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Research Idea

Towards a global sentinel plants research strategy to prevent new introductions of non-native pests and pathogens in forests. The experience of HOMED

Duccio Migliorini[‡], Marie-Anne Auger-Rozenberg[§], Andrea Battisti[|], Eckehard Brockerhoff[¶,#], René Eschen[¤], Jian-ting Fan[«], Hervé Jactel[»], Christophe Orazio[˄], Trudy Paap[˅], Simone Prospero[¦], Lili Ren[ˀ], Marc Kenis[¤], Alain Roques[§], Alberto Santini[‡]

‡ Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), Sesto Fiorentino, Italy
§ INRAE, URZF, Orléans, France
| 3Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Padova, Italy
¶ Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland
# SCION (New Zealand Forest Research Institute), Christchurch, New Zealand
¤ CABI, Delémont, Switzerland
« School of Forestry and Bio-technology, Zhejiang Agriculture and Forestry University, Lin'an, China
» INRAE, University of Bordeaux, BIOGECO, Cestas, France
˄ IEFI Institut Européen de la Forêt Cultivée, Cestas, France
˅ Department of Biochemistry, Genetics and Microbiology; Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa
¦ Swiss Federal Research Institute WSL, Birmensdorf, Switzerland
ˀ Beijing Key Laboratory for Forest Pest Control, College of Forestry, Beijing Forestry University, Beijing, China

Corresponding author: Duccio Migliorini (duccio.migliorini@ipsp.cnr.it)

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Abstract

The use of sentinel woody plants in experimental plantings, Botanical Gardens and Arboreta has been experimentally validated as a tool for identifying possible unknown future threats prior to their introduction into new countries. Sentinel Plantings were recently established in Italy, France, Switzerland, China and South Africa, using a common

experimental design. The plantings included various tree and shrub species of broadleaves and conifers. Two planting types were established, each with different objectives. *In-patria* plantings using native plants aim to estimate, in absence of any phytosanitary treatments, the associations and infestation rates of native insects susceptible to be exported to other countries with that particular commodity. *Ex-patria* plantings using non-native plants are relevant to identify native insect species capable of switching to the non-native plant that would otherwise be impossible to predict prior to its introduction. In the frame of the EU project HOMED, we have implemented this concept, widening the use of this tool simultaneously to many different countries and continents

Keywords

Sentinel Plantings, Sentinel Plants, Arboreta, Botanical Gardens, Invasive Pests, Prevention strategy

Overview and background

The ever-growing volume of intercontinental trade of living plants is considered responsible for the increasing introduction of seriously damaging non-native forest insects and fungal pathogens (hereafter "*pests*") in all parts of the world (Brockerhoff and Liebhold 2017, Liebhold et al. 2012, Roques et al. 2020), and efficient border biosecurity is necessary to face the risk of new introductions (Wingfield et al. 2015).

Many non-native woody plant pests are not known to science prior to their establishment in a novel environment (Brasier 2008, Seebens et al. 2018). If known, there may be no reports of them causing damage in the area of origin, and/or no prior reports of them as invaders outside the native range (Eschen et al. 2019b).Consequently, the biology of many of the newly discovered organisms is poorly known, and it may be challenging to obtain a rapid identification, even when using molecular tools (Vettraino et al. 2015, Roques et al. 2015). Morphological approaches like the systematic use of host's damage morphotypes in Entomology, following the recent categorization of Roques et al. (2017) that is adapted to the different regions of the world, have been efficiently adopted to overcome this problem. Its use, however, is possible in a limited number of case studies. Yet most prevention measures are focused on the knowledge of potential threats of pests known to Science (Eschen et al. 2015a), underlining that a different approach to contrast the introduction of pests associated with imported plants is needed (Eschen et al. 2015b). In particular, a preexisting identification of potentially harmful pests and characterization of their life cycle traits is essential for assessing an appropriate pest/commodity risk analysis, by ranking the relative risk of different host plants or commodities as sources of invasive pests and pathogens, allowing for more targeting inspections (Eschen et al. 2017, Liebhold and Kean 2019).

