Final Report

Project title (Acronym)
Lecanosticta - Brown spot disease of pines - spread in European forest ecosystems, impact on pines, predisposing and contributing factors, control (BROWNSPOTRISK)

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2. Short project report

2.1. Executive Summary

Brown-spot disease is a needle blight of conifers caused by the fungal pathogen *Lecanosticta acicola* (*LA*).
Screening of available literature revealed numerous gaps in knowledge, which impede both curative and preventive strategies. Effects of climate change on disease occurrence, the possibility of emergence of more aggressive strains, its biology and dissemination (also including anthropogenic activities) are some of the main questions that need to be addressed. In order to collect data on outbreaks of *LA*, a global database was established containing information on 3012 observations (879 records of these are officially confirmed by the National Plant Protection Organizations) from 44 countries. This database provides useful information for the (ongoing) modelling of the spread of *LA*. A global population genetic study conducted within the project showed that the pathogen was likely imported from North America in two major ingressions.
Nothing is known on the impact of eight species of *Lecanosticta* that are morphologically similar to *LA*, that were recently described from Central America but not yet recorded in Europe. *LA* European outbreaks indicate that climate change favours the spread and establishment of the fungus into new areas, in particular where temperatures and summer precipitation increased. On the contrary, dry and hot summers probably limit the spread of *LA*. As shown by a study conducted by project partners on pines in bogs, infection foci are predominantly located in vicinity of places of human activities indicating a spread caused by humans. Human activities in touristic hot-spots are observed also as a hot-spot for the brown-spot disease and its spread, as is reported by project partners.
Hygienic measures combined with increasing efforts to track outbreaks in early stages and removal of infected trees are the options to protect European forests as well as for urban sites. Inspection of nursery stocks by trained personnel followed by laboratory analyses and the destruction of infested plants are of foremost importance.
Preventing further unintended spread using a system implemented in Slovenia, where pines are preferably removed from sites that experience high touristic pressure is an option also for other countries. For infested bogs, preventing public access may limit the spread of the fungus by visitors and the need to close these sites is in discussion in Austria.
Artificial regeneration of trees should be done by using disease-free stocks only, sourcing high quality seedlings, planting trees at low density, and preferring planting sites where conditions are less favourable to *LA* such as good drained and aeriated sites.

2.2. Project aims

Brown-spot (needle blight) disease, caused by the fungal pathogen *Lecanosticta acicola* (*LA*), has rapidly spread in Europe, and especially in Northern European and some Central European countries. The lack of information regarding this disease can be regarded as the main obstacle to effective management.
The project contributed to develop and share knowledge on the fungus's biology, pathogenicity and epidemiology, focussing in particular on the European strains of *LA*. Such a knowledge will allow the development of efficient management strategies focussing both on control and preventive measures. The objectives of the BROWNSPOTRISK project were:

- To gather information on the pathogens' spread in Europe and produce maps showing the area of infestations
- To shed light on the influence of climatic and site conditions on the hosts affected by the disease
- To develop an efficient methodology to demarcate infestation areas
- To define the rates of natural spread and develop models to describe the spread of the fungus
- To define biotic and abiotic factors favouring the spread
- To characterize the factors pre-disposing host trees to decline
- To evaluate the susceptibility of different forest ecosystems and tree (clones) species
- To determine the strain genetic differences associated to differences in pathogenicity
- To develop strategies to control the disease (including biological measures)

2.3. Descriptions of the main activities

A worldwide literature search on *LA* was performed by the UK project partner that covered both peer reviewed publications and popular literature and a database, containing at present around 380 papers, was developed. The literature was reviewed for knowledge gaps and consequently research needs focussing on taxonomy, host sensitivity, geographic range and pathways of spreading and management practices. The database is accessible on request.

In order to assess the spread of *LA*, monitoring was done in Austria, Belarus, Czech Republic, Estonia, Lithuania, Slovenia, Spain, Poland and Turkey. Where trees with symptoms of LA were found, identification was performed by culturing and molecular tests (Ioos *et al*., 2010; EPPO PM 7/46). In Switzerland and Austria spore traps were validated and used to assess the spread of *LA* by wind. In bogs, the use of drones was tested to assess size and pattern of outbreaks (Austria).

