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# Risk assessment of the biological control product Limonica with the organism *Amblydromalus limonicus* Garman and McGregor

**Scientific Opinion of the Panel on Plant Health of the Norwegian Scientific  
Committee for Food and Environment**

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# **Risk assessment of the biological control product *Limonica* with the organism *Amblydromalus limonicus***

## **Preparation of the opinion**

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of two VKM members and a project manager from the VKM secretariat. Three referees commented on and reviewed the draft opinion. The Committee, by the Panel on Plant Health, assessed and approved the final opinion.

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The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Plant Health.

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## **Acknowledgment**

VKM would like to thank the referees Dr. Peter Dalin and Maria Björkman (both at the Swedish Environmental Protection Agency) and Dr. Lawrence R. Kirkendall (University of Bergen and member of the VKM Panel on Alien Organisms and Trade in Endangered Species (CITES)) for reviewing and commenting on the manuscript. VKM emphasises that the referees are not responsible for the content of the final opinion. In accordance with VKM's routines for approval of risk assessments (VKM, 2018), VKM received their comments before evaluation and approval by the Panel on Plant Health, and before the opinion was finalised for publication.

## **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

The product Limonica, with the predatory mite *Amblydromalus limonicus* as the active organism, is sought to be used as a biological control agent in Norway. Limonica is intended for use against western flower thrips (*Frankliniella occidentalis*), other thrips (e.g. *Thrips tabaci*), spider mites and whiteflies (e.g. *Trialeurodes*, *Aleyrodes* and *Bemisia* spp.) in protected horticultural crops such as cucumber, sweet pepper, strawberry and ornamentals. The product is not recommended for greenhouse-grown tomatoes.

## **VKM's conclusions are as follows**

### Distribution, especially if the organism is found naturally in Norway

*Amblydromalus limonicus* has a very wide natural distribution, being reported from New Zealand, Australia South America, Central America, and North America as well as Hawaii. It has also recently established populations in crop productions and non-crop vegetation in Catalonia, North Eastern Spain. *Amblydromalus limonicus* have not been observed in Norway. The species seems not to have the capability to enter diapause under unfavourable conditions and it is the view of VKM that it likely lacks the ability to survive and establish in areas with cold winters and chilly summers, as found in most parts of Norway under current climatic conditions.

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

The thermal preference of *A. limonicus* restricts its establishment, and the species has not been observed outdoors in Norway. As the species is incapable of entering diapause it is the opinion of VKM that it is unlikely that *A. limonicus* will be able to establish in outdoor areas in Norway. However, the lack of detailed information on temperature tolerance of the species constitutes an uncertainty factor. The risk of spread from greenhouses is low because no wind or vector are likely to carry the mites from the greenhouse to suitable outdoor habitats. However, mites that have escaped from a greenhouses to may spread in the nature.

All conclusions are uncertain due to lack of relevant information regarding the species' climate tolerance. Its origin and current distribution suggest that it cannot survive cold winters.

### Any ambiguities regarding taxonomy that hamper risk assessment

There are no taxonomic challenges related to the assessment of *A. limonicus*.

### Assessment of the product and the organism with regard to possible health risks

VKM is unaware of reports where harm to humans has been observed, whether by *A. limonicus* itself. Mites may, however, produce allergic reactions in sensitive individuals handling plant material with high numbers of individuals. There is reason to believe that this holds true also for *A. limonicus*.

**Key words:** VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, biological control, predatory mite



# Sammendrag på norsk

Produktet Limonica, med rovmidde *Amblydromalus limonicus* som aktiv organisme har blitt søkt godkjent som plantevernmiddel i Norge. Limonica ønskes brukt til å bekjempe Amerikansk blomstertrips (*Frankliniella occidentalis*), andre typer trips (for eksempel Nelliktrips (*Thrips tabaci*)), spinnmidd og ulike typer mellus (Veksthusmellus, Jordbærmellus, Bomullsmellus). *Amblydromalus limonicus* kan brukes i vekster, som for eksempel agurk, paprika jordbær og pryddplanter. Produsenten anbefaler ikke bruk i tomatproduksjoner.

## VKM konkluderer følgende

### Utbredelse, spesielt hvis organismen forekommer naturlig i Norge

*Amblydromalus limonicus* har aldri blitt observert i Norge. Den eneste dokumenterte ville forekomsten av arten i Europa er i nordøst Spania. Arten kan ikke gå i diapause under suboptimale forhold og det er derfor ikke grunn til å tro at den kan etablere seg under Norske forhold. VKM ser det derfor som lite sannsynlig at arten kan overleve og etablere seg i områder med kalde vintre og kjølige somre, forhold som kjennetegner store deler av Norge i dagens klima.

