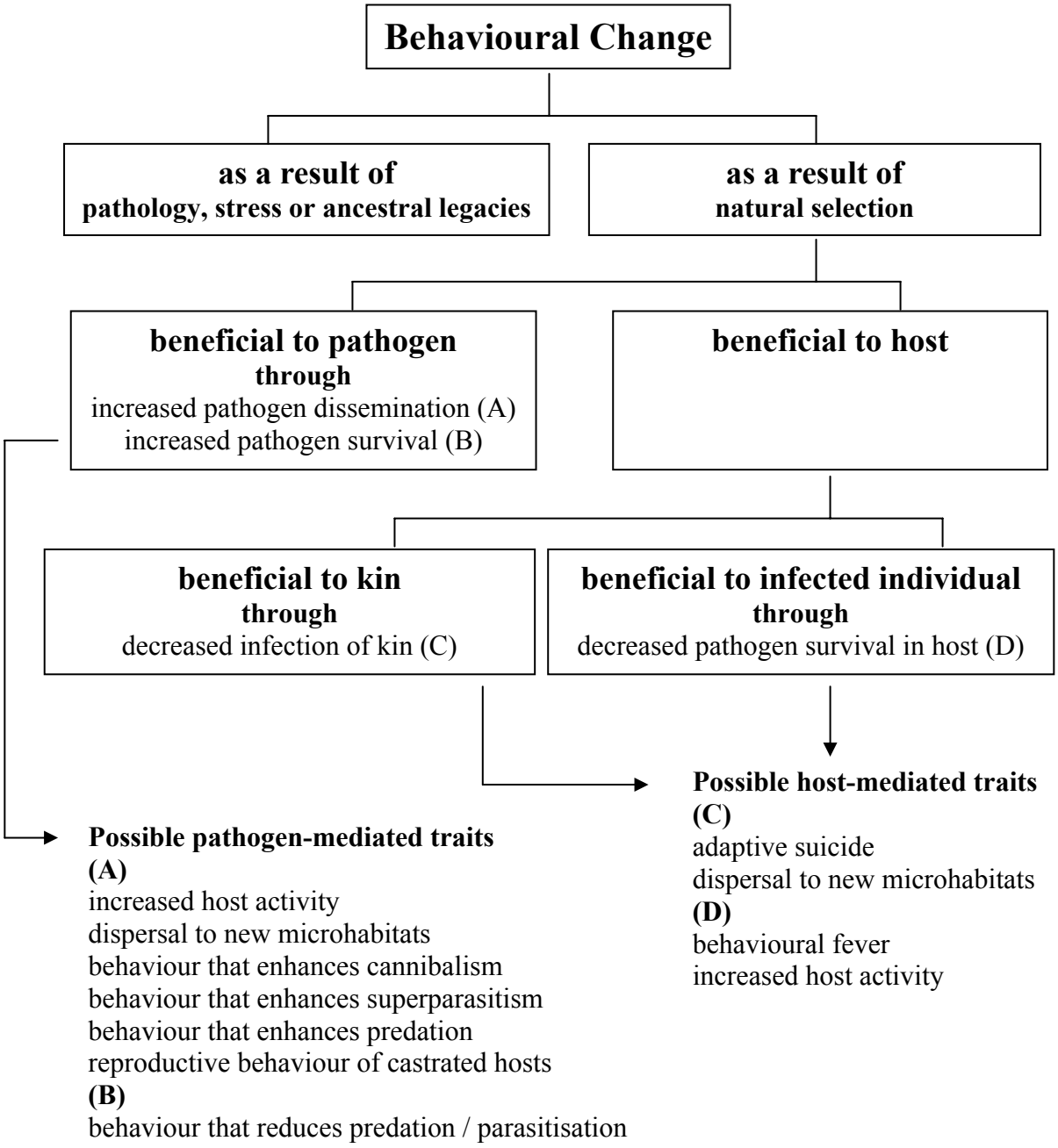


Figure 1: Flow diagram of pathogen- and parasite-induced behavioural changes, based on Horton and Moore (1993).

sult in contamination of more foliage with virus particles than after caterpillar lysis on the spot (Goulson, 1997), fungi that cause their insect host to die perched in a position that favours the dispersal of spores (Krasnoff *et al*., 1995) and viruses that stimulate superparasitism in solitary parasitoids what will result in horizontal transmission (Varaldi *et al*., 2003).

In the early 1990s Moore and Gotelli (1990) and Poulin (1995) demanded more rigorous evidence for the adaptive nature of a behavioural change in an infected individual. In his comprehensive review of the literature Poulin (1995) concluded that most known behavioural changes have not been demonstrated as resulting in fitness gains to either the host or the parasite. The author suggested that the following criteria should be met before one considers behavioural changes as adaptive: (1) they must be complex; (2) they must show signs of a purposive "design"; (3) the evidence is more convincing if the changes have arisen independently in several lineages of hosts or parasites; (4) they must be shown to increase the fitness of either the host or the parasite. How complicated an analysis of parasite-induced behaviour may be is shown by the system of aphids parasitized by aphidiine wasps. Parasitized aphids may disperse from feeding-sites, by dropping from the plants, shortly before death or mummification. Several studies on this behavioural change have led to very different hypotheses. It has been suggested that the behavioural change (1) is parasite-mediated and leads to reduction of hyperparasitism risk (Brodeur and McNeil, 1992); (2) is parasite-mediated and increases overwintering survival of the parasitoid (Brodeur and McNeil 1989, 1990); (3) is host-mediated and increases mortality of the infected individual while reducing the risk of parasitism of kin and thus results in an increase in inclusive fitness. This latter phenomenon is known as "adaptive suicide" (McAllister and Roitberg, 1987; McAllister *et al*., 1990); (4) is a consequence of pathology, differences between different parasites being caused by differences in larval development and growth between parasite species (Chow and Mackauer, 1999). This example illustrates that the question whether the behavioural changes recorded for *Acaricomes*-infected *P. persimilis* are adaptive or by-products of trauma and pathology, will not be answered easily.

Behavioural changes of *Acaricomes*-infected *P. persimilis*: who benefits?

Adult female *P. persimilis* infected by *A. phytoseiuli* show a lower degree of attraction to herbivore-induced plant volatiles than healthy conspecifics (chapters 3, 4, 5, 6, 7, 8) and disperse from prey patches before prey is eradicated (chapters 3, 4, 6, 7, 8), whereas uninfected conspecifics are attracted to herbivore-induced plant volatiles and will stay until eradication. Moreover they need less time to make a final choice in the olfactometer, caused by fewer stops and turns (chapter 4).

Before going into detail, regarding possible benefits of these behavioural changes, I first discuss the question, whether they are a mere **consequence of pathology/stress**. I hypothesized earlier, that the most obvious symptom of the present bacterial disease (shrinkage of adult female predators several days after mating) may be a consequence of starvation, as *Acaricomes*-infected predators cease feeding (chapter 4). Thus, the question arises whether star-

vation could induce the behavioural changes. The following effects of starvation have been reported for *P. persimilis*: (1) starved predators always move upwind (Sabelis and van der Weel, 1993; van Tilborg *et al*., 2004); (2) starved predators spend a longer time in prey patches than satiated predators (Zhang and Sanderson, 1993); (3) starvation did not have any effect on the response of *P. persimilis* to herbivore-induced plant volatiles (Sabelis and van de Baan, 1983; Krips *et al*., 1999b, chapter 3). Hence starvation could account for the short choice time in the Y-tube olfactometer, through straight upwind movement, but probably not for early dispersal and a lower degree of attraction to herbivore-induced plant volatiles. It would be interesting to investigate at which internal level of the individual the changes occur. I am aware of one study on a parasite-induced decreased response to volatile sex pheromones, where this question was addressed (Carmichael *et al.*, 1993). The authors found that male acanthocephalan-infected *Periplanata americana* did not show a behavioural response to components of the female sex pheromones, whereas the electroantennogram responses of infected and uninfected males did not differ significantly. Thus, in this case the change rather occurred at the central nervous system than at the peripheral level. Whether pathology and/or stress related factors induce the behavioural changes in *Acaricomes*-infected *P. persimilis* offers interesting questions for future research (for a recent review on this matter see Thomas *et al*., 2005).

In the following paragraph I will discuss the most obvious interrelations of behavioural traits and benefits to host or pathogen. With current knowledge several hypotheses concerning potential benefits to pathogen or host can be supposed (see also Table 4). It may be expected that the complex of behavioural traits reported for *Acaricomes*-infected predators will result in a greater area visited by the predator compared to when these traits are absent. This supports the hypothesis that the behavioural changes will lead to **increased dissemination of the pathogen** (column 1 in Table 4) and consequently to higher rates of horizontal disease transmission. Concerning the last conclusion it is necessary that healthy predators will come in contact with viable pathogens, by visiting the contaminated areas. Healthy predators show a higher degree of attraction to herbivore-induced plant volatiles than *Acaricomes*-infected conspecifics. Thus, *Acaricomes*-infected females are less likely to encounter new prey patches during dispersal than uninfected conspecifics, which may result in reduced encounters between healthy predators and contaminated faeces. However, this argument does not dismantle the hypothesis as also wind currents, rain or other species can contribute to contact between healthy host individuals and pathogens. Hence, this hypothesis cannot be rejected as long as these questions are not clarified.

The case could also be quite reverse, as one may argue that the behavioural changes **hamper disease transmission** and benefit the host. A lower response to herbivore-induced plant volatiles and early dispersal from prey patches still carrying prey should be detrimental for

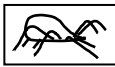
Table 4: Potential benefits (+) of behavioural changes induced by infection with *Acaricomes phytoseiuli* to pathogen or host.

behavioural trait 	**increased dissemination pathogen**	**increased survival pathogen**	**increased survival host kin**	**decreased survival pathogen**
Low degree of attraction to HIPV	+		+	
High dispersal rate	+		+	+
Increase in activity (short choice time)	+			+

the infected individual, as it will be more prone to death from starvation. However, the individual may gain in inclusive fitness when this behaviour will contribute to smaller infection risks in its kin. This is most probably the case here, as *Acaricomes*-infected predators leave places where kin is present and are not attracted to the same places as healthy kin. It is thus tempting to interpret these behavioural changes according to the concept of **"adaptive suicide"** as mentioned above (column 3 in Table 4). *P. persimilis* is a specialist predator of spider mites from the genus *Tetranychus* that have a clumped distribution pattern (Sabelis, 1981; Eveleigh and Chant, 1982; Zhang and Sanderson; 1997). Predators lay their eggs in prey patches in clutches and consequently highly related predator offspring become aggregated. A clumped distribution, which implies frequent interactions among conspecifics may be a driving force for evolution of kin recognition (Schausberger and Croft, 2001). Indeed kin recognition has been demonstrated for phytoseiid mites including *P. persimilis* (Faraji *et al*., 2000; Schausberger and Croft, 2001; Schausberger, 2004). It would thus be interesting to test whether the behavioural changes are more pronounced when kin are present compared to a situation were no kin is present (Kasuya, 2000).

One can also argue that an increased dispersal rate implies an increase of motor activity. *P. persimilis* had a short choice time in the Y-tube olfactometer, what was partly due to fewer numbers of stops during the experiment (chapter 4). Such behaviour may raise the body temperature in infected animals (Horton and Moore, 1993) and an increased temperature may be detrimental to pathogen success (Louis *et al*., 1987; Watson *et al*., 1993). Thus, high dispersal rates and an increased activity could eventually lead to a **decreased survival of the pathogen** in the host (column 4, Table 4). However, hosts generally raise their body temperature by seeking warmer microhabitats. This phenomenon is called behavioural fever (Horton and Moore, 1993; Watson *et al*., 1993). It would be interesting to investigate whether this is also the case for *Acaricomes*-infected *P. persimilis*.

It is obvious that at present the discussed hypothesis may neither be confirmed nor rejected and that many interesting questions are still to be answered.



Disease transmission in rearing systems: possible role of behavioural changes

As *Acaricomes*-infected female *P. persimilis* show a lower degree of attraction to herbivore-induced plant volatiles than healthy conspecifics they are less likely to colonize new prey patches (= fresh spider mite-infested leaves or plants added to the rearing) than uninfected conspecifics. This behavioural trait could therefore result in reduced encounters between infected and uninfected predators in newly colonized prey patches and this may delay disease spread in a rearing unit. The magnitude of these effects will of course depend on the spatial scale of the rearing. Moreover, *Acaricomes*-infected female *P. persimilis* disperse from freshly introduced spider mite-infested leaves or plants before prey is eradicated, whereas uninfected conspecifics will stay until eradication. Thus, infective faeces and dead predators are less likely to accumulate in a prey patch, or even in a rearing system, when the rearing system is open or semi-open. **This behavioural trait could therefore lead to a reduction of disease incidence in a rearing system,** the magnitude of these effects being dependent on the spatial scale and the degree of openness of the rearing.

