

Rapid Communication

First detection of invasive Douglas fir needle midges from the genus *Contarinia Rondani* (Diptera: Cecidomyiidae) in Switzerland

Simon Blaser, Beat Ruffner, Jana Mittelstrass, Vivianne Dubach and Valentin Queloz

Forest Health and Biotic Interactions, Federal Research Station for Forest, Snow and Landscape, WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

Corresponding author: Simon Blaser (simon.blaser@wsl.ch)

Citation: Blaser S, Ruffner B, Mittelstrass J, Dubach V, Queloz V (2023) First detection of invasive Douglas fir needle midges from the genus *Contarinia Rondani* (Diptera: Cecidomyiidae) in Switzerland. *BioInvasions Records* 12(4): 951–964, <https://doi.org/10.3391/bir.2023.12.4.08>

Received: 2 April 2023

Accepted: 4 August 2023

Published: 5 October 2023

Handling editor: Andrea Vannini

Thematic editor: Stelios Katsanevakis

Copyright: © Blaser et al.

This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](#)).

OPEN ACCESS

Abstract

Since 2015, North American Douglas fir needle midges of the genus *Contarinia Rondani* (Diptera: Cecidomyiidae) are known to spread across Europe. In 2022, these gall-inducing insects were detected for the first time in Switzerland, where their morphological identification was confirmed by DNA barcoding. Conducting a visual monitoring of Douglas fir stands in northern Switzerland, the current distribution range was delimitated and revealed the presence of Douglas fir needle midges in forest areas close to the borders of France and Germany, where their establishment has been reported earlier. Genetic analyses of five representative populations further proved the presence of two different mitochondrial haplotypes, of which one was only found in the westernmost population, indicating faint population structuring. When assessing co-occurrence of Douglas fir needle midges with other pests and pathogens originating from North America, Douglas fir stands were often found to be infested also by the insect *Adelges cooleyi* (Gillette) (Hemiptera: Adelgidae) and the fungus *Nothophaeocryptopus gaeumannii* (T. Rohde) Videira, C. Nakashima, U. Braun, Crous (Mycosphaerellales: Mycosphaerellaceae). The observed abundance of the newly established midges within the invaded area and their co-occurrence with other introduced pests and pathogens are an indication of the increasing biotic stress level on Douglas fir, which should be taken into account when considering Douglas fir as a potential future tree species for European forests.

Key words: Invasive forest insects, biological invasion, *Pseudotsuga menziesii*, monitoring, DNA barcoding

Introduction

Forest ecosystems are facing increasing challenges due to the impacts of climate change (Bennett et al. 2015; Gazol et al. 2022). Effects of this human-induced process include increasing temperatures along with more frequent and intensive weather extremes such as heat periods and droughts (Bennett et al. 2015; Seidl et al. 2017). Beside direct effects of these weather extremes such as defoliation and tree mortality, reduced water-availability can indirectly affect plants by reducing their defense mechanisms against disturbances such as infestations by pathogens and pests (Seidl and Kautz 2022).

One of the suggested solutions to maintain forest ecosystem services in a warmer and drier climate is the transition towards forest ecosystems composed of climate-adapted tree species. This can be achieved by combining natural regeneration and plantation of additional endemic and exotic tree species with elevated tolerance to drought (Pluess et al. 2016; Gazol et al. 2022). In Central Europe, Douglas fir (*Pseudotsuga menziesii* (Mirbel, 1825) Franco, 1950) is discussed as an attractive future tree species due to its remarkable growth performance and because it is well adapted to severe annual summer droughts in some areas of its natural distribution range in North America (Spiecker and Schuler 2019; Wohlgemuth et al. 2021).

First introduced from North America to Europe during the 19th century, Douglas fir nowadays accounts for the second most cultivated exotic conifer species in Europe covering approximately 0.8 million hectares of the forest area (Van Loo and Dobrowolska 2019). In Swiss forests, Douglas fir represents the second most common alien tree species with a stem share of 0.18% per hectare (Brändli et al. 2020; Frei et al. 2022).

Insect damages on Douglas firs are still moderate compared to those of other conifer species introduced to Europe (Roques et al. 2019). Host-switches from native insects may be prevented or slowed down by the phylogenetic distance of Douglas fir to native conifer tree species (Roques et al. 2006, 2019). However, since the arrival of the Douglas fir in Europe two centuries back, several insect pests and pathogens from the native distribution range have followed their host plant and successfully established in Europe such as the “Cooley spruce gall adelgid” *Adelges cooleyi* (Gillette, 1907) (Hemiptera: Adelgidae), and *Nothophaeocryptopus gaeumannii* (T. Rohde, 1938) Videira, C. Nakashima, U. Braun, Crous, 2017 (Mycosphaerellales: Mycosphaerellaceae), the fungal agent causing “Swiss needle cast” (Roques et al. 2006; Kimberley et al. 2011). *Adelges cooleyi* was introduced to Europe in the 1930’s (Essl et al. 2002) and *N. gaeumannii* was first identified in Switzerland in 1925 (Gäumann 1930). With the recent detection of Douglas fir needle midges (DFNMs) from the genus *Contarinia* (Rondani, 1860) (Diptera: Cecidomyiidae) in Western Europe, the list of Douglas fir associated insect pests introduced from North America has been again extended (Wilson et al. 2020).

In North America, three gall-inducing midge species on Douglas fir needles are described: *Contarinia pseudotsugae* (Condrashoff, 1961), *C. constricta* (Condrashoff, 1961), and *C. cuniculator* (Condrashoff, 1961) (Condrashoff 1961a). Out of the three species, *C. pseudotsugae* is reported to show the highest abundance, as well as the biggest damage potential in the native distribution area (Condrashoff 1962). Adult DFNMs are colored orange and have a body length of approximately 2 mm (Condrashoff 1961a), the life history and behavior of the three univoltine species are similar and described in detail by Condrashoff (1962).

