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Risk assessment of the biological plant protection product Montyline with the active organism *Amblyseius montdorensis*

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ISBN: 978-82-8259-259-8 Norwegian Scientific Committee for Food Safety (VKM) Po 4404 Nydalen N – 0403 Oslo Norway

Phone: +47 21 62 28 00 Email: <u>vkm@vkm.no</u>

www.vkm.no www.english.vkm.no

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Risk assessment of the biological plant protection product Montyline with the active organism *Amblyseius montdorensis*

Authors preparing the draft opinion

Torsten Källqvist (chair), Hubert Dirven, Edgar Rivedal, May-Guri Sæthre, Micael Wendell

(Authors in alphabetical order after chair of the working group)

Assessed and approved

The opinion has been assessed and approved by Panel on Plant Protection Products of VKM. Members of the panel are:

Torsten Källqvist (chair), Katrine Borgå, Hubert Dirven, Ole Martin Eklo, Merete Grung, Jan Ludvig Lyche, Marit Låg, Asbjørn M Nilsen, Line Emilie Sverdrup

(Panel members in alphabetical order after chair of the panel)

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May-Guri Sæthre from the Panel of Plant health of the Norwegian Scientific Committee for Food Safety is acknowledged for her valuable work on this opinion. Project manager from the VKM secretariat has been Edgar Rivedal

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

Montyline with the predatory mite *Amblyseius montdorensis* as the active organism is sought to be used as a plant protection product in Norway. Montyline is intended for use against whiteflies in ornamental plants, vegetables and herbs in greenhouses and plastic tunnels, as well as fruit and berries in greenhouses, plastic tunnels and open field.

VKM was requested by the Norwegian Food Safety Authority to consider the possible environmental and health risk related to the properties of Montyline; in particular to evaluate if the predatory mite is naturally occurring in Norway, the potential for establishing and spreading under Norwegian conditions, possible taxonomic challenges, and potential for health risk related to its use.

The assessment was finalized in January 2017 and adopted by VKM's Panel on Plant Protection Products.

VKM's conclusions are as follows:

Natural occurrence of the mite in Norway:

The predatory mite *Amblyseius montdorensis* has not been observed in Norway. It is the view of VKM that this is due to its lack of capability to survive and establish in areas with cold winters.

Potential for establishing and spreading of the predatory mite under Norwegian conditions:

The thermal preference of *Amblyseius montdorensis* restricts its establishing, and the mite has not been observed in northern parts of Europe. The species is incapable of entering diapause and it is the opinion of VKM that it is unlikely that *Amblyseius montdorensis* will be able to establish in outdoor areas in Norway.

Taxonomic challenges:

There are no taxonomic challenges related to the assessment of Amblyseius montdorensis.

Human health risk for operators:

VKM is unaware of reports where harm to humans by *Amblyseius montdorensis* itself, or associated pathogenic organisms have been observed. Insects and mites may however produce allergic reactions in sensitive individuals.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, *Amblyseius montdorensis,* Montyline, pesticide, biological control product

Sammendrag på norsk

Det er søkt om tillatelse for bruk av preparatet Montyline, med rovmidden *Amblyseius montdorensis* som virksom organisme, som plantevernmiddel Norge. Montyline er tenkt brukt som insektmiddel mot hvitfly i prydplanter, grønnsaker og urter i veksthus og plasttunneler, samt frukt og bær i veksthus, plasttunneler og utendørs.

VKM ble forespurt av Mattilsynet om å vurdere mulig miljø- og helserisiko knyttet til egenskapene til Montyline. VKM skulle spesielt vurdere om rovmidden *Amblyseius montdorensis* er naturlig forekommende i Norge, potensialet for etablering og spredning under norske forhold, mulige taksonomiske utfordringer, og mulig helserisiko knyttet til bruk av middelet.

Prosjektgruppens arbeid ble avsluttet i januar 2017 og rapporten godkjent av VKMs Faggruppe for plantevernmidler.

VKMs konklusjon er som følger:

Naturlig forekomst av Amblyseius montdorensis:

Rovmidden *Amblyseius montdorensis* har ikke vært observert i Norge. Det er VKMs oppfatning at dette skyldes middens manglende evne til å overleve og etablere seg i områder med kalde vintre.

