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Risk assessment of *Anagyrus vladimiri* as a biological control agent in the product Citripar

Johan A. Stenberg, Daniel Flø, Lawrence R. Kirkendall, Anders Nielsen, Selamawit Tekle, Beatrix Alsanius, Jorunn Børve, Paal Krokene, Christer Magnusson, Mogens Nicolaisen, Line Nybakken, May-Guri Sæthre, Iben M. Thomsen, Sandra A. I. Wright

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

VKM assessed potential environmental risk associated with use of the plant protection product Citripar containing the parasitic wasp *Anagyrus vladimiri* against mealybugs (family Pseudococcidae) in Norway. No observations of *Anagyrus vladimiri* have been reported from Norway. It is very unlikely that it will be able to establish or spread in Norway due to the absence of hosts and too low winter temperatures. Therefore, it is likely that the wasp will not affect local biodiversity.

VKM Report 2025: 01
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Cover photo: Collage by VKM with image *Anagyrus pseudococci* (female) by Ian Jacobs CC 2.0 and image of mealy bug from Mostphotos.

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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 three VKM members and two project managers from the VKM secretariat. Two referees commented on and reviewed the draft opinion. The Committee, by the Panel on Plant health, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2023). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and the VKM Panel on Plant health.

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Acknowledgement

VKM would like to thank the referees Peter Dalin, Swedish Environmental Protection Agency, and Mattias Jonsson, Swedish University of Agricultural Sciences, 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 the routines of VKM for approval of risk assessments (VKM, 2024), VKM received the referee comments prior to 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 regarding conflicts of interest apply to all work prepared by VKM.

Table of Contents

Summary

Citripar, a biological plant protection product containing the parasitic wasp *Anagyrus vladimiri,* is requested to be approved for use in Norway. The product is intended to be used against mealybugs, particularly *Planococcus citri* (citrus mealybug) and *Planococcus ficus* (vine mealybug) feeding on fruits, berries, vegetables and herbs in greenhouses and plastic tunnels, and on indoor plants. The Norwegian Food Safety Authority, therefore, asked the Norwegian Scientific Committee for Food and Environment to perform a risk assessment of the product.

Occurrence and distribution in Norway: No observations of *Anagyrus vladimiri* have been reported from Norway.

Potential for establishment and spread: VKM assesses that *Anagyrus vladimiri* will not be able to establish and spread in Norway under current conditions due to the absence of host organisms and too low winter temperatures, even in the warmest parts of the country.

Potential effects on biodiversity: VKM assesses that *Anagyrus vladimiri* will not affect biodiversity in Norway, as there are currently no known native hosts for the wasp to parasitize.

Taxonomic challenges that may affect the risk assessment: *Anagyrus vladimiri* belongs to the wasp family Encyrtidae, a family that includes the genus *Anagyrus*, many of which have quite tangled taxonomic histories. Individuals of what is now known as *Anagyrus vladimiri* were for many years identified as belonging to *Anagyrus pseudococci. Anagyrus pseudococci* and *A. vladimiri* are members of a complex of nearly indistinguishable species that are informally referred to as the *Anagyrus pseudococci* complex: *A. pseudococci*, *A. vladimiri*, *A. kamali*, *A. dactylopii*, *A. kivuensis*, and *A. callidus*. These species have been used for biological control of various mealybug species. Should incorrectly identified *Anagyrus* be imported to Norway, there would be no consequences for biological diversity, since the other species in the *Anagyrus pseudococci* complex are also host specific to mealybug genera that are not found in the Norwegian fauna, and they are physiologically unfit for the current Norwegian climate.

Key words: Mealybugs, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food and Environment, Parasitoid wasps

Sammendrag

Citripar, et biologisk plantevernmiddel med snyltevepsen *Anagyrus vladimiri*, er søkt godkjent for bruk i Norge. Produktet er ment å brukes mot ullskjoldlus, spesielt artene *Planococcus citri* og *Planococcus ficus,* på frukt, bær, grønnsaker og urter i veksthus og plasttunneler, samt på innendørs planter. Mattilsynet har derfor bedt Vitenskapskomiteen for mat og miljø om å utføre risikovurdering av produktet.

Forekomst og utbredelse i Norge: Det er ikke rapportert noen observasjoner av *Anagyrus vladimiri* fra Norge.

