

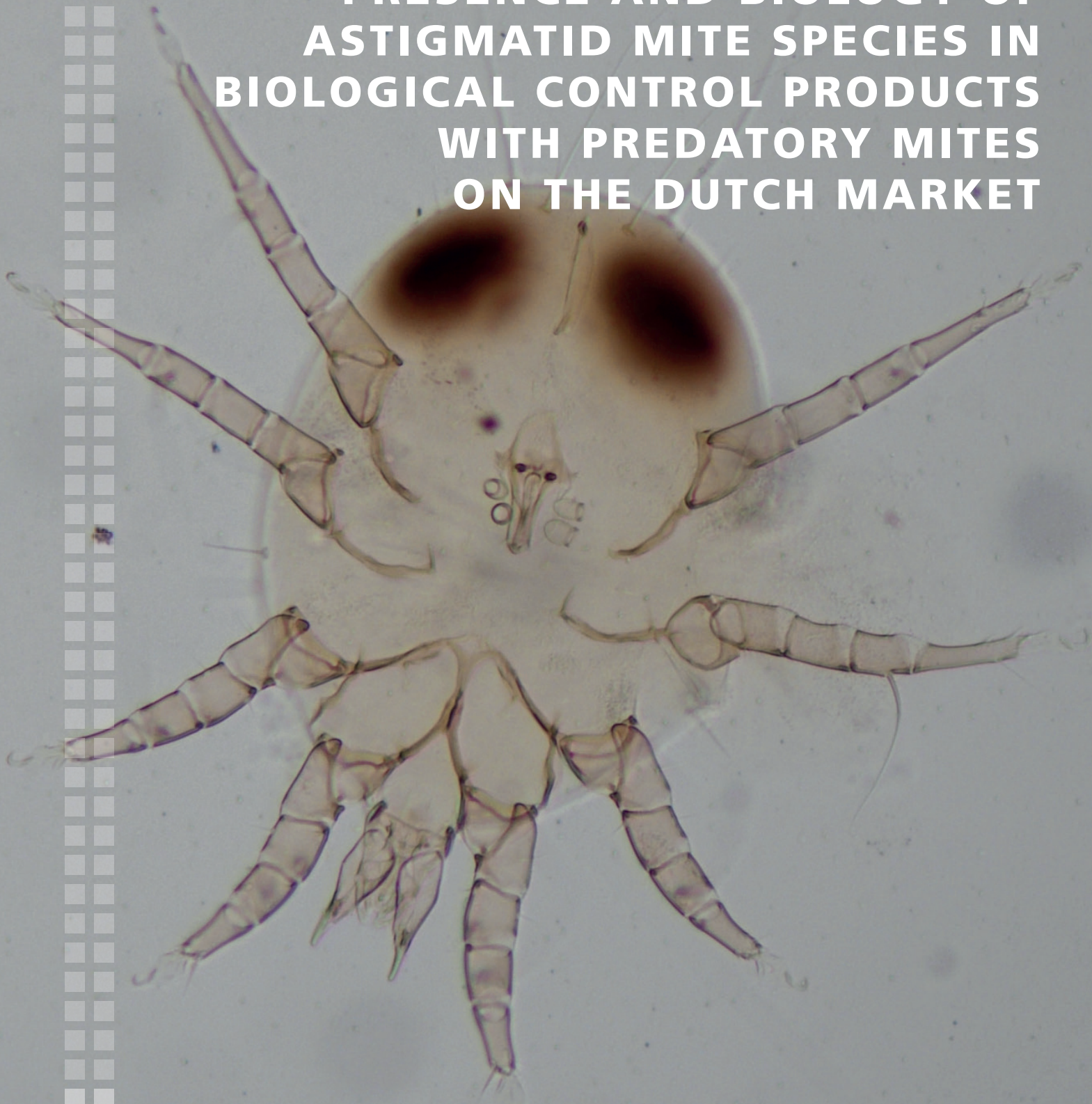


COMMISSIE  
**COGEM**  
GENETISCHE  
MODIFICATIE

# ONDERZOEKSRAPPORT

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## PRESENCE AND BIOLOGY OF ASTIGMATID MITE SPECIES IN BIOLOGICAL CONTROL PRODUCTS WITH PREDATORY MITES ON THE DUTCH MARKET



# Presence and biology of astigmatid mite species in biological control products with predatory mites on the Dutch market.

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## Voorwoord

Biologische bestrijding, met b.v. sluipwespen of roofmijten, is steeds meer de gangbare methode in de kasteelt om plagen terug te dringen, ook in kassen met genetische gemodificeerde (gg-) gewassen. De inzet van insecten als natuurlijke vijand van plaagorganismen kan echter gevolgen hebben voor de inperking van gg-planten. Pollen kunnen worden meegenomen door ontsnappende insecten en daarmee is verspreiding van de gg-plant of transgenen in het milieu mogelijk. De introductie van biologische bestrijders in ggo-kassen valt dan ook onder de ggo-vergunningplicht.

De COGEM heeft de noodzakelijke inperkingsmaatregelen voor biologische bestrijders, waaronder een aantal roofmijten op een rij gezet. In deze adviezen is de aanwezigheid van zogenaamde voermijten in de producten met roofmijten onderbelicht gebleven. Als de gebruikte voermijten door b.v. hun gedrag of voeding met pollen in aanraking komen en daarna buiten de kas terecht komen, dan zouden zij een extra risico voor het in het milieu terecht komen van gg-transgenen kunnen vormen. De introductie van voermijten in ggo-kassen is alleen toegestaan als dit vergund is, maar vaak is voor gebruikers onduidelijk of en welke voermijten aanwezig bij de roofmijten. Niet altijd staat op het etiket vermeld dat er voermijten aanwezig zijn, of welke soort het betreft. De gebruikte soorten variëren ook per leverancier of type roofmijt. De COGEM heeft alleen voor de voermijt *Carpoglyphus lactis* al eerder een advies afgegeven.

Om te komen tot een verdere verbetering en aanscherping van de risicoanalyse wilde de COGEM allereerst een goed overzicht krijgen van de algemeen gebruikte voermijtsoorten en eventuele vervuilingen (onbedoelde aanwezigheid van andere voermijten). Om dat doel te bereiken is geïnventariseerd welke voermijtsoorten worden meegeleverd bij roofmijten. De belangrijkste vragen die ten grondslag liggen aan dit onderzoek waren daarom: "Welke voermijtsoorten zijn aanwezig in zakjes met roofmijten en kunnen deze voermijten pollen uit de kas verspreiden?" en "Is op basis van het gedrag van de gebruikte/aangetroffen voermijten, b.v. het al dan niet foerageren op pollen, per voermijtsoort een inschatting te maken van het risico op doorbreking van de inperking in ggo-kassen?"

Zo mogelijk zouden de uitkomsten van deze studie kunnen worden gebruikt om per voermijtsoort de vergunningverlening te versnellen met generieke advisering over de eventuele risico's.

Voor u ligt nu het eindrapport van de uitvoerders van dit onderzoek. De begeleidingscommissie stelt vast dat het eindrapport aan de oorspronkelijke doelstellingen beantwoordt. Het rapport levert precies de informatie die nog ontbrak over een nog onderbelicht aspect van de risicobeoordeling voor toepassing van biologische bestrijding in kassen met gg-planten.

De begeleidingscommissie is ervan overtuigd dat dit rapport direct kan worden toegepast bij het formuleren van generieke adviezen in vergunningverlening voor de toepassing van roofmijt/voedermijt combinaties.

Ten slotte wil ik de leden van de begeleidingscommissie bedanken die met grote deskundigheid de plannen, tussentijdse resultaten, concepten en rapportages van de uitvoerders hebben besproken, becommentarieerd en tussentijds hebben bijgesteld.

Daarnaast wil ik de auteurs bedanken voor hun inzet, grote deskundigheid en getoonde flexibiliteit gedurende de onderzoeksfase. Alle overleggen waren open, bijzonder leerzaam en interessant, en hebben mede bijgedragen aan het bijzonder nuttige eindresultaat.

Prof. dr. Theo Elzenga  
Voorzitter van de Begeleidingscommissie

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## Summary

This study aimed to identify astigmatid mite species present in commercial biological control products containing predatory mites in the Netherlands and to evaluate their biological characteristics relevant to the potential spread of genetically modified (GM) plant material. Astigmatid mites are often used as factitious prey in augmentative biological control and may come into contact with pollen, creating a theoretical risk of dispersing GM material outside greenhouses. Seven astigmatid mite species; *Tyrophagus putrescentiae*, *Thyreophagus entomophagus*, *Carpoglyphus lactis*, *Acarus siro*, *Lepidoglyphus destructor*, *Suidasia pontifica*, and *Tyrolichus casei* are exempt from environmental regulation, but their presence in products is not always indicated on packaging.

A total of 15 biological control products from main Dutch suppliers (Biobest, Koppert, AgroBio, Bioline) were sampled in early spring and mid-summer. Mites were identified using a dual approach: morphological identification, examining the gnathosoma, idiosoma, dorsal setae, genital structures, and legs; and molecular identification, using species-specific SYBR Green qPCR primers based on COI and 28S sequences. Morphological identification revealed five of the seven exempted species, while *Tyrolichus casei* was detected only by DNA analysis and *Suidasia pontifica* was not detected. Variation in species detection between sampling periods and discrepancies between morphological and molecular methods were observed, highlighting the need for combined approaches for reliable identification.

A literature review provided insights into the biology, ecology, and dispersal potential of the seven species. All are cosmopolitan, generalist fungivores that exploit domestic, storage, and some non-domestic habitats. The developmental stages, including the phoretic hypopus in some species, can facilitate passive dispersal. Despite these traits, active dispersal is limited, and contact with pollen outside contained greenhouse environments is unlikely. Under standard confinement and hygiene measures, the risk of these mites dispersing GM pollen is minimal.

Overall, the study confirms that the seven exempted astigmatid mite species are commonly associated with predatory mite products and, when proper containment measures are followed, can be safely used in greenhouses with GM crops. The combined morphological, molecular, and ecological analysis provides a solid foundation for regulatory assessment and practical guidance on the safe application of astigmatid mites in GMO containment conditions.