Potensial for etablering og spredning av rovmidden under norske forhold:

Temperatur begrenser *Amblyseius montdorensis* sin etablering, og midden er ikke observert i nordlige deler av Europa. Arten er ikke i stand til å etablere dvaletilstand. Dette er trolig årsaken til at den ikke kan overleve og etablere seg utendørs i områder med kalde vintre som Norge.

Taksonomiske utfordringer:

Det er ingen taksonomiske utfordringer knyttet til vurdering av Amblyseius montdorensis.

Potensiell helserisiko for mennesker:

VKM kjenner ikke til at bruk av *Amblyseius montdorensis* har medført helserisiko, eller at midden inneholder patogene organismer. Midd og andre innsekter kan imidlertid gi allergiske reaksjoner hos følsomme individer.

Terms of reference as provided by the Norwegian Food Safety Authority/

Amblyseius montdorensis is the active organism in biological control product Montyline against whiteflies intended for use in ornamental plants, vegetables and herbs in greenhouses and plastic tunnels, indoor plantings, as well as fruits and berries in greenhouses, plastic tunnels and open fields.

In this regard, The Norwegian Food Safety Authority would like an assessment of the following:

- Prevalence, especially if the organism is found naturally in Norway.
- The potential of the organism for establishment and spread under Norwegian conditions specified for use in greenhouses and open field.
- Any ambiguities regarding the taxonomy, which hampers risk assessment.
- An assessment of the product and the organism with regard to possible health risk.

Assessment

1 Introduction

1.1 Product and trade name

The predatory mite *Amblyseius montdorensis* is used as biological control agent in a number of different plant protection products named Montyline. The different products consist of the same components but are packaged differently to suit specific uses. All life stages of *Amblyseius montdorensis* are included in the products.

Products containing *Amblyseius montdorensis* are intended for use in augmentative biological control as part of an integrated pest management (IPM) strategy. The products are available as either loose formulation for direct application to the crops, or in sachets, containing breeding populations for controlled release over time.

Amblyseius montdorensis are suited for use in crops grown under warm conditions, above 15-20°C. Lower temperatures will reduce or prevent its establishment. (See 2.2 Potential for establishment and dispersal)

1.1.1 Associated organism

Montyline am products contain the organism *Thyreophagus entomophagus,* which serves as food and carrier material. The host mite *T. entomophagus* has an ubiquitous distribution and is native throughout Europe (Hughes, 1976), including records from UK, Russia and Norway (Mehl, 1998). The species is also present in other widely used biological control products.

1.1.2 Original location of Amblyseius montdorensis

Amblyseius montdorensis was originally collected by Marilyn Steiner in the Atherton Tablelands inland of Cairns, Queensland, Australia in 1994. Steiner et al (2003) reported that the species is "widely distributed in un-forested coastal areas of Queensland, (from Brisbane in the south to Cairns in the north) and in irrigated and higher rainfall area inland including Biloela and the Atherton Tablelands". It is considered that the climate in these areas constitute optimum conditions for *A. montdorensis*.

Collections have later been done in other locations of Australia, and in the Mediterranean Sea area of Europe. The species has not been observed in northern parts of Europe (See 2.2 Potential for establishment and dispersal).

1.2 Properties for use as a plant protection product

Published literature has shown *A. montdorensis* to predate upon thrips and whiteflies (Cuthbertson et al., 2012; Manners et al., 2013; Medd and GreatRex, 2014; Rahman et al., 2011; Steiner et al., 2003). Predation has also been observed on eriophyid mites (Pijnakker and Leman, 2011; Steiner and Goodwin, 2005). Most literature is based on laboratory studies, but is also supported by field trials. Holmes & Greatrex (2011) published data from a replicated semi-field trial, demonstrating strong control of both thrips and whiteflies.

Plant protection products containing *A. montdorensis* is intended for use in greenhouse vegetables and ornamental crops. The producer (Syngenta) has provided information (unpublished studies) on the control of whiteflies and thrips in peppers, aubergines and cucumbers, as well as in Gerbera and Chrysanthemum.

