Natural enemies of mass-reared predatory mites (family Phytoseiidae) used for biological pest control

Susan Bjørnson

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Abstract Predatory mites of the family Phytoseiidae are valued natural enemies that provide effective pest control in greenhouses and on agricultural crops. Mass-reared phytoseiids are occasionally associated with microorganisms and although their effects are not always apparent, some are pathogenic and reduce host fitness. Invertebrate pathogens are encountered more frequently in mass production systems than in nature because rearing environments often cause overcrowding and other stresses that favour pathogen transmission and increase an individual's susceptibility to disease. Although unidentified microorganisms have been reported in phytoseiids, bacteria and microsporidia have been detected with considerable frequency. The bacterium *Acaricomes phytoseiuli* is associated with an accumulation of birefringent crystals in the legs of *Phytoseiulus persimilis* and infection reduces the fitness of this spider mite predator. *Wolbachia*, detected in *Metaseiulus occidentalis* and other phytoseiids, may cause cytoplasmic incompatibilities that affect fecundity. However, the effects of *Rickettsiella phytoseiuli* on *P. persimilis* are unknown. Microsporidia are spore-forming pathogens that infect *Neoseiulus cucumeris*, *N. barkeri*, *M. occidentalis* and *P. persimilis*. Microsporidia cause chronic, debilitating disease and these pathogens often remain undetected in mass-rearings until a decrease in productivity is noticed. Routine screening of individuals is important to prevent diseased mites from being introduced into existing mass-rearings and to ensure that mite populations remain free from pathogens. The means by which bacteria and microsporidia are detected and strategies for their management in phytoseiid mass-rearings are discussed.

Keywords *Amblyseius* · *Metaseiulus* · *Neoseiulus* · *Phytoseiulus* · Phytoseiids · Microorganisms · Bacteria · Microsporidia · Disease

S. Bjørnson (\boxtimes)

Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, NS, Canada B3H 3C3 e-mail: susan.bjornson@smu.ca

Introduction

The spider mite predator, *Phytoseiulus persimilis* Athias-Henriot, was the first mass-produced natural enemy to be made commercially available for biological pest control in Europe (van Lenteren et al. [1997\)](#page-7-0). Since its introduction almost 40 years ago, phytoseiids have gained recognition for their importance as natural enemies of thrips, white flies and spider mites. Several phytoseiids are now available for pest control.

Phytoseiids, like other mass-produced and field-collected arthropods, are occasionally associated with microorganisms. Although some microorganisms are known to affect host fitness, the role of others has yet to be determined. Diseases, and the microorganisms that cause them, are encountered more frequently in mass production systems than in nature because rearing environments often cause overcrowding and this favours pathogen transmission (Goodwin [1984](#page-6-0)). Overcrowding may also lead to temporary starvation or other stresses, which are thought to increase disease susceptibility (Goodwin [1984](#page-6-0); Kluge and Caldwell [1992](#page-7-1)).

Once detected, the identification of a particular microorganism is essential if one is to determine its significance. Not all microorganisms are capable of causing disease; therefore, the mere presence of a particular microbe is often insufficient for determining a cause and effect relationship. Depending on the microorganism that is detected, a conclusive diagnosis may involve simple or complex laboratory procedures and, in many cases, the satisfaction of Koch's Postulates.

This summary will focus on the types of natural enemies associated with phytoseiids, their effects on host fitness and efficacy, the means by which disease-causing microbes are detected, and strategies for their management in mass production systems. Further information may be found in comprehensive reviews regarding the parasites, pathogens and diseases of mites (Poinar and Poinar [1998](#page-7-2); van der Geest et al. [2000\)](#page-7-3) and pathogens of mass-produced natural enemies and pollinators (Bjørnson and Schütte [2003](#page-6-1)).