### Organismens potensiale for å etablere og spre seg under norske forhold spesifisert for bruk i drivhus og på friland

Temperaturforholdene begrenser hvilke områder hvor *A. limonicus* kan etablere seg i og arten er ikke registrert funnet i Norge. VKM anser det derfor usannsynlig at *A. limonicus* vil klare å kunne etablere seg utendørs i Norge. Konklusjonen er usikker på grunn av mangel på detaljert informasjon om artens temperaturtoleranse. Risikoen for spredning fra drivhus anses som lav, fordi hverken vind eller andre biologiske vektorer vurderes som effektive spredningsveier til egnede områder utendørs. Artens opphav og utbredelse tyder dog på at den ikke kan overleve kalde vintre.

### Uklarheter relatert til taksonomi som påvirker risikovurderingen

Det er ingen taksonomiske utfordringer relatert til vurderingen av *A. limonicus*.

### Vurdering av produktet og organismen opp mot mulig helserisiko:

VKM har ikke funnet rapporter som beskriver skade på mennesker fra *A. limonicus*. Midd kan utløse allergiske reaksjoner hos sensitive personer som håndterer plantemateriale med mye midd, og det er grunn til å tro at det også gjelder for *A. limonicus*.

**Nøkkelord:** VKM, risikovurdering, Vitenskapskomiteen for mat og miljø, Mattilsynet, biologisk kontroll, rovmidd

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

Limonica is a product containing the macroorganism *Amblydromalus limonicus*. The intended use is for biological control of mites and thrips in horticultural crops such as fruits, berries, vegetables, ornamentals and herbs.

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 Purpose and scope

This document presents a scientific opinion prepared by the Panel of Plant Health, in response to a request from the Norwegian Food Safety Authority. The opinion is an assessment of the biological control product Limonica and the predatory mite *Amblydromalus limonicus* (Acari: Phytoseiidae). The assessment area for this opinion is Norway.

### 1.2 Product and trade name

The predatory mite *Amblydromalus limonicus* is used as the biological control agent in Limonica.

The product Limonica contains *A. limonicus* individuals in nymph or adult stages and are mixed in an inert substrate containing millet husk bran and sawdust. The product does not contain any food source.

*Amblydromalus limonicus* is reportedly suited for use in crops grown in greenhouse conditions (optimum: temperature 25-27 °C, with relatively high humidity [ $>50\%$ ] (McMurtry and Scriven, 1965)) but also on ornamentals grown outdoors. The mite oviposits and is active between 10 and 33°C, but the oviposition rate is heavily reduced at the high and low end of this temperature range. It does not enter diapause at low temperatures or short days. It seems unable to survive air humidity below 50%.

#### 1.2.1 Associated organisms

Limonica contains no living organisms other than *A. limonicus*.

#### 1.2.2 Original location of *Amblydromalus limonicus*

In the early 1990s the species was collected from California and New Zealand and brought to Europe (Netherlands and England) for mass rearing and use as a biological control agent of thrips and whiteflies (Chorazy et al., 2016; van Houten et al., 1995).

The species has a very wide natural distribution, being reported from New Zealand, Australia, South America, Central America, and North America as well as Hawaii (Knapp et al., 2013). It has also recently established populations in agricultural fields and non-crop vegetation in Catalonia, North Eastern Spain (Chorazy et al., 2016; Escudero-Colomar and Chorazy, 2012).

## 1.3 Properties for use as a plant protection product

*Amblydromalus limonicus* is a generalist predator (McMurtry and Croft, 1997) feeding on whiteflies, thrips, moth eggs and all sorts of mites (Chorazy et al., 2016). It can also feed on and sustain populations on plant food including pollen and phytopathogens. The omnivorous lifestyle enables it to survive on host plants during periods of prey scarcity, which is considered an advantage for biocontrol agents. The extensive use of *A. limonicus* for biocontrol in Central and Southern Europe, and North America, has spurred a flurry of research papers (cited throughout this report) on how to maximize its effectiveness as a biocontrol agent. There is however, limited information on the species' climate tolerance and ecology in natural environments. As wild European populations of *A. limonicus* occurs only in Spain (as far as we know) the use of the species is normally classified as augmentation or inoculation biological control (for definitions of classifications, see Eilenberg et al. (2001).