Symptoms of an infestation by DFNMs become obvious from approximately August, when galls are formed due to the feeding activity of the larvae in

the current year's needles, which get discolored, frequently bent, and distorted (West et al. 1991). Due to the impaired needle surface as well as the premature drop of the infested needles, the photosynthesis potential of Douglas firs may be negatively affected (Condorshoff 1962; Ligot et al. 2020). In case Douglas firs are heavily infested for several consecutive years, they can suffer from considerable twig dieback (Condorshoff 1962; EPPO 2019). Combined with other pests and pathogens, severe attacks by DFNMs can cause significant defoliation (Wilson et al. 2020). In North America, *C. pseudotsugae* represents a destructive pest of Christmas tree plantations and can cause substantial economic damage by impeding tree aesthetics (West et al. 1991; Wilson et al. 2020). Generally not described as a mortality-inducing factor, recovery of heavily infested trees may take several years (West et al. 1991; EPPO 2019).

Adult DFNMs are capable to fly, but a gap of knowledge about their actual natural spreading capacity exists (EPPO 2019). It is presumed that the dissemination pathways include anthropogenic activities such as the movement of Douglas fir planting material, cut branches and Christmas trees (EPPO 2019). Another pathway is represented by the movement of soil containing larval or pupal development stages (EPPO 2019).

In Europe, *C. pseudotsugae* was detected simultaneously in Belgium, France, and the Netherlands in 2015 (Leroy et al. 2015; Département de la santé des forêts 2016; NPPO of the Netherlands 2016). One year later, the species was also recorded in Germany (Hielscher 2017). Additionally, it has been suspected that with *C. cuniculator*, a second DFMN species from North America could have arrived in the Netherlands (NPPO of the Netherlands 2016; EPPO 2019). However, because the morphological discrimination between the three closely related *Contarinia* species developing in Douglas fir needles is extremely challenging and due to the lack of species-specific DNA barcodes, the definitive confirmation of the presence of *C. cuniculator* in Europe is still missing (EPPO 2019).

Until now, there is not much information available about the current and expected damage potential of DFNMs in European forest (EPPO 2019). However, heavy infestations of Douglas fir seedlings and saplings with significant needle loss were recently reported from the Ardennes region of Belgium, where results of a long-term study also suggest a negative effect of DFNMs on the growth rate of Douglas fir saplings. In addition, their ability to outcompete saplings of other tree species such as Norway spruce seems to be negatively impaired by the presence of DFNMs (Ligot et al. 2020; Wilson et al. 2020).

In late summer 2022, the presence of DFNMs was detected for the first time in Switzerland. After this first discovery in the Canton of Basel-Land during phytosanitary evaluations of experimental plantations with exotic tree species, the current distribution area of the DFNMs in the North of Switzerland was delimited in the framework of a visual monitoring including

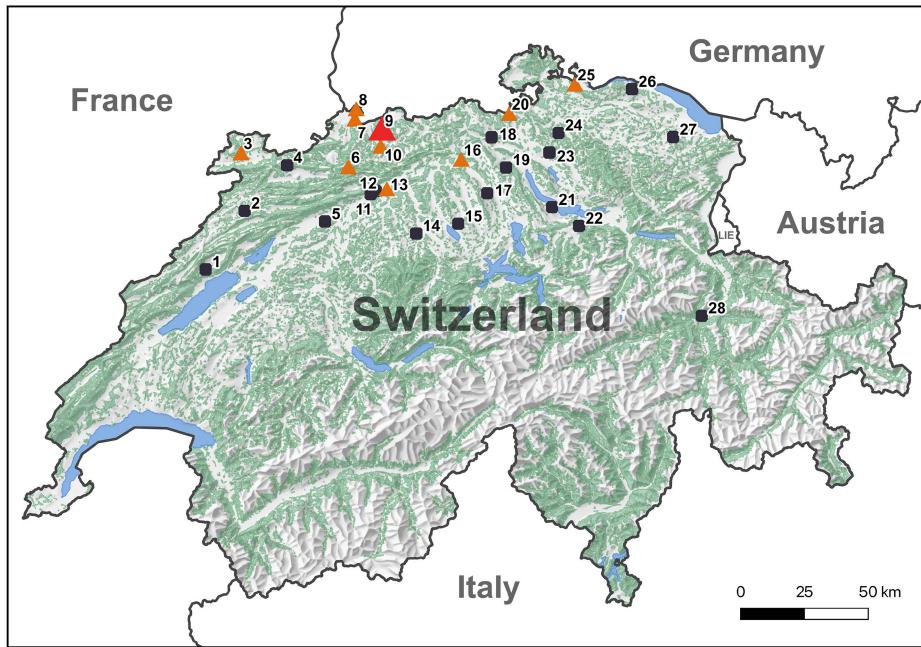


Figure 1. Monitoring locations in northern Switzerland controlled for presence of Douglas fir needle midges (DFNMs) of the genus *Contarinia*. Douglas fir stands located closer than 2 km to each other were considered as one monitoring location. The red triangle visualizes the site, where DFNMs were detected for the first time in Switzerland. Orange triangles represent monitoring locations where presence of DFNMs has been recorded, black squares indicate monitoring locations where DFNMs could not be detected. Detailed geographic information of the numbered monitoring locations is provided in Table S1. Forest areas are marked in green. LIE: Principality of Liechtenstein. © EuroGeographics for the administrative boundaries.

45 Douglas fir stands at 28 monitoring locations. Identification at the genus level was confirmed by DNA barcoding and genetic variability within and between five DFNM populations from different geographic regions has been assessed studying the mitochondrial cytochrome c oxidase I (COI) marker (Hebert et al. 2003). The first finding, as well as the results of the monitoring and genetic analyses are reported in the framework of this publication.

Materials and methods

Sampling and morphological identification

Monitoring was conducted in 2022 from August 15 to November 10 before onset of the first frost. Needles of youngest Douglas fir shoots were visually assessed for DFNM characteristic infestation symptoms such as discoloration and deformation (Condrashoff 1961b, 1962). To control needles of older trees, shoots were harvested using a pole shear and/or a ladder. Suspected shoots were stored in zip bags at 4 °C for further analyses. Monitoring locations (Figure 1) were identified using repository data from the Swiss Forest Protection group (WSL), Douglas fir distribution data published by Frei et al. (2021), as well as information from local foresters. Douglas fir stands within a range of 2 km were considered as one monitoring location to facilitate visualization. Detailed geographic information of the monitoring is provided in Supplementary material Table S1. Morphological identification

of infested needles and larvae was performed according to the key of (Condrashoff 1961b) using a stereomicroscope SteREO Discovery V8 (Zeiss, Göttingen, Germany). Co-occurrence of *A. cooleyi* was determined based on presence of insects (Albrecht 2017) and/or feeding symptoms on needles (chlorotic flecking and needle kinking) (Parry 1978).