## Samenvatting

Het doel van deze studie was het identificeren van astigmatide mijtensorten in commerciële biologische bestrijdingsproducten met roofmijten in Nederland en het beoordelen van hun biologische eigenschappen in relatie tot het risico van verspreiding van genetisch gemodificeerd (GM) plantmateriaal. Astigmatide mijten worden vaak gebruikt als factitieve prooimijten in augmentatieve biologische bestrijding en kunnen in contact komen met stuifmeel, wat een theoretisch risico op verspreiding van GM-materiaal buiten kassen met zich meebrengt. Zeven astigmatide mijtensorten; *Tyrophagus putrescentiae*, *Thyreophagus entomophagus*, *Carpoglyphus lactis*, *Acarus siro*, *Lepidoglyphus destructor*, *Suidasia pontifica* en *Tyrolichus casei* zijn vrijgesteld van milieuregulering, maar hun aanwezigheid wordt niet altijd vermeld op de verpakking.

In totaal werden 15 biologische bestrijdingsproducten van de grootste Nederlandse leveranciers (Biobest, Koppert, AgroBio, Bioline) bemonsterd in het vroege voorjaar en de late zomer. Mijten werden geïdentificeerd met een dubbele aanpak: morfologische identificatie, waarbij de gnathosoma, idiosoma, dorsale haren, geslachtsstructuren en poten werden onderzocht; en moleculaire identificatie, met soortspecifieke SYBR Green qPCR-primers gebaseerd op COI- en 28S-sequenties. De morfologische identificatie toonde vijf van de zeven vrijgestelde soorten aan, terwijl *Tyrolichus casei* alleen via DNA-analyse werd gedetecteerd en *Suidasia pontifica* niet werd aangetroffen. Seizoensvariatie in de detectie van soorten en discrepanties tussen morfologische en moleculaire methoden benadrukken het belang van een gecombineerde aanpak voor betrouwbare identificatie.

Een literatuuronderzoek gaf inzicht in de biologie, ecologie en dispersiepotentieel van de zeven soorten. Alle soorten zijn kosmopolitische, generalistische fungivoren die zowel binnen- als buitendomeinen kunnen benutten. Ontwikkelingsstadia, waaronder het foretische hypopusstadium bij sommige soorten, kunnen passieve dispersie bevorderen. Ondanks deze eigenschappen is actieve verspreiding beperkt en is contact met stuifmeel buiten kassen onwaarschijnlijk. Onder standaardbeheersmaatregelen en hygiënemaatregelen is het risico dat deze mijten GM-stuifmeel verspreiden minimaal.

Samengevat bevestigt de studie dat zes van de zeven vrijgestelde astigmatide mijtensorten voorkomen in producten met roofmijten en, mits de juiste beheersmaatregelen worden gevolgd, veilig kunnen worden gebruikt in kassen met GM-planten. De gecombineerde morfologische, moleculaire en ecologische analyse biedt een solide basis voor zowel regulatoire beoordeling als praktische richtlijnen voor het veilig gebruik van prooimijten onder GMO-containmentomstandigheden.

## 1 Introduction

Working with genetically modified (GM) crops in greenhouse horticulture is strictly regulated. Diseases and pests are increasingly controlled by using biological control agents, because the use of chemical pesticides in greenhouses with GM crops is often not possible or undesirable. However, the use of biological control agents may affect the containment of GM crops. Genetically modified material, such as pollen, may spread outside the containment and into the environment carried by escaping insects and mites. For this reason, the introduction of biological control agents into greenhouses with GM crops falls under the GMO permit requirement.

The necessary containment measures for the astigmatid mites present in products containing predatory mites have not yet been addressed by COGEM. Since astigmatid mites may come into contact with pollen and can easily spread outside greenhouses via workers' clothing, the introduction of astigmatid mites into greenhouses with GM crops is only permitted if a license has been granted. At present, COGEM has issued advice only for the astigmatid mite *Carpoglyphus lactis*.

Astigmatid mites are selected for their ease of cultivation and high nutritional value for predatory mites, and they vary in taxonomic and ecological background. The species of astigmatid mite used differs per supplier or type of predatory mite and this information is not always shown on the products. Article 4.30 of the Dutch Environmental Act and Annex VIIb provides an exemption from the environmental permit requirement for the release of specifically listed non-genetically modified biological control agents and their prey species into the environment. Currently, seven species of astigmatid mites (belonging to four families) are exempt from the Environmental Act (Annex VIIb of Article 4.30 of the regulation). The seven species are *Tyrophagus putrescentiae*, *Thyreophagus entomophagus*, *Carpoglyphus lactis*, *Acarus siro*, *Lepidoglyphus destructor*, *Suidasia pontifica*, and *Tyrolichus casei*. However, this exemption does not apply when released or used in greenhouses with GM crops, as strict containment of GM material is legally required to prevent unintended environmental release. As stated above, the intended presence of astigmatid mites in products containing predatory mites is often unclear, as information is often lacking on the packaging. In addition, astigmatid mites may also occur as contaminants in products containing predatory mites.

COGEM wishes to obtain an overview of the astigmatid mite species supplied together with various predatory mite species. In addition, COGEM also wants insight into the behavior of these astigmatid mites, such as whether or not they make use of pollen, in order to assess, for each species, the risk of breaching containment in greenhouses of genetically modified plant material.

Research questions:

1. Which astigmatid mite species are present in (biological) control products containing predatory mites sold in the Netherlands?
2. Which aspects of the species' ecology and biology, staff handling practices, and application methods are relevant for the assessment of the potential risks of pollen spread?

Objectives:

- To determine the astigmatid mite species present in biological control products with predatory mites through morphological identification and DNA analysis.
- To provide an overview of biological characteristics of the species relevant to pollen dispersal.

## **2 Material and methods**

To identify the astigmatid mites present in biological control products with predatory mites sold in the Netherlands, a list was first compiled of all products, providing information on the producer or supplier, predatory mite species used, application, and packaging material (see Table 1). At that time (October 2023), Biobest, Koppert, AgroBio, and Bioline were the main producers and suppliers of biological control products with predatory mites in the Netherlands. Other suppliers also existed, but they resold products originating from the aforementioned producers. Based on this inventory, a selection of product was made including all predatory mite species and type of packaging material used. In total, 15 products were included in the study of which also products intended for supplementary feeding of predatory mites.

Identification of the astigmatid mites was done, using both morphological and molecular DNA identification. This dual approach was chosen in case unknown astigmatid mite species were encountered, or the astigmatid mite gene bank proved to be incomplete. Furthermore, the identification of the astigmatid mites was conducted twice per product (ordered beginning of spring and mid-summer), as the species of astigmatid mites used may sometimes vary over time.

### **2.1 Morphological identification**

Sampling for the morphological identification proceeded as follows: five samples were taken after thoroughly mixing each product. Then from each sample 20 adult mites were mounted in Marc André medium on a microscopic slide and placed on a hot plate at 40 °C for three days (Hughes, 1976). Sampling took place once at the beginning of spring and again in mid-summer. Afterwards, the astigmatid mites were identified using in-house morphological reference slides and established identification keys (Hughes, 1976; Krantz & Walter, 2009; Melnyk et al., 2010). In total, approximately 100 astigmatid mites were identified per product.

For the identification of the astigmatid mites, the main morphological characteristics of the gnathosoma, specifically the chelicerae, and of the idiosoma, such as the dorsal setae, genital structures, and legs, including diagnostic features of the tarsus were examined.

### **2.2 Molecular identification**

For molecular DNA identification, five samples containing 40 or more astigmatid mites each (a total of 200 mites) were pooled. This was done separately for each product. The molecular identification of the astigmatid mites was carried out by first isolating DNA from the samples and then amplifying a PCR product using generic COI primers, which was subsequently sequenced. The DNA sequences of the samples were then individually compared with sequences available in GenBank. These DNA sequences were analysed using the BLAST search function in GenBank. However, in all samples only DNA of the predatory mites was detected. This suggests that the generic COI primers were probably not sufficiently specific for the identification of the astigmatid mites. For this reason, a more species-specific DNA analysis was selected for the astigmatid mites. The species-specific DNA analysis was carried out by developing specific SYBR Green qPCR primers for several astigmatid mite species. Specific SYBR Green qPCR primers were designed based on COI and 28S sequences of astigmatid mite species available on Gen Bank (See Table 1 for the accession numbers of the sequences) using software Primer-BLAST from NCBI (a tool for finding specific primers). Only the assays which amplified the PCR products with a size between 100 bp and 300 bp were chosen for further qPCR analysis. The qPCR of the cDNA went according to the following protocol: 3 min 95°C; 39 cycles of 10 sec 95°C, 10 sec 60°C, 30 sec 72 °C; 60 cycles of 31 sec 65 °C, 5 sec 65 °C (+0.5 °C per cycle).

### **2.3 Literature review**

In addition, a literature study was conducted on the biology and ecology of the astigmatid mites, focused on potential risks of (active or passive) pollen dispersal and the potential sources of risk for working under GMO-containment conditions.

Table 1 Specific sequences for detection of astigmatid mite species

Assay	Detection of	Acc. nr.	Size (bp)	qPCR ampl. (bp)
1	<i>Acarus siro</i>	KY922485 (COI)	1238	106
2	<i>Thyreophagus entomophagus</i>	OR640974 (COI)	1554	111
3	<i>Tyrolichus casei</i>	LC687593 (28S)	306	101
4	<i>Tyrophagus putrescentiae</i>	MH262544 (COI)	1217	157
5	<i>Carpoglyphus lactis</i>	KY922482 (COI)	1238	278
6	<i>Lepidoglyphus destructor</i>	KX228068 (COI)	581	130
7	<i>Suidasia pontifica</i>	PP734338 (COI)	317	133

Note: Description of the columns from left to right; Assay indicates the assay ID; Detection of denotes the target species; Acc. nr. refers to the GenBank accession number of the COI reference sequence; Size (bp) indicates the length of the reference sequence, and qPCR ampl. (bp) the amplicon size.