### 1.3.1 Sensitivity to pesticides

*Amblydromalus limonicus* is moderately sensitive to the carbamate insecticide Pirimicarb, and highly sensitive to spinosad (Fountain and Medd, 2015).

### 1.3.2 Target pests

*Amblydromalus limonicus* is an omnivorous phytoseid (Chorazy et al., 2016; McMurtry and Croft, 1997). It feeds on small arthropods including (but not limited to) mites from the families Tetranychidae (especially *Panonychus ulmi* and *Tetranychus urticae*) and Eriophyidae, as well as thrips (Thripidae), and whiteflies (Aleyrodidae). It also feeds on pollen and some phytopathogenic fungi. Its main target pest is western flower thrips (*Frankliniella occidentalis*).

### 1.3.3 Life cycle of target pests

***Frankliniella occidentalis*:** the western flower thrips, *Frankliniella occidentalis*, Thysanoptera: Thripidae (Pergande) is an important polyphagous pest in vegetables, fruits and ornamentals worldwide (Cloyd, 2009). This thrips inbreeds, so population establishment and spread are not hindered by the necessity of finding mates and population growth is enhanced by offspring being heavily female-biased (Ding et al., 2018). It causes direct damage to the plants and is also an important vector of viruses (Pappu et al., 2009) such as Orthotospovirus (tospovirus). *Frankliniella occidentalis* prefers flowers, if available, but oviposit wherever the thrips is located on the plant. At optimal temperatures (20° C) a female lays up to 40 eggs in a clutch and can produce 350 eggs during her lifetime (females live up to 90 days). Eggs hatch after ca. 4 days and goes through two larval instars, prepupal and pupal stages to finally reach the adult stage. The development time is dependent on temperature (McDonald et al., 1998) and is about 12 days at 30 °C and 20 days at 20°C. Adult females are active at least between

10 and 35 °C and the species can have up to five generations outdoors in the UK (McDonald et al., 1998) and considerably more generations in warmer areas. In greenhouses, they have multiple and overlapping generations. The two first instar stages and the adults feed on plant tissues and cause damage (Plantevernleksikonet.no).

***Trialeurodes vaporariorum***: the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) is an important polyphagous pest on herbs and woody plants. In greenhouses it attacks tomatoes, cucumbers and ornamentals, preferring younger leaves. It has four larval instars, where only the first is mobile. Asexual reproduction is common, and the females can reproduce after 1-2 days. Oviposition rate varies with host plant, temperature and density of conspecifics, while developmental time is temperature sensitive and survival is host plant specific. Nymphs and adults die after a few days at -3° C, while eggs can survive for 15 days at this temperature (Plantevernleksikonet.no).

***Bemisia tabaci***: the silverleaf whitefly, *Bemisia tabaci* (Grenadius) is a polyphagous pest, but is in Norway mainly found on imported poinsettia in greenhouses. The duration of its life cycle depends on temperature and host plant. Optimal temperature is 33° C, but it can complete its lifecycle at 15° C. It causes similar damage to *T. vaporariorum*, but can also act as a vector for several plant viruses.

***Tarsonemidae***: thread-footed mites or white mites are common on many fruit trees. Their diversity and biology in Norway are basically unknown, but in morphology they resemble strawberry mites, a common pest in strawberries (Plantevernleksikonet.no).

***Tetranychidae***: spider mites are a diverse group of mites with five life stages. Their development varies with temperature, host plant and mite species. Under dry and warm conditions, they can complete their life cycle in 10-12 days. Most species have sexual reproduction (Plantevernleksikonet.no).

## 1.4 Status in Norway

*Amblydromalus limonicus* has not been recorded in Norway. The only records in the wild in Europe are from north-eastern Spain. It is listed on the European and Mediterranean Plant Protection Organisation (EPPO) list of commercially used biological control agents (EPPO 2019; PM6/003(4)).

## 2 Data collection and literature search

Literature searches were performed in Medline, ISI Web of Science and Scopus. These databases were chosen to ensure comprehensive study retrieval. The literature search was performed by senior librarians at the Norwegian Public Institute of Public Health on 25.06.2020.

The main searches resulted in a total of 44 records after duplicates were removed, both automatically and during primary screening of the Endnote bibliography (Appendix I). In the primary screening, titles and abstracts of all publications retrieved were independently screened against the inclusion criteria.