Molecular genetic analysis of Douglas fir needle midges

From each of the monitoring locations no. 3, 8, 13, 20, and 25 (Figure 1), a total of 6 DFNM larvae were isolated from infested shoots by dissecting Douglas fir needles with sterile scalpels and toothpicks. Total genomic DNA was extracted from larvae using the NucleoSpin® Tissue XS Kit (Macherey-Nagel, Düren, Germany) according to manufacturer's instruction. DNA was diluted tenfold to amplify and sequence the COI barcode region with primers LCO1490 and HCO2198 (Folmer et al. 1994). Sequences were trimmed and assembled using the CLC Main Workbench Version 22.0.2 and checked manually before blasting against the "all barcode" database on BOLD (Ratnasingham and Hebert 2007). All sequences generated in this study are deposited on BOLD (dx.doi.org/10.5883/DS-PHPCON) and GenBank (Sayers et al. 2023), individual accession numbers are declared in Table S2. Sequence data of the COI barcode region were analyzed using the NGphylogeny.fr workflow PhyML+SMS (Lemoine et al. 2019). For phylogenetic comparisons, initial sequence selection was based on the 100 best matches by BLAST searches performed on NCBI (Sayers et al. 2023). Additionally, sequences from the databases BOLD and NCBI with concrete assignment of the genus *Contarinia*, as well as sequences of the Cecidomyiini tribe from the phylogenetic study of gall midges by Dorchin et al. (2019) were included. Except for the two representative sequences Hap1 and Hap2 generated in this study, the dataset was finally refined by excluding sequences generating an average branch length distance less than 0.01 nucleotide substitutions per site. Origin, identity with sequence of most common haplotype found in Switzerland (Hap1), and accession numbers of sequences used for the phylogenetic analyses are declared in Table S3.

Molecular genetic detection of N. gaeumannii

From each of five needles randomly chosen from one Douglas fir stand, a piece of the middle part measuring approximately 5 mm was cut. Needle pieces of one Douglas fir stand were subsequently pooled in one 2 ml Eppendorf tube. Before DNA extraction, needle samples were lyophilized and homogenized using a Retsch MM 200 ball mill (Retsch, Haan, Germany). Total genomic DNA was extracted using the Kingfisher 96 Flex (Thermofisher) and sbeadex plant kit (Biosearch Technologies, Hoddesdon, United Kingdom), according to an optimized protocol for needle samples (Schneider et al. 2019). To detect *N. gaeumannii*, a multiplex quantitative real-time PCR

(qPCR) with a specific primer pair targeting the beta tubulin gene and the corresponding dual-labelled probe was performed (Winton et al. 2002). Cycling conditions were adapted for using the qPCR Takyon core kit NO ROX (Eurogentec, Seraing, Belgium) as described by Stemmelen et al. (2023). The universal 18S primer pair with the corresponding dual-labeled probe 18S uni-P (Ioos et al. 2010) served as DNA quality control. All samples were run in triplicates on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, Zug, Switzerland).

Documentation

A map displaying monitoring locations was generated using QGIS version 3.16 based on free geodata from swisstopo (Wabern, Switzerland) and EuroGeographics (Brussels, Belgium). Pictures of Douglas fir needles and DFNM-larvae were documented using stereomicroscope SteREO Discovery.V8 (Zeiss, Göttingen, Germany) with camera Axiocam 506 color (Zeiss) and software ZEN 3.1 (Zeiss).

Results

First record and monitoring

Douglas fir needles infested with DFNM larvae were detected for the first time in Switzerland on 15 August 2022 in Arisdorf (Canton of Basel-Land) at monitoring location no. 9 during the evaluation of experimental plantation plots containing saplings of different coniferous and deciduous tree species (Figure 1, Table S1). After the first record, a total of 45 Douglas fir stands at 28 monitoring location in northern Switzerland were visually controlled for symptoms of DFNMs (Figure 1). Thereby, 17 Douglas fir stands at 10 monitoring locations ranging between an altitude of 271.7 m and 911.7 m above sea level were found to be infested by DFNM larvae (Figure 1, Table S1). The identified distribution area was mostly contiguous and extended mainly along the northern border of Switzerland (Figure 1). Monitoring location no. 3 in Porrentruy (Canton of Jura) was found to be the westernmost DFNM-infested Douglas fir stand, whereas monitoring location no. 13 in Neuendorf (Canton of Solothurn) and monitoring location no. 25 in Stammheim (Canton of Zurich) represented the southernmost, resp. easternmost DFNM-infested Douglas fir stands (Figure 1).

Discoloration of DFNM galls was visible on both upper and lower needle surface, the observed color range included light green, yellow, red, and brown (Figure 2A, B). No distinct dark spots were found in center of galls. Partially, needles were swollen or bent at site of injury (Figure 2A, B). Isolated larvae were colored white or light yellow (Figure 2C), lobes of sternal spatula were rather broad and bullet-shaped (Figure 2D). Per shoot, up to 36% of the youngest needles were found to be DFNM-infested (data not shown).

