VKM Report 2021: 16

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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 four VKM members and a project manager from the VKM secretariat and one student practicant. Two 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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VKM would like to thank the referees Dr. Bjørn Arild Hatteland (NIBIO) and Dr. Maria Viketoft (SLU) 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.

Table of Contents

Summary

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, biological control, Nematodes, *Phasmarhabditis californica, Moraxella osloensis.*

Parasitic nematodes and associated bacteria are increasingly being used for biocontrol of molluscs. Functionally, it is the bacteria that kill and thus control the targeted pests, but the function of the bacteria is dependent on the nematodes, which should be regarded as vectors of biocontrol. Although the nematodes and the bacteria have a symbiotic relationship within such biocontrol formulations, it should be noted that they are not dependent on each other in the wild, but can establish separate populations which can be free-living or hosted by other organisms.

The biocontrol product Nemaslug 2.0 contains the nematode *Phasmarhabditis californica* (strain P19D) and the bacterial symbiont *Moraxella osloensis* (unknown strain). The nematode was first described in 2016 and has never been reported in Norway. The lack of reports suggests that it is absent from Norway, but this conclusion comes with a high degree of uncertainty since there have been limited search efforts. The climatic thresholds of the nematode are not known, but its current distribution, spanning widely varying climates, suggests that it could survive and establish in Norway. Natural spread from currently known areas of establishment to natural habitats in Norway is ruled out due to the nematode's limited dispersal capacity. However, human-assisted spread (e.g. via the use of biocontrol products) and establishment would be likely if Nemaslug 2.0 is allowed for use in open fields in Norway. Use of Nemaslug 2.0 in greenhouses and other enclosed areas is not likely to facilitate spread to natural habitats in Norway provided that residues are properly handled. However, deposition of product residues from greenhouses to outdoor areas may result in local establishment of the nematode in the vicinity of the deposition.

Phasmarhabditis californica has a broad host range and may parasitize both rare/endangered and common mollusc species. However, there is no scientific evidence suggesting that the nematode can affect natural populations of molluscs in wild habitats, or otherwise have negative effects on biodiversity. The nematodes' association with the bacteria *Moraxella osloensis* is most likely lost, or at least weakened, in natural habitats, suggesting that the nematode becomes less capable of killing its hosts in the wild. *Phasmarhabditis californica* is not capable of harming or infecting humans.

The bacterial species *Moraxella osloensis* is already present in Norway in a few locations and at a low abundance, and it may be native to Norway. Little is known regarding its distribution in natural environments, but the literature shows that it can infect humans and other mammals. In humans with immunodeficiency or other comorbidities, *M. osloensis* can cause meningitis, vaginitis, sinusitis, bacteremia, endocarditis, and septic arthritis. The risk of infection in people handling Nemaslug 2.0 can probably be substantially reduced by

protective clothing and appropriate handling. We are not aware of any reported health issues arising from use of the previous version of Nemaslug, which also contains *M. osloensis*.

Different strains of *M. osloensis* are known to vary in their sensitivity to antibiotics, and likely in other traits too. Thus, the lack of information provided about the strain identity and specific characteristics of the strain used in Nemaslug 2.0 generates a high degree of uncertainty regarding its pathogenicity, climate tolerance, sensitivity to antibiotics etc.

Sammendrag på norsk

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, biological control, Nematodes, *Phasmarhabditis californica, Moraxella osloensis.*

Parasittiske nematoder og tilknyttede bakterier blir i økende grad brukt for biokontroll av bløtdyr. Funksjonelt er det bakteriene som dreper og dermed bekjemper skadedyrene, men bakterienes funksjon er avhengig av nematodene som kan betraktes som vektorer for biokontroll. Selv om nematodene og bakteriene har et symbiotisk forhold i slike biokontrollprodukter, bør det bemerkes at de ikke er avhengige av hverandre i naturen, men kan etablere separate populasjoner som kan være frittlevende eller tilknyttet andre organismer.

Biokontrollproduktet Nemaslug 2.0 inneholder nematoden *Phasmarhabditis californica* (stamme P19D) og bakterien *Moraxella osloensis* (ukjent stamme). Nematoden ble først beskrevet i 2016 og har aldri blitt rapportert funnet i Norge. Mangelen på rapporter tyder på at nematoden er fraværende fra Norge, men denne konklusjonen kommer med en stor grad av usikkerhet siden det har vært gjort få undersøkelser av nematoder tilknyttet snegler i Norge. Klimakravene til nematoden er ikke kjent, men dens nåværende utbredelse i områder med svært varierende klima antyder at den kan overleve og etablere seg i Norge. Naturlig spredning fra områder der nematoden er kjent i dag til naturlige habitater i Norge er utelukket på grunn av nematodens begrensede spredningskapasitet. Imidlertid vil menneskelig assistert spredning og etablering (for eksempel gjennom bruk av biokontrollprodukter) være sannsynlig hvis Nemaslug 2.0 tillates brukt utendørs i Norge. Bruk av Nemaslug 2.0 i drivhus og andre lukkede områder vil sannsynligvis ikke føre til spredning til naturlige habitater i Norge forutsatt at planterester og annet avfall håndteres riktig. Imidlertid kan deponering av avfall fra drivhus til utearealer føre til lokal etablering av nematoden i nærheten av deponeringen.