Potensial for etablering og spredning: VKM vurderer at *Anagyrus vladimiri* ikke kan etablere og spre seg i norsk natur under dagens forhold på grunn av mangel på vertsorganismer og for lave vintertemperaturer, selv i de varmeste delene av landet.

Potensielle effekter på biologisk mangfold: VKM vurderer at *Anagyrus vladimiri* vil ikke ha noen effekt på biologisk mangfold i Norge, da det ikke finnes kjente hjemmehørende vertsorganismer vepsen kan parasittere.

Taksonomiske utfordringer som kan påvirke risikovurderingen: *Anagyrus vladimiri* tilhører familien Encyrtidae, en familie som inkluderer slekten *Anagyrus*, hvor mange arter har en ganske komplisert taksonomisk historie. Individer av det som nå er kjent som *Anagyrus vladimiri* ble i mange år identifisert som tilhørende arten *Anagyrus pseudococci*. *Anagyrus pseudococci* og *A. vladimiri* tilhører et kompleks av arter som nesten er umulige å skille fra hverandre og som uformelt refereres til som *Anagyrus pseudococci*-komplekset: *A. pseudococci*, *A. vladimiri*, *A. kamali*, *A. dactylopii, A. kivuensis* og *A. callidus*. Alle disse artene har blitt brukt til biologisk bekjempelse av ulike arter av ullskjoldlus. Skulle feilidentifiserte *Anagyrus*-veps importeres til Norge, ville det ikke ha noen ytterligere konsekvenser for biologisk mangfold, da arter som *A. vladimiri* kan forveksles med også er vertsspesifikke til slekter som ikke finnes i norsk fauna, og heller ikke er fysiologisk tilpasset det nåværende norske klimaet.

Nøkkelord: Mattilsynet, snylteveps, ullskjoldlus, Vitenskapskomiteen for mat og miljø

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

Citripar is a new product that contains the new macroorganism *Anagyrus vladimiri*. The intended use is as a biological insecticide in crops in greenhouse and tunnel and indoor plants. In this regard, The Norwegian Food Safety Authority would like an assessment of the following:

- The fate and behaviour in the environment and the ecotoxicological effects and risks regarding the use of *Anagyrus vladimiri* as a plant protection product. More specifically, the committee is asked to make an assessment on the occurrence of this organism in Norway. If this organism is not found naturally in Norway, an assessment must be made on the potential for this organism to survive and spread in the environment under Norwegian conditions. The committee is also asked to make an assessment on the target species specific under Norwegian conditions. The risk to non-target organisms under Norwegian conditions should be assessed.
- Uncertainty about taxonomy which can have an influence on the risk assessment.

1 Introduction

1.1 Purpose and scope

This opinion presents a risk assessment of the parasitoid wasp *Anagyrus vladimiri* as a biological control agent in the product Citripar. The opinion was prepared as a response to a request from the Norwegian Food Safety Authority. The assessment area for this opinion is Norway.

1.2 The biological control agent and product

1.2.1 Taxonomy of the wasp

Kingdom Animalia, Phylum Arthropoda, Subphylum Hexapoda, Class Insecta, Order Hymenoptera, Family Encyrtidae, Genus *Anagyrus*, Species *Anagyrus vladimiri*

Anagyrus vladimiri Triapitsyn, 2019 is a parasitoid wasp in the family Encyrtidae, a family of parasitoid wasps, comprising more than 1,700 species in the Palearctic region (Noyes, 2019). Worldwide, over 400 species of Encyrtidae have been or are now being used for biological control of scale insects (species in the superfamily Coccoidea; in Norwegian *skjoldlus*) and of jumping plant lice (Psyllidae) (*sugere*; Soler et al., 2021). The family Encyrtidae includes the genus *Anagyrus*, 23 species of which are recorded in Europe, many of which have quite tangled taxonomic histories (Soler et al., 2021).

1.2.2 The product Citripar

The product Citripar is shipped to buyers in 100 ml bottles with parasitized host insects hatching 500 wasps. *Anagyrus vladimiri* is produced on live citrus mealybugs (*Planococcus citri*) and is supplied as parasitized mealybug mummies in sawdust.