Table 2 Overview of biological control products analysed

Supplier/Manufacturer	Products	Predatory mite species	Application	Packaging
Koppert	Limonica	<i>Amblydromalus limonicus</i>	Leaf	Bottle
Bioline	Andersoni	<i>Amblyseius andersoni</i>	Leaf	Sachets
Agrobio	SWIRScontrol Plus	<i>Amblyseius swirskii</i>	Leaf	Sachets
Biobest	Swirskii-Long-Life-System	<i>Amblyseius swirskii</i>	Leaf	Sachets
Koppert	Swirski-Mite LD	<i>Amblyseius swirskii</i>	Leaf	Sachets
Agrobio	ROBUScontrol	<i>Macrocheles robustulus</i>	Soil	Bucket
Koppert	Spical Ulti-Mite	<i>Neoseiulus californicus</i>	Leaf	Sachets
Biobest	ABS-System	<i>Neoseiulus cucumeris</i>	Leaf	Sachets
Koppert	Thripex-Plus	<i>Neoseiulus cucumeris</i>	Leaf	Sachets
Biobest	Hypoaspis-System	<i>Stratiolaelaps scimitus</i>	Soil	Bottle
Biobest	Montdorensis-Breeding-System	<i>Transeius montdorensis</i>	Leaf	Sachets
Bioline	Montyline	<i>Transeius montdorensis</i>	Leaf	Sachets
Agrobio	Power food 3.0	Unknown	Supplementary feed	Bag
Agrobio	Power food 3.0 Plus	Unknown	Supplementary feed	Bag
Agrobio	Power food 3.0 Sawdust	Unknown	Supplementary feed	Bag

### 3 Results

#### 3.1 Morphological and molecular identification

An overview of the morphological and molecular DNA identification results is presented in Table 2, showing products sampled in early spring (March–April 2025; round 1) and mid-summer (August–September 2025; round 2). Astigmatid mites were identified morphologically based on key features of the gnathosoma (especially the chelicerae) and the idiosoma, including dorsal setae, genital structures, and leg and tarsal characters. An extended overview of the morphological identification is presented in Appendix 1. Two species, *Tyrollichus casei* and *Suidasia pontifica*, were not identified morphologically in any of the samples. Products collected in mid-summer contained astigmatid mite species that differed from those found in early spring, and discrepancies were observed between morphological and molecular identifications, with some species detected by only one of the two methods. This emphasizes the importance of using both approaches to ensure species identification. The variation in species detected between early spring and mid-summer samples could be attributed to several factors. Differences in production batches, temporal changes in astigmatid mite populations during mass-rearing, or seasonal variations in environmental conditions such as temperature and humidity could influence species abundance.

Table 3 Complete overview of the astigmatic mite species found in predatory mite products through morphological and molecular DNA identification.

Product	Astigmata mite species					
	<i>Acarus siro</i>	<i>Thyreophagus entomophagus</i>	<i>Tyrollichus casei</i>	<i>Tyrophagus putrescentiae</i>	<i>Carpoglyphus lactis</i>	<i>Lepidoglyphus destructor</i>
Limonica					Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Andersoni	Morp. <sup>2</sup> +DNA <sup>2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>		DNA <sup>1:2</sup>	Morp. <sup>1:2</sup>	
SWIRScntrl Plus	DNA <sup>1</sup>	DNA <sup>1</sup>		DNA <sup>1</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Swirskii-Long-Life-System					Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Swirski-Mite LD			DNA <sup>1:2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
ROBUScntrl				Morp. <sup>1:2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Spical Ulti-Mite					Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	Morp. <sup>1:2</sup>
ABS-System				Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>		
Thripex-Plus	Morp. <sup>2</sup> +DNA <sup>2</sup>				Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Hypoaspis-System				Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>		
Montdorensis-Breeding-System		DNA <sup>2</sup>			Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Montyline	DNA <sup>2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>				
Power food 3.0	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>			Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Power food 3.0 Plus	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>			Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	
Power food 3.0 Sawdust	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>			Morp. <sup>1:2</sup> +DNA <sup>1:2</sup>	

Note: Morp. = Identified through morphological characteristics; DNA = Identified molecularly through DNA; The <sup>1</sup> or <sup>2</sup> or <sup>1:2</sup> indicate in which round the astigmatid mite species were found.

### 3.2 Literature review

According to the Environmental Act (Annex VIIIb, Article 4.30 Omgevingswet), seven species of astigmatid mites are exempted from registration and are free to be sold and released as prey mites for biological control agents in the Netherlands. These seven species are *Tyrophagus putrescentiae*, *Thyreophagus entomophagus*, *Carpolghyphus lactis*, *Acarus siro*, *Lepidoglyphus destructor*, *Suidasia pontifica* and *Tyrolichus casei*. All prey mite species that were encountered during sampling belong to these exempted species. *Suidasia pontifica* has not been encountered during sampling and another species *T. casei* was only identified based on DNA analysis and not in the morphological analysis. But since species that are exempted from regulation are allowed to be used commercially, we have included all seven exempted species in this literature study. While species may be safely used under regular growing practices, the possible risks of their use for the containment of genetically modified plants (GMO) material should be evaluated before they may be used in the biological control of arthropod pests in a GMO environment. Sources of risk to be considered under such conditions include the likelihood of a prey mite coming in contact with pollen of a GMO, disperse and transport GMO pollen outside the confinement and potentially bring it in contact with flowers of conspecifics in the wild or in another compartment. The biological characteristics that are relevant for the assessment by COGEM include the biology, resource use, ability to survive adverse conditions, the presence of developmental stages facilitating long-distance passive dispersal and their habitat use in domestic, storage and in outdoor environments. In addition, the way they are applied in augmentative biological control may differ. The purpose of this literature review was to give an overview of the biological characteristics and habitat use and elaborate on the likelihood of transport of GMO material by these species outside the confinement. This overview is based on the extended literature review presented in Appendix 2.

All seven prey mite species may occur in domestic environments and many of them are referred to colloquially as “dust mites” (Colloff, 2009). All seven species belong to the order Sarcoptiformes and the Astigmatina clade (formerly order Astigmata) and are widely distributed around the world and are considered as common pests in stored food. Although they share several biological traits and exploit habitats that overlap, there are also differences in their ecology (Colloff, 2009). The seven astigmatid prey mites share habitats and their biological characteristics make them easy to mass-produce on inexpensive substrates (bran, yeast, flour). This makes them suitable for use as supplemental prey to aid establishment of generalist predator populations in crops (Pirayeshfar, Safavi, Moayeri, & Messelink, 2020). Their use as factitious prey in the production of predatory mites is well studied (Pirayeshfar, Safavi, Moayeri, & Messelink, 2020) and the use of astigmatid mites in greenhouses, as a supplemental food source for predators, has also been explored in several studies (e.g. Hoogerbrugge et al., 2008; Messelink et al., 2014). However, there are some risks associated with using prey mites, which deserves attention in their application. Some astigmatid mites are sources of allergens that can persist and accumulate in the environment, and cause respiratory problems in workers (Pirayeshfar et al., 2020). Also, some of the astigmatid mites may, on occasion, feed on soft plant tissues resulting in damage.

Some of the species found, such as *A. siro*, *L. destructor* and *T. putrescentiae* are known to be facultative fungivores, which underscores that the interaction between mites and microfungi likely plays an important role in the occurrence of these mites in stored goods (Hubert et al., 2004). All of the seven species share the basic developmental stages: egg, larva, protonymph, deutonymph, tritonymph and adult. They share several ecological traits, exploiting domestic as well as non-domestic habitats that may overlap to different degrees (Colloff, 2009). However, regarding their developmental biology and ecology, there are several differences between the seven species. For example, depending on the species, the deutonymph is a developmental stage called a hypopus, which typically facilitates phoretic dispersal of mites by allowing them to adhere to hosts (e.g. insects, rodents, birds) and so access to distant new habitats (Reynolds, Reynolds, & Chapman, 2014; Corente & Knülle, 2003). The hypopal stage is a unique adaptation in astigmatid mites, but

it shows variation too, as in some astigmatids it may be sedentary and rather facilitate survival than phoresy, or may be absent in other species (Corente & Knülle, 2003; Mullen & OConnor, 2019). Species that are better adapted to storage conditions in human environments tend to tolerate suboptimal environmental conditions in their non-domestic habitats and these species tend to lack a phoretic hypopal stage (Colloff, 2009.). For instance *Tyrophagus putrescentiae* appears not to form hypopi (Champ, 1966.). While having a hypopus may facilitate dispersal, phoretic behaviour is not exclusive to this developmental stage and some mite species not having a hypopi can still be phoretic.

Other life-history traits, as well as the habitat use (both domestic and non-domestic) may vary between the seven species.

### **Considerations in the use of prey mites in biological control under confinement**

Important questions to be addressed are whether i) the biological characteristics of any of the species prey mites makes them unsuitable to use under restrictions in experiments with GMO plants, and ii) which confinement measures are necessary to eliminate potential risks of release to the environment of GMO material. In this context the specific risk relates to the probability of prey mites coming in contact with pollen of GMO plants and manage to escape confinement and transporting this genetic material to conspecific plants in the wild or in another compartment.

The following section provides an overview of the relevant mechanisms and considerations relevant to the use of astigmatid mite species in experiments involving GMO plants (Table 4). All seven dust mite species are cosmopolitan and share largely overlapping biological and ecological characteristics that render them suitable for use in biological control applications. Some species such as *T. putrescentiae* have broader habitat ranges, whereas others are more restricted to certain stored food and feed products. Despite the generalist nature of their habitat use, most of these mites are not obligately associated with living plant material. In the outdoor environment, all species (with the exception of *Suidasia pontifica* and *Tyrolichus casei*) have been recovered from plant (leaf) material or soil around plants. These cases do not pertain to feeding on plants, it may rather indicate feeding on fungi, or other organic material present on the plant. Although all the species are capable of developing on pollen and many of them occur in beehives, we have found no evidence that any of the astigmatid mites visits flowers in outdoor or indoor habitats. An exception may be made for *T. putrescentiae*, which under favorable conditions in the greenhouse may build up populations high enough to also feed on green plants plant parts and flower buds. However, for this to occur specific conditions apply, such as presence of soil or other organics material and very high levels of mites foraging for food, typically not relevant under general containment measures for experiments with GMOs. Moreover, even when high levels of prey mites are present in the compartment, these mites should come into contact with pollen, and be able to vector it. When living prey mites are spread indiscriminately on different plant parts (being dispensed with a carrier), the possibility of high prey mite abundance and direct contact with flowers cannot be excluded. Subsequently, if such mites escape confinements, they should be able to reach flowering plants of wild or cultivated relatives. Due to their small body size, all the mite species are seriously limited in their active dispersal. Given their limited capacity for dispersal, they are unlikely able to reach and come in contact with flowers by active movement. In this context, the possibility of phoretic vectoring and the passive dispersal with wind may be of relevance. Phoresy (either as hypopi or as an active feeding stage) and flying with wind currents make long-range dispersal of prey mites possible. If the phoretic host is a flower visitor, or the workers carrying mites come into contact with other flowering plants, then theoretically the escaped prey mite carrying a pollen may come in contact with a conspecific plant. However, when general measures recommended for PKb-I greenhouses and PC-I climate rooms are implemented, we estimate that the likelihood of this to happen is negligible. Provided that these measures are implemented, we think that the use of these prey mites in combination with GMO facilities entail minimal to negligible risk. Therefore, the general conditions spelled out earlier in the COGEM advise on *C. lactis* (CGM/220718-02) may be sufficient for all the seven species studied here.

