Published in final edited form as:

. 2011 August ; 130(4): 503–510. doi:10.1007/s10658-011-9769-3.

Is *Roesleria subterranea* a primary pathogen or a minor parasite of grapevines? Risk assessment and a diagnostic decision scheme

Sigrid Neuhauser,

Institute of Microbiology, Leopold–Franzens–University Innsbruck, Technikerstr. 25, 6020 Innsbruck, Austria, Sigrid.Neuhauser@uibk.ac.at

Lars Huber, and

Scientific Consulting Company, Am Grenzgraben 11, 55545 Bad Kreuznach, Germany

Martin Kirchmair

Institute of Microbiology, Leopold–Franzens–University Innsbruck, Technikerstr. 25, 6020 Innsbruck, Austria

Abstract

In the past the root rot pathogen *Roesleria subterranea* (Ascomycota) was generally considered as a minor parasite, a view with which we were often confronted during field work in German wine-growing regions where this ascomycete recently caused serious problems in established vineyards and at replant sites. To irrevocably demonstrate that *R. subterranea* is not a minor, but a primary pathogen of grapevines (and fruit trees) a pest risk analysis was carried out according to the guidelines defined by EPPO standard series PM 5, which defines the information needed, and contains standardised, detailed key questions and a decision support scheme for risk analysis. Following the provided decision scheme, it becomes apparent that *R. subterranea* must be considered as a serious, primary pathogen for grapevines and fruit trees that can cause massive economic losses. Based on the literature, the pathogen seems to be ubiquitous in wine growing regions in cool climates of the northern hemisphere. It is likely that because of its growth below ground, the small fruiting bodies, and ambiguous symptoms above ground, *R. subterranea* has been overlooked in the past and therefore, has not been considered as primary pathogen for grapevine. Available published information together with experience from field trials was implemented into a diagnostic decision scheme which will, together with the comprehensive literature provided, be the basis (a) to implement quick and efficient diagnosis of this pathogen in the field and (b) to conduct risk analysis and management in areas where *R. subterranea* has not established yet.

Keywords

Grapevine dieback; Pathogen detection; Root rot; Sampling; Soil-borne pathogen; Standard protocol

© KNPV 2011

Correspondence to: Sigrid Neuhauser.

Electronic supplementary material The online version of this article (doi:10.1007/s10658-011-9769-3) contains supplementary material, which is available to authorized users.

Introduction

Roesleria subterranea is a soil-borne ascomycete which is frequently found growing on wood of deciduous trees. It can grow saprobically on dead wood, but is also known to cause root rot of living and healthy plants. It is therefore a saprotroph rather than a biotroph. Over the last decade reports of losses in vineyards caused by *Roesleria* root rot accumulated in Germany and Luxemburg (Huber et al. 2006). Losses were also reported from vineyards in Michigan, USA (Miles and Schilder 2009). Although in the past *Roesleria* root rot was repeatedly reported to cause significant damage of *Vitis* spp. and fruit trees (Viala 1887; Beckwith 1924; Brendel and Hanff 1984; Végelyi 1987; Höfer 1992), in specialist literature for winegrowers *R. subterranea* is usually listed as a rare, minor, and weak pathogen. The reason for this may simply be that a significant amount of the available literature is quite old and/or published in small, local scientific journals in different languages (e.g. German, French, Hungarian and English), leading to a shortage of information available for experts, growers and researchers from different countries and educational backgrounds. To redress the imbalance between the specialised knowledge of scientific papers and practical literature, we collated the available information, translated it, and summarised this information together with current results from our field trials to evaluate the status of *R. subterranea* as plant pathogen using the EPPO standard PM 5 series.

The aim of this study is to provide an up-to-date document by (a) merging and reviewing the information available together with recent data on *R. subterranea* and (b) aiding on-site and in-the-lab detection of this pathogen. For this purpose a diagnostic decision scheme together with a summary of the available detection methods is provided.

Material and methods

To demonstrate that *R. subterranea* is an economically important pathogen of grapevine, a pest risk analysis (PRA) was carried out using data from literature and results from on-site inspections for *R. subterranea* made during the BISGRAM project (Biological Soilborne Grapepest Management, Germany) in the years 2005–2009.