A database of outbreaks was developed by the Estonian and Czech partners based on then existing distribution of *LA* (Global *LA* - database of outbreaks, http://www.portalofforestpathology.com/). Since the database was developed at end of 2020, work on models was initiated by the Slovenian partners and is ongoing.

Country level population genetic analyses was done for Estonia (Laas *et al*., 2019), Lithuania and Poland (Raitelaitytė *et al*., in prep.) and the work was developed by Estonian and Lithuanian partners. The studies give the first look into the population genetics of *L. acicola* in northern Europe.

A global population genetic study was done by the Estonian partner. The aim was to investigate the genetic diversity and the population structure of *LA* in Europe, and to study the links between European and North American *LA* populations to track possible migration pathways. This was performed with in total 650 isolates of *LA* from 27 countries.

Climatic, topographic and other factors such as the vicinity to human activities (roads, hiking paths, villages *etc*.) were assessed to define their influence on *LA* establishment and spread (Austria, Slovenia). In Austria, a country-wide monitoring was performed focusing on bogs with pine stands, selected mountainous or alpine stands of *Pinus mugo*/*uncinata* in vicinity of populated areas with special emphasis to settlements dominated by houses with gardens. To identify, which combination of factors causes the death of the trees affected by *LA*, symptoms and contributing agents were studied in an Austrian protection forest of *Pinus mugo*.

For the identification of further possible host species of *LA*, greenhouse tests were initiated (Estonia, Portugal and Spain) and this work is ongoing. In Estonia an inoculation test was conducted using two virulent *LA* haplotypes identified by the Estonian population study (haplotypes no. 10 and no. 31) (Laas *et al*., 2019). In total 13 of the best Estonian clones of *Pinus sylvestris* were inoculated with both haplotypes. *Pinus mugo* served as a positive control and in addition the susceptibility of *Picea abies* was also tested. From each *P. sylvestris* clone, *P. mugo* and *Picea abies* eight seedlings were inoculated with each *LA* haplotype, and two of each were kept in separate climate chambers as a negative control. Sixteen weeks after the first inoculation, 56% of the inoculated pine seedlings showed symptoms of needle infection. Since the presence of conidia of *LA* is still not observed, work is ongoing to molecularly characterise the different clones used for the experiments.

Managing *LA* can only be done by considering the ecosystems where *LA* occurs. For outbreaks in urban and localised forest sites an approach was implemented by the Slovenian partner to delimitate single outbreaks and prevent unintended spread by tourists. The measures are implemented around trees which are confirmed by laboratory test to be infected with LA, focusing on those areas with high pressure of human activities (camps, trails, *etc*.). All pine trees around the laboratory confirmed-LA infected trees (r = up to 2 tree heights) are cut and in the radius of 100m all pine trees with visually observed LA-symptoms are also cut. Logging residues with needles are burnt on site or transported in closed containers to a location of burning. Forest timber is allowed to be transported (except green chips). In order to apply these measures, collaboration between phytosanitary authorities, forest managers and forest owners is essential, therefore a considerable part of the strategy is based on information campaigns (radio, tv, newspaper, web).

Three plenary meetings for all participants were organised: A Kick-off meeting was held in December 2017 (Innsbruck, Austria, organised by project partner 1), a second meeting in May 2019 (Ljubljana, Slovenia, organised by project partner 10; Smolnikar *et al*., 2019[1]) and a final meeting in October 2020 (web-meeting[2], organised by project partner 8). Bilateral meetings were also organised (Slovenia/Austria, June 2018).

Communication, collaboration and engagement with external collaborators was pursued: forest protection authorities of the states as well as from provinces, nature protection authorities, forest schools, and forest owners.