Amblyseius montdorensis consume higher quantity of thrips per day than *Amblyseius cucumeris*, and it attacks and kills second stage as well as first stage thrips larvae. *Amblyseius montdorensis* also lay more eggs per day, and its population grows faster when provided with adequate food. The amount of eggs laid during its lifetime is around 50. The predator may consume about 20 first stage thrips larvae per day. Adult mite females lay eggs on leaf hairs, and the larvae emerge from them in 1–2 days. Thereafter they pass stages of molting and turn into proto-nymphs, and thereafter into deuto-nymphs. All motile stages of the mite are predatory.

1.2.1 Sensitivity to pesticides

Tests of the sensitivity of *A. montdorensis* to various commercial insecticides have shown that products such as Pirimor, Tilt and Bravo have low toxicity to *A. montdorensis*. Sulphur has intermediate toxicity, and Vertimec, Pyrethrum and Vapona are highly toxic for this organism. *Amblyseius montdorensis* is reported to provide good control on strawberry, cucumber, peppers, tomatoes and gerbera either alone, or together with a low dose application of low toxicity insecticides.

1.2.2 Target pests

The majority of the reported prey for *A. montdorensis* are pests of glasshouse crops and stored products. The organism is a generalist predator, and is likely to prey on a range of small insects. *Amblyseius montdorensis* is mainly used as biological control agent against whiteflies, thrips and eriophyid species.

Whitefly primary target species: *Trialeurodes* spp., *Aleyrodes* spp. and *Bemisia* spp. (Insecta; Hemiptera; Aleyrodidae).

Thrips primary target species: *Frankliniella occidentalis* (Insecta; Thysanoptera; Thripidae), *Thrips tabaci* (Insecta; Thysanoptera; Thripidae).

Eriophyid primary target species: Aculops lycopersici (Acari; Prostimata; Eriophyidae).

Common names for different prey species; Glasshouse Whitefly (*Trialeurodes vaporariorum*), Onion Thrips (*Thrips tabaci*), Western Flower Thrips (*Frankliniella occidentalis*), Rose Thrips (*Thrips fuscipennis*) and Tomato Russet Mite (*Aculops lycopersici*).

Other insects of glasshouse crops are also targeted, such as small arthropods on common bean, strawberry, cucumber and tomato.

Whiteflies, thrips and eriophyid mites are pests of glasshouse crops globally. Occurrence frequency of the particular species vary between countries.

1.2.3 Life cycle of target pests

Whitefly Life Cycle; Eggs are laid on the plant with a small pedicel holding the egg in place. The eggs hatch into the first instar larvae, called the crawler, which feeds on the plant and develop through three more larval stages until it develops into a pupae.

Thrips Life Cycle; Eggs are inserted into plant tissue; develop into first and second instar larvae - Pre-pupae (often in soil) - Pupa (often in soil) - Adult.

Aculops life Cycle; Eggs - first instar larvae - proto-nymph - Adult

The biology of the target pests have been extensively studied and documented in the scientific literature.

1.2.4 Damage by pest to plants

Whitefly; Direct sap feeding by adults and sessile juvenile stages weakens the plant. Honeydew (fecal matter rich in undigested sugars) leads to the growth of moulds (e.g. *Cladosporium* spp.) and to stickiness of harvested produce. The adult whitefly transmits several plant pathogenic viruses.

Thrips; Adult thrips insert eggs into the plant tissue, causing misshaping of the fruit. First and second instar larvae feed on the plant and damage the leaves, flowers and fruits. Adult life stages are vectors for plant pathogens and damage the plant by feeding on the leaves, fruits and flowers.

Eriophyid mites; Causes silvering, bronze colouring and wilting of leaves. The stems become rusty brown in colour and develop cracks on the surface with possible leaf desiccation and plant death.

1.3 Status in Norway

Two other species in the genus Amblyseius (*Amblyseius cucumeris* and *Amblyseius swirskii*) are approved in Norway for use against greenhouse whitefly, thrips and mites in different greenhouse crops (The current approval expires in 2017).