Table 4 Relevant characteristics of astigmatid mite species in relation to potential pollen dispersal

<b>Astigmatid mite species</b>	<b>Relevant biological traits</b>	<b>Likelihood of contact with pollen</b>	<b>Dispersal pathways relevant for exposure</b>	<b>Overall assessment of pollen dispersal risk</b>
<i>Carpoglyphus lactis</i>	Non-plant-feeding; associated with stored products, fungi, and beehives; rapid reproduction	Limited to stored pollen; no evidence for active flower visitation	Limited active dispersal; passive dispersal via phoretic hypopi, personnel, or insects	Low; exposure mainly possible following deliberate application to flowers
<i>Tyrophagus putrescentiae</i>	Generalist feeder; associated with fungi and organic matter; occasionally present in greenhouses	Limited to stored pollen; no active visitation of flowers	Limited active dispersal; phoretic associations with bumblebees documented	Low to moderate; phoretic dispersal via pollinators may contribute to indirect exposure
<i>Acarus siro</i>	Stored-product mite; facultative fungivore; non-plant-feeding	Limited to stored pollen in beehives	Passive dispersal via hypopal stage on arthropods or personnel	Low; pollen exposure unlikely without indirect passive transport
<i>Lepidoglyphus destructor</i>	Fungivore; common in stored products and beehives; non-plant-feeding	Associated with stored pollen; no flower visitation	Passive dispersal via air currents and personnel; hypopus non-phoretic	Low; dispersal largely restricted to passive pathways
<i>Thyreophagus entomophagus</i>	Broad saprophagous/fungivorous diet; not plant-feeding; no confirmed hypopal stage	Occurs in beehives but no evidence of flower visitation	Limited active dispersal; passive spread via air or handling	Very low; no biological traits indicating effective pollen transfer
<i>Suidasia pontifica</i>	Stored-product mite; allergenic; non-plant-feeding	Limited to stored pollen; no flower visitation	Passive dispersal via air currents or personnel	Low; no evidence for active or targeted pollen dispersal
<i>Tyrolichus casei</i>	Associated with cheese, stored products, and beehives; non-plant-feeding	Occurs on stored pollen only	Passive dispersal via air currents, personnel, or phoresy of feeding stages	Low; pollen exposure limited to accidental or indirect routes

Across all the astigmatid mite species found in this study, active pollen dispersal via flower visitation is not expected. Potential pollen contact is limited to stored pollen (e.g. beehives) or direct application to flowers during biocontrol use. Passive dispersal (via phoresy, air currents, or personnel) is the main relevant pathway. The use of sachets instead of sprinkling, combined with avoidance of flower-visiting pollinators, effectively minimizes pollen dispersal risk.

## Concluding remarks

The present study provides a comprehensive overview of astigmatid mite species associated with commercial biological control products containing predatory mites in the Netherlands, integrating morphological and molecular identification with a detailed review of their biology and ecology. Morphological analyses confirmed the presence of five of the seven astigmatid species exempted from environmental regulation: *Tyrophagus putrescentiae*, *Thyreophagus entomophagus*, *Carpoglyphus lactis*, *Acarus siro*, and *Lepidoglyphus destructor*, while *Suidasia pontifica* was not detected, and *Tyrolichus casei* was identified only through molecular DNA analysis. Variation in species detection between sampling periods, along with discrepancies between morphological and molecular methods, underscores the value of using both approaches to achieve reliable species-level identification, particularly given the limitations of generic COI primers for astigmatid mites. The literature review indicates that these species are primarily generalist fungivores with limited active dispersal. Although some exhibit phoretic stages, the likelihood of these mites transporting genetically modified pollen outside containment is negligible under standard greenhouse practices. When recommended hygiene and confinement specific measures are implemented, the use of these prey mites in combination with GMO crops poses minimal risk.

## Recommended specific measures

In addition to general measures recommended for PKb-I greenhouses and PC-I climate rooms a number of specific measures may be considered when working with these prey mites and GMO's.

- We do not recommend to dispense (living) prey mites, or combination of predatory and prey mites by blowing or sprinkling them on the plants. While dust mites may occur on stored pollen in the wild (beehives), no evidence suggests that they will actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers, or if they find a phoretic host that visits flowers.

- To minimize the chance of phoresy, we recommend avoiding the use of flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts.

- We do recommend to use release sachets when introducing predatory mites in the crop. In this case prey mites are confined within a sachet together with a preferred food substrate and predatory mites. Prey mites will reproduce in these sachets for a limited time and so will the predatory mites until all the prey mites are consumed. Prey mites will not readily abandon the favorable microclimate and food in the sachet and their majority will remain in the sachet. Using sachets therefore under these conditions has benefit of *i)* avoiding prey mite population build-up in the compartment and *ii)* avoiding contact of prey mites with flowering plant parts.

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## **Glossary of Terms**

### **Anal opening**

Posterior body opening of the digestive tract.

### **Anal suckers**

Paired ventral structures near the anus, often dome-shaped.

### **Apodeme**

Internal cuticular ridge serving for muscle attachment.

### **Bifid claw**

Claw divided into two terminal points.

### **Bursa copulatrix**

Female reproductive structure receiving sperm.

### **Chaetotaxy**

Arrangement and pattern of setae on the body or appendages.

### **Chelicerae**

Paired mouthparts, often chelate or toothed.

### **Chelate**

Pincer-like, with fixed and movable digits.

### **Coxae**

Basal leg segments articulating with the body.

### **Epigynium**

Sclerotized plate covering the anterior part of the female genital opening.

### **Epimeral plates / Epimerites**

Ventral sclerotized structures associated with leg bases and apodemes.

### **Famulus**

Minute sensory seta on the tarsus, near solenidia.

### **Genital opening (g)**

External reproductive opening, usually between coxae III–IV.

### **Gnathosoma**

Anterior body region bearing mouthparts.

### **Grandjean's organ**

Cuticular sensory fold, often serrate or pectinate, near the supracoxal seta.

### **Genu (pl. genua)**

Leg segment between femur and tibia.

### **Humeral setae**

Setae located near the shoulder region of the idiosoma.

### **Hysterosoma**

Posterior region of the idiosoma.

### **Idiosoma**

Main body region excluding the gnathosoma.

**Lamellar setae (le)**

Propodosomal setae associated with lamellae or shields.

**Opisthosoma**

Posterior portion of the idiosoma; sometimes bearing lobes.

**Opisthosomal lobe**

Posterior body extension, often present in males.

**Pedipalp**

Paired appendage near the mouthparts, sensory or manipulative.

**Pectinate**

Bearing comb-like projections.

**Pretarsus**

Terminal leg structure supporting the claw.

**Propodosoma**

Anterior dorsal region of the idiosoma.

**Scapular setae (sc)**

Paired dorsal setae near the posterior margin of the propodosoma.

**Seta (pl. setae)**

Hair- or spine-like cuticular projection.

**Sclerite / sclerotized**

Hardened cuticular structure.

**Solenidion ( $\omega$ ,  $\sigma$ ,  $\varphi$ )**

Modified sensory seta on legs.

**Sternum**

Ventral sclerotized structure formed by fused apodemes.

**Subtarsal scale**

Cuticular scale at the base of the tarsus, often pectinate.

**Supracoxal seta**

Modified seta located above the coxa.

**Tarsus (pl. tarsi)**

Distal leg segment bearing pretarsus and terminal setae.

**Tectal setae**

Dorsal terminal setae of the tarsus.

**Transverse dorsal groove**

Cuticular groove separating propodosoma and hysterosoma.

**Vertical setae (vi, ve)**

Anterior dorsal setae on the propodosoma.

## Appendices

### Appendix 1

#### Morphological identification

This section provides an overview of the mite species identified in this study using the identification keys of Hughes (1976) and Vacante and Flechtmann (2024).

*Table 5 Abbreviations of idiosoma dorsal setae*

<i>ve</i>	External ventral
<i>vi</i>	Internal ventral
<i>sc e</i>	External scapular
<i>sc i</i>	Internal scapular
<i>he</i>	External humeral
<i>hi</i>	Internal humeral
<i>d1 to d4</i>	Dorsals 1 to 4
<i>la</i>	Anterior lateral
<i>lp</i>	Posterior lateral
<i>sa e</i>	External sacral
<i>sa i</i>	Internal sacral
<i>pa</i>	Post anal
<i>a</i>	Anal setae

#### *Acarus siro* Linnaeus (Acaridae)

##### **Idiosoma and general characters**

Pedipalps without a conspicuously flattened distal segment. Chelicerae chelate. Ventral surface of idiosoma without prominent chitinous rings. At least one pair of vertical setae present in both sexes. External vertical setae *ve* arise near the anterior angles of the dorsal propodosomal shield, at the same level as *vi*, or slightly posterior. A dorsal transverse groove divides the propodosoma from the hysterosoma. Dorsal seta *d2* not more than twice the length of *d1*. Hysterosomal setae *hi*, *la*, *lp*, and *d1* to *d4* are short. In particular, the length of dorsal setae *d2* or *d3* does not exceed the distance between its base and the base of the seta immediately posterior to it (figure 1) .

##### **Male and female dorsal chaetotaxy**

The arrangement of the dorsal setae of the idiosoma is the same in males and females, but the setae are usually more sparsely pectinate. Ventrally, five pairs of setae surround the anal opening. Of these, *a2* are twice, and *a3* almost four times, the lengths of *a1*, *a4*, and *as*. Setae *pa1* and *pa2* extend well beyond the posterior margin of the body. The genital opening lies between coxae III and IV (figure 3).

##### **Legs**

The first pair of legs is no wider than the remaining ones (figure 3). The chaetotaxy is the same as that of the male, except that on tarsus I, the spines *u* and *v* are distinct and much narrower than *s*; seta *s* on all legs is large and curves backward. On genu I, sigma is more than three times longer than sigma<sub>2</sub>. On tarsus I, omega<sub>1</sub> arises from the base of the segment. Claws of the female are not bifid. The claw is attached to the end of the tarsus by paired sclerites surrounded by a short, cushion-like pretarsus (figure 2) .