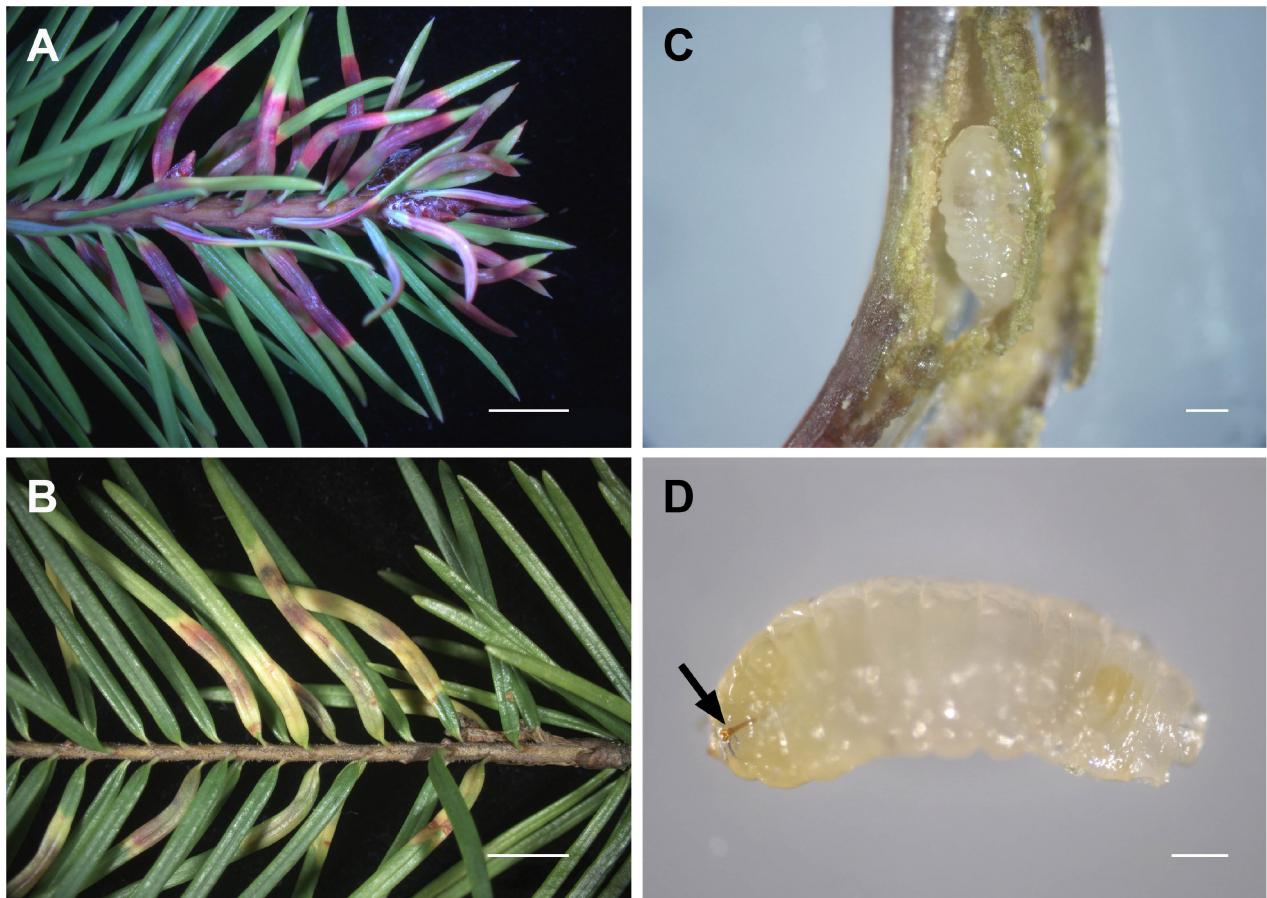


Figure 2. (A, B) Current year's needles with galls caused by the feeding activity of DFNM larvae. (C) DFNM larvae in an opened gall and (D) ventral view of a DFNM larvae with the sternal spatula (black arrow). Scale bars: (A, B): 5 mm; (C, D): 0.2 mm. Photographs by Simon Blaser.

Out of 17 Douglas fir stands found to be colonized by DFNMs, 16 were also controlled for co-occurrence of *A. cooleyi*. Thereby, the species was found to be present in 15 stands (93.8%) (Table S1). By performing qPCR analyses of needles from a total of 14 DFNM-colonized Douglas fir stands, the presence of the fungal pathogen *N. gaeumannii* was confirmed in 12 of the tested stands (86.7%) (Table S1). When morphologically assessing stands included for qPCR analyses using the stereomicroscope, infection symptoms characterized by the growth of fungal fruiting bodies were found in needles originating from 8 stands (57.1%) (Table S1). From a total of 12 stands with a qPCR-confirmed presence of *N. gaeumannii*, morphologically detectable infection symptoms were only identified in 8 stands (66.7%) (Table S1).

Genetic analyses

In order to support the morphological identification and to assess the genetic structure, the COI barcode region of six specimens collected from each of five representative DFNM populations (monitoring locations no. 3, 8, 13, 20, and 25) were sequenced (Figure 1). From 25 out of the 30 analyzed specimens, high-quality nucleotide sequences with a minimum sequence length of 633 bp were obtained, representing a minimum of three