Nematoden *Phasmarhabditis californica* har et bredt vertsspekter og kan parasittere både sjeldne og vanlige bløtdyrarter. Nematodens tilknytning til bakterien *Moraxella osloensis* blir mest sannsynlig brutt, eller i det minste svekket, i naturlige habitater, noe som trolig gjør nematoden mindre i stand til å drepe sine verter i naturen. *Phasmarhabditis californica* er ikke i stand til å skade eller smitte mennesker.

Bakterien *Moraxella osloensis* er allerede til stede i Norge, og den kan være hjemmehørende i Norge. Bakteriens utbredelse i naturlige miljøer er lite kjent, men litteraturen viser at den kan smitte mennesker og andre pattedyr. Hos mennesker som har immunsvikt eller lider av flere sykdommer samtidig kan slike infeksjoner forårsake for eksempel hjernehinnebetennelse, vaginitt, bihulebetennelse, bakteriemi, endokarditt eller septisk artritt. Risikoen for infeksjon hos brukere av Nemaslug 2.0 kan trolig reduseres vesentlig ved bruk av verneklær og hensiktsmessig håndtering. Vi kjenner ikke til noen rapporterte

helseproblemer etter bruk av den forrige versjonen av Nemaslug, som også inneholder *M. osloensis*.

Ulike stammer av bakterien *M. osloensis* er kjent for å ha ulik følsomhet overfor antibiotika, og varierer sannsynligvis også i andre egenskaper. Dette gjør at mangelen på informasjon om hvilken bakteriestamme som er brukt i Nemaslug 2.0 og de hvilke spesifikke egenskaper denne stammen har medfører en høy grad av usikkerhet i våre konklusjoner om viktige egenskaper ved *M. osloensis*, slik som patogenisitet, klimatoleranse, følsomhet overfor antibiotika med mer.

Background and terms of reference as provided by the Norwegian Food Safety Authority

The bacteria *Moraxella osloensis* and its nematode vector organism *Phasmarhabditis californica* (strain P19D) together make up the biocontrol agent Nemaslug 2.0. The intended use for Nemaslug 2.0 in Norway is as a biological control product in horticultural crops in greenhouses, tunnels, outdoors and in gardens.

In this regard, The Norwegian Food and Health Authority hereby ask for an assessment of the following, for both of the organisms *Phasmarhabditis californica* (strain P19D) and *Moraxella osloensis*:

- The prevalence of both species and whether they are naturally occurring in Norway.
- The organisms' potential to survive and spread in the environment under Norwegian conditions, specified for the use in greenhouses and outdoors.
- If the conclusion of the organisms' potential to survive and spread in the environment under Norwegian conditions is uncertain, then the organisms potential negative effects on biodiversity should be assessed.
- The human health risk by using the plant protection product Nemaslug 2.0 and its organisms.
- Uncertainties about the organisms' taxonomy which may complicate the risk assessment.

Regarding the bacteria *Moraxella osloensis* we also would like an assessment whether it has other vectors than *Phasmarhabditis californica*, and if so whether the other vectors occur naturally in Norway.

Methodology and Data

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 15.02.2021 and the 15.04.2021.

The main searches resulted in a total of 247 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 (terms of reference as provided by the Norwegian Food Safety Authority).

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 snow-balling, i.e. checking articles that were referred to in papers found in the main literature, as well as searches via Google, Google Scholar, and PubMed via EndNote.

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 Nemaslug 2.0, with the nematode *Phasmarhabditis californica* (Tandingan de Ley et al. 2016) (strain P19D) and the symbiotic bacterium *Moraxella osloensis* (Bovre and Henriksen 1967). The assessment area for this opinion is Norway.

1.2 Product and trade name

The product Nemaslug 2.0 (BASF Agricultural Specialities) contains individuals of the nematode *Phasmarhabditis californica* (strain P19D) as third stage infective dauers/juveniles. The product also contains the symbiotic bacterium *Moraxella osloensis* (unknown strain).

Functionally, the nematode can be viewed as a vector for biological control, as it carries the symbiotic bacterium that infects and thus control the target organisms. The bacterium should be viewed as the active biocontrol organism (Tan & Grewal 2001; Stenberg et al. 2021), although the mechanism of biocontrol is completely dependent on the vector.

