Grape leaf rust - *Phakopsora euvitis*

The rust fungus *Phakopsora euvitis* causes a disease of grape leaves. It is widely distributed in eastern and southern Asia and has been introduced into northern Australia. Its distribution in the Americas, other than a recent introduction into southwestern Brazil, is unclear, because other *Phakopsora* rust species on *Vitis* species have been identified in North and South America. The teleomorphic stages have not been compared. The means by which the recent introductions have occurred is not known; the fungus can persist in the uredinial form in warmer climates and dispersal of urediniospores by wind and air currents is a possible means of spread, in addition to transport of infected vines. Australia initiated an eradication program against this species in 2001.

*Phakopsora euvitis* Y. Ono 2000

Spermatogonia in clusters on the leaves of *Meliosma cuneifolia* (China) or *M. myriantha* (Japan, China) (Meliosmaceae), amphigenous, subcuticular, conical or hemispherical, 90-135 m x 60-80 µm.

Aecia mostly hypophyllous, opposite the spermatogonia, peridium well-developed, elongate-columnar or cupulate, rupturing at apex. Aeciospores catenulate, subglobose or broadly ellipsoid, often angular, 15-20 x 12-16µm. Spore wall evenly thin laterally (ca 1µm), thickened (-4µm) apically, colourless, minutely and evenly verrucose.

Uredinia on *Vitis* hypophyllous, minute, scattered or aggregated in small groups, subepidermal, becoming erumpent, surrounded by abundant paraphyses. Paraphyses cylindrical to weakly incurved, evenly thin-walled or dorsally thick-walled (1.5-4.0µm), 30-75 µm high. Urediniospores obovoid, obovoid-ellipsoid or oblong-ellipsoid, 15-29 x 10-18µm. Spore wall evenly ca. 1.5 µm thick, uniformly echinulate. Six or rarely four germ pores, equatorial.

Telia hypophyllous, crustose, brown to blackish brown, often confluent, subepidermal, applanate. Teliospores more or less regularly arranged in 3-5 layers, oblong to oblong-ellipsoid, 13-32 x 7-13µm. Spore wall evenly thin, slightly thickened and brownish in the spores of the uppermost layer. Basidiospores reniform, 8-11 x 5-8µm.

For additional description and illustrations, see Ono, 2000.

**Host range:** *Meliosma* spp. (Meliosmaceae), *Vitis* spp. (Vitaceae)

**Geographic distribution:** Africa, Asia, North America (Caribbean, USA), Oceania (Australia), South America (Brazil).

**Notes:** *Phakopsora uva* Buritica & Hennen in tropical America (Buritica, 1999) differs from *P. euvitis* in the thinner walls of the paraphyses and urediniospores. *Phakopsora muscadine* Buritica, reported from the southern USA and Mexico, is more like *P. euvitis*, but has fewer layers and shorter spores in the telia and its urediniospores have thicker apical walls (Buritica, 1999).

*Uredo caucensis* Mayor, also described from tropical South America, differs from the uredinial form of *P. euvitis* in having larger spores (Leu, 1988). Other rusts occurring on *Vitis* species include *Phakopsora cronartiiiformis* (Barcl.) Diet., an Asian species with brown verrucose urediniospores, and *Catenulopsora Vitis* (Butl.) Mund. and Thirum. that produces its teliospores in chains (Leu, 1988).

A fungus causing leaf rust disease of cultivated grapes was first described and named as *Uredo vitis* Thüm., based on a specimen on *Vitis vinifera* collected in Aiken, South Carolina, USA, in 1868 (Thümen, 1878a, b). Another uredinal rust fungus on cultivated grapes was found near Kingston, Jamaica, and named *U. vitiae* Lagerh. (Lagerheim, 1890). Massee compared the type materials of both *U. vitis* and *U. vitiae* and found the two identical (cited from Dale, 1955).