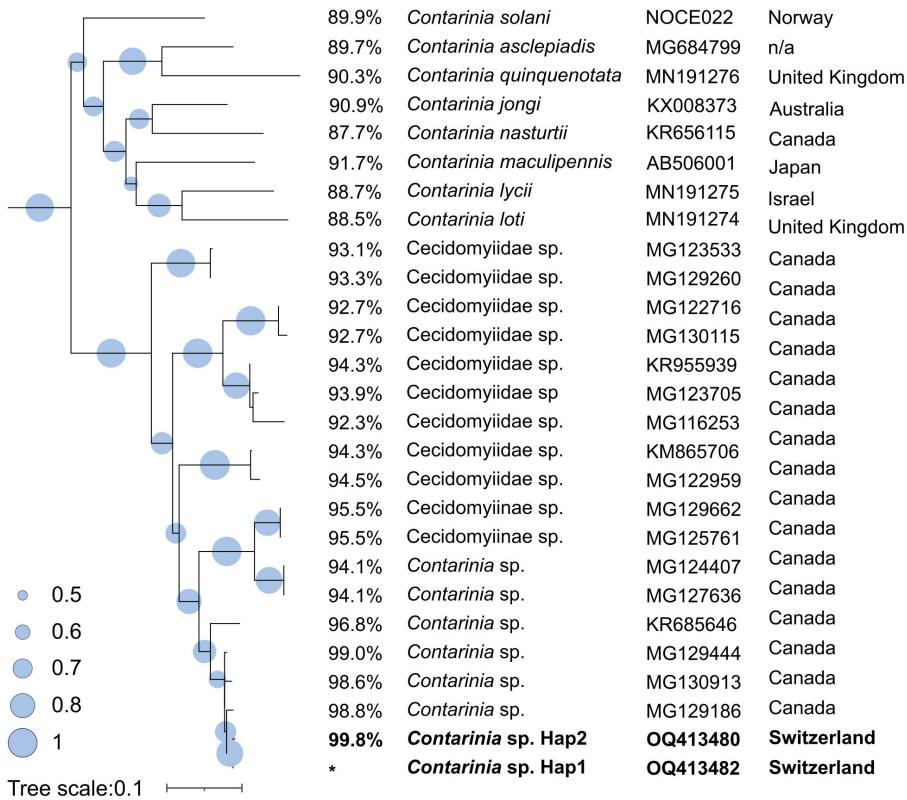


Figure 3. Sequence phylogeny of *Contarinia* sp. Hap1 and Hap2 based on the COI barcode region. Representative sequences were selected from NCBI and BOLD. Support values are drawn at scale and indicated next to the nodes. Shown is only the subtree with Hap1 and Hap2, and the sister clade including *Contarinia* spp. sequences with assigned species level identification. Branch lengths of phylogenetic tree are shown as nucleotide substitutions per site. Percentages represent identity with sequence *Contarinia* sp. Hap1 (OQ413482) originating from Switzerland.

larvae from each monitoring location (Table S2). Overall, two different haplotypes (Hap1 and Hap2), differing by one 1 bp, were identified. Hap1 ($N = 22$) was found at all genetically assessed monitoring locations, whereas Hap2 ($N = 3$) was detected only at the westernmost monitoring location (no. 3), where both haplotypes were found at an equal ratio (Table S2). Blast searches for Hap1 and Hap2 revealed 100%, resp. 99.8% identity to the COI barcode region of accession MG128474 on BOLD, designated as *Contarinia* sp., collected in British Columbia, Canada (Table S2). Conducting phylogenetic analyses, haplotypes Hap1 and Hap2 formed a larger clade together with sequence records lacking a species-level identification (Figure 3). This clade however shared a common node with a sister clade containing sequences of *Contarinia* spp. described at the species level. Of these, a sequence record of *C. maculipennis*, a polyphagous pest of vegetables and ornamental plants (Uechi et al. 2003), shared the highest similarity (91.7%) with Hap1 collected in Switzerland (Figure 3).

Discussion

Constantly growing trading volumes and human mobility during the last decades have drastically increased the spread and establishment of non-native forest insects and pathogens beyond their native ranges (Brokerhoff

and Liebhold 2017; Seebens et al. 2017; Jactel et al. 2020). In the present publication, we report on the first detection of invasive and non-native DFNMs of the genus *Contarinia* in Switzerland and present the results of monitoring activities to delimitate their current distribution area. Additionally, we studied the population genetic structure and assessed the co-occurrence of DFNMs with other non-native insect pests and pathogens on Douglas fir.

DNA barcoding analyses of isolated larvae revealed that the observed Douglas fir needle galls (Figure 2) clearly originated from gall midges of the genus *Contarinia*. However, due to the lack of reference sequences, an identification up to the species level was not possible. Earlier, Condrashoff (1961b) published a morphological key to distinguish between immature stages of the closely related DFMN species *C. pseudotsugae*, *C. constricta*, and *C. cuniculator* living in North America. Based on criteria such as the form of the sternal spatula from larvae, larval body color, as well as the characteristics of the detected galls, it has been suggested that the detected larvae from Switzerland belong to the species *C. pseudotsugae*. The assumption of the presence of this species is in accordance with the results from the near abroad (Seitz et al. 2018; San Martin and Durand 2021) and would be highly probable as *C. pseudotsugae* is the most abundant gall-inducing midge species on Douglas fir in North America (Condrashoff 1962). Nevertheless, due to the limiting discriminatory power of the available morphological traits of immature stages (Condrashoff 1961b) and the lack of high-resolution species-specific reference DNA sequences, the authors refrain from a species-level identification. To shed more light into the effective differentiation between the three DFMN species, future work should therefore intend the generation of DNA barcodes from North American paratypes, which would also simplify the unambiguous identification of the specimens collected in Europe.

Already established in southern German (Seitz et al. 2018; Metzger et al. 2021) and northeastern French (San Martin and Durand 2021) regions close to the Swiss borders, the detection of DFNMs in Switzerland was not surprising (Figure 1). The proximity of the finding sites in Switzerland with those from Germany and France indicates a continuous expansion of the distribution area rather than a new introduction event. However, to answer the question of origin conclusively, genetic analyses of DFMN populations from North America and Europe would be needed.

In Switzerland, the observed occurrence of DFNMs along the northern border was found to be mostly homogenous. Especially in the Cantons Basel-Stadt and Basel-Land, it was possible to detect DFMN symptoms on every Douglas fir stand controlled (Figure 1, Table S1). At monitoring location no. 4 in the Canton of Jura, DFNMs have not been detected so far suggesting an interruption of the distribution area between the most western population (monitoring location no. 3) and the populations of Canton Basel-Land (e. g. monitoring location no. 6) (Figure 1). One explanation for these findings

could be that the population at monitoring location no. 3 originates directly from the French infestation area, whereas the populations of southern Germany or France spread along the valley of Laufental (including monitoring location no. 6) and did not yet reach monitoring location no. 4 (Figure 1). The hypothesis of a slightly different origin of the DFNM population from monitoring location no. 3 is also supported by the presence of haplotype 2, which was solely detected at this study site. Due to the high sequence quality as well as the frequency of the variant, a sequencing artefact can be excluded. Despite the widespread distribution in the greater Basel area, DFNM populations have been only detected at one monitoring location (no. 13) in the south of the Jura Mountains. The spread may have slowed down somewhat at this natural barrier, which separates the Cantons of Basel-Stadt, Basel-Land, Jura, and parts of Aargau from the Swiss Central Plateau.