1.2.1 Associated organisms

Nemaslug 2.0 does not contain other organisms than the nematode *P. californica* and the symbiotic bacteria *M. osloensis*.

1.2.2 Natural distribution

1.2.2.1 Phasmarhabditis californica

Phasmarhabditis californica was first discovered in California (Tandingan de Ley et al., 2016) where it is most likely native. Thereafter, it has been found in several parts of the world, including Canada (Brophy et al. 2020), Ireland (Carnaghi et al., 2017), New Zealand (Wilson et al. 2016), South Africa (Pieterse et al 2017), and the UK (Andrus and Rae, 2018). This wide distribution of *P. californica* already within a few years of its detection indicates that the nematode is a cosmopolite or that it has dispersed and established by human assistance.

Previous genetic analyses of *P. hermaphrodita*, a related nematode species used in the first version of Nemaslug, shows that the wide distribution of this species most likely resulted from nematodes establishing in new areas following use of Nemaslug (Howe et al. 2020).

1.2.2.2 Moraxella osloensis

Moraxella osloensis is believed to be widespread and fairly common in most environments. This bacterial species has been identified in natural habitats worldwide, such as soda lakes in Russia (Foti et al., 2008), lake water in Germany (Probian et al., 2003) glacial meltwater on Mount Everest (Liu et al., 2009), Antarctic ice cores (Segawa et al., 2010) river water in Chile (Escalante et al., 2009), as well as in compost in Portugal (Vaz-Moreira et al. 2008). *Moraxella osloensis* has also been identified in household air in the UK (Yuan et al., 2007), restaurants in China (Chan et al., 2009), and surface areas of a public restrooms in the USA (Meschke et al., 2009). *Moraxella osloensis* has further been found in clothes or laundry, even after washing and drying, as well as on various hard surfaces in houses in Japan (Kubota et al., 2012). Thus, it can be regarded as a globally distributed species which is present in diverse environments.

1.3 Properties relevant to its use as a plant protection product

1.3.1 Fundamental biology of the functional organisms

1.3.1.1 Phasmarhabditis californica (partly inferred from *P. hermaphrodita*)

Many details of the biology and life cycle of *Phasmarhabditis californica* are not known but are expected to be similar to those of its close relative *P. hermaphrodita*. Both of these nematode species are bacterial feeders and detritivores living in leaf litter, compost, and organic soils. Here they consume dead invertebrates, slug feces and associated bacteria. They are also facultative parasites of slugs and snails, including those that are pests of agricultural and horticultural plants. However, the taxonomic relationship between the two nematode species is still unresolved.

During periods of food shortage, both nematodes can form developmentally arrested non-feeding juveniles (dauer-juveniles) that move locally in the soil in search for new food sources. When encountering a slug, the dauer-juvenile infects the slug by entering through natural openings at the rear of the slug's mantle. Once inside the body the dauer-juvenile may stay inactive until the slug dies and then molt to regular feeding juvenile stages, which commence their reproductive activities while feeding on bacteria flourishing on the slug cadaver (Wilson and Grewal 2005). The juveniles develop into self-fertilizing female hermaphrodites (males are uncommon or absent) which lay eggs and allow the population to increase. When the cadaver is depleted, new dauer-juveniles are formed which leave in search for new food sources.

For commercial production of Nemaslug 2.0, *P. californica* is reared in monoxenic cultures with the bacterium *Moraxella osloensis*. When the close relative *P. hermaphrodita* infects the slug *Deroceras reticulatum* it has been shown that *M. osloensis,* and not the nematode, is the killing agent (Tan & Grewal 2001). The food base of the nematodes is *M. osloensis* which is vectored by the infective dauer-juveniles. The feeding activity of infected slugs is reduced already from the early stages of the infection and the slugs may die in one to three weeks. The nematodes keep feeding on the cadaver until this food source is depleted.

Experiments in the laboratory have indicated that the body size of the slug host may be an important susceptibility factor to *P. hermaphrodita*, and that younger and smaller slugs are more vulnerable to the nematode and the bacterium. However, the importance of host body size is not known for *P. californica*.

1.3.1.2 Moraxella osloensis

Moraxella osloensis is a gram-negative, oxidase positive, aerobic bacterium within the family Moraxellaceae in the gamma subdivision of the purple bacteria (Vaneechoeutte et al., 2011; Bovre and Henriksen 1967). The bacteria-feeding nematode *Phasmarhabditis californica* acts as a vector that transports *M. osloensis* into the shell cavity of the slug. The bacterium is the killing agent within the nematode-bacterium complex (Tan and Grewal, 2001). *Moraxella osloensis* produces endotoxin(s) that are tolerant to heat and protease treatments and that kill the slug when it is injected into the shell cavity (Tan and Grewal, 2002). Purified lipopolysaccharide (LPS) produced by *M. osloensis* cultures are toxic to slugs, with an estimated 50% lethal dose (LD_{50}) of 48 µg per slug (Tan and Grewal, 2001). Interestingly, mucus from slugs attracts the parasitic nematode *P. hermaphrodita* (Hapca et al., 2007), thus making slugs more exposed to *M. osloensis*.