Some experimental evidence supports this hypothesis. Epizootics of highly virulent pathogens may destabilize host populations leading to eradication of local populations in case the pathogen is too efficient in its spread among hosts or too virulent. This has repeatedly been the case for the NR-population, when reared in a closed Petri-dish rearing (Schütte *et al*., unpublished data). Eradication occurred after only several generations. In this system predators cannot escape and infectious material is expected to accumulate (Figure 2A). In contrast, when reared in a semi-open rearing system the NR-population had never been eradicated, despite consistent fluctuations in predator densities and their behavioural response to herbivore-induced plant volatiles (chapter 3; Schütte *et al*., unpublished). This system consists of a heap of detached Lima bean leaves infested with spider mites. Leaves are placed on a plastic platform in a caged water basin. In this system a certain level of dispersal, for example to the platform or into the water is possible. This could lead to variable disease incidence, which will not reach 100% (Figure 2B).

In this case the timing of dispersal is a key factor. If an individual disperses before it is able to release infectious material, disease transmission will be impaired, whereas the effects will become less pronounced when dispersal starts during a later phase of the disease. In chapter 8 we report detection of *A. phytoseiuli* in infected female *P. persimilis* and their faeces as early as 2 days after the start of pathogen exposure, whereas clear differences in dispersal

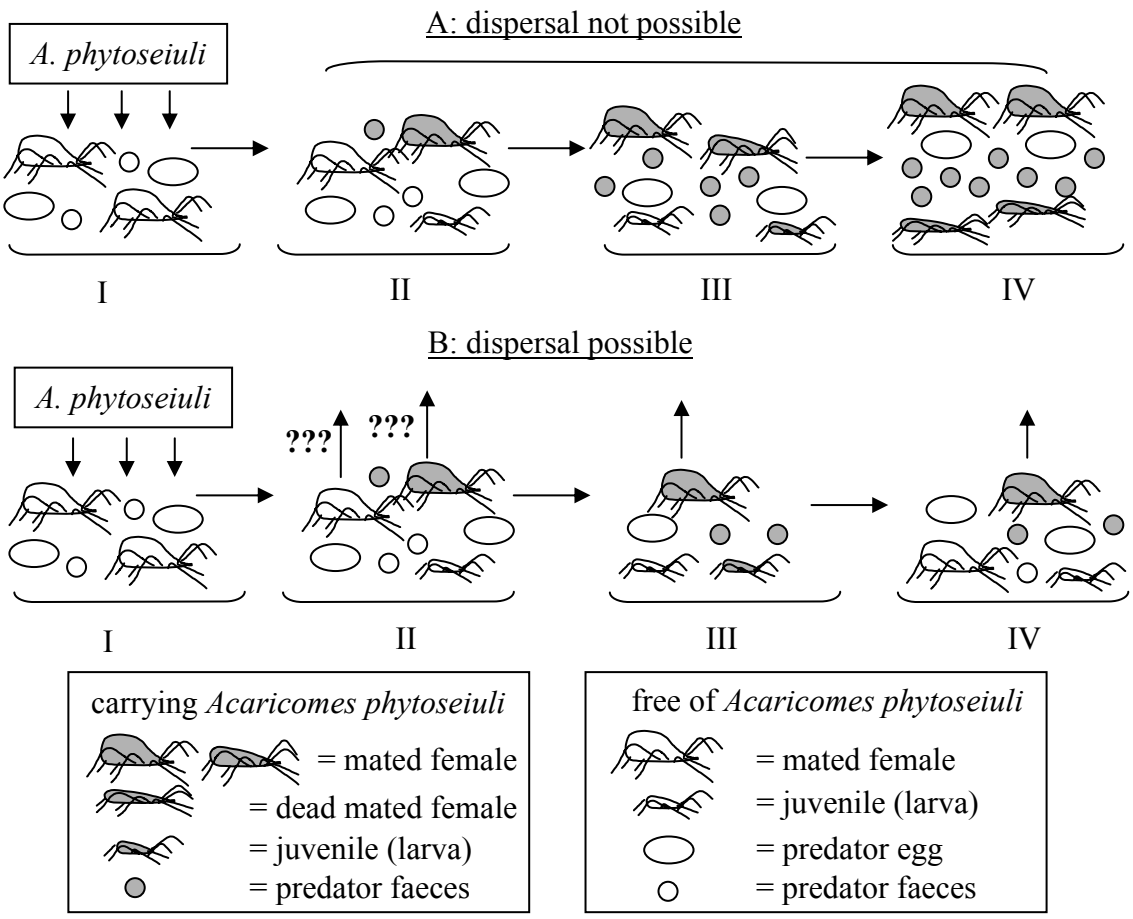


Figure 2: Schematic presentation for possible effects of *Acaricomes*-induced dispersal behaviour in *P. persimilis* on disease incidence in a (mass-) rearing.

behaviour between healthy and infected predators were only recorded 4 days after the start of pathogen exposure. However, we do not know whether the faeces of these 2 day old females was infectious and it also might well be that dispersal starts much earlier. The method of recording the position of the predator in the Petri-dish is not a very accurate method for the recording of predator dispersal. It has been applied in these studies as it could be incorporated into the general bioassay. During first experiments in a larger scale set-up we also found a higher dispersal rate for *Acaricomes*-exposed adult female *P. persimilis* compared to healthy females. Disease transmission from diseased to healthy predators occurred more frequently in a situation without escape possibility for the predatory mites (C. Schütte and M. Aveskamp, unpublished data).

Despite these promising results it is not possible to predict unambiguously whether the behavioural changes recorded here will indeed reduce pathogen incidence and transmission in rearing systems. More information about the timing of the recorded changes, other behav-

ioural characteristics and effects of behavioural changes in (semi-) field situations is required.

In this context it should also be mentioned that predators sold as biological control agents and predators used in experiments will have a high infection rate when (1) *A. phytoseiuli* is present in a *P. persimilis* population and (2) the applied rearing system is designed in such a way that mainly dispersing predators are caught for selling or experimental use. For the majority of commercial populations this is most probably the case, as "all moving stages" is indicated to be the collected stage when mites are sold in wheat bran (van Lenteren and Tommasini, 2003). In such a situation quality control tests would show good results for the rearing and bad results for the sold product. A lack of quality at the user side is generally explained by problems in storage and shipping (Bolckmans, 2003). Thus, in this way quality loss due to *A. phytoseiuli* could easily be overlooked over extended periods.

How can (micro-)climate affect disease transmission?

Several environmental factors, including moisture, temperature and solar radiation may directly affect the transmissibility of insect pathogens (Andreadis, 1987). Solar radiation and temperature extremes inactivate pathogens of all groups (Andreadis, 1987) whereas a high relative humidity and free water on leaf surfaces generally promotes pathogen infection and transmission (Jewett and Jarvis, 2001). It has even been suggested that plants may actively maintain insect pathogens in and on the plant surface. Such pathogens could then act as bodyguards for the plants by infecting harmful herbivores (Elliot *et al*., 2000). For the disease presented in this thesis humidity may be one of the most important factors mediating disease transmission. High levels of relative humidity may enhance pathogen release onto the plant surface and pathogen uptake by *P. persimilis* drinking *A. phytoseiuli*-contaminated water droplets (Gaede *et al.*, 1992, see for further discussion chapter 6). It may be expected that the role of solar radiation and temperature are less pronounced in this case, as *P. persimilis* and its spider-mite prey prefer to reside on the underside of the leaf and as extreme temperature levels that may inactivate bacteria would most probably also be lethal to *P. persimilis*.

All experiments of the present thesis have been conducted in closed Petri dishes with a high relative humidity (water droplets may have been present on the leaf – and dish surfaces), moderate temperatures (23±1 ^{o}C) and exclusion of solar radiation. Climatic conditions in open rearing systems and in greenhouse or field cultures, where *P. persimilis* is applied for biological control of spider mites, may be very different including the presence of solar radiation, higher and lower temperatures and a lower relative humidity. A high relative hu-

midity in the closed rearing system may, among others, explain why *A. phytoseiuli*-infected predator populations always died out when reared for several generations in a closed rearing system, whereas they never died out when reared on heaps of detached leaves in the climate chamber.

It is not likely that climatic conditions in greenhouses and field cultures will prevent any disease transmission because of the following arguments: (1) Solar radiation cannot inactivate all pathogen released by diseased predators, as *P. persimilis* prefers to reside on the underside of leaves. (2) Extreme temperatures of 40°C and higher will not be present during extended periods in greenhouses or field conditions. (3) Periods of high humidity and/or dew formation on the plants are a well-known phenomenon in both types of cultures. In greenhouses only small temperature differences between the leaves and the environment may cause the formation of dew (often during the night) and effective methods of expelling humidity from greenhouses such as simultaneous heating and ventilation are very cost intensive (Jewett and Jarvis, 2001). (4) Moreover, microclimate at the phylloplane where disease transmission processes occur is difficult, if not impossible, to measure and connections between the greenhouse macroclimate and the microclimate of the phylloplane have yet to be made (Jewett and Jarvis, 2001; Gaede, 1992). It has been suggested that there is a steep water vapour gradient from the saturated intercellular spaces, through the stomata to 1 or 2 mm beyond the leaf surface (Jewett and Jarvis, 2001). For epiphytic bacteria the boundary layer is only 2-3 µm thick in which there is presumed to be little or no air movement and a steep water vapour gradient. Thus, it may be expected that disease transmission should also be possible in greenhouse and open field cultures. Ambient humidity and temperature, air velocity, light regime, plant condition and plant characteristics (including leaf size, leaf shape, leaf position in plant, leaf thickness, leaf surface) could influence disease transmission in the present case, as these factors have a direct impact on the humidity within the boundary layer of the leaf (Gaede, 1992).

Pathogen isolation

The studies presented in this thesis led to the isolation and determination of a novel bacterial pathogen that causes serious disease, i.e. induction of the NR-syndrome, in the predatory mite *Phytoseiulus persimilis*. The novel pathogen has been described as ***Acaricomes phytoseiuli* gen. nov., sp. nov** (Pukall *et al*., 2006). This is the first record of a bacterial pathogen in phytoseiid mites.

In addition we isolated several other bacterial and fungal isolates from adult female predators of the NR-population and tested their effects on female predators. Only one bacterial isolate, determined as *Serratia rubidaea* induced a somewhat higher mortality in *P. persimi-*

lis females. None of the characteristic symptoms of the NR-syndrome were induced and *S. rubidaea* was not only re-isolated from predators inoculated with *S. rubidaea*, but also from control predators (Schütte *et al*., in prep.). Thus in contrary to *A. phytoseiuli*, S. *rubidaea* most probably represents an opportunistic bacterial pathogen, that is always present in *P. persimilis* and may cause disease only if conditions are favourable, i.e. when its host suffers from one or several stress factors (see also Lighthart *et al*., 1988).

Currently *A. phytoseiuli* is the only species known in the genus *Acaricomes*. Comparative analysis of the 16S rDNA sequence revealed that the strain was a new member of the family of the Micrococcaceae. Nearest phylogenetic neighbours were determined as *Arthrobacter globiformis* (94.8%), *Arthrobacter russicus* (94.6%) and *Renibacterium salmoninarum* (94.0%) (Pukall *et al., 2006).* As it appears that the **new genus *Acaricomes* is closely related to the genus *Arthrobacter***, I will refer to available literature on this genus in the following discussion. The genus *Arthrobacter* consists of a group of catalase-positive, strictly aerobic rod shaped micro-organisms that exhibit a coryneform morphology. Phylogenetically, species of this genus belong to the Actinomyces branch of the Gram-positive bacteria and are, among others, closely related to members of the genus Micrococcus (Stackebrandt *et al*., 1997). The genus *Arthrobacter* is phenotypically heterogeneous. A large number of studies have shown that soil is the usual habitat of members of the genus *Arthrobacter* and that they are a numerically important fraction of the indigenous soil flora from various parts of the world (Keddie *et al*., 1986).

The three species being most closely related to *Acaricomes phytoseiuli* deserve some extra attention in this discussion as they all represent quite peculiar cases. *Arthrobacter globiformis* has first been described as "*Bacterium globiforme*" by Conn (1928). As *Arthrobacter globiformis* this species had later become the type species of the genus *Arthrobacter*. In contrast, *Arthrobacter russicus* was described only very recently (Li *et al*., 2004). This species had been isolated in 1997 from air in the Russian space laboratory Mir. Interestingly, the third species closely related to *A. phytoseiuli* is a well-known pathogen. *Renibacterium salmoninarum* is the causative agent of bacterial kidney disease (BKD) in salmonid fishes (Sanders and Fryer, 1980). BKD is one of the most important diseases of wild and cultured salmonid fish and has been reported from many countries (see for a review Evenden, *et al*., 1993). Unlike some other fish pathogens, and like *A. phytoseiuli*, it is not an opportunistic pathogen but an obligate pathogen, causing systematic chronic infections. The bacterium is responsible for mortality in all age groups due to direct losses and poor growth rates in chronically infected fish. External disease symptoms are among others darkening of skin, a swollen grey-white kidney, ulcers and abscesses on the skin (see for a review Evenden, *et al*., 1993). *R. salmoninarum* can be transmitted vertically and horizontally and it has been recovered from faeces of wild and cultured salmonids. The expansion of salmonid culture

has assisted in the spread of the disease. Currently several isolates with different virulence types are known (Rhodes *et al*., 2004).