Unidentified microorganisms

Hess and Hoy [\(1982\)](#page-6-2) reported two unidentified microorganisms in *Metaseiulus occidentalis* (Nesbitt) that are associated with two, distinct pathologies. Some adult females have extruding rectal plugs that often stick to the substrate and prevent the affected mites from moving. Other mites become thin and translucent and high mortality is observed among immature mites. In both cases, affected females fail to oviposit or produce few eggs. One type of microorganism was found in all mites examined but it is not considered to be detrimental to *M. occidentalis*. However, a second, rickettsia-like microorganism found in the ovaries of some females is associated with rectal plug formation. Both pathologies are observed when mites are reared under crowded conditions.

Viruses

Sutáková and Rüttgen ([1978](#page-7-4)) observed virus-like particles in *P. persimilis* but these are present only in the cytoplasm of cells infected with *Rickettsiella phytoseiuli*. The authors conclude that the virus inadvertently infects *P. persimilis* when virus-contaminated food is ingested. Mites infected with *R. phytoseiuli* and the virus show no external signs associated with infection and host mortality is not affected (Sutáková and Rüttgen [1978;](#page-7-4) Sutáková

[1988\)](#page-7-5). In other studies, non-occluded viruses were observed in *Neoseiulus* (formerly *Amblyseius*) *cucumeris* (Oudemans) and *P. persimilis* (Steiner [1993](#page-7-6); Bjørnson et al. [1997](#page-6-3)) but their effects are unknown.

Bacteria

Hoy and Jeyaprakash ([2005\)](#page-6-4) sequenced four bacterial genomes from *M. occidentalis* and three additional genomes from the spider mite *Tetranychus urticae* Koch, which is often prey for *M. occidentalis* and other phytoseiids. Although *Wolbachia* are the only microorganisms detected in both *M. occidentalis* and *T. urticae*, there are no genetic differences in the rRNA sequences of these microorganisms. *Wolbachia* are thought to cause cytoplasmic incompatibility in *M. occidentalis* (Johanowicz and Hoy [1996\)](#page-6-5) and although other microorganisms have been detected in this predator (Hess and Hoy [1982](#page-6-2); Jeyaprakash and Hoy [2004;](#page-6-6) Hoy and Jeyaprakash [2005\)](#page-6-4), they may or may not affect host fitness. *Wolbachia* are also detected in other phytoseiids (Steiner [1993](#page-7-6)) but their effects on host fitness have not been established.

Rickettsiella phytoseiuli was detected in *P. persimilis* from a laboratory mass-rearing in the USSR (Sutáková and Rüttgen [1978\)](#page-7-4) but not in predators that were examined from other sources (Sutáková and Arutunyan [1990\)](#page-7-7). *R. phytoseiuli* is a pleomorphic (able to assume different forms) bacterium that was detected in all mites examined. It is present in most tissues of adult mites and is particularly abundant in the dorsal body region. However, this bacterium does not infect immature *P. persimilis*.

The bacterium *Acaricomes phytoseiuli* causes specific disease symptoms in adult female *P. persimilis* known as 'non-responding syndrome' (Pukall et al. [2006](#page-7-8); Schütte et al. [2006a\)](#page-7-9). Although molecular screening revealed that *A. phytoseiuli* is a rather common pathogen in European populations of *P. persimilis*, the bacterium has not been detected in mites from other continents (Gols et al. [2007](#page-6-7)). Mites with non-responding syndrome do not react as strongly to herbivore-induced plant volatiles as do uninfected mites (Schütte et al. [2006a\)](#page-7-9). This aberrant behaviour may develop in unaffected mites when they are exposed to live, non-responsive females or their faeces (Schütte et al. [2006b\)](#page-7-10).

Although normal in size after mating, the majority of female predators (76%) from the non-responding population becomes dorso-ventrally flattened, has reduced oviposition rates and dies prematurely. Affected mites consume few prey and tend to disperse from areas where prey is located. Mortality is higher for non-responsive mites than for *P. persimilis* that respond to herbivore-induced plant volatiles and some affected females have an accumulation of birefringent, dumbbell-shaped crystals in their legs. Unaffected (responsive) females may accumulate similar crystals in their bodies but these are restricted to the Malpighian tubules and rectum.