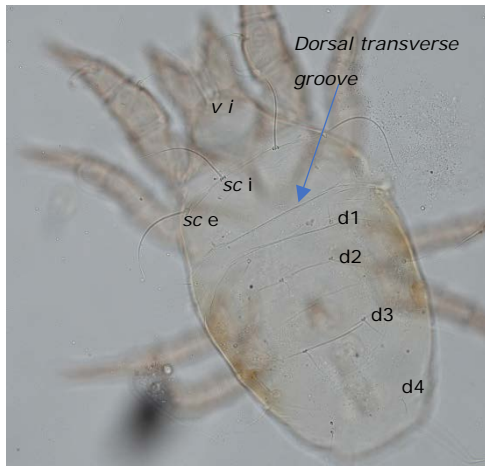


Figure 1 Dorsal side of *A. siro* ♀

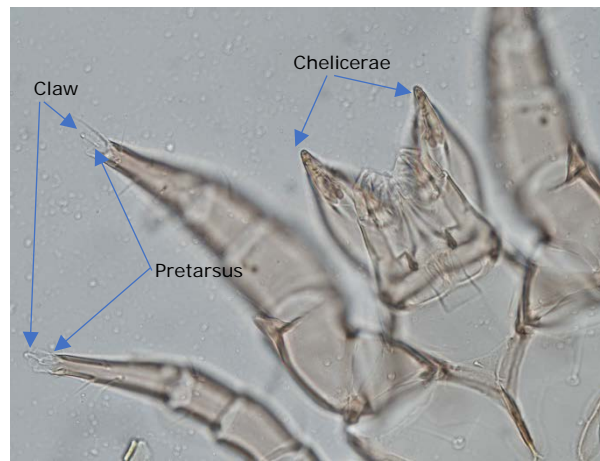


Figure 2 Gnathosoma and legs I and II ventral view of *A. siro* ♀



Figure 3 Ventral side of *A. siro* ♀

### *Tyrophagus putrescentiae* (Schrank, 1781) (Acaridae)

#### **Idiosoma and general characters**

Chelicerae and legs poorly tanned. Pedipalps without a conspicuously flattened distal segment. Ventral surface of the idiosoma without prominent chitinous rings. A dorsal transverse groove divides the propodosoma from the hysterosoma, with at least one pair of vertical setae present in both sexes (figure 4). The claw is attached to the end of the tarsus by paired sclerites surrounded by a short, cushion-like pretarsus. Female without bifid claws (figure 5).

#### **Propodosoma**

Internal setae *vi* reach beyond the tips of the chelicerae and, like all the other long setae, are sparsely pectinate. External vertical setae *ve* arise near the anterior angles of the dorsal propodosomal shield, slightly posterior to *vi*, and are longer than the length of the genu. Internal scapular setae *sc i* longer than external scapular setae *sc e*; both pairs longer than the propodosoma, *sc i* distinctly the longer. The anterior edge of apodemes I has an irregular outline (figure 4).

#### **Dorsal hysterosoma**

Setae *d1* and *la* short and almost equal in length (8–10% of the idiosoma). Seta *d2* always more than twice as long as *la* and 2–3.5 times the length of *d1*. Seta *d4* longer than *d1* and *la*. Seta *lp* very long, similar in length to *sa i*. Setae *hi* longer than *he* and projecting at right angles to the sides of the body. The remaining long setae together form a fan-shaped “train” (figure 4).

### Ventral surface

The genital (g) opening lies between coxae III and IV. The dome-shaped anal suckers extend slightly beyond the posterior extremity of the anus. Setae *pa1* are shorter and more slender than *pa2* and *pa3*, and arise from the end of the body (figure 6).

### Gnathosoma

Each chelicera is toothed and bears a spur-like outgrowth and a mandibular spine (figure 5).

### Legs

All legs terminate in a stalked claw and a well-developed pretarsus. The length of tarsus I does not exceed the combined length of the genu and tibia. On tarsus I,  $\omega_1$  is slightly expanded distally and arises close to the famulus; the origin of *aa* is distal to that of  $\omega_1$ . Solenidia  $\omega_3$  and *d* extend well beyond the end claw and are longer than *e*. On genu I,  $\sigma_1$  is only slightly longer than  $\sigma_2$  (figure 5).

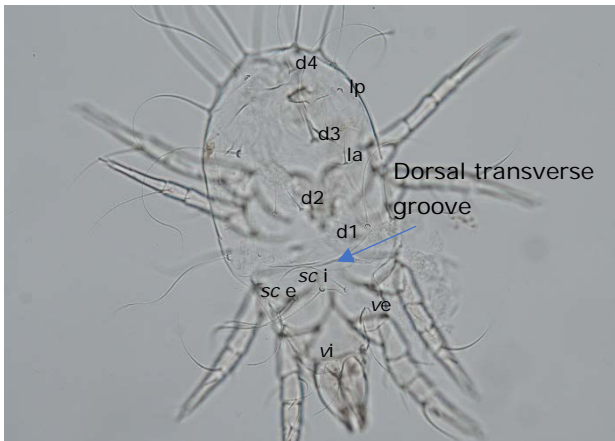


Figure 4 Dorsal view of *T. putrescentiae* ♀



Figure 5 Gnathosoma and legs I and II ventral view of *T. putrescentiae* ♀

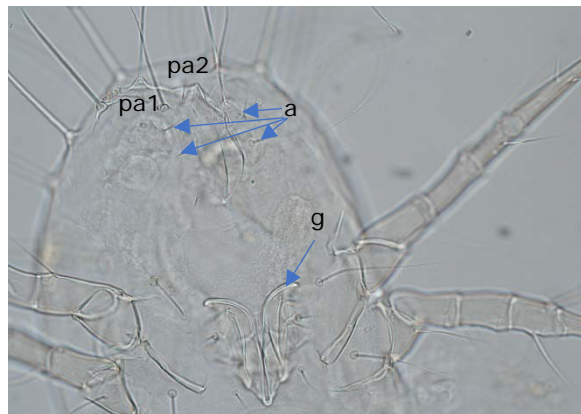


Figure 6 Ventral side of *T. putrescentiae* ♀

### *Thyreophagus entomophagus* (Laboulbène) (Acaridae)

#### Idiosoma and general characters

The male idiosoma is oval and elongated, with a colourless shining cuticle and pale brown stumpy legs. The opisthosomal lobe is flattened and concave ventrally. The dorsal opisthosomal plate has an irregular anterior margin and a thickened posterior margin. The propodosomal dorsal shield extends as far back as the scapular setae. Setae *vi* reach forward over the gnathosoma. Setae *sc e* are the longest setae arising from the body. Setae *he* and *lp* project from the sides of the hysterosoma, and *d4* is displaced to the base of the opisthosomal lobe. Setae *sc i*, *ve*, *d1*, *d2*, and *la* are absent (figure 7).

A dorsal transverse groove divides the propodosoma from the hysterosoma. Ventral surface of the opisthosomal lobe bears *pa1* and *pa2* as microsetae, and *pa3* are long and extend well beyond the posterior end of the body; *sa e* are external to *pa2*. Feebly sclerotized epimeral plates are developed in association with the apodemes. The genital opening lies between coxae IV (figure 10).

### Gnathosoma and chelicerae

Chelicerae have toothed shears (figure 8).

### Legs

All legs terminate in stalked claws whose bases are enveloped in a well-developed pretarsus. On tarsus I,  $\omega_1$  tapers toward its free end, and  $\omega_2$  is a blunt rod arising just in front of it. At the distal end of tarsus I, *d* extends beyond the end of the claw; *f*, *wa*, and *ra* are slender setae; *e* is a small spine; and *p*, *q*, *u*, *v*, and *s* are small spines lying at the base of the claw, with *p* and *q* smaller than *u*, *v*, and *s*.  $\omega_3$  occupies its normal position. On tarsus I,  $\omega_1$  arises from the base of the segment. On tarsi I and II, *ba* is slender. The claw is attached to the end of the tarsus by paired sclerites surrounded by a short, cushion-like pretarsus. If the pretarsus is elongated, then the claw is bifid in the female (figure 8).

### Female

The female body is longer and more slender than that of the male, with the posterior margin slightly pointed and not prolonged into an opisthosomal lobe. Seta *ve* is rudimentary or absent. Setae *sc i*, *hi*, and *d1* to *d3* are absent in the adult. The genital opening lies between coxae III and IV and is well separated from the anus, which extends back as far as the posterior edge of the body. Two pairs of long anal setae arise on either side of the anus (figure 9).



Figure 7 Dorsal view of *T. entomophagus* ♂



Figure 8 Legs I and II ventral view of *T. entomophagus* ♂



Figure 9 Ventral view of *T. entomophagus* ♂



Figure 10 Ventral view of *T. entomophagus* ♀ reproductive system

### *Carpoglyphus lactis* (Linnaeus, 1767) (Carpoglyphidae)

#### **Idiosoma and general characters**

Pedipalps without a conspicuously flattened distal segment. Chelicerae chelate. Ventral surface of idiosoma without prominent chitinous rings. At least one pair of vertical setae present in both sexes. On tarsus I,  $\omega_1$  arises from the base of the segment. A dorsal transverse groove dividing the propodosoma from the hysterosoma is absent (figure 12). The claw is attached to the end of the pretarsus and sometimes joined to the end of the tarsus by two thin tendons.

The idiosoma is slightly flattened, oval, and covered with a shining smooth cuticle. The shoulder region (sg) is well-defined, and the posterior margin is truncate or slightly concave (figure 11). No dorsal propodosomal shield is present, and no transverse groove separates the propodosoma from the hysterosoma. The apodemes of legs I and II are united with the sternum in both sexes. The distance between the *d1* setae is about the same as that between the *d2* setae. Most dorsal setae have rounded ends (figure 12).

Setae *ve* are almost in the same transverse line as coxae II. Three pairs of lateral setae (*l1* to *l3*) are present. With the exception of *ve* and two pairs of setae arising posteriorly, all idiosomal setae are short with rounded ends. Setae *vi* do not extend as far as the tips of the chelicerae; *ve* are more posterior and intermediate in position between *vi* and *sc i*. Dorsal setae *d1* to *d4* and *sa i* are arranged in two longitudinal rows down the middle of the idiosoma (figure 12). From the posterior end of the body project two pairs of long setae: *pa1* and *sa e* (figure 11).

#### **Gnathosoma and chelicerae**

The gnathosoma is shaped like a cone and very mobile. Chelicerae have thin narrow shears.

#### **Legs**

All legs terminate in well-developed pretarsi strengthened by two thin tendons which extend from the end of the tarsus nearly to the sickle-shaped claw. On tibia I, *phi* arises from the middle region of the segment. In females, the legs are more slender than those of the male, with less well-developed pretarsi.