2.4. Main results

Generally, most of the knowledge on *LA* derives from the USA. But this information is far from being complete and does not apply to the European situation. If it is known that the pathogen was imported via plant stock, the role of further anthropogenic or other means of dissemination (such as wind, vectors, clothes) is not sufficiently known. Furthermore, it is still not fully proved, that the pathogen is not seed-borne. Several gaps in knowledge concern symptomatology and infection biology. Disease expression may vary between commonly planted European tree species. Relations to climate change are not fully understood. This refers to the temperature and air humidity limiting spore release. Little is known on the variation in susceptibility of hosts affected by the different climates but also by the different populations of *LA*. Though the disease course takes commonly a couple of years until the host dies, this has to be checked for individual tree species under different infection

[1] Smolnikar P., Piškur B., Zidar S. (2019). Euphresco meeting in Ljubljana: *Lecanosticta acicola* in Europe (In Slovenian). Information available from https://www.gozdis.si/novice/euphresco-srecanje-v-ljubljani-2019-05-13/

[2] The final meeting program and presentations are available from http://ph.emu.ee/~drenkhan/brownspot/

conditions as well as for different strains of *LA* (*P. nigra*). Management and control techniques as practiced in North America (burning of affected tree stands, use of fertilisers and fungicides) can be adopted only partially in Europe. In addition, resistance studies with European hosts and silvicultural experiments are needed.

LA is currently known from about 20 European countries. Up to now, predominantly urban sites, but also forests are affected (Mullett *et al*., 2018). The disease is widespread in Estonia, Switzerland (highest recorded number of outbreaks), Belarus and Austria, while in Bulgaria, Slovenia, Slovakia, Spain, Sweden and Turkey local sites are affected (Adamson *et al*., 2018, Cleary *et al*., 2019, Georgieva 2020, Sadiković *et al*., 2019). Forest stands are infested in Estonia, Belarus, Austria, Spain, Slovenia, Poland, Czech Republic, Germany and Switzerland; bogs are infested in Germany, Switzerland, Czech Republic and Austria (Laas *et al*., 2019). In Austria 28% of all monitored bogs were affected. Alpine protection forests are affected in Austria. Drones tested in one Austrian bog delivered photos apt to distinguish between infested and not infested *P. mugo* stands, however early infection stages could not be differentiated (Fig. 1).

Figure 1. Bog in Austria (Styria) with mountain pines (*Pinus mugo*) affected by *Lecanosticta acicola*. Drone ©Mavic Pro, el.100m. Grey patches show heavily infested shrubs.

The global database of *LA* at present contains 3012 records from 44 countries. Of these, 879 were records of officially confirmed outbreaks of *LA* and 2104 were records where *LA* was not identified (using microscope, isolation and molecular testing were negatives) though tree symptoms were indicating *LA*-infections. Other *Lecanosticta* species were found (29 records) from 4 North and Central America countries.

Population genetic analyses of *L. acicola* were performed in Estonia. The analysis revealed low genetic diversity and a high number of clones that indicated *L. acicola* is an invasive species in northern Europe. Results suggest that several separate introductions have taken place and anthropogenic activity has apparently affected the spread of the pathogen. This is

the first time for Estonia when the pathogen's dispersal by human activity is evidently proved. Clonal reproduction is dominating and although sexual reproduction is possible, it probably takes place infrequently (Laas *et al*., 2019).
Population studies also revealed two genetically distinct populations of *L. acicola* in Lithuania and one population in the Polish coastal region. Both populations are clonal. The difference between two the populations could be explained with the possibility of two different *LA* introductions to Lithuania. The results indicate a high migration flow from the Lithuanian population to Poland, and it can be concluded that the pathogen was probably introduced from Lithuania. Analysis of mating type genes shows that asexual clonal reproduction of the MAT1-1 idiomorph dominates in Lithuania and Poland, but sexual reproduction is possible (Raitelaitytė *et al*., in prep).
LA microsatellite and mating type markers were used to study the population genetics, migration history and reproduction mode of the pathogen based on a collection of 650 isolates from three continents, 27 countries, and 27 hosts. Migration analyses indicate European populations as the probable source of the populations recently found in western Asia (Georgia and Turkey). The fragmented structure and several shared haplotypes between the various European populations indicate a mixed population history in Europe with several introduction events not only from North America but also between European countries. For the first time, genetic studies proved that a same haplotype of *L. acicola* is present in two continents: in North America (Canada) and Europe (Germany). This evidently proved that the pathogen was imported from North America into Europe. Another multilocus haplotype was found to be shared between West Asia and several countries in northern Europe. Data shows that sexual reproduction takes place in some populations in Europe and as in southeast USA, but the high proportion of clones indicates a clonal spread of the pathogen through Europe (Laas *et al*., in prep.).
Quite evidently the pathogen was imported from North America into Europe in two separate events. Most of the studied *LA* populations in Europe derive from the northern parts of the USA, while the populations in France and Spain derive from the southern regions of the USA. The population genetics study also confirmed that the genetic diversity is highest in the south of the USA (Laas *et al*., in prep.).
Nothing is known on the impact of eight newer *Lecanosticta* species that are morphologically similar to *LA*, and which were recently described from Central America (Evans, 1984; van der Nest *et al*., 2019). They represent a risk, and international concerted research and efforts are needed to prevent their spread. *LA* outbreaks indicate a relation between the fungus movement and climate change. For the mountain areas in Austria where protected forests are affected, aerial temperature increased during the past two decades, both during the vegetation period and the dormant season. Also spring and summer precipitation increased during that time (number of months with rainfall exceeding the 30 years average). This combination may explain the efficient establishment and steady spread of the pathogen in that area. On the contrary, dry and hot summers may probably limit the spread of *LA*: during the summer 2018 (a dry and hot season compared to previous ones), only very small amounts of *LA* conidia were caught using spore traps (Switzerland and Austria). From the bogs checked for *LA* outbreaks in Austria, the ones affected showed a significant shorter distance to mutual human inoculum sources (settlements, roads, parking places, hiking paths) compared to not affected bogs (Fig. 2).