In principle, Douglas fir is considered to have a relatively wide site spectrum due to its resistance to summer drought (Wohlgemuth et al. 2021). However, modeling approaches showed that in the long term and with higher temperatures expected in the context of climate change, its growth optimum will shift to altitudes above 1000 m (Schüler and Chakraborty 2021). In the presented work, DFNM infestations were found in Douglas fir stands at altitudes up to 912 m (Table S1). From North America it has been reported that only sparse populations were found at elevations above 900 m. Although not yet studied in detail, so far, no noticeable difference in the degree of infestation between lower and higher infestation sites could be detected in Switzerland. An assessment of differences in the infestation levels along the altitudinal gradient will be subject of further studies.

Although widespread in northern Switzerland, the magnitude of DFNM caused damage to Douglas firs in Switzerland was not comparable to that reported from Belgium, where in 2018 between 30–50% of the youngest needles from many Douglas fir stands were found to be DFNM-infested (Wilson et al. 2020). Despite infestation rates up to 36% of current year's needles per Douglas fir shoot (data not shown), large proportions of shoots without any symptoms have been observed at all Swiss monitoring locations with occurrence of DFNMs.

It was previously stated that the occurrence of DFNMs in combination with pathogens and other insect pests can cause a damage-increasing effect on Douglas firs (Seitz et al. 2018; Wilson et al. 2020). Infections by the fungal pathogen *N. gaeumannii* can impair photosynthesis and induce a loss of older needles (Kimberley et al. 2011), whereas attacks of sap-sucking *A. cooleyi* can cause symptoms including yellowing and deformation of needles (Saunders and Barstow 1970). Visual controls revealed the widespread of *A. cooleyi* across Switzerland (Table S1). The species was found to co-occur in most of the Douglas fir stands colonized by DFNMs (94%, Table S1). Performing qPCR analyses, it was furthermore possible to show that *N. gaeumannii* was present in needle samples of nearly all DFNM-positive Douglas fir stands

(87%, Table S1). However, when morphologically assessing *N. gaeumannii* qPCR-positive stands, only 67% were found to express detectable infection symptoms (Table S1). The partially inconspicuous occurrence of this fungus in some of the assessed Douglas fir stands is consistent with observations from other studies (Kimberley et al. 2011), as *N. gaeumannii* is known to also exhibit an endophytic lifestyle (Butin 2019). Especially in its native distribution range in the west of North America and prior to the 1980's, the fungus was often found in needles of healthy Douglas firs without greatly affecting the host plants (Kimberley et al. 2011; Bennett and Stone 2016). Nevertheless, since several years and probably due to increasing winter temperatures, "Swiss needle cast" caused by *N. gaeumannii* is observed more frequently also in its native range, evoking more and more defoliation and decreasing growth of Douglas firs (Lan et al. 2022). Altogether, the results of the co-occurrence analyses suggest that, at least in northern Switzerland, a combined occurrence and possible damage cumulation of *A. cooleyti*, *N. gaeumannii* and needle midges from the genus *Contarinia* on Douglas fir has to be expected.

Since it must be assumed that DFNMs will continue to spread, increase population sizes, and cause more severe infestations under favorable climatic conditions such as already seen in other European countries, biotic stress on Douglas fir will further increase in Switzerland. Combined with steadily growing abiotic stress from rising temperatures and more frequent and intense disturbances (e.g. summer droughts) expected in the context of climate change (Bennett et al. 2015; Seidl et al. 2017), this will lead to a serious test of suitability for this potential future tree species.

Acknowledgements

The authors are grateful to Gilles San Martin (Walloon Agricultural Research Centre, CRA-W, Gembloux, Belgium) for sharing his profound knowledge about Douglas fir needle midges in Europe. A special thanks goes also to Thomas Wohlgemuth (Federal Research Station for Forest, Snow and Landscape WSL, Birmensdorf, Switzerland) and Ester Frei (WSL) for providing occurrence data of Douglas fir stands in Switzerland, to the local foresters for their help in identifying suitable monitoring locations, as well as to the plant pathology laboratory for technical support. Finally, the authors are very grateful for the valuable comments of the two anonymous reviewers.

Funding declaration

Open Access Funding provided by WSL.

Author's contribution

Research conceptualization and methodology: SB, BR, JM, VD, VQ; sampling: SB, VD, VQ; laboratory work: SB, BR, JM, VD; data analyses and interpretation: SB, BR, JM, VD; Manuscript writing and final approval: SB, BR, JM, VQ, VD.

References

- Albrecht AC (2017) Illustrated identification guide to the Nordic aphids feeding on conifers (Pinophyta) (Insecta, Hemiptera, Sternorrhyncha, Aphidomorpha). *European Journal of Taxonomy* 338: 1–160, <https://doi.org/10.5852/ejt.2017.338>
- Bennett AC, McDowell NG, Allen CD, Anderson-Teixeira KJ (2015) Larger trees suffer most during drought in forests worldwide. *Nature Plants* 1: 1–5, <https://doi.org/10.1038/NPLANTS.2015.139>