In order to overcome this specific issue, the concept of sentinel plants used as an early detection method for non-native pests, has been recently implemented in two applicative approaches: Sentinel Plants and Sentinel Plantings (Eschen et al. 2019a, Vettraino et al. 2017).

The Sentinel Plants approach is generally carried out in high-risk sites to detect recently introduced non-native species soon after their arrival (Mansfield et al. 2019, Poland and Rassati 2019). The high diversity of woody plant species, the proximity to multiple high-risk sites and regular activities such as collection, cultivation, sharing and sale of plant materials, make Botanical Gardens (and toa lesser extent, Arboreta and Productive Plantations of non-native tree species) a unique resource of Sentinel plants across the World to identify recently introduced non-native pests and novel pest-host associations (Wondafrash et al. 2021). For instance, many pests' first entrances in new countries occurred via Botanical Gardens, including famous introduction events such as the chestnut blight, *Cryphonectria parasitica* in the United States (Rigling and Prospero 2018) and the root rot fungus, *Armillaria mellea* in South Africa (Coetzee et al. 2001) as well as less notorious cases (Morales-Rodríguez et al. 2021, Mukhina et al. 2015, Kirichenko et al. 2011). Non-native tree species introduced in a botanical garden can also be indicators of exotic species that are endangered by local pests, for example, *Agrilus planipennis* on *Fraxinus pennsylvanica* and *F. velutina* (Jianqiang 2011). According to the definition of *In-patria* and *Ex-patria* sentinel plants proposed by Eschen et al. (2019a), where the former aims to identify pests in the original native range of the plant itself and the latter targets the identification of pests in countries where the host plants are non-native, Sentinel Plants follows the *Ex-patria* approach, where pests are evaluated on non-native sentinels.

The Sentinel Plantings approach develops from the Sentinel Plants concept, representing a step forward in the prevention protocol (Britton et al. 2010). Sentinel Plantings consist of experimental plantations where the design, the young age and the high number of individuals aims at optimizing pests' collection. Sentinel plantings can be performed in an *Ex-patria* context thus, like Sentinel Plants, it is utilized as a post border surveillance tool. Alternatively, Sentinel Plantings can be also employed inside the indigenous range of the utilized woody plant species, following an *In-patria* approach aimed to a pre-border surveillance.

This aspect represents the main advantage of Sentinel Plantings over Sentinel Plants, as it allows pests that are likely to be harmful in a new, non-native environment, to be preventively identified in their country of origin prior to their possible introduction in other countries. According to this, pests would be already known if they are lately introduced into the rest of the distribution range of the sentinel host (Eschen et al. 2019a, Roques et al. 2015) (Suppl. material 1).

Practical applications of Sentinel Plantings were independently undertaken with both the *In-patria* and *Ex-patria* approaches (Eschen et al. 2019a and citations reported in the article), sketching few approaches and protocols for the diagnostic of pests in specific contexts (Morales-Rodríguez et al. 2019). Earliest studies, for instance, focused on singular topics such as fungal pathogens (Vettraino et al. 2015) or insects (Roques et al. 2015, Tomoshevich et al. 2013) in *Ex-patria* designs. During the last two decades, there has been a growing interest within the international community of plant pathologists and entomologists regarding sentinel methodology, which has led to the development of a well-defined legislative framework (Vettraino et al. 2020) and a solid experimental use (Kenis et al. 2018). Recently, the Sentinel Plantings approach was formalized as an EPPO standard, paving the way for its implementation (EPPO 2020). This activity was carried out due to the EU-funded projects focusing on early diagnosis in sentinel plants such as ALARM, PRATIQUE, ISEFOR, PERMIT and GLOBAL WARNING (Roques et al. 2015). Of these, however, only the first three also made use of experimental plantations, and they can be considered a test bed for the Sentinel Plantings approach in its current sense. Within this context and in order to produce a practical commonly recognized work protocol for the large-scale use of Sentinel Plantings, a dedicated task (Working Package 3 “Prevention and detection Tools”) was planned and validated within the frame of HOMED (Holistic Management of Emerging forest pests and Diseases) (EU Commission 2019). Within, a number of European woody plant species were experimentally planted (*Ex-patria* plantings) in areas of the world that are major suppliers of plants for planting (exporting countries) for Europe (importing countries). Reciprocally, trees and shrubs native to China, South Africa and North America were planted in Europe in a similar *Ex-patria* attempt, in collaboration with the the Ohio State University/USDA funded research project SENTINELS (*A global, reciprocal sentinel gardens approach to assess risk of invasion by alien pathogens and insect pests of important woody plant species*). At the same time, commonly traded woody plant species native to Europe (*In-patria*) were planted to assess the risk connected with these commodities. Alongside the work carried out in the experimental plantations created *ad hoc* for Sentinel Plantings, a massive monitoring activity on Sentinel Plants was also carried out in Botanical Gardens and Arboreta in the HOMED partner countries. In particular, an important collaboration was established with the REINFORCED network of arboreta and botanical gardens (Research infrastructure for adaptation to climate change) along the European Atlantic coast (Reinfforce.net 2018), where the presence of 4 European quarantine pest organisms was monitored in *In-patria* and *Ex-patria* woody plants.