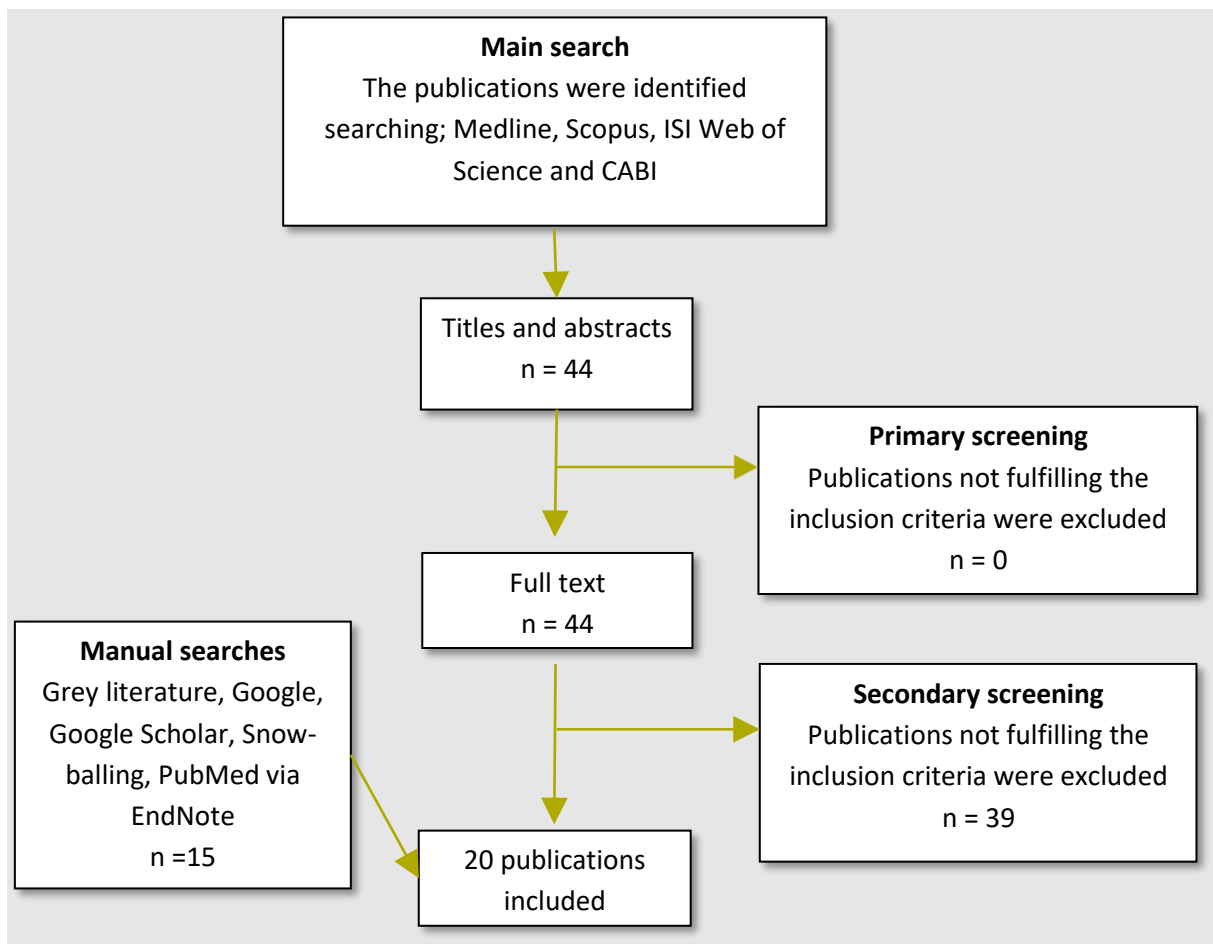
### 2.1 Inclusion and exclusion criteria:

- Inclusion criteria:
  - Publication type – primary research studies, review papers, systematic reviews, editorials and meeting abstracts addressing livestock/aquaculture, veterinary medicine or basic research with indirect applicability.
- Exclusion criteria:
  - Publications addressing other species or *A. limonicus* under environmental conditions not relevant for Norway.
  - Not relevant for answering the questions stated in the terms of references

Screenings and quality assessment of papers were performed independently by each member of the project group. Potential disagreements were solved in the project group.

Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study or not it was retained for further screening. Full text articles that passed the primary screening were retrieved and compared against the inclusion criteria and assessed for relevance and quality.