The PM 5 Standard series comprises three guideline documents for pest risk analysis published by the EPPO (European and Mediterranean Plant Protection Organization, <http://archives.eppo.org/EPPOStandards/pr.htm>). Although these three documents focus on the risk assessment of invasive pests, and are based on the “International Standards for Phytosanitary Measures: Risk analysis of quarantine pests – ISPM No.11” document (FAO 2005), they can be used to evaluate the risk from “old” but emerging pests, with some modifications. For the PRA we collected all relevant information needed to answer the questions using the structure specified by the EPPO standard PM 5/1 (1998, Supplementary data S1). Available information about the taxonomy, biology (including the Standard’s specified sections control, dispersal, host range, and geographic distribution), possible economic impact, and diagnoses and detection, were collected and summarised. The decision scheme provided in EPPO Standard 5/3 (2009, Supplementary data S2) was used as guideline to determine the risk of *R. subterranea* as a serious pathogen, ignoring questions relevant only for invasive pests. The format of the EPPO guidelines was not strictly adhered to, but all information needed is discussed in the sections below. The literature used to collect information for the sections specified by EPPO PM 5/1 is summarised in Supplementary document S1, the results of the decision support scheme of EPPO PM 5/3 are outlined in Supplementary document S2.

Results and discussion

Taxonomy

Roesleria subterranea (Weinm.) Readhead: Kingdom Fungi, Phylum Ascomycota, Class Pezizomycotina, Order Helotiales, Family Helotiaceae, Genus *Roesleria*.

Roesleria subterranea was originally described by Thümen (1878) from the roots of grapevine as *Roesleria hypogaea* Thümen & Passerini. At that time it was assumed to be a facultative parasite of grapevine. Prior to the development of molecular techniques, problems with the taxonomy and nomenclature of *R. subterranea* have been difficult to resolve. Readhead (1984) listed ten synonyms for this ascomycete. To further complicate the situation, *R. subterranea* has long been confused with the morphologically very similar lichen *Sclerophora pallida* (Pers.) Y.J. Yao & Spooner, a fact which was comprehensively discussed by Readhead (1984) and Yao and Spooner (1999).

The taxonomy of *R. subterranea* was recently revised based on both morphological and rDNA data (Kirchmair et al. 2008). According to molecular data *Hymenoscyphus pseudoalbidus* V. Quelos, C.R. Grüning, R. Berndt, T. Kowalski, TN Sieber & O. Holdenrieder (anamorph: *Chalara fraxinea* T. Kowalsky) is a relative of *R. subterranea*. *Hymenoscyphus pseudoalbidus* causes a rapidly spreading, devastating disease of ash (*Fraxinus* spp.). Symptoms of this disease are necrotic lesions in the bark and xylem leading to dieback of trees (Queloz et al. 2010).

Biology

The small ascomata of *R. subterranea* are formed on roots or dead wood 0.05–1.5 m belowground and only rarely above ground (Fig. 1–3). On standard culture media only sterile hyphae are formed. An anamorphic state was reported once (Viala and Pacottet 1910), but has not been observed since, despite considerable effort by Beckwith (1924), Véghelyi (1989), Höfer (1992) and our group to induce and identify an anamorphic state. The figures of the “anamorphic state of *Roesleria*” given by Viala and Pacottet (1910) can possibly be interpreted as a contaminating *Clonostachys* sp.

Cultures of *R. subterranea* are easily recognised by their characteristic green pigmentation (Fig. 3; see also section “diagnoses and detection”). The stipitate ascomata of *R. subterranea* are small (2–20 mm). Asci, are slender, septate, and sometimes branched paraphyses form a dry, powdery head (mazaedium, Fig. 1). The asci are cylindrical (up to 50 µm×10 µm) and eight-spored. Ascospores are uniseriate, lens-shaped and septate across the broadest plane (Fig. 2; Kirchmair et al. 2008). Ascomata are mainly formed from autumn to spring, but if the infestation is heavy and the climatic conditions favourable (i.e. wet, cool summer), they can be found during the whole year (Huber et al. unpublished). Like many fungi *Roesleria subterranea* can survive in soil for many years: the mycelia can stay active for years living on dead wood and dead plant material or on organic matter in soil (Höfer 1992) and it is very likely that survival structures like clamydospores or (mikro)sclerotia are formed.