This work contributed to address the spreading mechanisms of non-native pests between continents. Furthermore, it developed a shared conceptual and methodological approach for forest entomologists and forest pathologists, deepening a wide expertise in investigative tools (Jactel et al. 2020).

The main challenge of this part of the HOMED project was the assessment of diversity and distribution of potential newly introduced harmful pests from sample collections on woody plants distributed across a relevant number of different countries (Eschen et al. 2019b). The criteria utilized for the selection of woody plants for pest monitoring were taxonomy, age and abundance, three important factors behind pests' variation in host plants (Eschen et al. 2015a, Eschen et al. 2019a):

- Taxonomy of the species was determined according to the overall *Ex-patria* distribution in the non-European countries (China and South Africa).
- Age of the selected plant species was a consequence of their availability in preexisting *Ex-patria* tree stands like Arboreta, Botanical Gardens and Productive Plantations in the same countries; based on this differentiation the sample

collection was undertaken across the entire growing range of the plants, from the first years after planting (Sentinel Plantings) to the adult and senescent phases (Productive Plantations, Botanical Gardens, Arboreta).

- Density of the plants (here considered as a reference parameter for plant abundance in a certain area) was characterized by a relevant variation depending on the growing location. It ranged from 4 plants per m² in Sentinel Plantings to total isolation conditions in Botanical Gardens and Arboreta.

Objectives

This work aimed to provide a standardized approach for the identification of potential threats to European forests through the use of Sentinel Plants and Sentinel Plantings and it was structured in two operational protocols:

- Identifying native pests attacking those local tree species that may be exported with the trade in plants for planting (Commodity Risk Assessment for *In-patria* Sentinel Plant species).
- Identifying native pests attacking the non-native tree species and that may represent a hazard if accidentally introduced in the of country of origin of the host (Pest Risk Assessment for the *Ex-patria* Sentinel Plant species).

Implementation

The experiment was implemented across France, Italy, Switzerland, China and South Africa in the period 2019-2022 and New Zealand in the period 2012-2013. Woody plants used for the study belonged from 122 taxa, of which 27 were native in EU, 40 in East Asia, 7 in ZA, 9 in Oceania and 39 in America. A total of 115 different plant species were analyzed in *Ex-patria* conditions, of which 28 from Sentinel Plantings (Italy n=13; France n=9; South Africa n=4; China n=2), 4 from French Arboreta, 63 from Botanical Gardens (New Zealand n=62; South Africa n=1) and 24 from Arboreta (South Africa n=20; France n=4). A total of 26 different plant species were analyzed in *In-patria* conditions, of which 25 were from Sentinel Plantings (China n=12; Italy n=9; Switzerland n=5), 1 from Italian Botanical Gardens and 2 from French Arboreta (Suppl. materials 2, 3, 4).

Sentinel Plantings (France, Italy, South Africa, China)

Trees and shrubs in both *Ex-patria* and *In-patria* plantations of sentinel species were planted according to a randomized block design in square blocks of 25 plants each following the EPPO standard (EPPO 2020). Plants were sourced locally from ornamental nurseries or grown from commercially available seeds. Distance between plants within the block was 0.5 m. Project participants in each country established one plantation with a total of 50 plants per species, except Switzerland where two plantations with identical species and plant numbers were set up. Plants were watered during the dry season and weeded manually. No chemical treatments were used.