1.2.3 Distribution of Anagyrus vladimiri

*Anagyrus vladimir*i is an Old World species which has been spread widely via biological control programs and by imported plants hosting the mealybugs that the wasp parasitizes. The known distribution of *A. vladimiri* is summarized in Andreason et al. (2019; see also Triapitsyn et al., 2007). Confirmed records are from Israel, Italy, Russia, Spain, Turkmenistan, and the USA. There are older records of the similar species, *A. pseudococci* (Girault, 1915) from many other countries, many of whose records are likely to refer to be *A. vladimiri* (see section 3.4).

1.3 Properties relevant to its use as a biological control agent

1.3.1 Life cycle

Anagyrus vladimiri is a solitary endoparasitoid of juvenile mealybugs (nymphs). Much of its biology and life cycle has been studied and published under the name *A. pseudococci* (Daane et al. 2004, Avidov et al. 1967; see Andreason et al., 2019 for the corrected taxonomy). The female parasitoid oviposits a single egg into the third nymphal stage of the mealybug host. The survival and development time of the parasitoid larvae are highly temperature dependent (Daane et al. 2004), with no survival below 14 °C and above 36 °C. The parasitoid egg hatches after 1–2 days. The insect develops through three larval stages and a prepupal stage, before pupating within the host. The latter subsequently mummifies (Daane et al. 2004). The total development time from egg to adult ranges from ca. 10 days at 34 °C to 79 days at 14 °C. After pupation, the adult parasitoid chews an exit hole and emerges from the mummy. Adults with access to nectar resources live for approximately 1 week, depending on temperature.

1.3.2 Target organisms

Anagyrus vladimiri and its *Anagyrus* relatives only parasitize mealybugs (Pseudococcidae). Within the species complex that includes it*, A. vladimiri* appears to be comparatively generalized with respect to host use. A laboratory study of the host spectrum found that *A. vladimiri* successfully reproduced in all three Pseudococcidae host genera (*Planococcus, Pseudococcus* and *Phenacoccus*) to which it was presented (Bugila et al., 2013, 2014a). Four species of Pseudococcidae have been registered outdoors in Norway (Fjelddalen 1996), but as far as we know none of these are native to Norway; pseudococcids only occur in greenhouses, polytunnels and similar production systems, or on imported plants (see also: https://www.plantevernleksikonet.no/l/oppslag/23/).

The European and Mediterranean Plant Protection Organization (EPPO) considers *A. vladimiri* to be a safe biological control agent for use in the EPPO region (EPPO, 2021). As a biological control agent, *A. vladimiri* has been used primarily against the vine mealybug *Planococcus ficus* (Signoret), the most damaging mealybug of wine and raisin grape crops worldwide, and *Planococcus citri* (Risso), one of the most important pests of citrus crops. These two *Planococcus* species are considered to be the primary hosts of *A. vladimiri*, which is considered to be the main parasitoid of *P. citri* (Molina et al. 2024). (Note that *P. ficus* recently has been shown to be a species complex consisting of two species, one originating from the western and one from the eastern Mediterranean: Danne et al., 2018; Correa et al., 2023). *Anagyrus vladimiri* is attracted to the sex pheromones of its *Planococcus* hosts (Franco et al., 2007) Other hosts of *A. vladimiri* include the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) and another citrus mealybug, *Delottococcus aberiae* De Lotto (Tena et al., 2018; Andreason et al., 2019; but see Tena et al., 2016). In Italy, *A. vladimiri* has been reared from the Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Guerrieri & Pellizzari, 2009) and recently shown to be a potential biological control agent for this invasive mealybug from China (Ricciardi et al., 2021). The citrophilus mealybug *Pseudococcus calceolariae* (Maskell) is yet another citrus pest and one of the most common species on ornamental plants in Europe (Pellizzari & Germain, 2010); it, too, is parasitized by *A. vladimiri* (Suma et al., 2012).