Roesleria subterranea infections begin at the root surface and the fungus invades the cortex and the vascular cylinder, similar to the infection pathways of other soil-borne pathogens such as *Rosellinia necatrix* (Pérez-Jiménez 2006). The hyphae of *R. subterranea* aggregate particularly in the xylem and block the water-transport-vessels leading to root damage and dieback. Intense accumulations of dark compounds in uninfected cells can be observed (Höfer 1992; Kirchmair et al. 2008; Miles and Schilder 2009). Infected plants are usually found in groups and are patchy within vineyards. Symptoms do not appear on the plant parts above the ground until root decay is well advanced and the plant considerably damaged. The symptoms above ground are not distinctive and infections with *R. subterranea* can easily be

missed or confused with various other abiotic and biotic damages and diseases (e.g. chlorosis, nitrogen deficiency, phylloxera disease complex, and other secondary fungal parasites).

Roesleria subterranea can survive a wide range of eco-climatic conditions. The optimum soil temperature for growth is 15–20°C, but *R. subterranea* also grows at temperatures between -3°C and 35°C (Höfer 1992). It tolerates a wide range of pH values from 2.5 to 8.5 and grows well in both humid and dry soils (80–10% of maximum water holding capacity, Höfer 1992). The environmental conditions preferred, together with reports of the occurrence of this fungus (see below), provide evidence that *R. subterranea* may be abundant in cool climate wine producing regions such as Germany (Huber et al. 2006), Austria (Berger and Andert 2003), Canada (Anonymous 2007a), or the East Coast of the USA (Anonymous 1960; Miles and Schilder 2009).

Roesleria subterranea is a facultative parasite capable of infecting living plants as well as dead wood. In laboratory experiments, *Roesleria subterranea* was demonstrated to be a primary pathogen capable of infecting healthy plants (Höfer 1992; Miles and Schilder 2009). Miles and Schilder (2009) reported that artificially inoculated plants were 63% smaller than the control plants with a 77% reduction in fresh root weight, illustrating the severe effect of this pathogen on its host. The pathogen retains the potential to attack healthy plants even after growth outside of a living host (on dead wood or in agar culture) for extended periods. The pathogenicity was not reduced and *R. subterranea* was still able to infect healthy grapevines after having been cultivated on one single wood piece for 5 years (Höfer 1992).

Host range and distribution

Roesleria subterranea has been reported from **EUROPE: Austria** (Berger and Andert 2003; Reisenzein et al. 2007; Kirchmair et al. 2008), **Germany** (Brendel and Hanff 1984; Höfer 1992; Huber et al. 2006), **Hungary** (Véghelyi 1987; Véghelyi 1989), **Scotland** (Foister 1961), **Luxemburg** (Huber et al. unpublished), **France** (Viala 1887; Delatour and Guillaumin 1985). **NORTH AMERICA: USA** (Anonymous 1960; Beckwith 1924; Miles and Schilder 2009), **Canada** (Anonymous 2007a). Recently the pathogen was found to be present in **New Zealand** (Anonymous 2007b; Ho, personal communication). No records are known from Australia (Liberato et al. 2009), South Africa or South America. Given the known distribution, the pathogen is currently limited to vineyards in cool climates.

Roesleria subterranea was isolated frequently from *Vitis* spp. (eg. Thümen 1878; Brendel and Hanff 1984; Höfer 1992; Huber et al. 2006; Miles and Schilder 2009). The fungus was also isolated from *Cydonia* spp., *Malus* spp., *Pyrus* spp., *Prunus* spp., *Salix* spp., *Tilia* spp., *Rosa* spp., *Paliurus* spp., and *Populus* spp. (Beckwith 1924) and different fruit trees (Brendel and Hanff 1984; Véghelyi 1989).