What might be the origin of *A. phytoseiuli*?

Recent studies have shown that *A. phytoseiuli* is widespread among European commercial populations of *P. persimilis* (Gols *et al*., in prep.). Intriguing questions arising from this thesis are: where did this pathogen come from and how could it spread?

Origin from its prey *Tetranychus urticae* can be excluded, as pathogen presence was never established for this species: (1) *T. urticae* from our laboratory rearing did not transmit the NR-syndrome to *P. persimilis* (chapter 6), (2) *A. phytoseiuli* could not be isolated from *T. urticae* (chapter 8) (3) PCR tests with *A. phytoseiuli*-specific primers were never positive for *T. urticae* (Gols *et al*., in prep.).

A. phytoseiuli could be a **pathogen of native *P. persimilis*** populations from South-America or from Mediterranean countries, that has invaded commercial and/or laboratory populations by the introduction of new field material. An anonymous source informed me that such introductions have been done in the past as it is recommended for the maintenance of genetic variability (Nunney, 2003; Hoekstra, 2003). It should be stressed that our laboratory never introduced field material into the laboratory. However, we sometimes kept several populations of *P. persimilis* of different origin in the same room, divided by cages and water barriers.

A. phytoseiuli could be a **human-related species** that has been introduced into the rearing by human rearing activities, as humans may be one source of microbial contaminations in arthropod rearing (Sikorowski and Lawrence, 1994). A healthy human body harbours millions of micro-organisms, mainly bacteria, on the skin and in the mouth, respiratory tract, genitourinary tract and intestines (Sikorowski and Lawrence, 1994). It is striking that 41 cases of *Arthrobacter* species isolated from human clinical samples are reported in the literature (Bernasconi *et al*., 2004). Among the circa 40 validly described *Arthrobacter* species, as many as six were isolated exclusively from human clinical specimen, namely *Arthrobacter albus* (Wauters *et al*., 2000), *Arthrobacter creatinolyticus* (Hou *et al*., 1998), *Arthrobacter cumminsii* (Funke *et al*., 1996), *Arthrobacter luteolus* (Wauters *et al*., 2000), *Arthrobacter woluwensis* (Funke *et al*., 1996; Bernasconi *et al*., 2004) and *Arthrobacter scleromae* (Huang *et al*., 2005). In addition, some strains of *Arthrobacter oxydans* have been isolated from human blood (Wauters *et al*., 2000). In five occasions *Arthrobacter* species have been identified as causative agent for human disease (Bernasconi *et al*., 2004; Huang *et al*., 2005). It is likely that human infections with *Arthrobacter* species are cur-

rently underestimated, because of the difficulty of identifying these species with conventional biochemical assays (Bernasconi *et al*., 2004).

A. phytoseiuli might have **evolved under rearing conditions** by horizontal gene transfer between different bacterial species. It has been shown that horizontal gene transfers have effectively changed the ecological and pathogenic character of bacterial species (see for reviews on this matter Ochman *et al*., 2000; de la Cruz and Davies, 2000). Well-documented traits being introduced in bacteria via horizontal gene transfer include antibiotic resistance, virulence attributes and metabolic properties. Most pathogenicity genes are located in the bacterial chromosome, where they exist as discrete gene clusters named pathogenicity islands. Horizontally acquired pathogenicity islands are major contributors to the virulent nature of many pathogenic bacteria (Groisman and Ochman, 1996). Moreover de la Cruz and Davies (2000) proposed that horizontal gene transfers are also responsible for speciation and sub-speciation in bacteria. However, the frequency of homologous recombination between bacterial species decreases sharply with the extent of DNA divergence between the donor and the recipient (Majewski *et al*., 2000).

Many *Arthrobacter* species are soil inhabitants, where they form a numerically important fraction (Keddie et al., 1986), but they may also belong to bacterial communities on the plant surface (Padaga *et al*., 2000). It is thus possible that predatory mites are exposed to *Arthrobacter* species or *Arthrobacter*-related species in one or other way, and that they could acquire genes from other (opportunistic) pathogens. In this sense it is interesting that the type strain of *Arthrobacter protophormiae* has been isolated from the dipteran insect *Protophormia terraenovae*, whereas other strains were isolated from soil (cited in Keddie *et al*., 1986). A recent paper reports some evidence for horizontal transfer of several genes in *Arthrobacter* sp. (Garcia-Vallvé *et al*., 2002). The authors suggest that the *Arthrobacter* sp. genes encoding the L27 ribosomal protein and genes encoding the proteins responsible for the degradation of creatinine and sarcosine were acquired simultaneously from unknown *Bacillus* species, as these genes showed a phylogenetic relationship with *Bacillus* species. It would be interesting to study whether *A. phytoseiuli* acquired virulence genes from other bacterial pathogens such as *Bacillus thuringiensis*. Toxic effects of thuringiensin, a toxin produced by *Bacillus thuringiensis* have been reported for *P. persimilis* and other phytoseiid mites (cited in van der Geest *et al*., 2000). The oviposition of treated predators started to decline 2 days after treatment and ceased completely 3-4 days after treatment.

 **How could *A. phytoseiuli* invade numerous populations?**

It is a well-known fact that natural enemy producers regularly exchange material. Many small producers buy and sell material temporarily from other producers, as they may lack for a certain period rearing capacity to produce all natural enemies needed for an integrated control program in sufficient quantities. Moreover, research laboratories often exchange material with each other or introduce new material from a producer. Based on the high infectiousness of the disease (chapters 6, 7, 8), and the minute size of the host (body length of adult female *P. persimilis* ca. 0.45 mm; Gaede, 1992), it might be expected that the disease can easily spread from one population to the other, even if they are reared separately. After occurrence of the behavioural change in the NR-population we could only maintain a population with a high degree of attraction to herbivore-induced plant volatiles in our laboratory by rearing them under strict hygienic conditions: (1) predators were kept in closed Petri-dishes in another location than the NR-population, (2) during one day NR-populations were never handled before R-populations (3) all non-living material was sterilized or eliminated after first use. It can be assumed that the majority of commercial producers and research laboratories follow rearing protocols that are less rigid. Thus, infected predators or material contaminated by infected predators are likely to come in contact with healthy predators. We therefore recommend testing a *P. persimilis* population for the presence of *A. phytoseiuli* before introducing it into the rearing facilities.

Future perspectives

My findings as reported in this thesis are of importance to applied science as well as to fundamental science. This is due to the following facts: (1) the disease caused by the highly virulent pathogen *A. phytoseiuli* affects one of the most important organisms used in biological control, (2) *A. phytoseiuli* is the first bacterial pathogen found in phytoseiid mites, (3) *A. phytoseiuli* is the first pathogen of phytoseiid mites for which the effects on host behaviour have been described, and (4) *A. phytoseiuli* represents a new species and a new genus.

 **What may be the implications for applied science?**

Many characteristics that contribute to the effectiveness of *P. persimilis* as biological control agent are negatively affected by the disease caused by *A. phytoseiuli*: diseased mites are less effective in locating their prey, they leave prey patches even when ample prey is still present, they produce less offspring and die early (chapter 4). A serious problem is that the

disease seems to be present in several commercial and laboratory populations (Gols *et al*., in prep.). These findings match with confidential complaints from several producers concerning the lower production capacity of *P. persimilis* during the past years and of confidential comments by some researchers about the quality of *P. persimilis* sold to the market. Thus, it is most probable that the disease actually causes economic losses to growers and producers. Moreover, the presence of a widespread disease in a natural enemy in Europe can, on the long term, even have negative effects on the export and sale of biological control agents to other continents, as more strict risk assessment of mass-produced natural enemies may be expected in future. It has been suggested that a general framework for regulation of import and release of biological control agents should include the risk of vectoring diseases (van Lenteren *et al*., 2003b). In this context we should also keep in mind the possibility of disease transfer to mass-reared or natural populations of other phytoseiid species. In such a case the use of *P. persimilis* in biological control would no longer be environmentally sound. Thus, I suggest that the following points should receive attention in future applied scientific projects in order to obtain relevant knowledge about the disease and its potential negative effects: (1) effects of *A. phytoseiuli* on the fitness of predatory mites in mass production systems**,** (2) effects of *A. phytoseiuli* on effectiveness of biological control in greenhouse and farming systems, (3) spread of *A. phytoseiuli* among natural, commercial and laboratory populations of *P. persimilis*, (4) host range of *A. phytoseiuli* when offered commercial and native populations of other phytoseiid species, and (5) development and comparison of further methods of disease cure and disease prevention. Some of these questions have already been partly addressed by our group and will be published elsewhere (Silva *et al*., in prep.). Besides the abovementioned applied scientific projects, I here plead for more knowledge transfer between academia and producers, and among producers concerning potential diseases of their products. Points of attention should be: (1) development of fast and feasible test procedures for known diseases, (2) general inventory of disease related problems in mass production systems, (3) organisation of practical courses in disease recognition and curing of natural enemies with regard to the special needs of producers of natural enemies, (4) organisation of producer meetings to develop agreements on general disease prevention and certification standards and, (5) organisation of general meetings to develop guidelines for the prevention of diseases in natural enemies similar to what has been done successfully for the development of quality control guidelines. Knowledge transfer would essentially contribute to the production of pathogen-free natural enemies.

What may be the implications for fundamental science?

The fact that *A. phytoseiuli* represents a new species as well as a new genus and that it is the first bacterial pathogen described for phytoseiid mites makes it an interesting candidate for further fundamental research. Some points of interest are: (1) disease symptoms and disease transmission of juvenile and male *P. persimilis*, (2) histopathology of diseased mites, (3) presence of *A. phytoseiuli*-specific toxins, (4) genetic variability of different isolates, (4) pathogenicity islands, and (5) interaction with stress related factors and other microbes or pathogens.

The present study included behavioural symptoms in the experiments, which provided a consistent impression of distinct behavioural changes induced by *A. phytoseiuli*. This, together with the enormous amount of available knowledge on phytoseiid behaviour and predator-prey interactions makes the present disease an interesting candidate for fundamental research on the question whether the behavioural change is adaptive to host or pathogen. During the past two decades an enormous amount of work has been published on the role of herbivore-induced plant volatiles in the tritrophic system of Lima bean (*Phaseolus lunatus*) the spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis*) (Dicke *et al*., 1998; Sabelis *et al*., 1999; de Boer and Dicke, 2005). Herbivore-induced plant volatiles may evoke several behavioural traits in *P. persimilis* including (1) attraction to herbivore-induced plant volatiles (Sabelis and van de Baan, 1983); (2) right about turns at sharp odour gradients (Sabelis *et al*., 1984b); (3) suppression of the tendency to disperse aerially (Sabelis and Afman, 1994); (4) positive anemotaxis (=upwind walking) after starvation and walking in random directions at satiation (Sabelis and van der Weel, 1993; van Tilborg *et al*., 2004). Future studies concerning the pathogen-induced behavioural change should preferably include all these traits. Thus, at the very end of this discussion I am returning to the starting point of the work presented in this thesis, i.e. the sudden and permanent change in foraging behaviour in adult female *P. persimilis* by pleading in accordance with other authors (Dicke, 1996; Skorping and Högstedt, 2001): **Let's be aware of the hidden power of pathogens in food web interactions!**

Acknowledgements

I am grateful to Marcel Dicke and Joop van Lenteren for their helpful comments on an earlier version of this discussion.

Summary

The predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) is a specialist predator of spider mites. Since more than three decades *P. persimilis* has been successfully applied in biological control of the two-spotted spider mite *Tetranychus urticae* Koch (Acari, Tetranychidae) in several greenhouse and field crops. The importance of *P. persimilis* and other predatory mite species in integrated pest control has stimulated research activities, with special emphasis on predator prey-interactions and foraging behaviour.

During the past 20 years studies by different research groups have consistently demonstrated that adult female predatory mites are attracted to volatiles emanating from Lima bean plants infested with their prey *T. urticae*. These volatiles do not, as was first assumed, emanate from the prey organisms, but are produced by the plants after herbivore attack. That is why these volatiles have been designed as herbivore-induced infochemicals. Attraction was and is tested in a Y-tube olfactometer set-up, where an individual predatory mite may walk towards the odour of either prey-infested Lima bean plants or uninfested Lima bean plants, thereby making a choice for one of these two odour sources. About 80% of tested predators walk towards the odour of prey-infested Lima bean plants when tested in such an experimental set-up. However, in 1992 a sudden and permanent change in this behavioural response to prey-induced plant volatiles was recorded in our laboratory: adult female predators of our laboratory population, subsequently designated **non-responding (=NR-) population**, showed a lower degree of attraction to prey-induced plant odours than other populations of *P. persimilis*, designated **responding (=R-) populations**. The aim of my work was to understand the cause(s) of this behavioural change, as it may be expected that such a change negatively affects the quality of *P. persimilis* in biological control.