1.3.2 Sensitivity to chemical and antibiotic control

1.3.2.1 Phasmarhabditis californica

We have not found any studies investigating the effects of nematicides or other agrochemicals on *P. californica*. However, as it is a newly described species that probably has not been much exposed to agrochemicals, we assume that it has never been under selection to tolerate such chemicals. The use of nematicides is prohibited in Norway.

1.3.2.2 Moraxella osloensis

Gram-negative bacteria frequently evolve resistance to antibiotics, and some strains of *M. osloensis* have been shown to be relatively resistant to penicillin and streptomycin (e.g. Hansen et al. 1974). However, Tan & Grewald (2001) showed that co-injection of penicillin and streptomycin reduced the pathogenicity of certain *M. osloensis* strains to the slug, suggesting that at least some bacterial strains are sensitive to antibiotics.

1.3.3 Target pests

The target pests for Nemaslug 2.0 are airbreathing common land slugs in the order Stylommatophora, including dark-face arion (*Arion distinctus*), Spanish slug (*Arion vulgaris*), garden slug (*Arion hortensis*), tramp slug (*Deroceras invadens*) and grey field slug (*Deroceras reticulatum*).

The literature also includes reports of parasitism of Carnana's slug (*Deroceras panormitanum*; Pieterse et al 2017), white garden snail (*Theba pisana*; De Ley et al., 2020), and red slug (*Arion rufus*; Brophy et al., 2020).

1.3.4 Life cycle of target pests

The life span of targeted slug species is between 9 and 18 months. In Norway they typically have an annual life cycle, although some species can produce two generations per year under optimal conditions. For most target species, egg laying typically takes place in the summer or fall (South 1992, Hutchinson et al. 2017, Filipiak et al. 2020, Hatteland unpublished data).

1.4 Regulatory status in Norway

1.4.1 *Phasmarhabditis californica*

The nematode *Phasmarhabditis californica* has never been reported in Norway and is currently not regulated.

1.4.2 *Moraxella osloensis*

Moraxella osloensis is not listed in The Norwegian Labour Inspection Authority report on Biosafety; *Appendix 2: List of classified biological factors (infection risk groups)* (https://www.arbeidstilsynet.no).

Moraxella osloensis is also used in the first version of Nemaslug, which is approved for use in Norway.

2 Risk characterisation

2.1 Occurrence and distribution in Norway

2.1.1 Phasmarhabditis californica

Phasmarhabditis californica has never been reported from Norway (Artsdatabanken 2021, GBIF 2021). The lack of reports may be due to limited search efforts. However, assuming that *P. californica* is native to California and only disperses and establishes in new areas following the use of Nemaslug 2.0, we consider it likely that the lack of reported observations reflects a true absence of this species from Norway. This assumption, however, comes with high uncertainty.

A survey of nematodes associated with terrestrial slugs was conducted in Norway in 2015. The survey identified a total of five nematode species (*Alloionema appendiculatum, Agfa flexilis, Angiostoma limacis, Angiostoma sp.* and *Phasmarhabditis hermaphrodita*), but *P. californica* was not among them (Ross et al., 2015).

2.1.2 Moraxella osloensis

Carriage of *Moraxella* species in humans and human surroundings is quite common (Vaneechoeutte et al., 2011). The most frequent species encountered is *M. catharralis* (Vaneechoeutte et al., 2011). *Moraxella osloensis* may cause osteomyelitis as well as meningitis (Nidal et al., 2017). Case reports involving *M. osloensis* indicate that about 50% of the patients had a predisposing clinical condition (Shah et al., 2000). International reports highlight patients with immuno-deficiencies and patients who are immunosuppressed (Vaneechoeutte et al., 2011; Tiosejo et al., 1988; Shah et al., 2000; Nidal et al., 2017).

As far as we are aware, no survey on the carriage rate of *M. osloensis* has been published from Norway. Furthermore, as any findings of *M. osloensis* would constitute a non-reportable disease there are no available data in the Norwegian Surveillance System for Communicable Diseases (MSIS). However, a small examination of blood culture findings was made from patients at Oslo University Hospital – Ullevål during 2010–2014. Each year, ~1000 separate episodes of bacterial presence were encountered in a patient's bloodstream. Searching for *M. osloensis* in this archive, we found 0-1 cases per year. All age groups were represented among the cases with *M. osloensis* infection, and some of these probably had a disposing condition. Some of the patients were acutely hospitalized and some presumably suffered from respiratory tract infection and were diagnosed while hospitalized. Based on this limited data set we found no increase in the annual number of blood-stream findings between 2019 and 2021 compared to the period 2010-2014.