Product names:	Amblyseius-System, Tripsrovmidd, Tripex, Amblyseius cucumeris, Ambly-line cu (<i>Amblyseius cucumeris)</i>				
	SWIRSKI-MITE, SWIRSKI-MITE PLUS, Swirski Mite Plus, Swirski Mite, SWIRSKI-LINE/SWIRSKI-LINE PLUS, Swirskii-System, Swirskii- Breeding-System (<i>Amblyseius swirskii</i>)				
Species names:	<i>Amblyseius cucumeris</i> and <i>Amblyseius swirskii:</i> Predatory mite (Family: Phytoseiidae; Order: Parasitiforme)				
Target pests:	Greenhouse whitefly, thrips and mites				
Area for use:	Greenhouse crops				

2 Hazard identification and characterisation

2.1 Occurrence and distribution in Norway

Amblyseius montdorensis has not been observed in Norway.

2.2 Potential for establishment and dispersal

Amblyseius montdorensis is indigenous in Australia and is distributed to other sub-tropical and warm temperate regions such as Pacific islands. The organism has not been observed in northern parts of Europe.

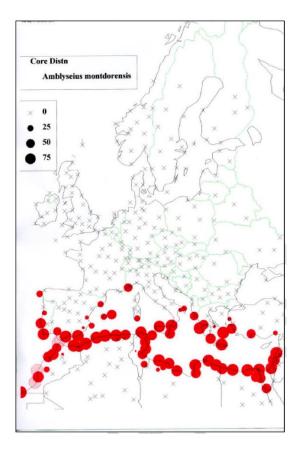


Figure 2-1. Predicted locations where suitable climates may allow *Amblyseius montdorensis* to establish. A climate envelope model was developed on CLIMEX by the applicant using published thermal biology data and reported locations for observation of *A. montdorensis* (Collection data from Steiner et al (2003), Schicha (1979), Beard (2001) and Moraes et al (2004). (Ecoclimatic indices greater than 75% are considered suitable for establishment by this CLIMEX modelling)

A climate envelope model has been developed in CLIMEX by the applicant using published thermal biology data, and the known distribution of *A. montdorensis* from collection data provided by Steiner et al (2003), and from locations reported by Schicha (1979), Beard (2001) and Moraes et al (2004). The model has been used to predict locations where suitable climates may allow the species to establish. Using CLIMEX (CLIMEX V 1.1 .) ecoclimatic indices greater than 75%, predicted locations where suitable climates may allow *A. montdorensis* to establish are shown in Figure 2-1. The map indicate a limited potential for establishment in southern Spain, Sicily, and parts of Greece, but no significant possibility of establishment further north in Europe.

2.2.1 Temperature dependence

The lack of observations and predicted capability to establish in Northern Europe may suggest that *A. montdorensis* is dependent on warm climate.

Hatherly et al (2004) have studied the species' temperature requirements. The observed effect of temperature on the duration of different developmental stages of *A. montdorensis* is shown in Table 2-1. The optimum temperature for reproduction is around 27°C. The average time for development from egg to adult is 6.3 days at 27°C, while 22.1 days are needed for the development from egg to adult at 15°C (See Table 2-1) (Hatherly et al., 2004).

Temperature	Egg hatch (days)	Larvae (days)	Protonymph (days)	Deutonymph (days)	Total days duration
15°C	7.1	2.9	5.8	6.3	22.1
20°C	4.5	1.9	3.6	2.9	12.9
25°C	2.7	0.9	2.0	1.9	7.5
27°C	2.4	0.9	1.5	1.5	6.3
30°C	2.7	0.9	1.9	2.5	8.0

Table 2-1. Development time (days) of *Amblyseius montdorensis* from egg to adult at different temperatures. (Data from Hatherly et al (2004))

Hatherly (2004) used the data in Table 2-1 to calculate the developmental threshold for the species. The minimum temperature for development of *A. montdorensis* colonies was determined to 10.7°C using simple regression, and 10.3°C using weighted regression. The thermal budget was calculated to 105.3 degree days (simple regression) or 108.7 degree days (weighted regression). The data also indicate that 30°C is approaching the upper developmental threshold, since the developmental time increases at temperature above 27°C.