Changes in the behavioural response to plant odours in mass-reared natural enemies may be caused by a variety of factors, including changes of (1) the environment, (2) the odour source, (3) the previous experience of the natural enemy (4) the physiological state of the natural enemy (5) the genetic constitution of the natural enemy or (6) by an infectious agent. As preliminary experiments indicated that the latter factor was the most probable explanation for the behavioural change (unpublished data), the following working hypothesis was formulated: ***The behavioural change in adult female P. persimilis of the NR-population is a symptom of an infectious disease*** (see for thesis outline Figure1).

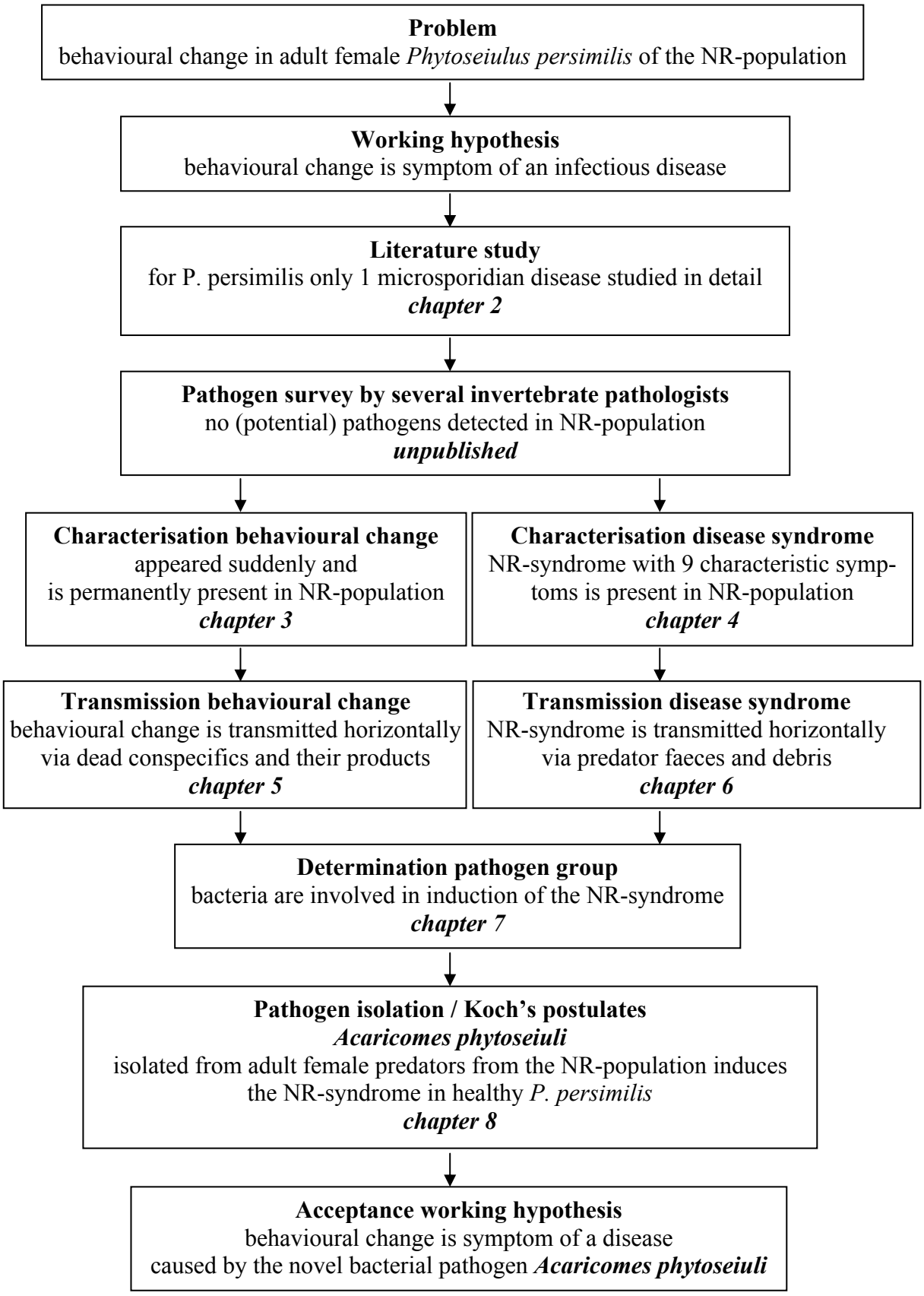


Figure 1: Outline of the research presented in this thesis

In **chapter 1** I give a short overview on the use of *P. persimilis* in biological control and the present standards of quality control in this sector, formulate the working hypothesis and explain the outline of this thesis.

A literature review on diseases in predatory mites (Acari, Phytoseiidae) is presented in **chapter2**. Despite recent reports on impaired quality of commercial and laboratory populations of *P. persimilis*, knowledge on pathogens is rather scarce. For only one microsporidium species, isolated from a European population of *P. persimilis* and assigned as *Microsporidium phytoseiuli*, clear pathological effects have been found, including reduced fecundity, reduced longevity, reduced predation rate and sex-ratio distortion. However, neither *M. phytoseiuli* nor any other suspicious entities were found in predators from the NR-population during microscopic and molecular studies executed by five invertebrate pathologists (unpublished data). This led me to the decision to follow a rather conventional research approach with the final aim to isolate the pathogen and satisfy the Koch's postulates (Figure1).

In all experiments mated adult female predators were used, as this stage is most important in biological control of spider mites. The experimental work described in **chapter 3** concentrated on the behavioural change of *P. persimilis* and was aimed at a characterization of this phenomenon and at the elimination of some of the possible explanations as listed above. The following results were found:

(1) In the second part of 1992 (after day 241) adult female predators of the NR-population show a degree of attraction to prey-induced plant odours (average percentage 50%, 15 olfactometertests with 20 females each) that is significantly lower than in the first part of 1992 (average percentage 78%, 14 olfactometertests with 20 females each). In the next years the percentage attraction varied considerably between olfactometertests (35%-75%) but the annual average of tests remained low (1993: 64%; 1994: 58%, 1995: 57%). The behavioural change thus occurred suddenly and was of a permanent nature.

(2) At the beginning of 1994 the same behavioural change occurred in a predator population from a commercial source. Also in this case the behavioural change occurred suddenly and was of a permanent nature.

(3) The attraction of predators from a commercial population with high response level decreased after being reared at our laboratory in a semi-open rearing set-up. When tested on the same day in the same set-up, the newly shipped predators were strongly attracted to prey-infested bean plants whereas those kept at our laboratory were not.

(4) A predator population with impaired response level consisted of two predator groups: predators that stayed on prey-infested plants in the rearing set-up were strongly attracted to

prey-infested bean plants while predators that had dispersed from the rearing set-up were not attracted.

(5) Isofemale lines consisting of predators showing a high degree of attraction could be selected from the NR-population and maintained for more than 20 generations at our laboratory when kept in a closed rearing set-up on the same food as the NR-population.

(6) Short-term starvation, which may occur among the predators in our rearing set-ups, did neither affect the degree of attraction of the predators from the NR-population neither of those from the R-population.

Conclusively the following factors could be excluded as possible explanation for the behavioural change: environmental variation and variation in the odour because of findings (3) and (4); previous experience (with respect to feeding) and short-term starvation of the natural enemy because of findings (5) and (6). Findings (4) and (5) on individual variation among predators suggest that genetic differences may be an explanation. However, findings (1) (2) and (3) suggest that the change invades an entire population at a high speed, which is a characteristic of an infectious agent rather than of a genetic change.

In **chapter 4** other characteristics of the NR-population were investigated in order to describe a distinct disease syndrome, designated **non-responding (=NR-) syndrome**. During this study I compared adult female *P. persimilis* from the NR-population with females of the same age originating from an R-population concerning several behavioural and non-behavioural characters.

The following symptoms were found in adult females from the NR-population:

(1) **Size change** by shrinkage to dorso-ventrally flattened form
(2) **Reduced fecundity** caused by oviposition stop after shrinkage
(3) **High mortality** caused by death several days after shrinkage
(4) **Presence of excretory crystals in the legs**
(5) **Low predation rate**
(6) **Low excretion rate**
(7) **Low degree of attraction to prey-induced plant odours** in Y-tube olfactometer
(8) **Short choice time** in Y-tube olfactometer
(9) **Early dispersal from prey-patches**

It may be expected that symptoms 2, 3, 5, 7 and 9 negatively affect the performance of *P. persimilis* in biological control of spider mites.

The crucial step towards verification of the working hypothesis was evidence of the infectious character of the behavioural change. In **chapter 5** I demonstrate that **the low degree of attraction is a contagious phenomenon** and that it cannot be explained by genetic differences between predator populations. Adult female *P. persimilis* that had been exposed to dead conspecifics of the NR-population and their products showed a lower degree of attraction and a higher mortality than predators that had been exposed to the products of live conspecifics of the NR-population. In a diseased population early dying individuals are likely candidates to carry and release pathogens and common routes of disease transmission consist of pathogen release prior to death or after death and cannibalism on dead conspecifics. However, in the present study we could not exclude that the same changes would be induced after contact to dead conspecifics of a R-population. Moreover, corpses are known to be colonized by secondary micro-organisms. I therefore designed a series of other infection experiments.

In **chapter 6** I tested several routes of syndrome transmission for six of the nine symptoms as listed above (numbers of tested symptoms: 1, 2, 3, 4, 7, 9). Pathogen transmission may be vertical or horizontal. Vertical transmission is defined as direct pathogen transfer from a parent organism to its offspring. Pathogens may be present on the egg surface (transovum transmission) or inside the egg (transovarial transmission). Horizontal transmission is defined as pathogen transfer from individual to individual but not directly from parent to offspring. This can occur among and between generations and between different host species.

I did **not** find evidence for symptom transmission:
(1) from parent to offspring directly via the egg (=vertical transmission)
(2) from *T. urticae* to female predatory mites (=interspecific horizontal transmission)
(3) from squashed females to females (=horizontal transmission via body fluids)

However, I found clear evidence for symptom transmission from female to female and mother to offspring **via faeces and debris** (=**horizontal transmission between and among generations**)

With knowledge about the main reservoir of the infectious agent I could determine to which group the pathogen in question belongs. In the first part of **chapter 7** I describe the development of a reliable bioassay for testing the infectiousness of predator faeces and debris fractions. This was done by keeping healthy adult female predators during a period of three days on prey-infested bean leaves, which had previously been sprayed with an aqueous suspension of faeces and debris. After exposure six of the nine symptoms as listed above were assessed (numbers of tested symptoms: 1, 2, 3, 4, 7, 9).

The following results were found:
(1) A faeces-and-debris-suspension collected from symptomatic females induced the NR-syndrome whereas a suspension collected from non-symptomatic females did not.
(2) The bacterial fraction of faeces-and-debris-suspension collected from symptomatic predators induced the NR-syndrome whereas the viral fraction of the same suspension did not.
(3) A faeces-and-debris-suspension collected from symptomatic predators induced the NR-syndrome whereas the same suspension treated with the antibiotic tetracycline did not.

These findings prove that **bacteria are involved in the induction of the NR-syndrome**.

The last step in this project was aimed at pathogen isolation and satisfying the Koch's postulates. Numerous bacterial isolates were isolated from adult female predators from the NR-population and their faeces and debris. In **chapter 8** I studied the effects of one of these isolates on adult female *P. persimilis*. The isolate represents a new bacterial species in a new genus, and is described as *Acaricomes phytoseiuli*. This genus is closely related to the bacterial genus *Arthrobacter.* The NR-syndrome was clearly induced in those predators that had been exposed to the bacterial inoculum (=treatment predators), whereas predators exposed to water (=control predators) did not show the NR-syndrome. Moreover, *A. phytoseiuli* was never isolated from control predators whereas it could be re-isolated from 60% of the treatment predators and from faeces of 41% of treatment predators. Light and electron microscopic studies of predators exposed to *A. phytoseiuli* revealed striking bacterial accumulations in the lumen of the alimentary tract together with extreme degeneration of its epithelium. In addition, bacterial foci also occurred in the fat body. These phenomena were not observed in control predators that had been exposed to sterile water. The present data prove that ***A. phytoseiuli*** **may infect the predatory mite** ***P. persimilis*** **and induce the occurrence of the NR-syndrome in adult female** ***P. persimilis***.

Thus, the present thesis led to the acceptance of the working hypothesis: **The sudden and permanent behavioural change in adult female *P. persimilis* of the NR-population is a symptom of an infectious disease**. This is the first record of a bacterial pathogen in phytoseiid mites. Moreover, the pathogen is a representative of a novel bacterial species and a novel bacterial genus. In **chapter 9** I discuss the results in the context of mite pathology, behavioural ecology, biological control and bacteriology.