The natural distribution of *Moraxella osloensis* in natural environments in Norway is not well known. However, as *M. osloensis* is the active organism also in the first version of Nemaslug, which has been used in Norway for many years, we assume that *M. osloensis* is present in Norwegian nature with a wide distribution. For general information about the occurrence of *M. osloensis* in natural environments, see section 1.2.2.2.

2.2 Potential for spread, establishment and dispersal

2.2.1 Climatic limitations

2.2.1.1 Phasmarhabditis californica

The climatic limitations (including temperature and humidity) of *P. californica* have never been studied. However, the known distribution of this nematode covers both cold (e.g. Canada, Brophy et al. 2020) and warm (e.g. South Africa, Pieterse et al. 2017) areas, suggesting that it is tolerant to temperatures that are more extreme than those found in Norway. Although climate change may lead to warmer/dryer summers and milder winters in Norway, we find it unlikely that such changes will affect the nematode's potential for establishment and spread.

Phasmarhabditis californica lives most of its life in the soil (except during host infection). Below-ground environments are typically more stable than above-ground conditions, thus sheltering the nematodes from extreme high or low temperatures. Generally, nematodes prefer a humid environment.

2.2.1.2 Moraxella osloensis

The climatic limitations of *Moraxella osloensis* have not been systematically investigated. However, aspects of tolerance to abiotic conditions have been investigated for some strains, some of which showed high tolerance to dry conditions, UV light irradiation and desiccation stress relative to *Escherichia coli* and *Staphylococcus aureus*. The optimal growth temperature for *M. osloensis* is reported to range from 33 to 37 °C under laboratory conditions (Public Health England 2015), but some strains can tolerate temperatures up to 42 °C (Welch & Maxcy, 1975).

2.2.2 Dispersal ability

2.2.2.1 Phasmarhabditis californica

Phasmarhabditis nematodes, as well as their slug hosts, disperse slowly unless they are vectored by humans (MacMillan et al. 2009). However, *P. hermaphrodita* seems to have spread extensively through the use of Nemaslug and become established in several areas around the world (Howe et al. 2020). This suggests that establishment following biocontrol may be possible also for *P. californica*. In addition, as slugs can be consumed by various predators, including some birds (Rae et al. 2005), we cannot exclude that predators may transport nematodes inside slugs and thereby facilitate nematode spread. However, this is highly speculative, partly because survival in the gut of predators may be limited.

The strain of *P. hermaphrodita* used in the first version of Nemaslug has severely restricted movement within sandy loam soil, and we expect this to be the case also for the closely related species *P. californica*. This limited movement restricts the nematode's ability to actively seek out host prey within soil (MacMillan et al. 2009). Both upward and downward dispersal of *P. hermaphrodita* in the soil is increased by the presence of the earthworm *Lumbricus terrestris*, which is common in Norway (Sveistrup et al. 1997). In the case of *P. hermaphrodita*, its use of earthworms as a natural carrier may give the nematode access to lower soil levels or to areas where potential slug hosts may be present, thus enhancing its capacity to spread.

2.2.2.2 Moraxella osloensis

Moraxella osloensis is carried (and probably spread) by humans (see section 2.1.1.2) and other mammals.

The occurrence of *M. osloensis* in streaming water (see section 1.2.2.2) suggests that this bacterial species also can disperse passively by water.

We cannot exclude the possibility that the dauer-juveniles of *Phasmarhabditis* nematodes contain and spread the bacterium. However, we have no indication that *M. osloensis* is being vertically transmitted successfully to new generations of nematodes that emerge from dead slugs, or that wild populations of *Phasmarhabditis* dauer-juveniles retain *M. osloensis* after they parasitize slugs. It therefore seems unlikely that *P. californica* nematodes that emerge from slugs in the wild would retain the bacterium and disperse it to new slug hosts. In any case, vectoring by nematodes is probably of minor importance for natural spread of the bacterium in the wild.

2.2.2.3 Preventive measures

As *Phasmarhabditis* nematodes seem to have a potential for establishing in almost all areas worldwide the only sure way to prevent their establishment in Norway would be to refrain from introducing them in outdoor soils. However, as the nematodes' active dispersal is limited, greenhouse use would still be possible if residues from greenhouses where Nemaslug has been used are sanitized before they come in contact with the natural environment. To prevent dispersal from greenhouses one could also make sure that residues on the floor are not spread via e.g. shoes to outdoor areas (e.g. by sanitizing the shoes or switching shoes when leaving the greenhouse).

2.3 Taxonomic challenges

2.3.1 Phasmarhabditis californica

Morphological identification of the nematode is based on female characters (Tandingan De Ley et al. 2016) and can be done under a light microscope. However, molecular identification using ribosomal RNA is recommended (Tandingan De Ley et al. 2016).