In order to strengthen the knowledge basis of the opinion, additional manual searches for papers and relevant grey literature were also performed. Manual searches included snowballing, i.e. checking articles that were referred to in papers found in the main literature, searches via Google, Google Scholar, and PubMed via EndNote. The manual searches resulted in 15 relevant papers and documents included in the opinion (Figure 1.)



**Figure 1.** Flowchart for the literature search on *A. limoniucus*

# 3 Risk characterisation

## 3.1 Occurrence and distribution in Norway

We have found no documentation that *A. limonicus* has been detected in Norway. The lack of reports does not necessarily mean that the organism is not present in Norway. However, considering the warm and humid climate in north-eastern Spain, the only place in Europe where *A. limonicus* has been observed in the wild we consider it unlikely that *A. limonicus* is present in Norway.

## 3.2 Potential for establishment and dispersal

### 3.2.1 Climatic limitations

*Amblydromalus limonicus* is native to areas with a warmer winter climate than Norway. It also seems to have strong humidity requirements as no eggs hatch below 50% relative humidity (McMurtry and Scriven, 1965). In Europe the only records from the wild are from north-eastern Spain (temperate climate with hot and dry summers). Despite its widespread use as a biocontrol agent in greenhouses also further north in Europe (e.g. Southern Sweden (Skytte af Sättra, 2013)) *A. limonicus* has not been found in the wild elsewhere in Europe. In Spain the species established before it was approved as a biocontrol agent, which suggest firstly that it was used before approval, and secondly that it dispersed from sites where it was used. However, the species seems unable to enter diapause, a common feature of mites originating from sub-tropical regions (van Houten et al., 1995, and references therein), suggesting it will not be able to survive Norwegian winters outside protected conditions (e.g. greenhouses).

Experimental data on effects of climatic factors on survival and life history traits of *A. limonicus* are limited, but one laboratory study (McMurtry and Scriven, 1965) showed that the oviposition rate is substantially reduced already at 10 °C. The application for approval of Limonica states that the species is effective between 13 and 30 °C. Therefore, it is unlikely that the species will be able to establish in Norway under current climatic conditions, particularly during winter. However, there is no information about its potential to survive freezing temperatures.

### 3.2.2 Other factors affecting survival

The availability of food is unlikely to constrain the survival of *A. limonicus* in Norwegian orchards or wild habitats as the species is omnivorous, feeding on a range of prey, as well as pollen (Knapp et al., 2013; McMurtry and Scriven, 1965).

### 3.2.3 Reproduction

*Amblydromalus limonicus* reproduces sexually, but there is little published data on its life



history traits, specifically the number of successive matings needed to maximize female reproductive success (Samaras et al., 2015). Its generation time is unknown, but females can produce 0.1-~4 eggs per day depending on temperature, humidity and food source (Knapp et al., 2013).

### 3.2.4 Means of dispersal

Mites are wingsless and long-distance dispersal depend on wind or vectors (e.g. humans). There are no data suggesting that long-distance dispersal by wind is common, and generally we expect *A. limonica* to have limited capability to disperse from e.g. greenhouses to wild habitats.

## 3.3 Taxonomic challenges

It is the opinion of VKM there are no taxonomic problems concerning the identification of *A. limonicus*.

Knapp et al. (2013) states that *Amblydromalus limonicus* was described as *Amblyseius limonicus* by Garman and McGregor (1956) from citrus trees in California. The following names and synonyms can be found in the literature: *Typhlodromus (Amblyseius) limonicus* Chant; *Amblyseius (Typhlodromalus) limonicus* Muma; *Typhlodromus imonicus* Hirschmann; *Amblyseius (Amblyseius) limonicus* Wainstein; *Typhlodromalus limonicus* DeLeon; *Typhlodromalus garmani* Chant and *Typhlodromalus rapax* (De Moraes et al., 2004). According to Steiner and Goodwin (2005) *A. limonicus* is suspected to be the senior synonym of *Amblydromalus lailae* (Schicha), so far only reported from Australia. Braun et al. (1993) described a similar phytoseiid mite from tropical South America and named it *Amblyseius limonicus* sensu lato. Detailed morphological examination and cross breeding experiments have later led to the definition of this as a separate species now named *Amblydromalus manihoti* (Chant and McMurtry, 2005). As written above, this mite has been reported under a number of names which now are known to be synonyms of *A. limonicus*. With one exception (*A. manihoti*), it is clear that these are all the same species. However, *A. manihoti* is a tropical mite and thus not going to create any taxonomical challenges Norway.