#### **Ventral structures and genitalia**

Ventrally, the apodemes are well-sclerotized, and apodemes I join in the midline to form a sternum, which bifurcates at its posterior end to articulate with the apodemes of legs II. On the ventral side of the female idiosoma, the sternum and apodemes II are fused to form an epigynium covering the anterior end of the genital opening. The genital (*g*) folds are not well-sclerotized and lie between coxae II and III. The genital opening is situated between coxae III and IV. Two pairs of

genital setae are present, approximately equal in length. The male genital organs are unusually long (figure 13). The bursa copulatrix is a round opening on the posterior dorsal end of the body. The anal opening extends almost to the posterior margin of the body, with only one pair of anal setae (figure 11).



Figure 11 Ventral view of *C. lactis* ♀



Figure 12 Dorsal view of *C. lactis* ♀



Figure 13 Ventral view of *C. lactis* ♂

*Lepidoglyphus destructor* (Schrank) (Glycyphagidae)

**Idiosoma and general characters**

Pedipalps without a conspicuously flattened distal segment. Chelicerae chelate. Ventral surface of idiosoma without prominent chitinous rings. At least one pair of vertical setae present in both sexes. On tarsus I, omega arises from the base of the segment. A dorsal transverse groove dividing the propodosoma from the hysterosoma is absent.

The idiosoma is long and pear-shaped, constricted behind the fourth pair of legs. The dorsal setae are stiff and densely pectinate, standing away from the surface of the body. Setae *vi* extend well beyond the tips of the chelicerae, *ve* are more posterior in position and well-separated from *vi*, at the same distance apart as *sc i*. The scapular setae are arranged in a transverse row across the idiosoma behind legs II, *sc i* being almost the same length as *vi*. Two pairs of humerals are

present. Dorsal setae *d1* are longer than *d2*, which barely reaches the posterior margin of the body; *d3* are posterior to *d2* and almost in linear series with *d1* and *d4* (figure 14). Three pairs of lateral setae (*l1* to *l3*) are present, increasing in length toward the posterior end of the body. From the hind margin of the body project *sa i*, *sa e*, and three pairs of postanal setae, one pair of which is short and smooth. Setae *d3*, *d4*, *l3*, and *sa i* are the longest setae arising from the idiosoma.

### Ventral structures and genitalia

Ventrally, the apodemes of legs I meet to form a short sternum, and apodemes II are well-developed (figure 16). Apodemes III and IV are much reduced, their function of muscle attachment being taken over by epimerites II, each with a strong anteriorly-directed process. The genital opening lies between coxae III; its anterior end is marked by a triangular plate (figure 15). Two pairs of genital setae lie on either side, and a third pair arises behind the posterior edge of the genital opening. In females, the genital folds meet for the greater part of their length and a crescentic epigynium covers their anterior end; the posterior pair of genital setae is on a level with the hind end of the genital opening, between apodemes III and IV. The bursa copulatrix is a short tube with a partially lobed edge. The anal opening extends to the posterior margin of the body. In males, there is one pair of setae at its anterior end; in females, two pairs of setae are inserted on either side of the anterior end of the anus, the outer pair longer than the inner.

### Gnathosoma and chelicerae

The chelicerae are slender, with the movable limb bearing four large teeth and five on the fixed arm (figure 17). The pedipalp terminates in three small processes.

### Legs

The legs are long and thin, particularly the third and fourth pairs, each terminating in a pretarsus and small claw. Tibia, genua, and femora are not expanded or produced into a thin rim at their distal ends. Each tarsus is enveloped in a pectinate subtarsal scale (*wa*) attached at the base; in natural position, this scale gives the tarsus a ciliated appearance. At the distal end of the tarsus, the pretarsus is encircled by three setae (*d*, *e*, *f*), three small spines, and a solenidium omega; close behind these arise *la*, *ba*, and *ra*. At the base of the tarsus, omega1, omega2, and the famulus arise close together, omega1 a curved rod and omega2 less than half the length of omega1. On genu I, sigma is expanded distally, and sigma<sub>2</sub> is more than four times longer than sigma<sub>1</sub>. The ventral setae of genu and tibia are pectinated. Tarsi are long and slender, without a dorsal keel, but with two ventral setae on tibiae I and II. Setae of tibia and genu are pectinated. On genu II, sigma is not thickened. On tarsus I, setae *la*, *ra*, and *ba* arise in the distal third of the segment. Tarsal scales are present.



Figure 14 Dorsal view of *L. destructor* ♀



Figure 15 Ventral view *L. destructor* ♀



Figure 16 Legs I and II ventral view of *L. destructor* ♀



Figure 17 Gnathosoma ventral view of *L. destructor* ♀

*Tyrolichus casei* Oudemans, (Acarididae)

**Gnathosoma and general characters**

The pedipalps lack a conspicuously flattened distal segment. The chelicerae are chelate. The ventral surface of the idiosoma is without prominent chitinous rings.

**Idiosoma and dorsal chaetotaxy**

At least one pair of vertical setae is present in both sexes. A distinct dorsal transverse groove divides the propodosoma from the hysterosoma. External vertical setae (*ve*) arise near the anterior angles of the dorsal propodosomal shield, at the same level as *vi* or slightly posterior. Internal scapular setae (*sc i*) are longer than the external scapular setae (*sc e*). The chelicerae and legs are poorly tanned.

Setae *ve* are shorter than the genu and arise posterior to *vi*, or are as long as or longer than the genu and arise almost on a level with *vi*. Seta *la* is four to six times longer than *d1*, or more than twice its length. Seta *d1* is the only short seta on the hysterosoma; *d2* are two to three times longer than *d1*. The remaining dorsal setae are long and arranged in a characteristic fan-shaped 'train'.

**Legs and tarsal structures**

On tarsus I, solenidion arises from the base of the segment. On genu I, solenidion is less than three times longer than  $\sigma 2$ . The femur of the male is not enlarged and lacks a ventral conical process. The claw is attached to the end of the tarsus by paired sclerites surrounded by a short, cushion-like pretarsus. If the pretarsus is elongated, the claw is bifid in the female; females otherwise lack bifid claws. Homomorphic males are usually found, although heteromorphic males also occur. The dorsal terminal tarsal seta *e* is a short, thick spine. All five ventral terminal tarsal setae (*p, q, s, u, v*) are thickened to form spines of equal size.

*Suidasia medanensis* (= *pontifica*) Oudemans (Suidasiidae)

**Idiosoma and general characters**

The adult body is distinctly longer than wide and flattened, broadly oval in outline. The cuticle is longitudinally grooved and at the same time thrown into scale-like wrinkles; this wrinkling extends onto the ventral surface of the opisthosoma and gives the skin an iridescent appearance when the animal is active, but tends to disappear in mounted specimens. The body cuticle is often ornamented with rounded protuberances. A smooth dorsal propodosomal shield is present, extending as far back as the hysterosoma. A full complement of setae is present. The dorsal setae are filiform and nude. External vertical setae (*ve*) are present. The propodosoma possesses lamellar setae (*le*). Setae *vi* extend forward over the gnathosoma; *ve* are short and arise midway along the lateral edge of the propodosomal shield. The supracoxal seta is a flattened structure with

well-marked lateral pectinations, and Grandjean's organ is a fold of cuticle with a serrate edge. Setae *sc e* are more than four times longer than *sc i*; *sc i* are inserted near *sc e* and separated from each other by the width of the propodosomal shield. With the exception of *he* and *sa e*, all hysterosomal setae are short, about the same length as *sc i*, and difficult to distinguish against the wrinkled cuticle. Setae *d1* to *d4* are arranged almost in linear series. Setae *sa e* are long, limp setae exceeding half the length of the idiosoma. On the ventral surface, the apodemes are broad, stout structures. The anal opening reaches the posterior margin of the body and is surrounded by three pairs of anal setae.

#### **Gnathosoma and chelicerae**

Each chelicera bears distinctly toothed shears and a mandibular spine on its ventral surface.

#### **Legs and tarsal chaetotaxy**

The legs are rather short and stumpy, with the same chaetotaxy in both sexes. The tarsi bear enlarged proral setae (*p* and *q*), which are spine-like or claw-like. The unguinal setae (*u* and *v*) are reduced or absent; when present, they are shorter than the proral setae. The tarsi bear both tectal setae (*e* and *f*), which are filiform and similar in length. On leg I, *d* extends beyond the end of the claw; *e* and *f* are shorter setae. Ventrally, *p*, *q*, and *s* are conspicuous bent spines, *s* arising in the middle of the tarsus; *u* and *v* are long and slender and pressed tightly against the pretarsus, so that only their bases are clearly visible. On genu I,  $\sigma_2$  is more than three times longer than  $\sigma_1$ ; the setae of this and the remaining segments of leg I are not thickened to form spines. On tarsus IV, the copulatory suckers are nearer to the base and apex of the tarsus than to each other.

#### **Genital characters**

The support of the penis is curved inwards, while the penis itself is almost straight.

## Appendix 2

### Description of biology and ecology of the prey mite species

In this section, an overview of the differences in the biology and ecology of the different mite species is provided, with focus on information that may be relevant for containment measures for working with GMO material under greenhouse conditions.

#### *Carpoglyphus lactis* (Linnaeus, 1767) (Carpoglyphidae).

**Common names:** dried fruit mite or confiture mite.

**General description, distribution** -Adults are about 0.38-0.4 mm long. *Carpoglyphus lactis* can reproduce very rapidly: under optimal conditions, a female can produce 500 to 600 eggs in her lifetime, and it takes only 14 days for the mite to develop from egg to adult (COGEM, 2022.).

**Habitat use** - *Carpoglyphus lactis* is one of the most common storage mite on dried figs in storage. *Carpoglyphus lactis* is known to be one of the most important pest species from dried apricots, in dried fig, apricot, and raisin, dairy products (milk and cheese), fruits preserved in sugar, fermenting pulp and stored honey (Dizlek, Karagoz, Faraji, & Cakmak, 2019), (Güldali & Çobanoğlu, 2011). *Carpoglyphus lactis* is likely a facultative fungivore as this was shown in case of other closely-related species (Hubert et al., 2004) (Dizlek et al., 2019). It often infests products following substantial development of yeast (P.B. Klimov et al., 2016.). As a result of feeding on stored food products occurs as well as contamination by mold, which results in undesirable flavors and odors. The mite can also cause allergic reactions in humans (Bakr, Rezk, Abd El-Hamid, & Osman, 2021). *Carpoglyphus lactis* is considered a pest not only in the domestic environment but also in some non-domestic ones. For example, *C. lactis* feeds on pollen and is a pest in beehives (IDtools.org), occurring in the debris on the bottom boards of beehives, honeycombs, dead bees, honey, and especially on the bee bread (bee pollen with added honey and bee secretions that is stored in brood cells). (Nguyen et al., 2024) notes that *Apis mellifera*, especially weak ones, are highly vulnerable to *Carpoglyphus lactis* mites, which can rapidly infest and consume stored pollen, leading to weakened colonies and potential colony collapse. Aside from beehives, *C. lactis* is also found in old honeycombs, wine barrels in cellars, dried sweet fruits, canned fruits, fruits preserved in sugar, fermenting pulp, dairy products (milk and cheese), and stored honey. In the field, this species is found in fermenting tree sap flows, burrows of moles, and as phoretic deutonymphs on butterflies, moths, and scarabaeid beetles (e.g., *Gnorimus*) (P.B. Klimov et al., 2016.). In the Netherlands the species has been collected from candied cherries, dried apricot and apple molasses, and from non-domestic habitats such as from branch canker, from *Rhododendron* leaves and has been recovered from greenhouses (Siepel, Cremers, & Vierbergen, 2016.).