2.3.2 Moraxella osloensis

Hansen et al. (1974) reported contrasting responses to antibiotic treatments in different strains of *Moraxella osloensis*, suggesting intra-specific variability in some important traits. Because we have no information about which strain of *M. osloensis* that is used in Nemaslug 2.0 we cannot say if this strain has any differences in e.g. cell wall composition that may affect its virulence.

2.4 Health hazards

2.4.1 Human health

2.4.1.1 Phasmarhabditis californica

There is no scientific evidence suggesting that the nematode *P. californica* inflicts any health hazards to humans.

2.4.1.2 Moraxella osloensis

Moraxella osloensis may be isolated from the respiratory tract of healthy humans. Although *M. osloensis* is a rare human pathogen, it has been found to cause several diseases and infections such as meningitis, vaginitis, sinusitis, bacteremia, endocarditis, and septic arthritis (Bilyk & Moghrabi, 2020). Patients with cancer, leukemia, as well as organ transplant recipients and other immunocompromised individuals, may be susceptible to *M. osloensis* infection (Maruyama et al., 2018).

Carriage of *Moraxella spp.* in humans and human surroundings is quite common (Vaneechoeutte et al., 2011). The most frequently encountered species is *M. catharralis* (Vaneechoeutte et al., 2011). *Moraxella osloensis* does also occur, but probably quite rarely (Vaneechoeutte et al., 2011; Bovre and Hendriksen 1967; Zhou, 2020; Berger and Falsen, 1976; Walls and Wald, 2005). Case reports indicate that more than 50% of the patients that are infected by this unusual pathogen have predisposing factors (Shah et al., 2000).

Moraxella osloensis carriage/infections in humans occur at a low level in Norway. Various organ systems may be infected (Bennett et al., 1995; Berger and Falsen, 1976; Bovre and

Hendriksen, 1967; Butzler 1974; Hapca et al., 2007; Maruyamaa et al., 2018; Nidal et al., 2017; Shah et al., 2000; Strojnik 2020; Tiosejo et al., 1988; Walls and Wald 2005; Vaneechoeutte et al., 2011).

2.4.1.3 Possible measures for health risk mitigation

Nemaslug 2.0 is mixed with water before it is applied to the soil. Users of the product could use protective clothing and glasses to reduce the risk of splash reaching e.g. the eyes.

2.4.2 Animal health and biodiversity

2.4.2.1 Phasmarhabditis californica

In general, *P. californica* is believed to have a broad host range and, until proven otherwise, we assume that it can parasitize many slug species that are present in Norway. However, in controlled bioassays Andrus et al. (2019) showed that the nematode strongly discriminates between different host species, suggesting that, in practice, some slug species will probably not be parasitized by *P. californica* in the wild.

Very few studies have yet investigated potential side effects of *P. californica* on non-target slugs. In the first study undertaken in Europe, Carnaghi et al. (2017) found that it may parasitize the EU-protected slug *Geomalacus maculosus* in the field in Ireland. However, parasitism did not increase the mortality of *G. maculosus*.

Data provided by BASF (the producer of Nemaslug 2.0) do not give reasons to expect adverse side effects on other organism groups than slugs. Studies on birds and mammals carried out in the 1990's, using the first version of Nemaslug (with *Phasmarhabditis hermaphrodita* as a vector), showed no lasting adverse effects.

Although the limited existing data do not show alarming effects of Nemaslug on non-target species one should note that introductions of exotic species can have unforeseen, and sometimes very serious, effects on biodiversity (reviewed by Mack et al. 2000). Such negative effects could potentially include outcompeting native niche nematodes and reducing native populations of slug species (which carry out important ecosystem services related to decomposition of organic material). Such negative effects may not become evident until several years after introduction. Thus, with the limited information available today it is not possible to completely exclude negative consequences of introducing *P. californica* to Norway. See also Chapter 3 (Uncertainties) for further elaboration on this topic.

2.4.2.2 Moraxella osloensis

Some *Moraxella* species have been found to inhabit the conjunctival eye sac of clinically healthy horses (Johns et al., 2011), dogs (Wang et al., 2008), and rabbits (Cooper et al., 2001), but it is unknown if this also is true for *M. osloensis*. *Moraxella osloensis* has been detected in living organisms such as insects (Yamoah et al., 2008) and in the oral cavity and eyes of cats (Büttner et al., 2019), as well as in kidney tissue of the Chinook salmon (Evans & Neff, 2009). *Moraxella osloensis* has also been identified in animal products such as beef (Takahashi et al., 2008), milk and cheese (Delbès et al., 2007). These reports indicate that *M. osloensis* can be present in many animals without necessarily causing infection. Pathogenicity and virulence of *M. osloensis* in mammals seems to require co-existing trauma or immune system disturbance that facilitate host infection.