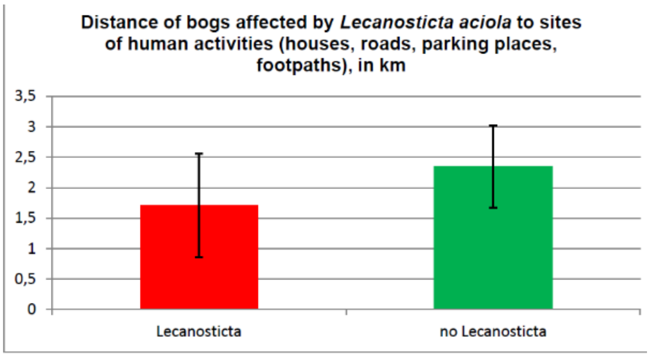

Figure 2. bogs in Austria with mountain pines (*Pinus mugo*) affected by *Lecanosticta acicola* and vicinity to sites with human activities

Needle loss due to *LA* infection increased with the tree height up to a maximum in trees of 1.5 m height but decreased in trees that are shorter. In the studied protection forests, there was no indication that thinned stands were less affected by *LA* than dense ones.
In Austria, mortality due to *LA* did not exceed 10% of infected trees on average (Fig. 3 and 4).

Figure 3. dead *Pinus mugo* in an Alpine protection forest following heavy infestation by *Lecanosticta acicola* (Austria, Tyrole).

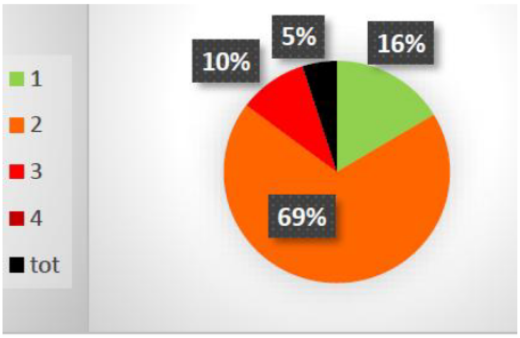

Figure 4. Lecanosticta-infestation intensities of *Pinus uncinata* in an Alpine protection forest (Austria, Tyrole).

% needle loss	condition	Codes
0 % - 10 %	healthy	1
11 % - 30 %	weak	2
31 % - 60 %	medium	3
61 % - 90 %	strong	4
91 % - 100 %	virtually dead	5

Infected trees dry and this appears as a continuous loss of needles. Simultaneously deep longitudinal cracks above the stem base were recorded. Such cracks were found more frequently on heavily infested individuals than on slightly diseased or healthy ones. Since cracks are commonly caused by frost, this might indicate a reduction in frost resistance by the needle loss. Mortality is also reported from Belarus (*P. mugo*), Estonia (single *P. mugo*) and Slovenia, where Austrian Black Pines (*P. nigra austriaca*) are affected by a probably geographically still separate population of *LA* hypothesized to be more aggressive to *P. nigra* trees (Sadiković *et al.*, 2019).
Work conducted in Austria revealed few biotic factors involved in the decline process at a very late stage (*Scolytidae*, *Curculionidae*, *Anobiidae* and *Cerambicidae*). In needles infected by *LA*, other pathogens (*Dothistroma* spp., *Lophodermella conjuncta*, *Lophodermium seditiosum*, *Neocatenulostroma germanicum*, *Diplodia sapinea*, *Cenangium ferruginosum*,

Desmazierella acicola, *Cyclaneusma minus*) contributed to the decline (Austria, Belarus, Czech Republic).