Saplings were monitored every month during the growing season. Invertebrates and pathogens spotted on the plants and that were supposedly plant pests (e.g., not predators or other organisms that are likely to be on the plant by chance) were collected and identified. More precisely, this included:

Invertebrates

When insects were observed:

1. aphids at all different development stages and adult insects were collected, put in ethanol 95% for morphological identification and further DNA barcoding;
2. insect larvae were placed half in ethanol 95% (morphological identification and barcoding) and half in rearing cages in situ, to obtain adults treated as above;
3. insect damage without associated agent was documented by photograph and classed into damage morphotypes (e.g., foliar feeding, leaf miner, stem borer, etc.) using the classification of Roques et al. (2017). The extent of damage was visually estimated and classified to the nearest 10% of the plant affected.

Pathogens

When damage by pathogens (fungi) was observed:

1. photos were taken and symptoms severity on the plants was quantified and classified according to a rank;
2. symptomatic plant material was collected for isolation and identification of the causal agent by processing them for fungal isolation after surface disinfestation with 80% ethanol, and consequent plating on 2% malt extract agar (MEA, DIFCO, Detroit, Michigan, USA) amended with streptomycin (0.05 gr/l) and incubation at 20 °C in the dark for 2-10 days. Pure colonies were sub-cultured on MEA and their taxonomy was assessed with a multigene molecular approach. Samples were not pooled and each plant was considered as a replicate.

Arboreta (France, South Africa)

Ex-patria woody plants from Arboreta including *Eucalyptus globulus*, *E. nitens*, *E.* ‘Gundal’ (*E. gunnii*×*E. dalrympleana*), *Pseudotsuga menziesii* and *In-patria* plants including *Castanea sativa* and *Quercus robur* were monitored during this study in 14 French Arboreta distributed across the entire western range of the country (Reinfforce.net 2018). Presence/absence of specific plant associated invasive pests was weekly investigated, including *Contarinia pseudotsugae* on *P. menziesii*, *Gonipterus scutellatus* on the Eucalypt species, *E.* ‘Gundal’, *E. nitens*, *Dryocosmus kuriphilus* on *C. sativa* and *Corythucha arcuate* on *Q. robur*. Insect taxonomy was assessed by morphological characteristics.

Plants from 20 *Ex-patria* species were monitored in multiple South African Arboreta for the detection of associated fungi and insects as described for “Sentinel Plantings” above.

Botanical Gardens (Italy, New Zealand, South Africa)

Asymptomatic samples of twigs and leaves were collected from *Quercus suber* plants across three Botanical Gardens in central Italy (*In-patria*) and three in the Western Cape province of South Africa (*Ex-patria*). Specimens were processed both for fungal endophytes' isolation in pure cultures as described for "Sentinel Plantings" above and for the metabarcoding sequencing of fungal environmental DNA (eDNA).

A survey of tree-associated aphid species based on morphological identification was undertaken during summer 2012-2013 at Christchurch Botanical Gardens in New Zealand on the following *Ex-patria* plant categories:

Pinus (28 species, 39 trees), *Picea* (12 species, 20 trees), *Abies* (11 species, 15 trees), *Cedrus* (2 species, 3 trees) and nine other genera represented by one tree of one species (*Calocedrus*, *Cryptomeria*, *Cunninghamia*, *Cupressus*, *Fitzroya*, *Juniperus*, *Larix*, *Podocarpus* and *Sequoia*).

Discussions of outcomes

The project is approaching its final stage. An impressive database of fungal organisms and insects have already been collected and partially analyzed by the research groups of the different countries involved in the project. Taxonomical results will be presented to the international scientific communities in peer review journals in the near future. Such an outcome represents a step forward in the study of non-native forest tree pests. Furthermore, it successfully tests the double approach based on *In-patria* and *Ex-patria* concept, validating its use for the early detection of insects and fungal pathogens potentially harmful for the examined trees.