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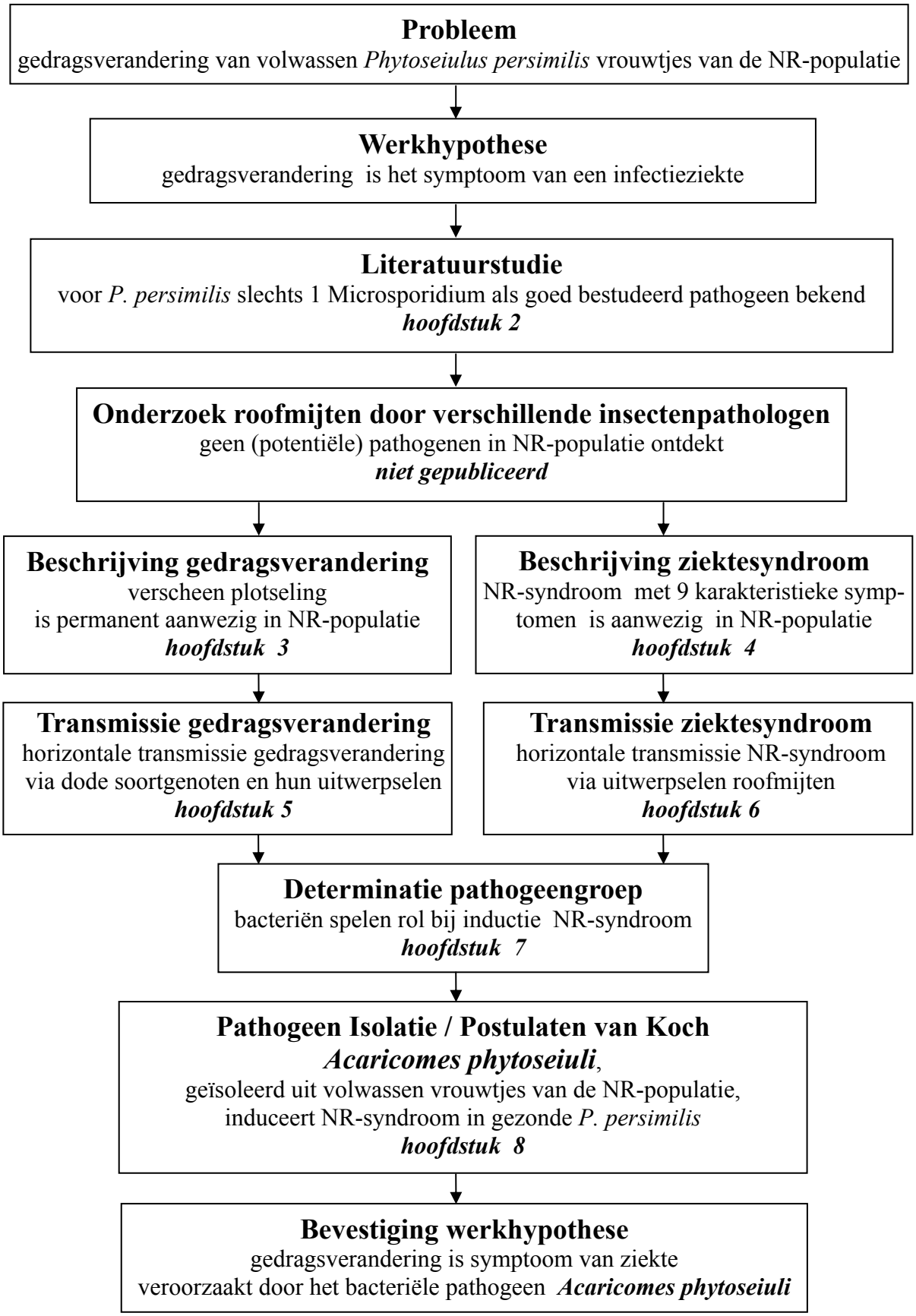
Samenvatting

De roofmijt *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) is een op spintmijten gespecialiseerde rover. Sinds meer dan 30 jaar wordt *P. persimilis* met succes ingezet in de biologische bestrijding van de spintmijt *Tetranychus urticae* Koch (Acari,Tetranychidae). Dit gebeurt in meerdere gewassen, zowel in de kasteelt als in de volle grondteelt. Sindsdien spelen *P. persimilis* en andere roofmijtensoorten een belangrijke rol in de geïntegreerde bestrijding van verschillende plaagorganismen. Dit heeft over de hele wereld het onderzoek betreffende roofmijten gestimuleerd, waarbij de thema's predator-prooi interactie en zoekgedrag centraal stonden.

Gedurende de laatste 20 jaar liet onderzoek van verschillende onderzoeksgroepen herhaaldelijk zien dat roofmijten aangetrokken worden door vluchtige stoffen afkomstig van door spintmijten aangetaste Lima boon planten. De vluchtige stoffen zijn niet, zoals men eerst dacht, afkomstig van de spintmijten, maar ze worden door de plant geproduceerd. Men kan dus zeggen dat een aangevallen plant door de productie van geurstoffen de natuurlijke vijand te hulp roept, want de roofmijt zal als hij eenmaal een prooihaard heeft gevonden deze ook uitroeien en daardoor de plant voor verdere schade behoeden. De geurstoffen zullen in het volgende met de term herbivoor-geïnduceerde plantengeuren aangeduid worden. De aantrekkingskracht van geurstoffen werd en wordt nog steeds getest in een Y-buis olfactometer opstelling. In deze opstelling kan een individuele roofmijt naar de geur van door spint aangetaste Lima boon planten toegaan of naar de geur van schone planten. Op deze manier maakt een roofmijt een keuze tussen de twee haar aangeboden geurbronnen. In een dergelijke proefopstelling kiest ca. 80% van de geteste roofmijten voor de herbivoor-geïnduceerde plantengeuren.

In 1992 vonden wij echter een opmerkelijke verandering in het keuzegedrag van de roofmijten in ons laboratorium: gepaarde roofmijtenvrouwtjes uit een van de in ons laboratorium gekweekte populaties werden in duidelijk mindere mate aangetrokken door herbivoor-geïnduceerde plantengeuren. Deze populatie wordt in het volgende met de term **NR-populatie** aangeduid (uit het Engels "**non-responding**", wat "**niet reagerend**" betekent), terwijl populaties met een normaal keuzegedrag **R-populatie** genoemd worden (uit het Engels "**responding**", wat "**reagerend**" betekent). Men kan aannemen dat roofmijten met een veranderd keuzegedrag minder goed hun prooi kunnen vinden dan roofmijten met een normaal keuzegedrag. De gedragsverandering zou daarom negatieve gevolgen kunnen hebben voor de biologische bestrijding van spintmijten, als deze ook bij commerciële populaties



Figuur 2: Opbouw van het proefschrift

optreedt. Het doel van mijn werk was daarom de oorzaak van de opmerkelijke gedragsverandering te begrijpen.

Verandering in het keuzegedrag ten opzichte van herbivoor-geïnduceerde plantengeuren kan door verschillende factoren veroorzaakt worden, bij voorbeeld door een verandering (1) in de omgeving (luchtdruk, aanwezigheid andere geurstoffen) (2) in de geurbron (3) met betrekking tot voorafgaande ervaringen van de carnivoor (4) in de fysiologische toestand van de carnivoor (5) van de genetische constitutie van de carnivoor of (6) door een infectie met een ziekteverwekker. Omdat in eerste voorlopige experimenten de laatst genoemde factor als de meest waarschijnlijke verklaring voor de gedragsverandering naar voren kwam (niet gepubliceerde data), ontwikkelde ik de volgende werkhypothese voor mijn onderzoek: ***De gedragsverandering van volwassen P. persimilis vrouwtjes uit de NR-populatie is het symptoom van een infectieziekte*** (zie ook Figuur 1 voor opbouw van het proefschrift).

In **hoofdstuk 1** geef ik een kort overzicht van het gebruik van *P. persimilis* in de biologische bestrijding. Daarnaast presenteer ik de huidige stand van zaken in de kwaliteitsbewaking binnen deze sector, formuleer ik de werkhypothese en leg ik de opbouw van dit proefschrift uit.

In **hoofdstuk 2** presenteer ik de resultaten van een literatuurstudie betreffende ziektes in roofmijten (Acari, Phytoseiidae). Momenteel is de kennis over ziektes in roofmijten niet bijzonder groot. Dit is verbazingwekkend gezien het feit, dat in de laatste jaren rapporten over een slechte kwaliteit van commerciële en laboratorium populaties van *P. persimilis* toenamen. In de wetenschappelijke literatuur zijn pathologische symptomen in *P. persimilis* slechts voor één ziekteverwekker beschreven. Het gaat daarbij om een microsporidium, genoemd *Microsporidium phytoseiuli*. Microsporidia zijn sporenvormende Protozoa die bij insecten vaak een chronische ziekte veroorzaken. Infectie van *P. persimilis* met *M. phytoseiuli* leidt tot gereduceerde reproductie, gereduceerde predatie, minder vrouwelijke nakomelingen en een vroegtijdige dood. Terwijl vijf insectenpathologen roofmijten van de NR-populatie met microscopische en moleculair-biologische technieken onderzochten, kon helaas noch *M. phytoseiuli* noch een andere (potentiële) ziekteverwekker ontdekt worden. Daarom besloot ik in het vervolg, om een conventionele weg voor mijn onderzoek in te slaan, met als einddoel de isolatie van het onbekende pathogeen en de bevestiging via de postulaten van Koch voor oog (Figuur 1).

In al mijn experimenten onderzocht ik gepaarde vrouwelijke roofmijten, omdat dit stadium vanwege zijn grote predatiesnelheid en haar rol in het stichten van nieuwe kolonies het belangrijkste stadium voor de biologische bestrijding is. Het experimentele werk van **hoofdstuk 3** was gericht op een karakterisering van de gedragsverandering en op het uitsluiten

van een aantal van de boven genoemde mogelijke verklaringen voor de gedragsverandering. De resultaten zijn als volgt:

(1) In het tweede deel van 1992 (na dag 241) was de fractie van de gepaarde roofmijt vrouwtjes van de NR-populatie die koos voor herbivoor-geinduceerde plantengeuren significant kleiner (gemiddelde van 15 olfactometertests = 50%, elke test uitgevoerd met 20 roofmijten) dan in het eerste deel van hetzelfde jaar (gemiddelde van 14 olfactometertests = 78%, elke test uitgevoerd met 20 roofmijten). Gedurende de volgende 3 jaar varieerde de mate van aantrekking sterk tussen testdagen (35%-75%) maar het jaarlijkse gemiddelde van alle tests bleef laag (1993: 64%; 1994: 58%, 1995: 57%). De gedragsverandering trad dus plotseling op en bleef permanent aanwezig in de NR-populatie.

(2) In het begin van 1994 trad dezelfde gedragsverandering op in een roofmijtenpopulatie afkomstig van een commercieel bedrijf. Ook in dit geval trad de verandering plotseling op waarna ze permanent in deze roofmijten populatie aanwezig bleef.

(3) De mate van aantrekking door herbivoor-geïnduceerde plantengeuren van een roofmijtenpopulatie met de kenmerken van een R-populatie (zie voor uitleg boven) nam af zodra deze populatie in ons laboratorium in een semi-open kweekopstelling werd gehouden. Als wij vers aangeleverde roofmijten samen met de door ons gekweekte roofmijten van dezelfde populatie op dezelfde dag in dezelfde opstelling met dezelfde geurbronnen testten, dan werden de vers aangeleverde roofmijten in hoge mate door herbivoor-geïnduceerde plantengeuren aangetrokken terwijl dit niet het geval was voor de in ons laboratorium gekweekte roofmijten.

(4) Een roofmijtenpopulatie met de kenmerken van een NR-populatie (zie voor uitleg boven) bestond uit twee groepen roofmijten: Roofmijten die op door spint aangetaste planten in de kweekopstelling verbleven werden in hoge mate aangetrokken door herbivoor-geïnduceerde plantengeuren terwijl roofmijten die de kweekopstelling hadden verlaten niet aangetrokken werden.

(5) Meerdere roofmijtenlijnen, die in hoge mate aangetrokken werden door herbivoor-geïnduceerde plantengeuren, konden uit de NR-populatie geselecteerd werden. Deze lijnen behielden de kenmerken van een R-populatie gedurende 20 generaties, als zij gekweekt werden in een gesloten kweekopstelling in ons laboratorium. Deze lijnen ontvingen hetzelfde voedsel als de NR-populatie.

(6) Korte periodes van voedseldeprivatie, zoals die in onze kweekopzetten kunnen voorkomen, hadden geen effect op het keuzegedrag van de roofmijten. Dit was het geval zowel voor R-populaties als voor NR-populaties.