2.4.3 Potential for damage to plants

The nematode *P. californica* does reportedly not feed on live plants. The bacterium *M. osloensis* does not infect plants.

3 Uncertainties

Several of the conclusions in this report are uncertain due to lack of data about the natural distribution of *Phasmarhabditis californica* and *Moraxella osloensis*, as well as their tolerance to abiotic stress (e.g. temperature and drought). In addition, the uncertainty about the strain identity of *M. osloensis* makes it difficult to evaluate the pathogenic potential of Nemaslug 2.0 to humans and other animals, as well as the bacterium's sensitivity to antibiotics.

The nematode *P. californica* was described as late as in 2016. Thus, little is known about its general biology and ability to infect and harm non-target species in Norway. A more robust analysis of the potential of *P. californica* to harm non-target species and biodiversity can only be carried out in a number of years from now, assuming that in-depth primary research is carried out in the meantime. However, clues about potential negative effects of *P. californica* can be obtained much earlier by investigating effects of the related nematode *P. hermaphrodita* (used in the first version of Nemaslug) on non-target species and biodiversity in Norway.

Due to the lack of specific data, some of our assumptions regarding the basic biology of *P. californica* are based in the closely related species *P. hermaphrodita*. These assumptions have some degree of uncertainty as the biological similarities between the two nematode species are yet to be confirmed.

There have been almost no surveys for *P. californica* or *M. osloensis* in the natural environment in Norway. This implies that there is a high degree of uncertainty relating to their potential distribution in Norway.

The sensitivity of *P. californica* to agrochemicals used in Norway is completely unknown.

4 Conclusions (with answers to the terms of reference)

4.1 The prevalence of both species and whether they are naturally occurring in Norway

The nematode *Phasmarhabditis californica* has never been reported in Norway. The lack of reports suggests that it is absent from Norway, but this conclusion comes with a high degree of uncertainty due to limited search efforts.

The bacteria *Moraxella osloensis* is present in Norway. It occurs regularly in humans but at a low incidence. Little is known about the distribution of *M. osloensis* in the natural environment in Norway.

4.2 The organisms' potential to survive and spread in the environment under Norwegian conditions, specified for the use in greenhouses and outdoors

Although no experimental data is available regarding the abiotic limitations of the nematode *Phasmarhabditis californica*, we conclude that it probably can survive and establish in most parts of Norway. This conclusion is based on recent establishments in areas with similar environmental conditions as Norway in Canada and the UK. The natural spread of the nematode is considered to be slow, but human-assisted spread (e.g. via use of Nemaslug 2.0) is likely to lead to its establishment in most parts of Norway where the product is used. Provided that its natural spread is limited, the nematode is not likely to spread naturally from greenhouses to wild habitats. However, deposition of soil and other substrate from greenhouses to outdoor areas may result in local establishment in the vicinity of such depositions.

The bacterial species *Moraxella osloensis* is already present in humans in Norway and is probably present in various natural habitats throughout Norway, thus showing that it can establish and spread under Norwegian environmental conditions. The bacterium is considered to be relatively tolerant to abiotic stress such as drought and UV radiation.

4.3 If the conclusion about the organisms' potential to survive and spread in the environment under Norwegian conditions is uncertain, then the organisms' potential negative effects on biodiversity should be assessed

The nematode *Phasmarhabditis californica* has a broad host range and is known to parasitize at least one EU-protected mollusc. However, parasitism does not necessarily kill the non-target slug in this case. In general, available data is very limited, but do not suggest that this nematode would constitute a major threat to biodiversity in Norway. However, the lack of data concerning potential negative effects of the nematode on non-target species and biodiversity implies a high degree of uncertainty.

The nematode *P. californica* is likely exotic to Norway, and introduction of exotic species can sometimes lead to unforeseen and serious consequences that become evident only several years after the introduction. This uncertainty can ideally be reduced by initiating new primary research on environmental effects of similar species under Norwegian conditions. Clues about potential negative effects of *P. californica* could be obtained by analyzing how the related nematode *P. hermaphrodita* (which was introduced to Norway several years ago with the previous version of Nemaslug) has affected non-target species and biodiversity in Norway.

4.4 The human health risk by using the plant protection product Nemaslug 2.0 and its active organisms

There is no evidence suggesting that the nematode *Phasmarhabditis californica* can infect or harm humans.

The bacterium *Moraxella osloensis* may infect humans in various ways, but mostly in patients with predisposing clinical conditions. The lack of knowledge regarding the exact bacterial strain used in Nemaslug 2.0 causes a high degree of uncertainty regarding its pathogenic potential in humans.

The risk of infection to users of Nemaslug 2.0 can probably be substantially reduced by protective clothing and appropriate handling. We are not aware of any reported health issues arising from use of the previous version of Nemaslug, which also contains *M. osloensis*.