Economic impact

According to Höfer (1992) grapevines infected with *R. subterranean* can die within 2 to 3 years. Similar death rates have been reported on other host plants. In Hungary, 46% of the infected fruit trees (mainly apple) died in the first year and within the first 3 years a total of 60% of the infected trees died (Véghelyi 1989). Current field observations from German vineyards indicate that the rate of dieback is influenced by various environmental factors and/or the location of the infection(s). Plants with infections of primary roots or trunks will die much faster than plants infected only on small, lateral roots. Moreover, dieback rate and severity are influenced by the general plant health and/or the total amount of infected roots. Consequently, damage can vary substantially between infected sites. In Germany we observed that the rate of dieback caused by *R. subterranea* was up to 46% of the total stock

of inspected vineyards. Up to 80% of the vines from our test sites showed reduced vigour or were found to be stunted within vineyards, and yield losses reached 80% and an annual increase in dead or stunted vines of up to 35% was observed. There was no difference in the rate of dieback in old vineyards and replanted sites, which were also severely affected.

The actual extent of economic loss is difficult to assess because of a paucity of available data and natural annual fluctuations in yield and quality, and because the real prices of products have to be considered. Also climatic fluctuations and the influence of other pathogens and pests must be taken into account. But given the data on the rate of dieback observed during our monitoring period in Germany it becomes evident that *R. subterranea* is able to cause massive losses (sometimes complete), especially if favourable conditions for pathogen growth and propagation occur.

Currently, no efficient control methods are available. *Roesleria subterranea* is difficult to control with classical fungicides because they have a restricted potential to percolate the soil. It has been repeatedly recommended that all possible substrates be removed, including infected rootstocks together with every piece of wood in the soil, similar to the recommendations for other soil-borne fungi (e.g. Pérez-Jiménez 2006). As vine roots grow deep into the soil (several metres) and *Roesleria*-infected roots break very easy, these recommendations are not sufficient.

The application of fungal antagonists is a promising method to control *R. subterranea*, since these microorganisms actively disperse throughout the soil (Kirchmair et al. 2007). The first *in-vitro* experiments using dual cultures of *R. subterranea* and fungal antagonists (e.g. *Trichoderma* spp., *Clonostachys* spp.) produced promising results (Neuhauser et al. 2010a). Selected antagonists are currently being evaluated in *in-vitro* systems to identify the most promising strains for field tests.

Diagnoses and detection

The following diagnostic guidelines were designed and approved for the pathosystem *R. subterranea* - *Vitis* spp. If fruit trees or plantations of other deciduous trees are screened, the following protocols should be adopted to the specific needs defined by the crop to ensure reliable results. Generally, the most reliable results are obtained when direct observations in the field and PCR-based detection methods are combined. A decision scheme for the diagnoses of *R. subterranea* is provided in Fig. 4, and the analyses necessary are described below.

Within vineyards, infected plants are usually found in patchy distributed groups. Above-ground disease symptoms are reduced vigour and stunted appearance of the plant. Characteristically a weak chlorosis of the leaves can be seen, but with the progress of the disease, apoplexy of single shoots or the whole plant can be observed regularly. When clear symptoms are visible on plant parts above ground, root decay is usually well advanced and a considerable part of the root system is already damaged. Because the symptoms above-ground are ambiguous, it is important not to mistake *R. subterranea* infection for other abiotic and biotic damages and diseases like chlorosis, nitrogen deficiency, the phylloxera disease complex, or other fungal parasites causing similar symptoms.

Because the symptoms seen above-ground are located distant from the true cause of the disease, they are only indicative of an infestation with *R. subterranea*, but not diagnostic. To definitively identify *Roesleria* root rot, the root system must be inspected. The best diagnostic characters are the typical fruiting bodies formed on the roots (Fig. 1, for a detailed description of the ascomata see section “Biology”). To find these fruiting bodies a hole of at least 0.5 m in depth and width should be dug next to the trunk of a plant suspected

to have the disease. The exposed main and lateral roots should be inspected for ascomata, primordial ascomata or other suspicious mycelial aggregations. The typical green colour that is formed in pure culture (Fig. 3) cannot be considered as diagnostic character in the field, because it is usually impossible to recognise in field samples.