1.3.3 Sensitivity to chemical pesticides

Several studies have investigated the effects of pesticides on *Anagyrus* spp. Some studies show increased mortality of *A. pseudococci* that have fed on nectar containing the neonicotinoid insecticide imidacloprid (Krischik at al., 2007). Contact toxicity from the butanolide insecticide flupryadifurone, the neonicotenoid acetamiprid, and fungicide spiroxamine resulted in 100% mortality of adult wasps (Mansour et al. 2023). The pyrethroid insecticides lambda-cyhalothrin and diamide insecticide cyantraniliprole did, however, appear to have a limited effect on the species (Mansour et al., 2023). Mansour et al. (2011) found that the organophosphate insecticide chlorpyriphos-methyl caused 100% mortality of *Anagyrus* sp. near *pseudococci* (the name used for *A. vladimiri* before it had been formally described), while the biopesticide Prev-Am® and the systemic keto-enol insecticide spirotetramat had neglectable effect on the parasitoid.

Molina et al. (2024) investigated the effects on adult *A. pseudococci* mortality and offspring production of six systemic pesticides—acetamiprid (neonicoteniod), dimethoate (organophosphate), cyantraniliprole (diamide), flupyradifurone (butanolide), spirotetramat (keto-enol) and sulfoxaflor (sulfoximine). The pesticides were either ingested directly by the parasitoid or indirectly via feeding on nectar of buckwheat plants that had been watered with a pesticide solution. All six pesticides negatively affected *A. vladimiri* by one or both routes of ingestion. Singh et al. (2023) studied the mortality of *A. vladimiri* by application of pesticides commonly used for killing mites—spirodiclofen (tetronic acid), spirotetramat (keto-enol), sulfur, fenpyroximate (pyrazole), and abamectin (avermectin). Only abamectin and fenpyroximate caused significant mortality of the wasps (the latter only if sprayed directly on them).

Moreover, certain pesticides are applied in vineyards specifically to control ants whose tending behavior increases the severity of mealybug infestations. Mgocheki & Anderson (2009) found that fipronil (phenylpyrazole) and a-cypermethrin (pyrethroid) caused acute toxicity to "*Anagyrus* sp. near *pseudococci*" (syn. *A. vladimiri*) and to a second encyrtid parasitoid in the study, *Coccidoxenoides perminutus* (Timberlake).

Based on the above reports, VKM concludes that species of *Anagyrus* are sensitive to many insecticides. Integrated pest management regimes should carefully select insecticides that are to be used in combination with products based on *A. vladimiri*.

2 Data and literature search

Literature searches were performed in ISI Web of Science and Scopus, by using the search terms “*Anagyrus vladimiri*” and “*Anagyrus pseudococci*”. Searching for “*Anagyrus* sp. nr. *pseudococci*” and “*Anagyrus* sp. near *pseudococci*”, the names used for *A. vladimiri* before it was formally described, did not add any new references. The literature searches were performed on September 30, 2024. The searches resulted in a total of 75 records after removing duplicates. Publications addressing other species, research conducted under environmental conditions not relevant to Norway, and publications that did not address the questions in the terms of references were excluded. Screening and quality assessment of publications were performed independently by each member of the project group. In addition, manual searches were also performed, including “snowballing”, i.e., checking articles that were referred to in papers found in the main literature list, as well as searches in Google Scholar.

3 Hazard identification and characterization

3.1 Occurrence and distribution in Norway

There are no reported observations of *Anagyrus vladimiri* in Norway, nor in neighboring countries.

3.2 Potential for establishment and spread

Anagyrus vladimiri reproduces by parasitizing mealybugs (family Pseudococcidae) but appears to be relatively generalized within this group of hosts (Bugila et al., 2013, 2014a, b). As far as we know, there are no Pseudococcidae in the native Norwegian fauna, but non-native pseudococcids can be found in greenhouses, polytunnels and similar production systems, and on imported plants (Fjelddalen, 1996) (see also: https://www.plantevernleksikonet.no/l/oppslag/23/).

Temperatures under which *A. vladimiri* can develop from egg to adult have been shown to span from 11.6 °C to 36.0 °C (optimal temperature = 24.7 °C), but overwintering individuals seem to be able to survive temperatures as low as -5°C (Daane et al. 2004). However, a semi-field experiment in Switzerland (Gilliéron et al., 2024) showed that no adults survived, and no pupae hatched when exposed to mean daily temperatures of 2.7 °C and 3.7 °C respectively. Eggs exposed to ambient Swiss winter temperatures did not hatch at all (Gilliéron et al., 2024).