Derhalve konden de volgende verklaringsmogelijkheden voor de gedragsverandering uitgesloten worden: variatie van de omgeving en van de geurbron vanwege de resultaten (3) en (4), voorafgaande ervaring (wat voedsel betreft) en fysiologische toestand (korte tijd van voedseldeprivatie) van de carnivoor vanwege de resultaten (5) en (6). De resultaten (4) en (5) betreffende de individuele variatie tussen roofmijten suggereren dat mogelijk genetische verschillen de gedragsverandering kunnen verklaringen. Daartegen spreken echter de resultaten (1) (2) en (3) die suggereren dat de gedragsverandering een populatie met een hoge snelheid binnendringt, wat eerder wijst op een besmettelijke ziekte dan op een genetische verandering.

In **hoofdstuk 4** werden andere eigenschappen van de NR-populatie onderzocht met als doel een exact ziektesyndroom te beschrijven. Dit syndroom wordt in het vervolg met de term "**non-responding**" **(=NR-) syndroom** aangeduid. In deze studie vergleek ik gepaarde *P. persimilis* vrouwtjes van de NR-populatie met gepaarde roofmijtenvrouwtjes van dezelfde leeftijd afkomstig van een R-populatie. Hierbij werden verschillende gedragskenmerken en andere eigenschappen onderzocht.

De volgende symptomen werden gevonden voor gepaarde roofmijtenvrouwtjes afkomstig van de NR-populatie:

(1) **Verandering in lichaamsgrootte** door krimpen tot een dorso-ventraal platte vorm
(2) **Gereduceerde vruchtbaarheid** door plotselinge stop van eileg na het krimpen
(3) **Hoge mortaliteit** door dood een aantal dagen na krimpen
(4) **Aanwezigheid van excretorische kristallen in de poten**
(5) **Lage predatiesnelheid**
(6) **Geringe productie van uitwerpselen**
(7) **Lage mate van aantrekking door herbivoor-geïnduceerde plantengeuren**
(8) **Rechtdoor lopen met weinig omkeringen** in Y-buis olfactometer
(9) **Vroegtijdig verlaten van prooihaarden**

De symptomen 2, 3, 5, 7 en 9 zullen waarschijnlijk de effectiviteit van *P. persimilis* in de biologische bestrijding van spintmijten negatief beïnvloeden.

Een belangrijke stap voor bevestiging van de werkhypothese was het bewijs van de overdraagbaarheid van de gedragsverandering. De resultaten van **hoofdstuk 5** laten zien dat de **verminderde mate van aantrekking door herbivoor-geïnduceerde plantengeuren een besmettelijk fenomeen is** en niet door genetische verschillen tussen individuen verklaard kan worden. Gepaarde roofmijtenvrouwtjes die eerder in contact waren met dode soortgenoten van de NR-populatie en hun uitwerpselen werden in geringere mate door plantengeuren

aangetrokken en hadden een hogere mortaliteit dan roofmijten, die eerder in contact waren met levende soortgenoten van de NR-populatie.

In een zieke populatie zijn vroegtijdig stervende individuen de meest waarschijnlijke bron voor aanwezigheid van pathogenen. Bekende manieren van ziekteoverdracht bij geleedpotigen zijn het vrijkomen van pathogenen net voor of na overlijden en kannibalisme van dode individuen. Maar helaas kon ik in deze experimenten niet uitsluiten dat de gedragsverandering en mortaliteit eveneens geïnduceerd zouden worden na contact met dode soortgenoten van de R-populatie. Ook zijn op kadavers veel secundaire micro-organismen aanwezig die de effecten zouden kunnen verklaren. Daarom voerde ik een serie van verdere infectie-experimenten uit.

In **hoofdstuk 6** testte ik verschillende routes van ziekteoverdracht waarbij ik zes van de negen bovengenoemde ziektesymptomen observeerde (nummers van geteste symptomen: 1, 2, 3, 4, 7, 9). Overdracht van een pathogeen kan horizontaal of verticaal zijn. Verticale transmissie is gedefinieerd als directe pathogeenoverdracht van ouders op nakomelingen. Pathogenen kunnen dan aanwezig zijn op het eioppervlak (transovum transmissie) of binnen het ei (transovariale transmissie). Horizontale transmissie is gedefinieerd als pathogeenoverdracht van het ene individu op het andere, waarbij directe transmissie van ouder op nakomeling uitgesloten wordt. Horizontale transmissie kan plaatsvinden binnen en tussen generaties en tussen verschillende soorten.

Ik kon **geen** aanwijzingen vinden voor overdracht van symptomen:
(1) van ouder op nakomelingen direct via het ei (=verticale transmissie)
(2) van de prooi *T. urticae* op volwassen roofmijtenvrouwtjes (=horizontale transmissie tussen verschillende soorten)
(3) van dode geplette roofmijtenvrouwtjes op andere roofmijtenvroutjes (horizontale transmissie via lichaamsvloeistof).

Daarentegen vond ik duidelijk bewijs voor de transmissie van symptomen van het ene roofmijtenvrouwtje op het andere en van moeder op nakomelingen via **uitwerpselen en andere uitscheidingen (horizontale transmissie binnen en tussen generaties)**

Met kennis over het hoofdreservoir van het pathogeen kon ik nu vaststellen tot welke pathogeengroep het onbekende pathogeen behoort. In het eerste deel van **hoofdstuk 7** beschrijf ik de ontwikkeling van een bioassay waarmee getest kan worden of roofmijtenuitwerpselen het onbekende pathogeen bevatten of niet. De bioassay ziet er als volgt uit: gezonde, gepaarde roofmijtenvrouwtjes worden gedurende een periode van 3 dagen op bonenbladeren met prooi gezet, die vooraf met in water opgeloste roofmijtenuitwerpselen besproeid werden. Na blootstelling werden de roofmijten met betrekking tot zes van de negen

boven genoemde ziektesymptomen onderzocht (nummers van geteste symptomen: 1, 2, 3, 4, 7, 9).

De volgenden resultaten werden gevonden:
(1) Een oplossing van uitwerpselen induceerde alleen dan het NR-syndroom in gezonde roofmijten als de uitwerpselen van symptomatische roofmijtenvrouwtjes uit de NR-populatie verzameld waren. Als de uitwerpselen van niet-symptomatische roofmijtenvrouwtjes van de R-populatie verzameld waren, dan werd niet één van de geteste symptomen geïnduceerd.
(2) Werd een oplossing opgedeeld in twee fracties, dan induceerde alleen die fractie het NR-syndroom in gezonde roofmijtenvrouwtjes, die bacteriën bevatte. De virusfractie induceerde niet één van de symptomen van het NR-syndroom.
(3) Een oplossing van uitwerpselen van roofmijtenvrouwtjes uit de NR-populatie induceerde het NR-syndroom niet als ik van tevoren het antibioticum tetracycline aan de oplossing toegevoegd had.

Deze resultaten bewijzen dat **bacteriën een rol spelen bij de inductie van het NR-syndroom.**

De laatste stap van dit project was de isolatie van het pathogeen en de bevestiging van de postulaten van Koch. Talrijke bacteriesoorten werden geïsoleerd uit gepaarde roofmijtenvrouwtjes van de NR-populatie en uit hun uitwerpselen. In **hoofdstuk 8** onderzocht ik de effecten van één van deze bacteriën op volwassen roofmijtenvrouwtjes. Het gaat hierbij om **een tot dusver niet bekende soort in een nieuw genus, nu beschreven als *Acaricomes phytoseiuli***. Het NR-syndroom werd geïnduceerd in roofmijten na contact met *A. phytoseiuli* (=behandelde roofmijten) terwijl roofmijten die alleen met water in contact waren geweest het NR-syndroom niet ontwikkelden (=controle roofmijten). *A. phytoseiuli* werd nooit geïsoleerd uit controle roofmijten, terwijl het geïsoleerd kon worden uit 60% van de behandelde roofmijten en uit uitwerpselen van 41 % van de behandelde roofmijten. Licht- en elektronen-microscopisch onderzoek van behandelde roofmijten liet de aanwezigheid zien van opvallende bacteriële accumulaties in de spijsverteringsorganen en de degeneratie van diens epithelium. Ook werden bacteriën in het vetlichaam ontdekt. Geen van deze symptomen werd waargenomen in controle roofmijten. De voorliggende resultaten bewijzen dat ***A. phytoseiuli* de roofmijt *P. persimilis* infecteert en het NR-syndroom in gepaarde roofmijtenvrouwtjes induceert.**

Dit proefschrift leidt dus tot de bevestiging van de werkhypothese: ***De gedragsverandering van volwassen P. persimilis vrouwtjes uit de NR-populatie is het symptoom van een infectieziekte***. Dit is de eerste keer dat een bacterieel patghogeen in een roofmijt wordt beschreven. Ook representeert het tot dusver onbekende pathogeen niet alleen een nieuwe soort

maar ook een nieuw geslacht. In **hoofdstuk 9** bediscussieer ik de resultaten in de context van de pathologie van mijten, biologische bestrijding en bacteriologie.

Dankwoord

Ik bedank Marcel Dicke en Joop van Lenteren voor hun waardevol commentaar op een eerdere versie van deze samenvatting.

Zusammenfassung

Die Raubmilbe *Phytoseiulus persimilis* Athias-Henriot (Acari, Phytoseiidae) ernährt sich ausschließlich von den für den Pflanzenanbau schädlichen Spinnmilben. Seit mehr als drei Jahrzehnten wird *P. persimilis* erfolgreich in der Biologischen Schädlingsbekämpfung der Spinnmilbe *Tetranychus urticae* Koch (Acari, Tetranychidae) angewandt. Die Raubmilbe findet Einsatz in mehreren Gewächshaus- und Feldkulturen. Die große Bedeutung von *P. persimilis* und anderen Raubmilben in Biologischer und Integrierter Schädlingsbekämpfung hat zahlreiche wissenschaftliche Studien zu diesen Milben in Gang gesetzt. Räuber-Beute Interaktionen und Fouragierverhalten galt hierbei besonderes Interesse.

Studien verschiedener wissenschaftlicher Gruppen aus den letzten 20 Jahren haben wiederholt bewiesen, dass bestimmte flüchtige Stoffe, sogenannte Signalstoffe, adulte weibliche Raubmilben anziehen, wodurch die Raubmilben ihre Beute gut lokalisieren können. Diese flüchtigen Signalstoffe werden nicht, wie man erst annahm, von den Beutetieren produziert. Sie werden vielmehr von den Pflanzen, zum Beispiel der Limabohne, *Phaseolus lunatus,* produziert, nachdem diese durch die Beutetiere, zum Beispiel der Spinnmilbe *T. urticae*, befallen sind. Die Pflanzen rufen sozusagen die Raubmilben zu Hilfe, sobald sie durch deren Beute befallen sind. Die flüchtigen Stoffe werden im Folgenden als beute-induzierte Signalstoffe bezeichnet. Die Anziehungskraft solcher flüchtigen Signalstoffe wird in einer speziell dafür entwickelten Versuchsaufstellung (="Y-tube olfactometer") getestet. In dieser Aufstellung hat eine individuelle Raubmilbe die Wahl zwischen zwei ihr angebotenen Gerüchen. In diesem Fall sind das der Geruch von Blättern der Limabohne und der Geruch von durch Spinnmilben befallenen Blättern der Limabohne. Die Raubmilbe trifft die Wahl dadurch, dass sie in die Richtung einer der Geruchsquellen läuft. In einem solchen Test wählen ungefähr 80% der getesteten Raubmilben beute-induzierte Signalstoffe.