Arutunjan ([1985\)](#page-6-8) described dumbbell-shaped entities in the gut of *P. persimilis* as bacteria. These entities are similar in morphology to dumbbell-shaped crystals that were reported by Bjørnson et al. ([1997\)](#page-6-3) and Schütte et al. ([2006a\)](#page-7-9). The accumulation of crystals in the digestive tract is associated with white coloration of the opisthosoma and is observed when live mites are examined by stereomicroscopy. Mites may have a white dot in the distal opisthosoma (when crystals accumulate in the rectum) or white stripes along the sides of the body in the region of the Malpighian tubules. Occasionally predators with both symptoms are observed (Bjørnson and Raworth [2003\)](#page-6-9) and in some cases, crystals accumulate in the legs (Schütte et al. [2006a\)](#page-7-9). The proportion of white opisthosomal coloration in *P. persimilis* increases when predators are fed prey mites (*T. urticae*) that fed on plants treated with high concentrations of 20–20–20 (N–P–K) fertilizer. Furthermore, some symptomatic mites become asymptomatic after wastes are egested from the anus (Bjørnson and Raworth [2003](#page-6-9)). Birefringent crystals are thought to be excreted under normal circumstances but in some cases, crystal accumulation is linked to reduced fecundity and poor performance (Bjørnson et al. [2000;](#page-6-10) Schütte et al. [2006a\)](#page-7-9), particularly when crystals accumulate in the legs (Schütte et al. [2006a\)](#page-7-9).

Discoloration of the distal opisthosoma has been observed in *Euseius hibisci* (Chant) when fed a diet consisting only of citrus red mites, *Panonychus citri* (McGregor) (see Tanagoshi et al. [1981](#page-7-11)). The guts of affected female mites are dark red and this discoloration is attributed to incomplete digestion of prey. Predators become less robust at successive moults and adult females are dorso-ventrally flattened and produce few or no eggs. The authors conclude that *E. hibisci* is not an obligate predator of *P. citri* and requires pollen as well as prey mites for food.

In many cases, bacteria are readily detected by light microscopy. Simple and differential staining provides some information regarding bacterial shape, size and morphology but fur-ther tests are required for taxonomic identification (see Pukall et al. [2006\)](#page-7-8). Transmission electron microscopy and molecular techniques can be used to detect and identify bacteria (*Wolbachia* and *Rickettsia*) that are too small to be observed by light microscopy (see Jeyaprakash and Hoy [2004](#page-6-6); Hoy and Jeyaprakash [2005](#page-6-4)). Antibiotics are used to eliminate *Wolbachia* from insect parasitoids (Dedeine et al. [2001](#page-6-11); Lundgren and Heimpel [2003\)](#page-7-12) but the efficacy of such remedies in mass-reared phytoseiids has not been investigated.

Microsporidia

Microsporidia are spore-forming, intracellular pathogens that cause sub-lethal and debilitating disease. Microsporidian spores may be transmitted both horizontally (from one individual to another) or vertically (from parent to offspring) and are somewhat resistant to harsh environmental conditions (Maddox [1973\)](#page-7-13). Mass-reared arthropods are often confined to small areas and high host population densities favour pathogen transmission. Microsporidia may remain undetected in mite colonies because symptoms are not usually associated with infection. These pathogens may be detected once predatory mites fail to thrive and a decrease in their productivity is noticed.

Microsporidia reduce the productivity of mass-reared *N. cucumeris* and *N. barkeri* (Hughes) (Beerling and van der Geest [1991\)](#page-6-12). These predators are commercially available for controlling western flower and onion thrips [*Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman], respectively. Symptoms of infection (sluggishness, swollen and whitish bodies) are observed only in heavily infected individuals when spores are abundant. The organs of these individuals are occluded when whole mounts of mites are examined by light microscopy. Prey mites (*Acarus siro* L. and *Tyrophagus putrescentiae* Schrank) are also infected. Symptoms are similar to those observed for *N. cucumeris* and *N. barkeri* but less pronounced. Based on spore dimensions and the hosts infected, three microsporidia are thought to infect both predatory and prey mites of this production system (Beerling and van der Geest [1991;](#page-6-12) Beerling et al. [1993](#page-6-13)).