**Use in augmentative biological control** – Its biological characteristics makes *C. lactis* very easy to mass rear, which makes it one of the most often used prey mites for the mass production of predatory mites. In addition, *C. lactis* does not feed on green plant parts, which makes it a widely-used supplementary food for the release of predatory mites in greenhouses (Knapp, van Houten, van Baala, & Groot, 2018; Vangansbeke et al., 2024). *Carpoglyphus lactis* is also sold as a (living) feed product separately from predatory mites as supplementary food to support predatory mite populations at times of scarcity of the target pest (“standing army strategy”).

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Like all species reviewed here, *C. lactis* has a very limited active dispersal, but the presence of hypopi can facilitate passive dispersal by biocontrol agents or pollinators or spread by attaching them to personnel.

We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid

using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *C. lactis*.

*Tyrophagus putrescentiae* (Schrank, 1781) (Acaridae)

**Common names:** mould mite, copra mite, cheese mite, ham mite

**General description, distribution** - Large mite, idiosoma 432 µm long and 285 µm wide (Colloff, 2009.; P. Revainera, Lucia, Abrahamovich, & Maggi, 2014). Development is fast, depending on the type of substrate, egg to adult developmental time (at 25°C) lasts about 15 and 17 days (Kheradmand, Kamali, Fathipour, & Goltapeh, 2007). Total female longevity varies between 23 and 30 days, the daily fecundity of females was observed between 4 to 11 eggs per female per day, with 23 days of mean oviposition period resulting in an average of 255 eggs of lifetime fecundity (Colloff, 2009.; Kheradmand et al., 2007).

**Habitat use** - *Tyrophagus putrescentiae* is found in a large number of foodstock, infests various stored products, from dried figs, dried bananas, wheat spillage, oats, barley and flour (Kheradmand et al., 2007), to plant seeds (grass and sugar beet seeds), medicinal plants and mushroom beds (Dizlek et al., 2019), (Barker, 1967), (James, 1993). It is often associated with decaying organic matter in stored food with relatively high fat and protein content, such as linseed, dried egg, groundnuts, cheese, ham, herring meal and copra, and has been reported to be associated with over 140 commodities (Mullen & OConnor, 2019); (Kheradmand et al., 2007), (Michalczyk-Wetula et al., 2021; X. Zhang et al., 2018). *Tyrophagus putrescentiae* is a facultative fungivore and its interaction with microfungi likely plays an important role in their occurrence and distribution in storage ecosystems (Hubert et al., 2004). *Tyrophagus putrescentiae* can feed and slime moulds (e.g. *Didymium* sp). can positively interact with *Didymium* sp. by spreading its spores through the digestive system. (Michalczyk-Wetula et al., 2021). Due to its close association with fungi, *T. putrescentiae* is a potential vectors of parasitic fungi such as *Aspergillus* spp, *Mucor* and *Nectria* (Kheradmand et al., 2007). It has also been recorded as a predator on storage insect pests (Kheradmand et al., 2007).

In addition to being a stored product pest, *T. putrescentiae* exhibits various feeding behaviour in a number of non-domestic environments. It may be found in habitats such as soil, haystack, and grasslands (Colloff, 2009.). *Tyrophagus putrescentiae* are predaceous on plant and soil nematodes, belonging to different trophic categories such as saprophagous, plant parasitic and predaceous nematodes (Bilgrami, 1994). *T. putrescentiae* has been able to reach many new habitats, which is likely due to their ability to tolerate a wide range of temperature and low humidity (Kheradmand et al., 2007), (Michalczyk-Wetula et al., 2021). Despite the absence of a hypopal stage, *T. putrescentiae* has been found to be phoretic on four bumble bee (*Bombus*) species in Latin America (P. Revainera et al., 2014; P. D. Revainera et al., 2019) and in nests of *B. terrestris* in European greenhouses (Rozej et al., 2012). Since *T. putrescentiae* is typically associated with fungi that develop in stored products, they likely prefer the conditions prevalent in bumble bee nests, especially when these are poor of health and hence the phoretic association with (bumble)bees is not surprising (P. D. Revainera et al., 2019). (P.B. Klimov et al., 2016.).

In the Netherlands, frequently encountered on imported products (Siepel et al., 2016). The species has been recovered from different ornamentals (*Acidanthera* sp., *Haemanthus katherinae*, *Chamaedorea*, *Hyacinthus* bulb), from mushroom substrate, from roots of Cactaceae, grass (*Festuca*) tussock, in greenhouses from *Solanum dulcamara*, from Tulipa, *Allium* bulbs and potato peels (Siepel et al., 2016.).

**Presence in greenhouse crops** - *Tyrophagus putrescentiae* is also common and can be an important contaminant in laboratory environments. Since *T. putrescentiae* carries bacteria and fungi on its body, it may contaminate plant tissue cultures and on occasions it has been found to cause direct damage to plants (Murillo, Arias, & Aguilar, 2021). On occasion, *T. putrescentiae* has been found to cause damage to greenhouse crops, such as Begonia, Cyclamen, Viola and Gerbera. Under regular conditions *T. putrescentiae* causes no problems to greenhouse crops (Buxton, 2009.). However, favourable soil conditions, such as high humidity levels and the presence of organic material, their numbers may increase resulting in damage to plants (Buxton, 2009; Z.

Zhang, 2003). (Buxton, 2009.) observes that in such cases *T. putrescentiae* (and other *Tyrophagus* species) can be found feeding inside the growing points, flower buds and young leaves of plants, piercing the leaf tissue with its mouth parts. As leaves expand, damage typically remains limited to small holes or irregular cavities appear often going unnoticed or unrecognized (Buxton, 2009.). However, feeding on the flower buds of viola and cyclamen can damage the anthers and cause flowers to become infertile (Z. Zhang, 2003.). On other crops such as kalanchoë, mite damage to shoots results in brown streaks of corky tissue, and damage to growing tips and rudimentary can result in a 'broomy' appearance.

**Use in augmentative biological control** – *Tyrophagus putrescentiae* can be easily mass-produced on various inexpensive diets and are therefore used in the mass production of predatory mites (Pirayeshfar et al., 2020).

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Although like all species reviewed here, *T. putrescentiae* has limited active dispersal, it is known to exhibit phoretic behaviour (despite absence of hypopi), and thus passive dispersal by biocontrol agents or pollinators or spread by attaching them to personnel is relevant here.

We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *T. putrescentiae*.

#### *Acarus siro* Linnaeus (Acaridae)

**Common names:** flour mite, grain mite

**General description, distribution** - Large mites (females ca. 400µm long), a females lays on average 24.4 eggs per day (Colloff, 2009.). Mean oviposition period is 17 days and total fecundity is 365 eggs (Colloff, 2009.).

**Habitat use** - *Acarus siro* is a common stored - product pest, most prevalent in stored agricultural products like cereals, flours, and pulses, and also infest other dried foods and animal feed. (Clemmons & Taylor, 2016). The species is typically associated with starch-type substrates and maltose (Erban, Erbanova, Nesvorna, & Hubert, 2009). Cheese in cool stores (Melnyk, Smith, Scott-Dupree, Marcone, & Hill, 2010.), nut-in-shell peanuts in silos (Champ, 1966.). Causes damage to stored seeds and grains by feeding on the germ (the embryo part of the seed), leading to a loss of germination viability and a reduction in nutritional value. *Acarus siro* is a facultative fungivore and its interaction with microfungi likely plays an important role in their occurrence and distribution in storage ecosystems (Hubert et al., 2004). These mites contaminate products, creating odors from their lipid secretions, and their presence can lead to allergic reactions like dermatitis and asthma in humans handling infested products.

Outside domestic environments, *A. siro* lives in various habitats, such as haystacks, bird nests and bee nests, where they feed on decomposing organic matter and fungi (Chmielewski, 1995.; Colloff, 2009.; P.B. Klimov et al., 2016.). *Acarus siro* may occur on combs, wax, dead brood and bees, and mold (P.B. Klimov et al., 2016.). The dispersal success of *A. siro* over large distances is facilitated by the phoretic deutonymphs (hypopus), which opportunistically attach themselves to any arthropod carrier they can find nearby. Phoretic hosts include various arthropods (e.g., beetles, flies, fleas, and large mites). Phoresy on adult bees has not been documented but is possible (P.B. Klimov et al., 2016.).

*Acarus siro* is widely distributed across the Netherlands, it has been found on a variety of substrates such as stored flour, birdcage (*Serinus canaria*), poultry litter, dog bread, pig feed grains, stored barley, mushroom substrate, from roots of Gerbera, corn stevedoring, from apple tree (Siepel et al., 2016.). The species is not known to feed on greenhouse crops, but can be likely encountered in the greenhouse environment in association with soil substrates and plant debris.

**Use in augmentative biological control** – *Acarus siro* is used for the mass-production of predatory mites (Pirayeshfar et al., 2020).

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Like all species reviewed here, *A. siro* has a very limited active dispersal, but the presence of hypopi can facilitate passive dispersal by biocontrol agents or pollinators or spread by attaching them to personnel.

We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *A. siro*.

#### *Lepidoglyphus destructor* (Schrank) (Glycyphagidae)

**Common names:** storage mite, sugar mite

**General description, distribution** - *Lepidoglyphus destructor* is smaller than *A. siro* or *T. putrescentiae*. With a mean fecundity of 4.9 eggs per day. The mean oviposition period can last 29 days, and the total fecundity of females is on average 140 eggs (Colloff, 2009.).