A wide, intercontinental use of Sentinel Plantings was extensively performed during this project, aiming to prove its reliability and efficiency as investigation method in invasion science.

The main advantage of Sentinel Plantings was represented by the high number of individuals per species available for sampling, in our case 50 trees per sentinel species. This was a safety element with regard to the occasional death of young sentinel trees during the duration of the project and it guaranteed the continuity of specimens' collection for all the species.

The age of the individuals was a further point in favor of the success of the project, since it included sampling from young woody plants in the Sentinel Plantings, but also adult and senescent trees in Botanical Gardens and Arboreta. Monitoring conspecific trees of different ages can facilitate pest detection (Eschen et al. 2019b): in our case, the high number of young individuals per species available in the experimental plantations was a strong advantage, making the random presence of individuals more naturally attractive to pests more likely. These types of plants characterized by a higher susceptibility are not found in different contexts such as Botanical Gardens and Arboreta because they tend to disappear before reaching adulthood due to their lack of adaptability to pests.

The overall good survival rate of Sentinel Plantings *Ex-patria* plants during the few growing seasons included in the project duration was an undoubted advantage over older *Ex-patria* plants in Botanical Gardens and Arboreta. In this situation, the prolonged stay in non-ideal vegetative conditions may bring to repeated seasonal stress conditions in trees (Kirichenko and Kenis 2016), making both monitoring and diagnosis difficult and confusing.

Botanical Gardens and Arboreta have played a relevant role in this trial maximizing the spacial distribution of sampling site. In particula, many non-native taxa considered in our work were retrieved in Botanical Gardens and Arboreta across a scattered sites distribution within the same country, providing a consistent contribution to the monitoring of *Ex-patria* plant species. Differently to planted saplings, the individual adult trees already present in Botanical Gardens and Arboreta did not require any management effort, basically represented a zero-cost item in the project budget. While detection of crown pests was inconvenient or even not possible in many occasions, these trees were best exploited to observe the susceptibility to certain pests, such as the polyphagous shot hole borer in the *Ex-patria* plants of South Africa and to spot the annual incidence of damaging invasive pests on *In-patria* and *Ex-patria* plants in France.

Moreover, the choice to include numerous mature and generally isolated woody plants in our sampling also greatly increased the detection of wood boring and bark beetles, which are more attracted to mature trees and cannot usually develop in young ones (Eschen et al. 2019a). In this sense, the project outcomes are likely to demonstrate that the monitoring of *Ex-patria* plants using Sentinel Plantings must not be separated from the simultaneous use of preexisting adult trees from the same area of study. In our project this has given a strong advantage in the detection of potentially harmful pests within the same plant species, both in terms of time, due to the variable age of the individuals, and in terms of space, due to the widespread position of Botanical Gardens and Arboreta. It has been suggested, indeed, that different plants suboptimal conditions in multiple sampling locations within and across countries affect the identity and relative abundance of associated organisms, increasing the likelihood of disease symptoms developing and pests' detection (Eschen et al. 2019a).

In conclusion, the development of a stable sentinel network in the current context of increasing global trade of plants for planting is presented in this work as a promising tool against further spread of woody plant pests.

This trial was the most advanced practical application of the EPPO standards (EPPO 2020) in the identification of potential threats to European forests through the use of woody plants from different growing contexts. By the identification of native pests attacking native tree species, that may be exported with the trade in plants for planting, the results are expected to provide sufficient elements for the implementation of Commodity Risk Assessment for *In-patria* plants species. At the same time, the characterization of native pests attacking non-native tree species and that may represent a hazard if accidentally introduced in the of country of origin of the host, is fundamental knowledge for the production of Pest Risk Assessment for the *Ex-patria* plant species.

The implementation of this project and its practical realization were achieved thanks to the strong local expertise of the research teams involved and the close collaboration between the respective researchers, who were able to reach a shared agreement on the design of the experiment, the costs and the practicality of monitoring. The standardized detection and identification methods developed during the project facilitated the establishment of a more inclusive and a truly unified interdisciplinary framework in the international community of plant pathologists and entomologists, reducing costs and facilitating the interpretation of the results.