If fruiting bodies of *R. subterranea* are found, the apparently healthy plants close to the infected ones should also be examined for ascomata to estimate the spatial distribution of the pathogen. If no ascomata can be found, suspected diseased root parts (e.g. roots with rot symptoms, the characteristic glass-like breaking behaviour, roots covered by mycelia) should be transferred into the laboratory, incubated in a humid chamber in the dark at 15–20°C, and checked for the formation of ascomata regularly during the next 8 weeks. But some *R. subterranea* isolates do not form fruiting bodies in the field or after incubation in the lab (Höfer 1992). Therefore, roots from suspected plants with no visible fruiting bodies should be tested with PCR-based methods.

DNA extraction methods for *Vitis*-rootstocks and soil, and *R. subterranea* specific primers were described by Neuhauser et al. (2009). Root samples from suspicious plants collected as described above should be analysed according to these methods. If a whole site needs to be screened for *R. subterranea*, extensive sampling strategies for root and soil samples are needed, and best results are obtained when both root and soil samples are processed. The most reliable and convenient way of taking root samples is to follow the guidelines for grape-phylloxera quantification (Porten and Huber 2003): root samples are taken with a spade 10–20 cm beside the grapevine-trunk underneath the row from the upper 50 cm of the soil horizon. At least 15 samples per hectare should be collected from the population of suspected plants. During transport to the laboratory the roots should be stored at or below room temperature (4–25°C), depending on shipping time. In the laboratory, soil should be removed as much as possible without damaging the roots, and the roots screened for ascomata of *R. subterranea*. If not processed immediately, root samples should be air dried overnight and stored at – 20°C until use.

To evaluate the infection risk it can be useful to analyse samples from soil with no vines planted (e.g. at replant sites). Soil samples are taken according to standard procedures (e.g. RAFBCA Standard BCA1/2, Längle et al. 2005). A soil corer (minimum diameter 1 cm) is used to sample the soil. From sites cleared plots at least 40 soil cores per hectare should be taken randomly (if possible down to a depth of 50 cm, otherwise as deep as possible) and are pooled into plastic bags. Preliminary results indicate that taking well-selected, individual samples with a spade from the top 20 cm of the soil horizon gives more reliable results than analysing one pooled sample (Neuhauser et al. 2010b). Soil samples are transported to the lab (as described for root samples), allowed to air-dry overnight, passed through a 2 mm mesh sieve, and stored at – 20°C until use.

Internal controls for DNA extraction and PCR should be used to exclude cross contaminations and false negative results. A parallel experiment with fungal, bacterial or eukaryote universal primers to test the integrity of DNA extracts is advised as indicated in Fig. 4. Reference cultures of *R. subterranea* can be obtained from culture collections (e. g. Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre, Utrecht, the Netherlands) or can easily be established by touching a mazaedium to the surface of an agar plate supplemented with antibiotics (e.g. malt extract agar, potato dextrose agar supplemented with streptomycin and tetracycline). *Roesleria subterranea* is identified rapidly by the characteristic green pigment (26E4-E8, 26 F4-F8, 27E4-E8, 27 F4-F8; Methuen Handbook of colour, Kornerup and Wanscher 1978). This green pigment is the only diagnostic character, as no distinct anamorphic stage is formed.

Conclusion

Following the assessment scheme provided in EPPO standard PM 5/3, it becomes apparent that *R. subterranea* is a serious pathogen with the potential to cause considerable economic losses. Because of its hypogeous growth *Roesleria subterranea* has very likely often been overlooked and, because of the difficulty of accessing the literature, has not been considered as a real concern for the viticulture industry. On summarising the available information, it is evident that the ascomycete *R. subterranea* is a serious threat for grapevine and fruit trees because: (a) its pathogenicity was repeatedly proven by the application of *Koch's* postulate (Viala 1887; Höfer 1992; Miles and Schilder 2009); (b) disease symptoms develop slowly and an infection is usually spotted only when the plants are irreversibly damaged; (c) transmission pathways are largely unclear and diverse (machines, plant material, soil water, vectors); (d) infection results in reduced yield and dieback with potentially severe economic consequences; (e) no control strategies are available and infected plants do not recover; (f) it seems to be widely distributed and abundant in regions with moderate soil temperatures and high humidity (i.e. cool climate vine growing regions); and (g) it can tolerate very wide pH and temperature ranges, combined with a saprophytic ability which enables it to persist until environmental conditions favour the expression of disease symptoms.