4.5 Uncertainties about the organisms' taxonomy which may complicate the risk assessment

The nematode *Phasmarhabditis californica* is well defined taxonomically and can be identified using molecular and morphological traits. However, it is not known to what degree environmental and ecological adaptations differ between strains.

By contrast, we did not receive information about the specific strain of *Moraxella osloensis* that is used in Nemaslug 2.0. This is a serious weakness, as different strains may have different pathogenic potential and differ in their sensitivity to antibiotics and abiotic stress. Although the species *M. osloensis* is widespread in Norway, it is not known if the specific strain used in Nemaslug 2.0 is present in, or native to, Norway.

4.6 Other vectors of *Moraxella osloensis* in Norway

Moraxella osloensis is widespread in Norway and occurs in many environments, including various animals, although almost all reported observations are from humans. Due to lack of data we cannot determine if any particular animal species are important for natural spread of *M. osloensis* in Norway.

5 Data gaps

Several of the uncertainties highlighted in this report (see Chapter 3) are due to lack of data about the natural distribution of the two species used in Nemaslug 2.0, as well as their tolerance to abiotic stress (e.g. temperature and drought), and effects on non-target species and biodiversity. The lack of biological data for *P. californica* is a fundamental data gap, but is not surprising given that this species was described as late as in 2016. Screenings of both species in the natural environment are needed to determine their distribution with a lower degree of uncertainty. Experimental investigations of the tolerance levels of both species to high/low temperatures and drought are needed to more accurately model their ability to establish in different areas of Norway.

Potential negative effects of *P. californica* and *M. osloensis* on non-target species and biodiversity are yet to be studied, both in Norway and internationally. Data on potential negative effects on e.g. non-target slugs (via parasitism) and native niche nematodes (via competition) would be crucial to evaluate the consequences of using Nemaslug 2.0 outdoors in Norway.

In addition, identification and characterization of the specific *M. osloensis* strain used in Nemaslug 2.0 is needed to more precisely evaluate its pathogenic potential to humans and other animals, as well as its sensitivity to antibiotics.

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Appendix I

Literature search strategy

PHASMARHABDITIS CALIFORNICA

Kontaktperson:	Per Hans Micael Wendell
Søk:	Ragnhild Agathe Tornes
Dublettsjekk i EndNote:	Før dublettkontroll: 30 Etter dublettkontroll: 24

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to February 01, 2021>**

Dato: 03.02.21

Antall treff: 1

1	"phasmarhabditis californica".tw,kf.	1

Database: **Embase**

Dato: 03.02.21

Antall treff: 1

1	"phasmarhabditis californica".tw,kw.	1

Database: **Web of Science**

Dato: 03.02.21

Antall treff: 4

# 1	4	TS="phasmarhabditis californica" Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years

Database: **Scopus**

Dato: 03.02.21

Antall treff: 24

1	"phasmarhabditis californica"	24 document results

Database: **Cinahl**

Dato: 03.02.21

Antall treff: 0

S1	TI ("phasmarhabditis californica") or AB ("phasmarhabditis californica")	0

Database: **Cochrane Database of Systematic Reviews**

Issue 2 of 12, February 2021

Cochrane Central Register of Controlled Trials

Issue 2 of 12, February 2021

Dato: 03.02.21

Antall treff: 0

#1	"phasmarhabditis californica":ti,ab	0

Database: **Epistemoniskos**

Dato: 03.02.21

Antall treff: 0

"phasmarhabditis californica"

Moraxella osloensis

Kontaktperson:	Per Hans Micael Wendell
Søk:	Marita Heintz
Dublettsjekk i EndNote:	Før dublettkontroll: 531 Etter dublettkontroll: 223

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to February 01, 2021>**

Dato: 15.04.21

Antall treff: 102

1	"Moraxella osloensis".tw,kf.	102

Database: **Embase**

Dato: 15.04.21

Antall treff: 131

1	"Moraxella osloensis".tw,kw.	131

Database: **Web of Science**

Dato: 15.04.21

Antall treff: 106

# 1	106	TS="Moraxella osloensis" Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years

Database: **Scopus**

Dato: 15.04.21

Antall treff: 184

1	"Moraxella osloensis"	184 document results

Database: **Cinahl**

Dato: 15.04.21

Antall treff: 8

S1	TI ("Moraxella osloensis") or AB ("Moraxella osloensis")	8

Database:	**Cochrane Database of Systematic Reviews**	
	Issue 4 of 12, April 2021	
	Cochrane Central Register of Controlled Trials	
	Issue 3 of 12, March 2021	
Dato:	15.04.21	
Antall treff:	0	

#1	"Moraxella osloensis":ti,ab	0

Database:	**Epistemoniskos**
Dato:	15.04.21
Antall treff:	0

"Moraxella osloensis"