Beerling and van der Geest ([1991\)](#page-6-12) conclude that vertical transmission plays an important role in pathogen transmission in mass-rearings of *N. cucumeris* and *N. barkeri* and suggest several possible means for successful horizontal transmission. These include: direct contact with spores that are liberated into the environment, direct contact between healthy and diseased individuals, and transmission through cannibalism or grooming.

Host fitness of the spider mite predator, *M. occidentalis*, is reduced when laboratory colonies are infected with the microsporidium, *Oligosporidium occidentalis*. Microsporidiainfected mites do not live as long or produce as many eggs as uninfected mites and microsporidiosis results in male-biased sex ratios (Olsen and Hoy [2002](#page-7-14)). *O. occidentalis* produces two types of spores: one is common in immature and young adult mites whereas the other is found in eggs and in older adults. The first spore type is slightly smaller than the latter and is thought to be important for autoinfection (re-infection of the same host) and transovarial (vertical) transmission of the pathogen. The second spore type is thought to be transmitted horizontally (Becnel et al. [2002\)](#page-6-14). Cannibalism of eggs and immatures by *M. occidentalis* adult females provides a route for pathogen transmission (Olsen and Hoy [2002\)](#page-7-14). Microsporidian spores and other developmental stages infect several host tissues but there are no external signs associated with infection (Becnel et al. [2002\)](#page-6-14). Spider mites (*T. urticae*) are not infected with the pathogen.

Three microsporidia were found in the spider mite predator *P. persimilis* from three commercial sources (Bjørnson and Keddie [2000\)](#page-6-15). Microsporidia reduce the fecundity, longevity and prey consumption of infected *P. persimilis* females (Bjørnson and Keddie [1999\)](#page-6-16). As is the case for *M. occidentalis*, sex ratios of microsporidia-infected *P. persimilis* are male-biased and although several host tissues are infected, there are no overt external signs or symptoms associated with infection (Bjørnson and Keddie [2001\)](#page-6-17). In some cases, microsporidia may reduce the performance of predators (Bjørnson and Keddie [1999](#page-6-16); Olsen and Hoy [2002](#page-7-14)) and may ultimately prevent predator populations from becoming established in new environments.

One of the microsporidian pathogens in *P. persimilis* is transmitted vertically and may become prevalent within mass-rearings over a short time (Bjørnson and Keddie [2001](#page-6-17)). Horizontal transmission occurs through direct contact but this is not observed frequently under laboratory conditions. Prey mites (*T. urticae*) are not infected with microsporidia and are unlikely to play a role in pathogen transmission (Bjørnson and Keddie [2001\)](#page-6-17).

Microsporidia are also known to infect *A. siro* and *T. putrescentiae* in phytoseiid massrearings (Beerling et al. [1993;](#page-6-13) Larsson et al. [1997\)](#page-7-15) but horizontal transmission of microsporidia from prey to predatory mites has not been demonstrated. Even if microsporidia from infected prey mites prove to be host specific and are not transmitted to predators, it is important to ensure that prey mite colonies remain free from these pathogens. Microsporidiosis may affect the vigour of prey mites and the sustainability of phytoseiid colonies that depend on prey mites for food.

Routine examination of infected phytoseiids by light microscopy can be labour-intensive and require some expertise but it is a reliable and relatively inexpensive means of detecting microsporidian spores. Microsporidia may be present in only a few individuals when pathogen prevalence is low; therefore, the examination of many individuals from a particular colony may be necessary to detect the pathogen. Smear preparations are typically made from whole mites that are air-dried, fixed in methanol and stained in buffered Giemsa prior to their examination by light microscopy.