- Bennett P, Stone J (2016) Assessments of population structure, diversity, and phylogeography of the Swiss needle cast fungus (*Phaeocryptopus gaeumannii*) in the US Pacific Northwest. *Forests* 7: 14, <https://doi.org/10.3390/f7010014>
- Brändli UB, Abegg M, Allgaier Leuch B (ed) (2020) Schweizerisches Landesforstinventar. Ergebnisse der vierten Erhebung 2009–2017. Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft WSL, Birmensdorf, Schweiz, Bundesamt für Umwelt, Bern, Schweiz, 341 pp, <https://doi.org/10.16904/envidat.146>
- Brokerhoff EG, Liebhold AM (2017) Ecology of forest insect invasions. *Biological Invasions* 19: 3141–3159, <https://doi.org/10.1007/s10530-017-1514-1>
- Butin H (2019) Krankheiten der Wald- und Parkbäume. Ulmer Eugen Verlag, Stuttgart, Germany, 303 pp
- Condrashoff SF (1961a) Three new species of *Contarinia* Rond (Diptera: Cecidomyiidae) in Douglas fir needles. *The Canadian Entomologist* 93: 123–130, <https://doi.org/10.4039/Ent93123-2>
- Condrashoff SF (1961b) Description and morphology of the immature stages of three closely related species of *Contarinia* Rond (Diptera: Cecidomyiidae) from galls on Douglas fir needles. *The Canadian Entomologist* 93: 833–851, <https://doi.org/10.4039/Ent93833-10>
- Condrashoff SF (1962) Bionomics of three closely related species of *Contarinia* Rond. (Diptera: Cecidomyiidae) from Douglas fir needles. *The Canadian Entomologist* 94: 376–394, <https://doi.org/10.4039/Ent94376-4>
- Département de la santé des forêts (2016) Département de la santé des forêts (2016) Bilan sylvosanitaire de l'année 2016. La Lettre du DSF 51. Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt, Paris, France, pp 1–15
- Dorchin N, Harris KM, Stireman JO (2019) Phylogeny of the gall midges (Diptera, Cecidomyiidae, Cecidomyiinae): Systematics, evolution of feeding modes and diversification rates. *Molecular Phylogenetics and Evolution* 140: 106602, <https://doi.org/10.1016/j.ympev.2019.106602>
- Essl F, Rabitsch W, Breuss O (2002) Neobiota in Österreich. Umweltbundesamt, Wien, Austria, 432 pp
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299
- Frei ER, Moser B, Hafner J, Faessler F, Glanzmann A, Wohlgemuth T (2021) Ausbreitungstendenzen und Konkurrenzkraft der Douglasie in der Schweiz. *Schweizerische Zeitschrift für Forstwesen* 172: 94–105, <https://doi.org/10.3188/szf.2021.0094>
- Frei ER, Moser B, Wohlgemuth T (2022) Competitive ability of natural Douglas fir regeneration in central European close-to-nature forests. *Forest Ecology and Management* 503: 119767, <https://doi.org/10.1016/j.foreco.2021.119767>
- Gazol A, Valeriano C, Cantero A, Vergarechea M, Camarero JJ (2022) Douglas fir growth is constrained by drought: delineating the climatic limits of timber species under seasonally dry conditions. *Forests* 13: 1796, <https://doi.org/10.3390/f13111796>
- Gäumann E (1930) Über eine neue Krankheit der Douglasien. *Schweizerische Zeitschrift für Forstwesen* 81: 63–67
- Hebert PDN, Cywinska A, Ball SL, Deward JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270: 313–321, <https://doi.org/10.1098/rspb.2002.2218>
- Hielscher K (2017) *Contarinia pseudotsugae* (Condrashoff, 1961) (Diptera, Cecidomyiidae): a North American gall midge on Douglas fir in the northeast German lowlands. *Journal für Kulturpflanzen* 69: 351–358
- Ioos R, Fabre B, Saurat C, Fourrier C, Frey P, Marçais B (2010) Development, comparison, and validation of real-time and conventional PCR tools for the detection of the fungal pathogens causing brown spot and red band needle blights of pine. *Phytopathology* 100: 105–114, <https://doi.org/10.1094/PHYTO-100-1-0105>
- Jactel H, Desprez-Loustau M-L, Battisti A, Brokerhoff E, Santini A, Stenlid J, Björkman C, Branco M, Dehnen-Schmutz K, Douma JC, Drakulic J, Drizou F, Eschen R, Franco JC, Gossner MM, Green S, Kenis M, Klapwijk MJ, Liebhold AM, Orazio C, Prospero S, Robinet C, Schroeder M, Slippers B, Stoev P, Sun J, Van den Dool R, Wingfield M, Zalucki MP (2020) Pathologists and entomologists must join forces against forest pest and pathogen invasions. *Neobiota* 58: 107–127, <https://doi.org/10.3897/neobiota.58.54389>
- Kimberley MO, Hood IA, Knowles RL (2011) Impact of Swiss needle-cast on growth of Douglas fir. *Phytopathology* 101: 583–593, <https://doi.org/10.1094/phyto-05-10-0129>
- Lan YH, Shaw DC, Lee EH, Beedlow PA (2022) Distribution of a foliage disease fungus within canopies of mature Douglas fir in Western Oregon. *Frontiers in Forests and Global Change* 5: 743039, <https://doi.org/10.3389/ffgc.2022.743039>