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Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Firenze, Italy.

INRAE, URZF, 45075, Orléans, France.

Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Padova, Italy.

Scion (New Zealand Forest Research Institute), Christchurch, New Zealand.

Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903, Birmensdorf, Switzerland

CABI, Delémont, Switzerland.

School of Forestry and Bio-technology, Zhejiang Agriculture and Forestry University, Lin'an, China

INRAE, University of Bordeaux, BIOGECO, Cestas, France.

IEFI Institut Européen de la Forêt Cultivée, 69, Route de Arcachon, F-33610, Cestas, France.

Department of Biochemistry, Genetics and Microbiology; Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.

Beijing Key Laboratory for Forest Pest Control, College of Forestry, Beijing Forestry University, Beijing, China

Ethics and security

I, Duccio Migliorini, consciously assure that for the manuscript "**Towards a global sentinel plants research strategy to prevent new introductions of non-native pests and pathogens in forests. The experience of HOMED**":

- is the authors' own original work, which has not been previously published elsewhere;
- is not currently being considered for publication elsewhere;
- reflects the authors' own research and analysis in a truthful and complete manner and it does not present any ethical and security issue.

Author contributions

All co-authors equally contributed to the preparation of the manuscript. I, Duccio Migliorini, as first author, took care of the first draft and submission.

Conflicts of interest

I, Duccio Migliorini, as first author, declare that the submitted work does not present any kind of problem related to conflicts of interest.

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Supplementary materials

Suppl. material 1: File 1 doi

Authors: Duccio Migliorini, Marie-Anne Auger-Rozenberg, Andrea Battisti, Eckehard Brockerhoff, René Eschen, Jian-ting Fan, Hervé Jactel, Christophe Orazio, Trudy Paap, Simone Prospero, Lily Ren, Marc Kenis, Alain Roques, Alberto Santin
Data type: Conceptual scheme
Brief description: Scheme of the approaches
Download file (28.37 kb)

Suppl. material 2: File 2 doi

Authors: Duccio Migliorini, Marie-Anne Auger-Rozenberg, Andrea Battisti, Eckehard Brockerhoff, René Eschen, Jian-ting Fan, Hervé Jactel, Christophe Orazio, Trudy Paap, Simone Prospero, Lily Ren, Marc Kenis, Alain Roques, Alberto Santini
Data type: Geographical
Brief description: Number of plant species used per categories in each of the countries involved in the project
Download file (15.07 kb)

Suppl. material 3: File 3 doi

Authors: Duccio Migliorini, Marie-Anne Auger-Rozenberg, Andrea Battisti, Eckehard Brockerhoff, René Eschen, Jian-ting Fan, Hervé Jactel, Christophe Orazio, Trudy Paap, Simone Prospero, Lily Ren, Marc Kenis, Alain Roques, Alberto Santini
Data type: Geographical
Brief description: Summary of the countries, sentinel plant categories and sentinel plants taxonomy used in HOMED. Data reported are the same of Supplementary file 4. Both files present an overview of the experiment design according to the countries (file 3) and sentinel plants (file 4) prospective. Name of the Countries are reported as ISO codes: China (CN), France (FR), Italy (IT), New Zealand (NZ), South Africa (ZA), Switzerland (CH). Geographic indications (Northern, Southern, Eastern and central) are replaced with the letters N, S, E, and c, respectively.
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Suppl. material 4: File 4 doi

Authors: Duccio Migliorini, Marie-Anne Auger-Rozenberg, Andrea Battisti, Eckehard Brockerhoff, René Eschen, Jian-ting Fan, Hervé Jactel, Christophe Orazio, Trudy Paap, Simone Prospero, Lily Ren, Marc Kenis, Alain Roques, Alberto SantinDuccio Migliorini, Marie-Anne Auger-Rozenberg, Andrea Battisti, Eckehard Brockerhoff, René Eschen, Jian-ting Fan, Hervé Jactel, Christophe Orazio, Trudy Paap, Simone Prospero, Lily Ren, Marc Kenis, Alain Roques, Alberto Santin
Data type: Geographical
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