Screening may be used as a means to isolate healthy individuals and establish microsporidia-free colonies. First, parent females are isolated and allowed to produce eggs and progeny, which are also isolated. When vertical transmission of a microsporidium is high, as may be the case in *M. occidentalis* and *P. persimilis* mass-rearings (Bjørnson and Keddie [2001;](#page-6-17) Olsen and Hoy [2002](#page-7-14)), random examination of some of these progeny over a prolonged period will help determine if the parent female is infected. As a final measure, each parent female is examined for microsporidian spores to verify that her remaining progeny are pathogen-free. In this way, uninfected mites may be isolated from infected ones and microsporidia-free colonies may be established from the uninfected progeny. This technique of separating uninfected individuals from infected ones is referred to as Pasteur's method (Tanada and Kaya [1993](#page-7-16)). Although other methods for removing microsporidia from arthropods have proven successful (Olsen and Hoy [2002](#page-7-14)), the methodology introduced by Pasteur remains the only means for definitively removing microsporidia from arthropod colonies with low levels of infection.

Another means of detecting microsporidia in phytoseiids involves the use of monoclonal antibodies for ELISA testing (Beerling et al. [1993\)](#page-6-13). Although immunoassays may be efficient for detecting pathogens in mass-reared arthropods, the use of ELISA testing requires specialized equipment and may be costly to develop.

In some cases, microsporidia may be reduced or eliminated by treating infected arthropods with chemicals or heat treatments (Hsiao and Hsiao [1973;](#page-6-18) Geden et al. [1995;](#page-6-19) Olsen and Hoy [2002\)](#page-7-14). Although antimicrosporidial agents (benzimidazole) have been used for controlling microsporidia in insects with variable success, chemical compounds do not provide effective control of microsporidia in *P. persimilis* (Bjørnson [1998](#page-6-20)). Further studies may prove fruitful; however, chemical compounds may not be well suited for controlling microsporidia in phytoseiids. Chemicals are usually added to artificial diets or sugar solutions but some arthropods (particularly phytoseiids) cannot be reared successfully on artificial diets. Furthermore, it is difficult to determine how much of the chemical agent is consumed when chemicals are added to food that is eaten.

Heat treatments are successful for reducing infection in *M. occidentalis* (Olsen and Hoy [2002\)](#page-7-14). The number of viable microsporidian spores is reduced when microsporidia-infected mites are reared at high temperatures $(32-35^{\circ}C)$ for several days. Under these conditions, spores that remain in the host tissues are thought to become non-viable because all subsequent eggs deposited by heat-treated females are microsporidia-free. Although heat treatments are successful for eliminating microsporidia from *M. occidentalis*, this technique may be of limited value when used to control microsporidia in other phytoseiids. The mortality of *M. occidentalis* was low $(\sim 20\%)$; Olsen and Hoy [2002](#page-7-14)) when individuals were reared at high temperatures; however, *P. persimilis* does not survive well at high temperatures and heat treatments are not effective for controlling microsporidia in this predator (Bjørnson [1998\)](#page-6-20). Spore viability is dependent on environmental factors, including temperature, humidity, and exposure to ultraviolet light (Maddox [1973\)](#page-7-13). The use of heat treatments as a means to reduce microsporidiosis in *M. occidentalis* (Olsen and Hoy [2002\)](#page-7-14) provides evidence that pathogen development and spore viability may be reduced when rearing conditions are altered. Sanitation of rearing facilities and equipment also helps reduce pathogen transmission.

Conclusion

Although many factors influence the outcome of a particular biological control program, the use of pathogen and parasitoid-free natural enemies is the foundation for success. Invertebrate pathogens are often overlooked in scientific studies and in mass-production systems when things go awry. It is essential to use pathogen-free beneficial arthropods in scientific studies if quality control testing is to have meaning and to avoid the misinterpretation of data (Goodwin [1984\)](#page-6-0).

Not all microorganisms are pathogenic; therefore, it is important to correctly identify all microorganisms and determine their impact on host fitness. Both bacteria and microsporidia have been reported from mass-reared phytoseiids and some of these cause subtle symptoms that may be overlooked. Quarantine of introduced or newly-acquired arthropods, in combination with routine microscopic examination of field-collected specimens (or specimens otherwise introduced into a mass rearing), is recommended so that invertebrate pathogens are not inadvertently introduced into existing arthropod colonies (Goodwin [1984;](#page-6-0) Bjørnson and Keddie [1999\)](#page-6-16).

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