In summary, *R. subterranea* must be considered a serious pathogen for grapevine, for which at this time no efficient control is available, and eradication is hardly possible when this pathogen becomes established. In order to avoid excess losses in production due to this root rot pathogen, both phytopathologists and winegrowers need to be aware that this pathogen is capable of causing tremendous harm in their vineyards.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Frank H. Gleason for constructive comments and suggestions during the preparation of this paper. The authors are indebted to Reinhold Pöder for valuable discussions, Mark Hoffman for sampling, Kathrin Bartl-Lecker for help with translating the French texts, and our waitress Lisa for help with Hungarian. We thank all growers for access to vineyards and orchards as well as for their help and support. Special thanks are due to the German Winegrowers' Association for their help and support. The work was partly financially supported by the Hessian Ministry for Environment, Agriculture and Forestry (BISGRAM). Sigrid Neuhauser was supported by a Hertha-Firnberg research grant of the Austrian Science Fund (FWF, grant T379-B16).

Abbreviations

PRA Pest Risk analyses

References

- Anonymous. Index of Plant Diseases in the United States. U.S.D.A. Agriculture Handbook. 1960; 165:1–531.
- Anonymous. [Retrieved September 20, 2010] British Columbia Ministry of Agriculture and Lands. Grape root rot and decline (n.d.). 2007a. from <http://www.agf.gov.bc.ca/cropprot/grapeipm/rootrot.htm#roesleria>
- Anonymous. Pest watch Directory. Biosecurity. 2007b; 80:23.
- Beckwith AM. The life history of the grape root rot fungus *Roesleria hypogaea* Thüm. et Pass. Journal of Agricultural Research. 1924; 27:609–616.
- Berger N, Andert J. Schäden durch *Roesleria hypogaea*. Der Winzer. 2003; 3(03):17–19.