The family Phytoseiidae is, however, a not very well studied group of organisms. It is therefore likely that the genus *Amblydromalus* could contain several unknown species yet to be described. Considering the species *A. limonicus* wide distribution in America and Australasia, it is likely to be physiological and ecological variations across the species distribution range, which could imply also the species consist of several (cryptic) species. The product Limonica may however contain a stable strain of *A. limonicus*.

## **3.4 Health hazards**

### **3.4.1 Human health**

Some mites, including *Amblyseius cucumeris* which is approved for use in Norway, can sometimes cause allergic reactions in sensitive individuals, especially after long-term exposure to the organisms (de Jong et al., 2004). However, to the best of our knowledge there are no published studies reporting adverse effects of *A. limonicus* to humans.

### **3.4.2 Animal health**

*Amblydromalus limonicus* is omnivorous (Chorazy et al., 2016; McMurtry and Croft, 1997) Chorazy, A., et al. 2016) and is known to feed on all sorts of mites and other small arthropods (Chorazy et al., 2016; McMurtry and Croft, 1997). There are no reports of *A. limonicus* negatively affecting other animal populations, although intra-guild predation is possible.

### **3.4.3 Potential for damage to plants**

*Amblydromalus limonicus* is omnivorous and can feed on several plant tissues including pollen of different plant species (McMurtry and Scriven, 1965). There are however, no reports of direct damage to plants caused by *A. limonicus*.

# 4 Uncertainties

## 4.1 Summary of uncertainties

The main uncertainties with regards to *A. limonicus* are related to its temperature tolerance and thus its ability to survive Norwegian winters. The species has never been reported in Norway and the closest occurrence in the wild is north-eastern Spain. The exact lower temperature thresholds for survival, development, and reproduction are not known.

# 5 Conclusions (with answers to the terms of reference)

All conclusions are attached with uncertainty due to lack of relevant information regarding climatic suitability.

## **5.1 Distribution, especially if the organism is found naturally in Norway**

The predatory mite *A. limonicus* has not been observed in Norway. In Europe, it has been observed in the wild only in north-eastern Spain and does not enter diapause under unfavourable conditions. It is therefore the view of VKM that it lacks the ability to survive and establish in areas with cold winters and chilly summers, as found in most parts of Norway under current climatic conditions.

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

The thermal preferences of *A. limonicus* restrict its establishment and the species has not been observed in Norway. The species seems to have very limited activity level below 13 °C and is not capable of entering diapause when facing unfavourable conditions. It is therefore the opinion of VKM that it is very unlikely that *A. limonicus* will be able to establish outdoors in Norway. However, the lack of information on temperature tolerance of the species constitutes an uncertainty factor. The risk of spread from greenhouses is low because dispersal by wind or biological vectors is unlikely to carry the mites from the greenhouse to suitable outdoor habitats.

## **5.3 Ambiguities regarding taxonomy that hamper risk assessment**

There are no taxonomic challenges related to the assessment of *A. limonicus*.

## **5.4 Assessment of the product and the organism with regard to possible health risk**

VKM is unaware of reports where harm to humans by *A. limonicus* itself or associated pathogenic organisms have been observed. Mites may however produce allergic reactions in sensitive individuals handling plant material with a high number of mites.

## 6 Data gaps

Some of the uncertainties mentioned in chapter 4 depends on lack of data, especially data on the lower temperature limitation of *A. limonicus*.

## 7 References

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- Plantevernleksikonet.no

# Appendix I

## AMBLYDROMALUS LIMONICUS

**Kontaktperson:** Micael Wendell  
**Søk:** Ragnhild Agathe Tornes  
**Dublettsjekk i EndNote:** Før dublettkontroll: 108  
Etter dublettkontroll: 44

**Database:** Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) 1946 to June 23, 2020

**Dato:** 25.06.2020

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**Database:** Web of Science

**Dato:** 25.06.2020

**Antall treff:**

# 1	40	TOPIC: ("amblydromalus limonicus")
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**Database:** Scopus

**Dato:** 25.06.2020

**Antall treff:** 38

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**Database:** Cinahl

**Dato:** 25.06.2020

**Antall treff:** 0



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**Database:** Cochrane Database of Systematic Reviews  
Issue 6 of 12, June 2020,  
Cochrane Central Register of Controlled Trials  
Issue 6 of 12, June 2020  
**Dato:** 25.06.2020  
**Antall treff:** 0

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**Antall treff:** 0

"amblydromalus limonicus"