Hatherly et al (2005a; 2005b) has predicted the development of maximum six generations of *A. montdorensis* in the UK, all of which would occur during the summer months.

The data from Hatherly et al (2005a; 2005b) indicate that *A. montdorensis* is not tolerant of low temperatures. UK field studies indicate that no reproduction occurs within the field in winter (November to March) and that 100% mortality of eggs, larvae and adults occurs within two weeks of release during this period (Hatherly et al., 2004). This could be related to the finding that the species is incapable of entering diapause (Hatherly et al., 2004; Steiner et al., 2003).

Data from Steiner et al (2003) indicate that the eggs of *Amblyseius montdorensis* are sensitive to low humidity. Both the temperature and humidity characteristics are not unexpected for an organism originating from sub-tropical and warm temperate regions of Queensland, Australia and Pacific islands, and may suggest that the species is not well adapted to cool or dry climates.

2.2.2 Other factors affecting survival

Availability of suitable prey species are unlikely to constrain survival of *Amblyseius montdorensis* since it is a generalist predator reported to occur on a wide range of plants.

2.2.3 Reproduction

Amblyseius montdorensis reproduces sexually. Egg laying is more frequent and continuous when males are present, indicating that females must mate more than once to reach their maximum egg laying potential. Sex ratio has been shown to vary between 1.68:1 to 2.24:1 (Females:Males) in different trials performed by Steiner et al (2003).

Total egg laying over a 30 days life span is approximately 50 eggs, laid at rates of up to 3.5 eggs/day. Egg hatching is strongly dependent upon temperature and humidity. Steiner et al (2003) demonstrated that the percentage egg hatch declines rapidly below 75% relative humidity.

2.2.4 Means of dispersal:

Amblyseius montdorensis may be dispersed by the wind, or by contact with humans, animals or plants. Adult individuals are also quite mobile and capable of dispersal from plant to plant when there is contact between plants.

2.2.5 Release and post-release monitoring

Amblyseius montdorensis has previously been released in Finland, UK, Netherlands, France, Germany, Denmark, Italy, Spain, Portugal, Russia, Ukraine and Poland. Releases in these countries have taken place as part of a biological control strategy. Post-release monitoring

has reportedly been carried out following releases in Almeria, Spain; France and UK. These studies have not shown that *A. montdorensis* can establish or spread outside of the release structure.

VKM assessment of prevalence, establishment and spread

Occurrence of *Amblyseius montdorensis* has not been reported in Norway. It is the view of VKM that this is due to its lack of abilities to survive and establish in areas with cold winters. The species is incapable of entering diapause and it is the opinion of VKM that this, combined with the thermal requirements of the species makes it unlikely that *A. montdorensis* will be able to establish outdoors in Norway.

2.3 Taxonomic challenges

Origin of parent individuals

Amblyseius montdorensis was originally provided to Syngenta Bioline by Marilyn Steiner and Stephen Goodwin of New South Wales Agriculture, Australia. The individuals originated from collections made in Atherton Tablelands, inland from Cairns, Queensland, Australia.

Species identification was originally performed by Steiner prior to supplying the mites to Syngenta Bioline (formerly Novartis BCM) in 1999. In additions to experts within Syngenta, reference specimens have been identified by independent experts such as Ian Hatherly, Birmingham University and Les Shipp, Agriculture and Agrifood, Canada. The independent experts have verified the identity of the species as *A. montdorensis*, although they have not provided formal identifications.

Amblyseius montdorensis (Alternative name: *Typhlodromips montdorensis*) is a predatory mite in the family Phytoseiidae (Acari: Parasitiformes), used for the control of whiteflies, thrips and spider mites. Following a taxonomic review of the Phytoseiidae by Chant and McMurtry (2007), the species was transferred to the genus *Transeius*. Thus, the following species names are used in the published litterature: *Amblyseius montdorensis, Typhlodromips montdorensis* and *Transeius montdorensis*.

The description of the species has not changed despite variations in the taxonomic classification. Conclusive morphological identification is possible when using the original description of Schicha (1979) and Beard (2001).