- Brendel G, Hanff B. Wurzelschimmelpilze, mögliche Mitverursacher von Absterbeerscheinungen im Weinbau. *Die Weinwissenschaft*. 1984; 39:407–418.
- Delatour PC, Guillaumin JJ. Importance des pourridiés dans les régions tempérées. *European Journal of Forest Pathology*. 1985; 15:258–263.
- FAO. International standards for phytosanitary measures 2005 edition. ISPM No. 11 Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms (2004). Rome: 2005. p. 135–160. from <http://www.fao.org/docrep/008/y5874e/y5874e00.HTM>
- Foister CE. The economic plant diseases of Scotland. Technical Bulletin of the Department of Agriculture and Fishery Scotland. 1961; 1:1–210.
- Höfer M. Untersuchungen über *Roesleria hypogaea* Thüm. & Pass. als Erreger des Wurzelschimmels der Weinrebe. *Geisenheimer Berichte*. 1992; 13:1–137.
- Huber L, Eisenbeis G, Hoffmann M, Rühl EH, Neuhauser S, Porten M. Wurzelschimmelerreger *Roesleria subterranea* (Weinm.) Redhead: Absterbeerscheinungen und Kümmerwuchs—Gefahr im Verborgenen. *Das Deutsche Weinmagazin*. 2006; 6:25–31.
- Kirchmair M, Hoffmann M, Neuhauser S, Strasser H, Huber L. Persistence of GRANMET®, a *Metarhizium anisopliae* based product, in grape phylloxera-infested vineyards. *IOBC/WPRS Bulletin*. 2007; 30(7):137–142.
- Kirchmair M, Neuhauser S, Buzina W, Huber L. The taxonomic position of *Roesleria subterranea*. *Mycological Research*. 2008; 112:1210–1219. [PubMed: 18693105]
- Kornerup, A.; Wanscher, JH. *Methuen handbook of colour*. 3rd ed. Eyre Methuen; London: 1978.
- Längle T, Pernfuss B, Seger C, Strasser H. Field efficacy evaluation of *Beauveria brongniartii* against *Melolontha melolontha* in potato cultures. *Sydowia*. 2005; 57:54–93.
- Liberato, JR.; Sholberg, P.; Reizenzein, H. [Retrieved September 20, 2010] Grape root rot (*Roesleria subterranea*) Pest and Diseases Image Library. 2009. Updated on 02/09/2009 1:38:37 PM <http://www.padil.gov.au>
- Miles TD, Schilder AMC. First report of grape root rot caused by *Roesleria subterranea* in Michigan. *Plant Disease*. 2009; 93:765–765.
- Neuhauser S, Held R, Kirchmair M. Natural antagonists of the grapevine root rot fungus *Roesleria subterranea*. *IOBC bulletin*. 2010a (in press).
- Neuhauser S, Held R, Huber L, Kirchmair M. Sampling strategies for PCR based detection of *Roesleria subterranea*. *Phytopathologia Mediterranea*. 2010b; 49:116.
- Neuhauser S, Huber L, Kirchmair M. A DNA based detection method for *Roesleria subterranea* in grapevine roots and soil samples. *Phytopathologia Mediterranea*. 2009; 48:59–72. [PubMed: 21442023]
- OEPP/EPPO. EPPO standards. PM 5/3 (4). Guidelines on pest risk analysis. OEPP/EPPO; France, Paris: 2009. from http://archives.eppo.org/EPPOStandards/PM5_PRA/PRA_scheme_2009.doc
- OEPP/EPPO. EPPO standards. PM 5/1 (1). Guidelines on pest risk analysis. OEPP/EPPO; France, Paris: 1998. from http://archives.eppo.org/EPPOStandards/PM5_PRA/pm5-01-e.doc
- Pérez-Jiménez RE. A review of the biology and pathogenicity of *Rosellinia necatrix*—the cause of white root rot disease of fruit trees and other plants. *Journal of Phytopathology*. 2006; 154:257–266.
- Porten M, Huber L. An assessment method for the quantification of *Daktulosphaira vitifoliae* (Fitch; Hemiptera: Psyllorixidae) populations in the field. *Journal of Applied Entomology*. 2003; 127:157–162.
- Queloz V, Grünig CR, Berndt R, Kowalski T, Sieber TN, Holdenrieder O. Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology*. 2010 doi:10.1111/j.1439-0329.2010.00645.x.
- Redhead SA. *Roeslerina* gen. nov. (Caliciales, Caliciaceae), an ally of *Roesleria* and *Coniocybe*. *Canadian Journal of Botany*. 1984; 62:2514–2519.
- Reizenzein H, Pfeffer M, Aust G, Baumgarten A. The influence of soil properties on the development of grape phylloxera populations in Austrian Viticulture. *Acta Horticulturae*. 2007; 733:13–24.
- Thümen, F. Monographische Bearbeitung der sämtlichen bisher bekannten, auf den Arten der Gattung *Vitis* L. vorkommenden Pilze. Wilhelm Braunmüller; Wien: 1878. *Die Pilze des Weinstockes*.

- Véghelyi K. A *Roesleria pallida* (Fries) Sacc. szerpepe a gyümölcsfák fiatlalkori puszulásában. (The role of *Roesleria pallida* (Fries) Sacc. in the early death of fruit trees). Növényvédelem. 1987; 23:405–412.
- Véghelyi K. The isolation and characteristics of *Roesleria hypogaea* Thüm. et Pass. Acta Phytopathologica et Entomologica Hungarica. 1989; 24:293–299.
- Viala, P. Les Maladies de la Vigne. Aux Bureaux de Progès agricole et viticole a Montpellier; Montpellier: 1887.
- Viala P, Pacottet P. Sur la culture du Roesleria de la vigne. Comptes rendus hebdomadaires des séances de l'Académie des sciences. 1910; 151:1770–1771.
- Yao YJ, Spooner BM. Roesleriaceae, a new family of Ascomycota, and a new species of Roeslerina. Kew Bulletin. 1999; 54:683–693.

Fig. 1-3.
Roesleria subterranea **1:** Ascomata on *Vitis* spp. root. **2:** Photograph of a young ascus and septate ascospores. **3:** Pure culture on PDA; characteristic green pigment formed

Fig. 4. Diagnostic decision scheme. Diagnostic procedures that can be applied direct in the field are not shaded, laboratory based diagnostic procedures are grey shaded