VKM concludes that *A. vladimiri* will likely not be able to establish and spread in Norwegian nature, due to the absence of host organisms and too low winter temperatures, under current climate conditions, even in the warmest parts of the country.

3.3 Potential effects on biodiversity

VKM assesses that *Anagyrus vladimiri* would not affect native biodiversity in Norway under current conditions, as there are no known native or naturalized hosts that it can parasitize. Adults feed on nectar and honeydew, but VKM does not see any potential for competition with native pollinators (VKM, 2024). Mass-reared *A. vladimiri* reportedly have a much higher prevalence of bacteria (mainly *Wolbachia*) and viruses as compared to wild individuals (Izraeli, 2023), but to what extent pathogens carried by *A. vladimiri* would constitute a threat to Norwegian species is beyond the scope of this report.

3.4 Taxonomic challenges

Individuals of what is now known as *Anagyrus vladimiri* were identified for many years as belonging to the widely used biological control agent *Anagyrus pseudococci*. Triapitsyn et al. (2007) documented morphological and DNA sequence differences between populations of true *A. pseudococci* and a new species temporarily designated as "*Anagyrus* sp. near *pseudococci*" and found that the two species were reproductively isolated. The new species was later formally described as *A. vladimiri* Triapitsyn, 2019 (in Andreason et al., 2019). The best morphological feature separating *A. vladimiri* from *A. pseudococci* is a color difference in the first funicle segment of the female antenna (e.g. Soler et al., 2021)—a difference ascribed to intraspecific variation before the work of Triapitsyn et al. (2007).

Anagyrus pseudococci and *A. vladimiri* are members of a complex of nearly indistinguishable species that are informally referred to as the *Anagyrus pseudococci* complex: *A. pseudococci*, *A. vladimiri*, *A. kamali* Moursi, 1948; *A. dactylopii* (Howard, 1898); *A. kivuensis* Compere, 1939;

and *A. callidus* Triapitsyn, Andreason & Perring, 2019 (Andreason et al., 2019). All these species have been used for biological control of various mealybug pests (Andreason et al., 2019). Projects that have reared what they believed to be *A. pseudococci* for biological control have been bedeviled by the difficulties of correctly identifying the wasps, especially since closely related species that are nearly identical in appearance can be reared from the same hosts (e.g. Triapitsyn et al., 2007; Andreason et al., 2019). Molecular tools exist now, enabling producers to properly identify their species (Triapitsyn et la, 2007; Andreason et al., 2019; Correa et al., 2023). Nonetheless, should incorrectly identified *Anagyrus* wasps be imported to Norway, there would be no additional consequences for biological diversity in Norway, as species with which *A. vladimiri* could easily be confused are also host specific to genera not present outdoors in Norway (Andreason et al., 2019). All *Anagyrus* species are also physiologically poorly adapted to the Norwegian climate, since they are originally from much milder and warmer climates.

4 Uncertainties

Both observational and experimental studies from other countries have shown that *A. vladimiri* is unable to survive Norwegian winter conditions (Daane et al. 2004, Gilliéron et al. 2024), and no native host species are found in Norwegian nature. Despite the absence of studies carried out in Norway, our conclusions come with minimal uncertainty.

5 Conclusions (with answers to the terms of reference)

5.1 Occurrence in Norway

There are no reported observations of *Anagyrus vladimiri* in Norway, nor in its neighboring countries.

5.2 Potential for establishment and spread, if the organism does not occur naturally in Norway

VKM assesses that *Anagyrus vladimiri* will not be able to establish and spread in Norwegian nature under current conditions due to the absence of host organisms and too low winter temperatures, even in the warmest parts of the country.

5.3 Target species specificity and risk to non-target organisms under Norwegian conditions

Anagyrus vladimiri can only parasitize mealybugs (Pseudococcidae). Thus, VKM assesses that *Anagyrus vladimiri* would have no negative effects on biodiversity in Norway, as there are no known native hosts that could be parasitized by the wasp.

5.4 Uncertainty about taxonomy which may influence risk assessment

Though it is difficult to correctly identify parasitoid wasps of the genus *Anagyrus*, VKM sees no uncertainty that would influence the present risk assessment (see section 3.4).

6 Data gaps

There are no data gaps that have an effect on the risk assessment.

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