The life stages of *A. montdorensis* consists of egg, larvae, protonymph, deutonymph and adult. Morphological identification is best done on adult females. It is also possible to morphologically identify adult males or deuto-nymphs of either sex, although this is more challenging. It is not possible to identify proto-nymphs, larvae or eggs using morphological descriptions. The identifications of these life stages requires molecular techniques.

Syngenta has sequenced the Cytochrome Oxidase 1 gene, which has made it possible to perform a molecular identification of *A. montdorensis.*

The sequence of loci 1 - 349 is:

There are no relevant special characteristics of this strain, and the strain is not expected to differ from wild populations.

VKM assessment of taxonomy

Amblyseius montdorensis was originally characterized by Marilyn Steiner prior to supplying the mites to Syngenta Bioline in 1999. Later, reference specimens have also been verified as *A. montdorensis* by independent experts, in additions to experts within Syngenta although no formal identification was performed. Conclusive morphological identification has been described by Schicha (1979) and Beard (2001), and is best done on adult females.

Following a taxonomic review by Chant and McMurtry (2007), the species was transferred to the genus *Transeius*. The following species names are therefore used in the published litterature: *Amblyseius montdorensis, Typhlodromips montdorensis* and *Transeius montdorensis*.

Identification of *A. montdorensis* may also be performed using molecular techniques since the Cytochrome Oxidase 1 gene has been sequenced.

It is the opinion of VKM that no taxonomic problems exist for the identification of *A. montdorensis.*

2.4 Health hazards

2.4.1 Human health

No report of adverse effects to humans by *A. montdorensis* has been found. A number of pathogenic organisms are known to affect Phytoseiid mites, for example microsporidian infection (Bjørnson, 2005). None associated pathogens have however been identified in the current stock of *A. montdorensis*.

A possible potential for harm would be if individuals would become allergic to the mites. The majority of insects and mites are capable of producing allergic reactions in

sensitive individuals. Sensitisation is more likely to occur after prolonged exposure to large numbers of the organism. Specific allergies to *Amblyseius* spp. have been reported (de Jong et al., 2004), although the applicant claims that this has not been the case for the present population of *A. montdorensis* (in culture since 1999).

Montyline products contain the organism *Thyreophagus entomophagus* as fodder for the active organism. *Thyreophagus entomophagus* is native throughout Europe as well as in Norway and is not considered a hazard to human health.

2.4.2 Animal health

There are no reports of *A. montdorensis* causing harm to animals.

2.4.3 Potential for plant damage

There are no reports of plant damage caused by A. montdorensis.

VKM assessment of health hazard

VKM is unaware of reports where harm to humans by *A. montdorensis* itself, or associated pathogenic organisms have been observed. The applicant states that associated pathogens have not been identified in the current population of *A.montdorensis*.

Many types of insects and mites are capable of producing allergic reactions in sensitive individuals. Use of personal protective equipment during handling of the product should therefore be considered.

3 Uncertainties

In general the predatory mite *Amblyseius montdorensis* is well studied, and the conclusions on prevalence, establishing capability and taxonomy are well funded.

When it comes to possible associated pathogenic organisms, less data is available, and the conclusion is mainly based on statements provided by the applicant.

4 Conclusions (with answers to the terms of reference)

Prevalence, especially if the organism is found naturally in Norway.

The predatory mite *Amblyseius montdorensis* has not been observed in Norway. It is the view of VKM that this is due to its lack of capability to survive and establish in areas with cold winters.

The potential of the organism for establishment and spread under Norwegian conditions specified for use in greenhouses and open field.

The thermal preference of *Amblyseius montdorensis* restricts its establishing, and the mite has not been observed in northern parts of Europe. The species is incapable of entering diapause and it is the opinion of VKM that it is unlikely that *Amblyseius montdorensis* will be able to establish in outdoor areas in Norway.

Any ambiguities regarding the taxonomy, which hampers risk assessment.

There are no taxonomic challenges related to the assessment of *Amblyseius montdorensis*.

Assessment of the product and the organism with regard to possible health risk.

VKM is unaware of reports where harm to humans by *Amblyseius montdorensis* itself, or associated pathogenic organisms have been observed. Insects and mites may however produce allergic reactions in sensitive individuals.

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