In unserem Laboratorium wurde im Jahr 1992 eine bemerkenswerte Veränderung des Orientierungsverhaltens von *P. persimilis* konstatiert: Gepaarte weibliche Raubmilben einer der Züchtungen zeigten einen deutlich verminderten Grad der Orientierung zu beute-induzierten flüchtigen Signalstoffen. Diese Population wird im Folgenden als **NR-Population** bezeichnet (vom Englischen "**non-responding**", was soviel bedeutet wie "**nicht-reagierend**"). Populationen mit einem normalen Orientierungsverhalten werden in der vorliegenden Arbeit als **R-Populationen** bezeichnet (vom Englischen "**responding**" was übersetzt werden kann mit "**reagierend**"). Da die Lokalisierung von Beutetieren für

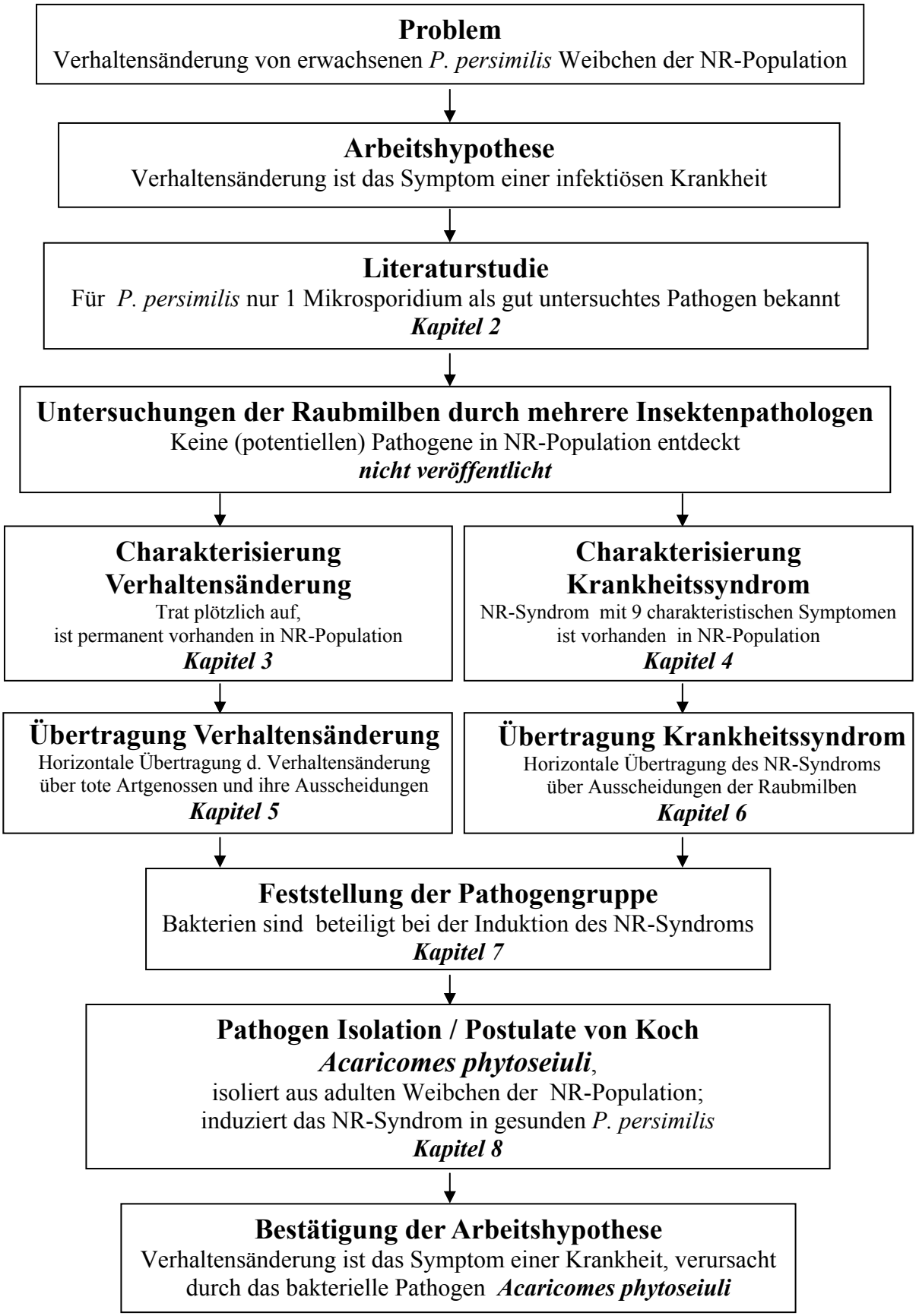


Abbildung 1: Aufbau der Dissertation

Raubmilben mit einem derart veränderten Orientierungsverhalten erschwert ist, könnte eine solche Verhaltensänderung negative Auswirkungen auf die kommerziell wichtige Biologische Bekämpfung von Spinnmilben haben, wenn sie sich auf kommerziell angewandte Populationen ausbreiten würde. Das Hauptanliegen der vorliegenden Dissertation war darum, die Gründe für die bemerkenswerte Veränderung zu erforschen.

Veränderungen des Orientierungsverhaltens zu flüchtigen pflanzlichen Signalstoffen können durch unterschiedliche Faktoren hervorgerufen werden, zum Beispiel durch Veränderungen (1) in der Umgebung (Luftdruck, Präsenz von anderen Signalstoffen) (2) der Geruchsquelle (= Pflanze) (3) der vorrausgehenden Erfahrungen der Raubmilben (4) des physiologischen Zustandes der Raubmilben (5) der genetischen Konstitution der Raubmilben oder (6) durch einen infektiösen Erreger. Da erste Experimente den letztgenannten Faktor als den wahrscheinlichsten auswiesen (nicht veröffentlichte Daten), wurde die folgende Arbeitshypothese formuliert: ***Die Verhaltensänderung von gepaarten* P. persimilis *Raubmilbenweibchen der NR-Population ist das Symptom einer infektiösen Krankheit*** (siehe auch Aufbau der Dissertation in Abbildung 1).

Kapitel 1 beinhaltet eine kurze Übersicht zur Anwendung von *P. persimilis* in der Biologischen Schädlingsbekämpfung und zu den gegenwärtigen Qualitätsstandards in diesem Industriesektor. Darüber hinaus wird die Arbeitshypothese formuliert und der Aufbau dieser Dissertation präsentiert.

In **Kapitel 2** finden sich die Resultate einer ausführlichen Literaturstudie zu Krankheiten von Raubmilben (Acari, Phytoseiidae). Zum gegenwärtigen Zeitpunkt ist der Wissensstand bei Raubmilbenkrankheiten nicht sehr groß. Dies ist umso mehr verwunderlich, als sich in der letzten Zeit Berichte von verminderter Qualität von sowohl kommerziellen als auch Laborpopulationen der Raubmilbe *P. persimilis* häuften. In der wissenschaftlichen Literatur sind deutliche pathologische Symptome nur für einen Krankheitserreger beschrieben und zwar für die Mikrosporidie *Microsporidium phytoseiuli*. Mikrosporidien sind sporenformende Protozoen, die oftmals chronische Krankheiten bei Insekten verursachen. Eine Infektion von *P. persimilis* mit *M. phytoseiuli* führt zu reduzierter Fruchtbarkeit, einem reduzierten Beutefang, zu relativ wenigen weiblichen Nachkommen und zum vorzeitigen Tod. Obwohl 5 Insektenpathologen Raubmilben unserer NR-Population mit mikroskopischen als auch molekularbiologischen Methoden untersuchten, wurde weder *M. phytoseiuli* noch irgendein anderer Krankheitserreger entdeckt (nicht veröffentliche Resultate). Dies führte zu einer eher konventionellen Herangehensweise mit dem Ziel, den unbekannten Krankheitserreger zu isolieren und die Postulate von Koch zu erfüllen, da diese den ultimativen Beweis der Arbeitshypothese repräsentieren würden.

In allen der im Folgenden beschriebenen Experimenten, wurden gepaarte weibliche Raubmilben verwendet, da dieses Entwicklungsstadium wegen des großen Beutekonsums und der bedeutenden Rolle in der Gründung neuer Kolonien das wichtigste Stadium für die Biologische Schädlingsbekämpfung ist. Im **Kapitel 3** werden die Ergebnisse von Untersuchungen zu der Veränderung des Orientierungsverhaltens von *P. persimilis* dargelegt. Die Experimente waren so konzipiert, dass sie zum Ausschluss einiger der oben genannten Erklärungsmöglichkeiten führen würden.
Die Resultate sind wie folgt:

(1) In der zweiten Hälfte des Jahres 1992 (nach Tag 241) war die Rate der adulten weiblichen Raubmilben der NR-Population, die im Olfaktometertest die flüchtigen Signalstoffe von beute-befallenen Pflanzen wählten, signifikant niedriger (gemittelt 50% von 15 Olfaktometertests, jeder Test ausgeführt mit 20 Raubmilben), als in der ersten Hälfte desselben Jahres (gemittelt 78% von 14 Olfaktometertests, jeder Test ausgeführt mit 20 Raubmilben). Während der folgenden drei Jahre variierten die Raten der Anziehung stark zwischen den verschiedenen Testtagen (35%-75%), jedoch blieb das Jahresmittel niedrig (1993: 64%; 1994: 58%; 1995: 57%). Die Verhaltensveränderung in der NR-Population trat also plötzlich auf und blieb permanent vorhanden.

(2) Zu Beginn des Jahres 1994 trat dieselbe Verhaltensänderung in einer Raubmilben-Population eines kommerziellen Produzenten auf. Auch in diesem Fall trat die Verhaltensänderung plötzlich auf und war anschließend permanent in dieser Population zu verzeichnen.

(3) Der Grad der Orientierung zu beute-induzierten flüchtigen Signalstoffen einer kommerziellen Population mit den Kennzeichen einer R-Population (siehe oben) nahm schnell ab, wenn diese Population in unserem Labor in einer semi-offenen Aufstellung gezüchtet wurde. Wenn eine solche Population simultan (=am gleichen Tag in derselben Versuchsaufstellung) mit einer neu vom Produzenten kommenden Population getestet wurde, so war die Rate der gepaarten weiblichen Raubmilben, die im Olfaktometertest die flüchtigen Signalstoffe von beute-befallenen Pflanzen wählten, bedeutend niedriger für die Population, die im eigenen Labor gezüchtet wurde.

(4) Eine Raubmilbenpopulation mit einem niedrigen Grad der Orientierung zu beute-induzierten Signalstoffen bestand aus zwei Gruppen: Raubmilben, die auf beute-befallenen Pflanzen verblieben, wurden in hohem Maße durch Signalstoffe angezogen, während Raubmilben, die frühzeitig die Beutekolonien verlassen hatten, nicht auf Signalstoffe reagierten.

(5) Mehrere Linien von Raubmilben mit einem hohen Grad an Orientierung zu beute-induzierten Signalstoffen konnten aus der NR-Population selektiert und über mehr als 20

Generationen im Labor in einer geschlossenen Aufstellung gezüchtet werden. Diese Populationen erhielten dasselbe Futter wie die NR-Population.

(6) Kurzzeit-Hungerperioden, die gelegentlich in den Züchtungen auftreten können, hatten keinen Effekt auf den Grad der Anziehung. Das galt sowohl für NR-Populationen als auch für R-Populationen.

Aus diesen Ergebnissen konnte geschlossen werden, dass die folgenden Faktoren als mögliche Erklärung für die Verhaltensveränderung von *P. persimilis* nicht in Frage kommen: Veränderungen der Umgebung und der Geruchsquelle aufgrund der Resultate (3) und (4); vorrausgehende Erfahrungen (betreffend der Beutetiere) und physiologischer Zustand der Raubmilben (Kurzzeit-Hungerperioden) aufgrund der Resultate (5) und (6). Die Resultate (4) und (5) betreffenden individuellen Variationen zwischen den Raubmilben lassen vermuten, dass genetische Veränderungen als Erklärungsmöglichkeit eine Rolle spielen. Dem widersprechen jedoch die Resultate (1), (2) und (3), die suggerieren, dass sich die Veränderung mit hoher Geschwindigkeit in einer Population verbreitet, was eher auf einen infektiösen Erreger hinweist als auf genetische Veränderungen.

Im **Kapitel 4** wurden verschiedene weitere Eigenschaften der NR-Population untersucht, mit dem Ziel, ein genaues Krankheitssyndrom zu beschreiben, das im Folgenden mit der Bezeichnung "**non-responding**" **(=NR-) Syndrom** angedeutet wird. In dieser Studie wurden gepaarte weibliche *P. persimilis* der NR-Population mit Weibchen desselben Alters einer R-Population verglichen. Hierbei wurden verschiedene Verhaltensmerkmale sowie andere Eigenschaften untersucht.

Adulte weibliche Raubmilben der NR-Population zeigten die folgenden Symptome:

(1) **Veränderte Körpergröße** durch Schrumpfung zu einer dorso-ventral platten Form.
(2) **Verminderte Fruchtbarkeit** durch abrupten Stopp der Eiablage direkt nach dem Schrumpfen.
(3) **Erhöhte Mortalität** durch frühzeitigen Tod einige Tage nach dem Schrumpfen.
(4) **Anwesenheit von exkretorischen Kristallen in den Beinen**.
(5) **Niedriger Beutefang**.
(6) **Niedrige Exkretionsrate**.
(7) **Niedriger Grad der Orientierung zu beute-induzierten flüchtigen Signalstoffen**.
(8) **Geradliniges Laufen mit wenigen Umkehrungen** im Olfaktometer.
(9) **Frühzeitiges Verlassen der Beutekolonien**.

Es ist anzunehmen, dass sich die Symptome 2, 3, 5, 7 und 9 negativ auf die Effektivität von *P. persimilis* in der Biologischen Bekämpfung von Spinnmilben auswirken.