LA is currently known from 53 pine species and hybrids, as well as *Picea glauca* and *Picea omorika*. Among the pine species in Europe, *P. mugo* and *P. uncinata* are especially susceptible, high susceptibility is also reported for *P. radiata* in Spain and France. *P. sylvestris* shows different degrees of susceptibility depending on the provenance. The pathogen was confirmed by the Turkish project partner to cause severe damage to native endangered endemic forms of Turkish *Pinus nigra* subsp. *pallasiana* (Oskay *et al.*, 2020). Even more, *L. acicola* were found on non-*Pinus* species *Cedrus libani* (Oskay *et al.*, 2020).

Legal constraints limit the application of chemical compounds against fungal diseases in forests. The cultural method of burning affected sites (*P. palustris* in USA) is hardly an option for an effective measure against *LA* in Europe as European host species are not as tolerant to fire as *P. palustris* and burning as a means of pest control is largely banned for environmental reasons. Consequently, hygienic measures combined with increased surveillance to track outbreaks in early stages are the options for European forests as well as urban sites.

Preventing further unintended spread using the same approach which was developed and implemented by the Slovenian partner is an option for other countries. In Austria, discussions with authorities, forest owners and nature protection people are ongoing. For infested bogs, a closure to public access is in discussion.

Artificial regeneration should comprise the use of disease-free stock only, sourcing high quality seedlings, planting at low density, and respecting site conditions that are less favourable to *LA* e.g. good drained and aeriated sites. The implementation of phytosanitary protocols such as ensuring that plants are sourced from areas free of disease, inspection of nursery stock by trained personnel and destruction of infested plants are of outmost importance. For infected trees in urban sites, annual surveys (as done in Switzerland) to check symptomatic trees followed by the removal of the infected tree are an option to keep further spread limited.

A study on mycoviruses of *LA* by the Czech project partner as an option to control *LA* revealed the presence of members of the *Partitiviridae* virus family. However possible effects have to be tested in dual cultures, testing biological interactions between fungi in planta.

2.5. Conclusions and recommendations to policy makers

According to the new EU Plant Health Regulation, *LA* is a regulated non quarantine pest, and obligations concern only plant production in order to limit the risk of spread of *LA* by plant stocks. This focus on plants for planting aims to reduce the dissemination of *LA* via trade but does little to help detecting and managing the organism in the wider environment, as no statutory action is required by EU law following a report in the wider environment although action may be taken at country level.

The project highlights our incomplete understanding of the potential impact of *LA* infection on different host species in Europe, and of the possible influences of climate on pathogen behaviour. The multiple genotypes/populations of *LA* identified during the course of this project, and the recent discovery of multiple species of *Lecanosticta* in Central America rise concerns. Hence, an extremely conservative approach should be taken following the detection of *LA* in the wider environment, and is the consortium recommends to increase the surveillance of European forests and trees.

Outbreaks in the public green can only be tracked by expert people that have awareness of the risks and knowledge of the symptoms. Identification of symptomatic trees can be achieved by regular monitoring or by a system to report trees with suspicious symptoms. This requires collaboration between researchers and professionals from urban authorities, tree carers, garden associations, but also garden owners and the general public. To achieve this comprehensive information, campaigns are needed as well as workshops and a reporting system for woody plant pests as currently used in Slovenia (the mobile app LIFE ARTEMIS that can be downloaded from www.invazivke.si) or in the United Kingdom (OBSERVATREE, https://www.observatree.org.uk/).

The consortium recommends that the diagnostic protocol for *Lecanosticta* is reviewed to consider the 8 recently discovered species from Central America, as they present a real risk to European conifers.