- Lemoine F, Correia D, Lefort V, Doppelt-Azeroual O, Mareuil F, Cohen-Boulakia S, Gascuel O (2019) NGPhylogeny.fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Research* 47: W260–W265, <https://doi.org/10.1093/nar/gkz303>
- Leroy Q, San Martin G, Schmitz S (2015) *Contarinia pseudotsugae*: un nouvel insecte détecté sur Douglas. Observatoire Wallon de la Santé des Forêts, Gembloux, Belgium, 5 pp
- Ligot G, Balandier P, Schmitz S, Claessens H (2020) Transforming even-aged coniferous stands to multi-aged stands: an opportunity to increase tree species diversity? *Forestry: An International Journal of Forest Research* 93: 616–629, <https://doi.org/10.1093/forestry/cpa004>
- Metzger J, Wallerer G, Nannig A, Strasser L (2021) Douglasien-Gallmücke(n) auch in Bayern. LWF aktuell 3-2021. Bayerische Landesanstalt für Wald und Forstwirtschaft (LWF), Freising, Germany, pp 48–49
- NPPO of the Netherlands (2016) The presence of two species, *Contarinia pseudotsugae* and *Contarinia cuniculator* is suspected in the Netherlands. European and Mediterranean Plant Protection Organization (EPPO) Reporting Service no. 04–2016. <https://gd.eppo.int/reporting/article-5541> (accessed 27 March 2023)
- Parry WH (1978) Studies on the factors affecting the population levels of *Adelges cooleyi* (Gillette) on Douglas fir. *Zeitschrift für Angewandte Entomologie* 86: 8–18, <https://doi.org/10.1111/j.1439-0418.1978.tb01905.x>
- Pluess AR, Brang P, Augustin S (ed) (2016) Wald im Klimawandel: Grundlagen für Adaptationsstrategien. Haupt Verlag, Bern, Switzerland, 447 pp
- Ratnasingham S, Hebert P (2007) BOLD: the barcode of life data system. *Molecular Ecology Notes* 7: 355–364, <https://doi.org/10.1111%2Fj.1471-8286.2007.01678.x>
- Roques A, Auger-Rozenberg M-A, Boivin S (2006) A lack of native congeners may limit colonization of introduced conifers by indigenous insects in Europe. *Canadian Journal of Forest Research* 36: 299–313, <https://doi.org/10.1139/x05-277>
- Roques A, Auger-Rozenberg M-A, Capretti P, Sauvard D, La Porta N, Santini A (2019) Pests and diseases in the native and European range of Douglas fir. In: Spiecker H, Lindner M, Schuler J (eds) Douglas fir – an option for Europe. European Forest Institute, Joensuu, Finland, pp 63–72
- San Martin G, Durand T (2021) La cécidomyie des aiguilles du douglas. Une présence qui s'est étendue à la majeure partie de la région Bourgogne-Franche-Comté en 2020. Information technique DSF Avril 2021. Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt, Paris, France, pp 1–3
- Saunders JL, Barstow DA (1970) *Adelges cooleyi* (Homoptera: Phylloxeridae) control on Douglas fir Christmas trees. *Journal of Economic Entomology* 63: 150–151, <https://doi.org/10.1093/jee/63.1.150>
- Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, Farrell CM, Feldgarden M, Fine AM, Funk K (2023) Database resources of the National Center for Biotechnology Information in 2023. *Nucleic Acids Research* 51: D29–D38, <https://doi.org/10.1093/nar/gkac1032>
- Schneider S, Jung E, Queloz V, Meyer JB, Rigling D (2019) Detection of pine needle diseases caused by *Dothistroma septosporum*, *Dothistroma pini* and *Lecanosticta acicola* using different methodologies. *Forest Pathology* 49: e12495, <https://doi.org/10.1111/efp.12495>
- Schüler S, Chakraborty D (2021) Limitierende Faktoren für den Douglasienanbau in Mitteleuropa im Klimawandel. *Schweizerische Zeitschrift für Forstwesen* 172: 84–93, <https://doi.org/10.3188/szf.2021.0084>
- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn Ingolf, Lenzner B, Liebhold AM, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, Van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications* 8: 14435, <https://doi.org/10.1038/ncomms14435>
- Seidl R, Kautz M (2022) Impacts of climate change on disturbances. In: Wohlgemuth T, Jentsch A, Seidl R (eds), *Distrubance Ecology*. Springer International Publishing, Cham, Switzerland, pp 377–389, <https://doi.org/10.1007/978-3-030-98756-5>
- Seidl R, Thom D, Kautz M, Martin-Benito D, Peltoniemi M, Vacchiano G, Wild J, Ascoli D, Petr M, Honkaniemi J, Lexer MJ, Trotsiuk V, Mairotta P, Svoboda M, Fabrika M, Nagel TA, Reyer CPO (2017) Forest disturbances under climate change. *Nature Climate Change* 7: 395–402, <https://doi.org/10.1038/nclimate3303>
- Seitz G, Delb H, Grüner J, Mitze S, Wussler J (2018) Die Douglasien-Gallmücken (*Contarinia* spp.) in Südwestdeutschland. FVA Waldschutz-Info 1-2018, Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg (FVA), Freiburg Br, Germany, 8 pp
- Spiecker H, Schuler J (2019) Introduction. In: Spiecker H, Lindner M, Schuler J (eds), Douglas fir – an option for Europe. European Forest Institute, Joensuu, Finland, pp 17–19
- Stemmen A, Castagnéyrol B, Ponette Q, Prospero S, San Martin G, Schneider S, Jactel H (2023) Tree diversity reduces co-infestation of Douglas fir by two exotic pest and pathogen. *Neobiota* 84: 397–413, <https://doi.org/10.3897/neobiota.84.94109>

- Uechi N, Tokuda M, Yukawa J, Kawamura F, Teramoto KK, Harris KM (2003) Confirmation by DNA analysis that *Contarinia maculipennis* (Diptera: Cecidomyiidae) is a polyphagous pest of orchids and other unrelated cultivated plants. *Bulletin of Entomological Research* 93: 545–551, <https://doi.org/10.1079/ber2003266>
- Van Loo M, Dobrowolska D (2019) History of introducing Douglas fir to Europe. In: Spiecker H, Lindner M, Schuler J (eds), Douglas fir – an option for Europe. European Forest Institute, Joensuu, Finland, pp 21–25
- West KJ, DeAngelis JD, Simko BC (1991) The biology and control of the Douglas fir needle midge in Christmas trees. Oregon State University, Corvallis, USA, 2 pp
- Wilson E, San Martin G, Ligot G (2020) The Douglas fir needle midge (*Contarinia pseudotsugae*). A potential threat to Douglas fir in the United Kingdom and Ireland? *Quarterly Journal of Forestry* 114: 244–250
- Winton LM, Stone JK, Watrud LS, Hansen EM (2002) Simultaneous one-tube quantification of host and pathogen DNA with real-time polymerase chain reaction. *Phytopathology* 92: 112–116, <https://doi.org/10.1094/phyto.2002.92.1.112>
- Wohlgemuth T, Gossner MM, Rigling A (2021) Chancen und Risiken der Douglastie im Waldbau. *Schweizerische Zeitschrift für Forstwesen* 172: 62–65, <http://dx.doi.org/10.3188/szf.2021.0062>

Web sites and online databases

EPPO (2019) Mini data sheet on *Contarinia pseudotsugae*. Online database of the European and Mediterranean Plant Protection Organization – EPPO. <https://gd.eppo.int/taxon/CONTPS/documents> (accessed 22 December 2022)

Supplementary material

The following supplementary material is available for this article:

Table S1. Geo-referenced sampling data and results of Douglas fir monitoring in Switzerland.

Table S2. Detailed information on the genetically analyzed Douglas fir needle midge specimens.

Table S3. Accession numbers and origin of sequences included in the phylogeny (Figure 3).

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Blasler_etal_SupplementaryMaterial.xlsx