**Habitat use** - *L. destructor* is a common pest of granaries.

It is universally distributed and occurs in large numbers in storage and agricultural premises, barns, stables, etc. Seeds of crops such as oilseed rape, linseed, carrot, beet, and maize are particularly vulnerable to this type of infestation. Trophic niches of astigmatic mites are certainly determined to a high degree by fungi. *L. destructor* is a facultative fungivores and its interaction with microfungi likely plays an important role in their occurrence and distribution in storage ecosystems (Hubert et al., 2004). *L. destructor* thrives on 12 species of fungi (Corente & Knülle, 2003). The ability of *L. destructor* to use various common fungi is probably one of the main reasons for its wide distribution in all kinds of stored products and plant materials.

*Lepidoglyphus destructor* may also be found in a wide variety of non-domestic habitats such as rodent and bird nests, stored products (as a pest), house dust, grass and hay and soil (e.g. (Colloff, 2009.)). This mite has also been found in nests of social insects, and is one of the most common species found in bee nests and beehives (P.B. Klimov et al., 2016.). Often there are hundreds of thousands of mites in a single beehive. They feed on decomposing organic matter, stored pollen, beehive debris, and honey, but often prefer dead bees (P.B. Klimov et al., 2016.). *Lepidoglyphus destructor* is widely distributed across the Netherlands and has been found on a wide variety of substrates such as meat meal, stored corn, company car seats, *Coriandrum* (seeds), potato peels, on legs of horse, in crusts round mouth of dwarf goats (Siepel et al., 2016.).

Although a hypopus is present it is non-phoretic. Rather, it is adapted for dormancy to enable *L. destructor* to survive adverse conditions and tolerate dry environments and can passively disperse by air currents (Corente & Knülle, 2003).

**Use in augmentative biological control** – *Lepidoglyphus destructor* is used for the mass-production different predatory mite species (Knapp et al., 2018; Pirayeshfar, Safavi, Moayeri, & Messelink, 2021).

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact

with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Although like all species reviewed here, *L. destructor* has limited active dispersal, it can passively spread by air currents and by personnel. We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *L. destructor*.

*Thyreophagus entomophagus* (Laboulbène) (Acaridae)

**Common names:** flour mite

**General description, distribution** - A cosmopolitan species (Champ, 1966.). Adults ca 400 µm long.

**Habitat use** – The mite has a broad habitat range. *Th. entomophagus* has been found in stored food products and barn dust (Champ, 1966.). The species may attack entomological collections, for example specimens have been found on a museum specimen of *Bombus lapidarius*. Although not considered as a pest in beekeeping, it is frequently encountered in beehives and in nests of stingless bees, where they presumably feed on decomposing organic matter and/or associated fungi, and their presence in hives is not considered harmful (P.B. Klimov et al., 2016.). Species of this genus inhabit different habitats and as phoretic deutonymphs use a large array of animals for dispersal: collembolans, beetles, ichneumonids, ants, fleas, birds, and mammals. Adults and other feeding stages are found in decomposing plant material, forest litter, soil, stored food including flour, sporocarps of bracket fungi, flowers, house dust, nests of birds, or on rodents themselves (P.B. Klimov et al., 2016.). In the Netherlands, *Th. entomophagus* occurs in different indoor and outdoor habitats. The species has been encountered on linseed, south slope soil under *Hieracium pilosella*, leaves of *Polypodium vulgare* and *Dryopteris carthusiana* (Siepel et al., 2016.). Dispersal of feeding stages is known on rodents (P.B. Klimov et al., 2016.). Phoretic deutonymphs are not known from bees but are known from other insects (P.B. Klimov et al., 2016.). The hypopal form (heteromorphic deutonymph) has been described from specimens collected from sparrow's nest (Fain, Knülle, & Wurst, 2000.). However, mass reared lines in the laboratory seem to entirely lack the hypopal form. Recent re-examination of the species underlines that *Th. entomophagus* lacks a heteromorphic deutonymph (hypopal) form and that previous descriptions of hypopi from outdoor habitats may relate to incorrect identification of a closely-related species *Th. holda* (P. B. Klimov et al., 2025). *Thyreophagus entomophagus* has not been recorded feeding on plants.

**Use in augmentative biological control** – Compared to *T. putrescentiae*, *Th. entomophagus* is less allergenic and less harmful in stored goods. In addition is very suitable as a factitious prey for predatory mites, making it a popular candidate for mass rearing of biological control agents (P. B. Klimov et al., 2025; Pirayeshfar et al., 2021). As a result of the successful combination of having less allergenic effect, feasibility to use in mass - production and suitability as feed for predatory mites, sterilized eggs of *Th. entomophagus* is also being sold as supplementary food to support predatory mite establishment in greenhouses ("standing army strategy").

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Although like all species reviewed here, *Th. entomophagus* has limited active dispersal, it may passively spread by air currents and by personnel, even though a (phoretic) hypopal form has not been found. We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM

advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *Th. entomophagus*.

*Suidasia medanensis* (= *pontifica*) Oudemans (Suidasiidae)

**Common names:** scaly grain mite

**General description, distribution** - Two species, *S. pontifica* and *S. medanensis*, were described by Oudemans first as separate species and he later synonymized them (Balmes-Pacia & Corpuz-Raros, 1998.). *Suidasia pontifica* appears to be cosmopolitan with a mainly tropical and subtropical distribution and common in Central and South America and Southeast Asia, New Guinea, India, Pakistan, New Zealand, Australia, across Africa (Balmes-Pacia & Corpuz-Raros, 1998.; Colloff, 2009.; Fain & Philips, 1978.; VKM et al., 2025.). Records in northwest Europe such as in Norway and the Netherlands are from indoors (Siepel et al., 2016.; VKM et al., 2025.). Thus, although *S. pontifica* is listed as a species native to the Netherlands (Nederlandse soortenregister), the remark of (Fain & Philips, 1978.) that the species may have been introduced to Europe in the past with shipments of storage goods from the (sub)tropics seems plausible.

The body length of *Suidasia pontifica* averages 0.63 mm in females and 0.47 mm in males (Balmes-Pacia & Corpuz-Raros, 1998.). Egg to adult development of females (26°C, 85% relative humidity) 12.6 days on average. The reproductive period is about 20 days and lifetime fecundity between 62-177 eggs per female (Mercado, Puerta, & Caraballo, 2001).

**Habitat use** - The species can cause great damage to commodities and is an important food pollutant and of allergenic significance (Colloff, 2009.; VKM et al., 2025.). *Suidasia pontifica* has mainly been reported from human environments, having been collected from a wide variety of dry foodstuffs such as dried fish, shrimp, stored agricultural products such as flour, cereal grains, oilseeds, animal feed, cheese and nuts, and is commonly found in house dust (Balmes-Pacia & Corpuz-Raros, 1998.; VKM et al., 2025.) (Colloff, 2009.). It is also reported to infest nests of honeybees and solitary bees (*Xylocopa*) in the tropics (P.B. Klimov et al., 2016.; VKM et al., 2025.). *Suidasia pontifica* is not known to damage living plant tissue.

**Use in augmentative biological control** – *Suidasia pontifica* is used to mass-rear *Amblyseius swirskii* and other predatory mites (Pirayeshfar et al., 2020; Vangansbeke et al., 2024).

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Although like all species reviewed here, *S. pontifica* has limited active dispersal, it may passively spread by air currents and by personnel. We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *S. pontifica*.

*Tyrolichus casei* Oudemans, (Acarididae)

**Common names:** Cheese mite

**General description, distribution** -

Similar in habitus to *T. putrescentiae*. Length of males (idiosoma) 450–550  $\mu\text{m}$ , that of females 500–700  $\mu\text{m}$  (Melnyk et al., 2010.). Likely cosmopolitan species, records from Holarctic and Oriental regions (Canada, England, Germany, the Netherlands, Poland, Ukraine, Azerbaijan, Russia, and India), Australia, New Zealand (P.B. Klimov et al., 2016.); (Champ, 1966.).

**Habitat use** - In some countries considered a major pest of the cheese industry. In some cases *Tyrol. casei* is either naturally allowed to colonize some “gourmet cheese” with characteristic taste, such as “Cantal” (France), or even intentionally added to produce “mite cheese” or “Milbenkäse” (Germany) or “Mimolette” (France) (P.B. Klimov et al., 2016.; Melnyk et al., 2010.); (Colloff, 2009.). The mite is known to cause respiratory diseases to individuals handling infested cheese as well as dermatitis (Colloff, 2009.; P.B. Klimov et al., 2016.).

Except for cheese (see below), *Tyrol. casei* may be found in stored foods, grains, flour, cheeses, dog meal, old honeycombs, and insect collections (Mullen & OConnor, 2019.), but infestation rates in these stored food other than cheese are typically relatively low (P.B. Klimov et al., 2016.). In non-domestic habitats, *Tyrol. casei*. has been collected from under bark, soil, decaying plant materials, bird and mammal nests, and stored food. *Tyrol. casei* often lives in honey bee hives, where they feed on pollen and beehive debris and although it can reproduce in considerable numbers, it is not considered harmful to beehives (P.B. Klimov et al., 2016.). In non-domestic habitats, the species in the Netherlands Amsterdam has been collected from cleanings from cage of mink (*Neovison vison*) (Siepel et al., 2016.). *Tyrol. casei* is not known to damage living plant tissue.

Phoretic deutonymphs (hypopus) are absent and feeding stages may disperse phoretically, or via air currents or active movement.

**Use in augmentative biological control** – Since *Tyrol. casei* is highly suitable as factitious prey for predatory mites and easy to culture, it makes it a popular candidate for mass rearing of biological control agents. However, it is unclear to what extent the species is currently used as prey mite for commercial biocontrol purposes.

**Remarks on risk of pollen transmission** – The species may occur on stored pollen in the wild (beehives), but no evidence suggests that it would actively visit flowers and thus come in contact with pollen. The only way it can come to contact with pollen is by directly spreading mites over the flowers such as sometimes applied by sprinkling them from bottles or blowing them in the crop. When released in the crop, consider therefore using sachets, as prey mites will not disperse in the crop, rather than directly sprinkling them in the vegetation. Although like all species reviewed here, *Tyrol. casei* has limited active dispersal, it may passively spread by air currents and by personnel. We advise general measures relevant for PKb-I greenhouses and PC-I climate cells to be used (see earlier COGEM advise on *C. lactis* CGM/220718-02). In addition, we recommend the use of sachets (combination prey and predatory mites) rather than inundative sprinkling. We recommend to avoid using flower visiting biocontrol agents and pollinators (hover flies, bees and bumble bees) that can act as phoretic hosts for *Tyrol. casei*.