During the project, partners reviewed the available management strategies based on *LA* literature from the USA and Canada, where foresters have had more than a century of managing this pathogen. The results of this study will be published in a peer-review journal (in preparation). Ultimately, no 'silver bullet' has been found to manage existing infections within European forests and woodland areas. Workable options include those general silvicultural techniques which one would employ against a range of foliar pathogens (such as *Dothistroma* spp.), namely, ensuring that plants for planting are healthy and not pre-stressed by inappropriate storage or wounds, and maintaining trees at low stocking density to increase airflow through the stands and keep the disease inoculum levels low. Sites should be selected to minimise the risk of *LA* outbreaks: damp sites (ditches), sites temporarily or permanently waterlogged as well as sites subject to extensive periods of fog should be avoided. Other techniques used in North American are either inappropriate for tree species grown in Europe, or involve use of chemical agents no longer supported in the EU. Further research into new techniques and new plant protection products is strongly recommended. If, some host species turn out to be highly susceptible to *LA* (this has occurred in North America), it may be necessary to consider species change or species diversification at a forest level to reduce the impacts of infection.

Outbreaks of *LA* in woodlands, including bogs and swamps with pines, can be demarcated and isolated to prevent or slow down further spread. The best approach for a successful isolation of infection-foci is to build a barrier around the site, where all potential hosts are removed. On the outbreak site, thinning of the stand, achieved by removing infested trees, can be done to reduce the infection potential and to slow down the spread of the disease on the site itself. Wherever possible, the trees should be replaced by not susceptible tree species in high diversity. These measures should be followed by regular inspection and check for new symptomatic trees for ten years. The system developed and under practice in Slovenia is aimed at the prevention of spread by the public on clothes, shoes and car tires. After identifying sites subject to intense touristic activities (campsites, hiking paths, parking places etc..), all potential host trees, whether infected or not, should be removed from the sites and the material (needles, twigs) should be burnt either on site or transported in sealed containers to an appropriate place for burning. Nevertheless, the awareness among campsite owners, tourists and visitors needs to be strengthened in order to prevent further establishment and spread.

2.6. Benefits from trans-national cooperation

The widespread occurrence of *LA* on various pine species in different ecosystems and climatic zones and the increasing concern about the future impact of this disease are the reasons for an international approach to coordinate research efforts. For the exhaustive collection of both scientific and popular literature worldwide a structured cooperation was needed involving researchers from many European countries to enable a critical review of the knowledge gaps. The same refers to the establishment of a global database containing records of outbreaks of *LA*, which is the base for the ongoing modelling of the future spread of the disease. And last not least, global population genetic studies as also performed in this project, require a strong international cooperation.
A particularly useful outcome from the Euphresco project was showcasing the large eradication efforts which are ongoing in Slovenia. This case study highlighted a close collaboration between scientific institutes, local government, arboricultural workers and the local community, and can be used as an example for other EU countries to follow. Although complete eradication has not yet been achieved, huge efforts have been put in place to isolate this infection and slow down further spread.

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3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

None.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

Cleary M., Laas M., Oskay F., Drenkhan R. (2019). First report of *Lecanosticta acicola* on non-native *Pinus mugo* in southern Sweden. For Path. 2019; e12507. https://doi.org/10.1111/efp.12507

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Oskay F., Laas M., Mullett M., Lehtijärvi A., Doğmuş-Lehtijärvi H.T., Woodward S., Drenkhan R. (2020). First report of *Lecanosticta acicola* on pine and non-pine hosts in Turkey. Forest Pathology. 00: e12654. https://doi.org/10.1111/efp.12654

Sadiković D., Piškur B., Barnes I., Hauptman T., Diminić D., Wingfield M.J., Jurc D. (2019). Genetic diversity of the pine pathogen Lecanosticta acicola in Slovenia and Croatia. Plant Pathol, 68: 1120-1131. https://doi.org/10.1111/ppa.13017

Manuscripts in preparation

Raitelaitytė *et al*. Genetic diversity, population structure and reproductive mode of *Lecanosticta acicola* in Lithuania and Poland

Laas *et al*. Global populations of *Lecanosticta acicola* indicate fragmented structure and shared haplotypes in Europe

Global spread of *Lecanosticta acicola* and other *Lecanosticta* species.

4. Open Euphresco data

None.