Ein wichtiger Schritt hin zur Bestätigung der Arbeitshypothese war der Beweis, dass die Verhaltensveränderung ansteckend ist. **Kapitel 5** zeigt, dass der niedrige Grad der Orientierung zu beute-induzierten flüchtigen Signalstoffen der NR-Population ein ansteckendes Phänomen ist, das folglich nicht durch eine genetische Veränderung der Raubmilben erklärt werden kann. Adulte *P. persimilis* Weibchen zeigten eine erhöhte Mortalität und die besagte Verhaltensänderung, nachdem sie mit toten Artgenossen der NR-Population und deren Ausscheidungen in Kontakt gebracht worden waren. Dies war dagegen bei gepaarten *P. persimilis* Weibchen, die mit den Ausscheidungen von lebenden Artgenossen der NR-Population in Kontakt gebracht wurden, nicht der Fall. In einer erkrankten Population sind es vor allem die frühzeitig versterbenden Individuen, die Krankheitserreger in sich tragen und in die Umgebung entlassen. Das Freiwerden von Pathogenen kurz vor oder nach dem Tod des infizierten Individuums sowie Kannibalismus an verstorbenen Artgenossen ist eine weit verbreitete Art von Krankheitsübertragung bei Insekten und Milben. In der hier beschriebenen Studie konnte nicht ausgeschlossen werden, dass vergleichbare Effekte nach Kontakt mit toten Artgenossen der R-Population auftraten. Darüber hinaus bevölkern sekundäre Mikroorganismen abgestorbene Körper, so dass die in diesem Kapitel gefundenen Resultate ebenso auf solche sekundären Mikroorganismen zurückzuführen sein könnten. Diese Überlegungen führten daher zur Ausführung der folgenden Experimente.

Im **Kapitel 6** wurde eine Anzahl von möglichen Arten der Krankheitsübertragung getestet, wobei jeweils sechs der neun oben genannten Symptome des NR-Syndroms einbezogen wurden (Symptome 1, 2, 3, 4, 7 und 9). Man unterscheidet bei der Übertragung von Insektenpathogenen zwei Formen, die vertikale und die horizontale Übertragung. Man spricht von vertikaler Krankheitsübertragung, wenn der Erreger direkt von den Eltern auf die Nachkommen übertragen wird. In diesem Fall befinden sich die Pathogene entweder auf der Oberfläche des Eis (transovum Übertragung) oder aber im Innern des Eis (transovarielle Übertragung). Dagegen spricht man von horizontaler Krankheitsübertragung, wenn der Erreger von einem Individuum auf das andere übertragen wird, jedoch nicht direkt über das Ei. Dieser Fall schließt Übertragung innerhalb und zwischen Generationen ein sowie Übertragung zwischen verschiedenen Arten.

Es konnten **keine** Beweise oder Anhaltspunkte für die Übertragung von Symptomen gefunden werden:
(1) Von den Eltern auf die Nachkommen direkt durch das Ei (=vertikale Übertragung),
(2) von den Beutetieren *T. urticae* auf gepaarte weibliche Raubmilben (=horizontale Übertragung zwischen verschiedenen Arten),

(3) von zerquetschten Weibchen auf lebende Weibchen (=horizontale Übertragung über Körperflüssigkeiten).

Dagegen konnten **deutliche** Beweise für die Übertragung von Symptomen gefunden werden: Von Weibchen auf Weibchen und von Mutter auf Nachkommen über **Exkremente und andere Ausscheidungen** (=**horizontale Übertragung innerhalb und zwischen Generationen**).

Nachdem deutlich war, dass die Exkremente erkrankter Raubmilben ein wichtiges Pathogenreservoir darstellten, war der folgende logische Schritt nötig, um festzustellen, welcher Pathogengruppe der hier vorliegende Erreger angehört: **Kapitel 7** beschreibt zunächst die Entwicklung eines Testverfahrens, mit dem einfach und zuverlässig festgestellt werden kann, ob Pathogene, die das NR-Syndrom verursachen, in den Ausscheidungen von *P. persimilis* Weibchen vorhanden sind. Das Verfahren sieht wie folgt aus: Gesunde adulte *P. persimilis* Weibchen werden während einer Periode von drei Tagen auf Beute tragenden Limabohnenblättern gehalten, die zuvor mit einer wässrigen Lösung von Raubmilbenausscheidungen besprüht wurden. Danach wird täglich überprüft, ob die gesunden Raubmilben das NR-Syndrom entwickeln. Hierbei wurden wiederum sechs von den neun oben genannten Symptomen des NR-Syndroms beobachtet (Symptome 1, 2, 3, 4, 7 und 9).

Mit diesem Verfahren wurden die folgenden Resultate erlangt:
(1) Eine wässrige Lösung von Ausscheidungen induzierte nur dann das NR-Syndrom in gesunden Raubmilben, wenn die Ausscheidungen von symptomatischen Raubmilben stammten. Stammten die Ausscheidungen von gesunden, nicht-symptomatischen Raubmilben, wurde nicht eines der getesteten Symptome des NR-Syndroms induziert.
(2) Wurde eine wässrige Lösung von Ausscheidungen symptomatischer Raubmilben in eine Bakterien enthaltende Fraktion und eine Virus enthaltende Fraktion geteilt, dann induzierte nur die Fraktion das NR-Syndrom in gesunden Raubmilben, die Bakterien enthielt.
(3) Eine wässrige Lösung von Ausscheidungen symptomatischer Raubmilben induzierte die Symptome des NR-Syndroms nicht, wenn ihr zuvor das Antibiotikum Tetracycline zugefügt worden war.

Diese Resultate beweisen, dass **eine oder mehrere Bakterienarten eine Rolle bei der Induktion des NR-Syndroms spielen**.

Der letzte Schritt dieses Projektes bestand dann auch darin, das unbekannte Bakterium zu isolieren und die Postulate von Koch zu erfüllen. Es wurden viele unterschiedliche Bakterienisolate aus gepaarten Raubmilbenweibchen der NR-Population und aus ihren Ausscheidungen erhalten. In **Kapitel 8** werden die Resultate einer Studie über die Effekte

eines dieser isolierten Bakterienarten auf gesunde gepaarte *P. persimilis W*eibchen präsentiert. **Das verwendete Isolat repräsentiert eine bis dahin unbekannte Bakterienart einer neuen Gattung, die inzwischen als *Acaricomes phytoseiuli* beschrieben wurde**. Raubmilben, die mit einer wässrigen Lösung dieser Bakterienart in Kontakt gebracht worden waren (behandelte Raubmilben), entwickelten deutlich das NR-Syndrom, während dies bei Raubmilben, die mit Wasser in Kontakt gebracht worden waren (unbehandelte Raubmilben) nicht der Fall war. Darüber hinaus konnte *A. phytoseiuli* aus unbehandelten Raubmilben niemals isoliert werden, während es von 60% der behandelten Raubmilben und aus Exkrementen von 41% der behandelten Raubmilben isoliert werden konnte. Licht- und elektronenmikroskopische Studien behandelter und unbehandelter Raubmilben ergaben die folgenden Resultate: Bei behandelten Raubmilben fanden sich auffällige Akkumulationen von Bakterien im Innern des Verdauungstraktes dessen Epithelium deutlich degeneriert war. Ferner wurden Bakterienherde auch im Fettkörper aufgefunden. All diese Phänomene wurden nicht in unbehandelten Raubmilben festgestellt. Diese Resultate beweisen deutlich, dass ***A. phytoseiulus* die Raubmilbe *P. persimilis* infiziert und in ihr das Auftreten des NR-Syndroms induziert.**

Die vorliegende Dissertation führte folglich zur Bestätigung der zu Anfang aufgestellten Arbeitshypothese: ***Die plötzliche und permanente Verhaltensänderung von gepaarten P. persimilis Raubmilbenweibchen der NR-Population ist das Symptom einer infektiösen Krankheit***. Dies ist die erste Beschreibung einer bakteriellen Krankheit in Raubmilben (Acari: Phytoseiidae). Außerdem gehört *A. phytoseiuli* einer neu beschriebenen Art und einer neu beschriebenen Gattung an. In **Kapitel 9** werden die Resultate dieser Dissertation im Zusammenhang mit der Milbenpathologie, der Verhaltensökologie und der Bakteriologie diskutiert.

Danksagung

Ich danke Regina G. Kleespies für ihre hilfreichen Anmerkungen zu der ersten Version dieser Zusammenfassung.

Ever tried. Ever failed. No matter.
Try again. Fail again. Fail better.
Samuel Beckett

This motto perfectly expresses what I repeatedly felt during the past decade. Finally, a seemingly never-ending chain of drawbacks, diseases and failures has come to its end. This was possible with the help of many people, whom I would like to thank, each in his/her own language:

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Conny
Wageningen, januari 2006

Curriculum vitae

Conny (Cornelia) Schütte werd op 31 augustus 1963 te Volmerdingsen (BRD) geboren. In 1983 behaalde zij het "Abitur" (=VWO-diploma) aan het Immanuel Kant Gymnasium te Bad Oeynhausen (BRD). Na een oriëntatiejaar, waarin zij stage liep op een regionaal bureau voor milieuhygiëne (Umweltschutzamt des Kreises Minden-Lübbecke) en twee scholen (Schule für Sprachbehinderte, Hille-Eickhorst; Bernart-Schule, Bad Oeynhausen), begon zij haar studie pedagogie (Duitse lerarenopleiding voor speciaal onderwijs, met onderwijsvak biologie) aan de Universiteit Hamburg (BRD). Haar passie voor de biologie werd echter zo groot dat zij in 1985 overstapte naar de studie Biologie eveneens aan de Universiteit Hamburg. In 1988 behaalde zij het "Vordiplom" (=kandidaats) en ontving in 1989 een studiebeurs van de "Deutsche Akademischer Austauschdienst", waardoor zij haar studie Biologie aan de Wageningen Universiteit kon voortzetten. Hier deed zij onderzoek bij het Laboratorium van Entomologie aan het foerageergedrag van een sluipwesp. Deze sluipwespsoort is een belangrijke natuurlijke vijand van een in bonenopslag schadelijke keversoort in Afrika. Haar stageonderzoek voerde zij uit aan het Département de Formation en Protection des Végétaux (DFPV) te Niamey (Niger). Hier onderzocht zij de populatiedynamica van dezelfde sluipwespsoort in natuurlijke opslagomstandigheden. In 1991 behaalde zij haar doctoraaldiploma, waarna ze werkzaam was als docente biologie aan het C.E.S. te Lagdo (Kameroen). In 1992 begon zij als assistente in opleiding aan een promotieonderzoek bij het Laboratorium van Entomologie van de Wageningen Universiteit. In 1998 kon zij haar onderzoek voortzetten in een postdoc-project gefinancierd door de Stichting Technische Wetenschappen (STW). Het onderzoek richtte zich op een onbekende bacteriële ziekte van een roofmijtensoort die commercieel geproduceerd wordt voor de biologische bestrijding van spintmijten. Zowel resultaten uit het AIO-project als ook resultaten van het post-doc project zijn onderdeel van dit proefschrift. Sinds 2004 is zij werkzaam als docente aan diverse middelbare scholen.

List of publications

Some chapters of this thesis are or will be published (in a slightly different form) as:

Chapter: Publication:

2 **Schütte C**, Dicke M. An overview of diseases of predatory mites (Acari, Phytoseiidae). To be submitted.

3 Dicke M, **Schütte C**, Dijkman H (2000) Change in behavioral response to herbivore-induced plant volatiles in a predatory mite population. Journal of Chemical Ecology, 26: 1497-1514.

4 **Schütte C**, Kleijn PW, Dicke, M (2006) A novel disease of the predatory mite *Phytoseiulus persimilis* (Acari, Phytoseiidae): symptoms in adult females. Experimental and Applied Acarology, in press.

5 **Schütte C**, van Baarlen P, Dijkman H, Dicke M. (1998) Change in foraging behaviour of the predatory mite *Phytoseiulus persimilis* after exposure to dead conspecifics and their products. Entomologia Experimentalis et Applicata, 88: 295-300.

6 **Schütte C**, Poitevin O, Negash T, Dicke M (2006) A novel disease of the predatory mite *Phytoseiulus persimilis* (Acari, Phytoseiidae): disease transmission by adult females. Experimental and Applied Acarology, in press.

7 **Schütte C**, Poitevin O, Dicke M. A novel disease of the predatory mite *Phytoseiulus persimilis* (Acari, Phytoseiidae): evidence for the involvement of bacteria. Submitted.

8 **Schütte C**, Gols, R, Kleespies RG, Poitevin O, Dicke M. Novel bacterial pathogen *Acaricomes phytoseiuli* isolated from *Phytoseiulus persimilis* (Acari, Phytoseiidae) causes severe disease symptoms. To be submitted.

Additional publications related to this thesis:

Schütte C, Hulshof J, Dijkman H, Dicke M (1995) Change in foraging behaviour of the predatory mite *Phytoseiulus persimilis*: some characteristics of a mite population that does not respond to herbivore-induced synomones. Proceedings of the section Experimental and Applied Entomology, N.E.V. Amsterdam, 6: 133-139.

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Bjørnson S, **Schütte C** (2003) Pathogens of mass-produced natural enemies and pollinators. In: Van Lenteren JC (ed), Quality Control and Production of Biological Control Agents: Theory and Testing Procedures. CABI Publishing, Wallingford, UK, pp. 133-165.

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Other publications:

Sagnia SB, **Schütte C** (1992) Le système de stockage du Niébé en milieu villageois dans l'état de Kano, Nigéria. Sahel PV Info, 46: 6-15.

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