After the two *Uredo* species were published, two other rust fungi, collected on *Ampelopsis brevipedunculata* and *Parthenocissus tricuspidata* in Tokyo, Japan, were described as *Phakopsora amapelopsis* (Dietel, 1898) and *P. vitis* (Sydow, 1899). Hiratsuka (1900) found no disjunction in morphological variations among the host-genus limited fungal groups when comparing Japanese isolates on *A. brevipedunculata, P. tricuspidata, vitis coignetiae, V. flexuosa* and *V. vinifera*. He concluded that all rusts on vitaceous plants in Japan belonged to the species described, but considered *U. vitis* different from *P. amapelopsis*. In his monographic work on the Japanese species of *Phakopsora*, Hiratsuka (1935) also concluded that *P. vitis* and *P. amapelopsis* were conspecific and then included *U. vitis* and *U. vitiae* under the synonym of *P. amapelopsis*. Since this monograph, rusts parasitic on vitaceous plants including cultivated grapes usually have been referred to as *P. amapelopsis*. 
Ono (2000) showed that fungal isolates on the three host genera were distinct in their host specificity and life cycle and that the host-delimited rusts were consistently different in number and distribution of urediniospore germ pores, shape and size of uredinial paraphyses, aeciospore wall thickness, teliospore arrangement in the sorus and shape and size of basidiospores. The host-delimited and morphologically different rusts were determined to be distinct species. The fungi retained the names *Phakopsora ampelopsidis* on *Ampelopsis* and *P. vitis* on *Parthenocissus*. As a result, the fungus on *Vitis* lacked a teleomorph name and was subsequently named *P. eu* _vitis_ (Ono, 2000). This taxonomy is supported by results of the molecular study of Chatasiri and Ono (2008), which placed the isolates of the three species in separate phylogenetic clades within the genus, based on sequences of the D1D2 and ITS2 regions of rDNA.

Ono (2000) put the anamorphic names of *Uredo*, *U. vialae* and *U. Vitis*, which had been described from the Americas, in synonymy with *P. eu*vitis. Buriticá (1994, 1999), however, identified two new species in the Americas: *Phakopsora uva* on *Vitis* in tropical regions, and *P. muscadinae* in subtropical areas of the southern USA and Mexico. He considered the first to be the teleomorph of *U. vialae* and the second to be the teleomorph of *U. vitis*.

**DISTRIBUTION**

Ono (2000) discovered *Phakopsora eu*vitis on *Vitis* throughout eastern Asia. Distribution of the rust found on *Vitis* spp. in Asia may be considered to be *P. eu*vitis, although attributed to *P. ampelopsidis* or *Physopella Vitis* at the time of the reports (Punithalingam, 1968; CABI/EPPO, 2007). This rust appears to have a wide distribution in China and Southeast Asia, as well as India and Sri Lanka. It was recently reported from northern Australia (Weinert et al., 2003). The Australian isolate differed genetically from Japanese isolates of *P. eu*vitis (Chatasiri and Ono, 2008), thus its introduction may have occurred earlier.

The occurrence of *Phakopsora eu*vitis in the Americas is inferred from the taxonomic identification of *U. vitis* on *V. vinifera* from North Carolina, USA, *U. vialae* on cultivated grapes from Jamaica (Ono, 2000), and examination of additional specimens collected from the southern USA (deposited in the Arthur Herbarium (PUR), Purdue University, USA). However, only seven telial specimens from Florida and Georgia, USA, Mexico, Guatemala, Honduras and Colombia have been deposited in PUR (Ono, 2000). No teliospore germination was observed and basidiospore morphology remains unknown. Host ranges and life cycles of the American isolates of grape rust fungi have not yet been studied sufficiently to confirm the occurrence of *P. eu*vitis in the USA and its geographic distribution in the Americas.

An additional fungus, *Phakopsora uva*, that also causes rust of grapes was described by Buriticá (1994) based on a specimen collected in Mexico. Diagnostic characters were stated to include long, basally united paraphyses, with hyaline and evenly thin walls that encircle the entire sorus, and apically thick-walled urediniospores. *Phakopsora uva* was reported to occur on unidentified species of *Vitis* in Colombia (six specimens) and in Mexico (the holotype). Its host range and geographic distribution is not clear.

Grape leaf rust, identified as *P. eu*vitis according to the description of Ono (2000), has recently spread into southwestern Brazil. It was first identified in the state of Parana (Tessman et al., 2003), but was soon found also in the states of Mato Grosso (Gava et al., 2003; Souza, 2004), Mato Grosso do Sul (Papa et al., 2003; Naruzawa et al., 2006), Sao Paulo (Tessmann et al., 2004), and Santa Catarina (Sonego et al., 2005). It is not known whether the fungus arrived from a location in the Americas or from elsewhere.

**DETECTION AND INSPECTION METHODS**

The lower surfaces of leaves of *Vitis* species must be examined for minute yellowish powdery uredinia and flat brown subepidermal telia.

**DIAGNOSTIC METHOD**

This fungus seems to be highly host-specific, being restricted to the genus *Vitis* (Ono, 2000). Thus, correct identification of the host will facilitate the identification of this species within *Phakopsora*.

*Phakopsora eu*vitis forms cylindrical or weakly incurved uredinial paraphyses with a moderately thickened dorsal wall (1.5-4 µm) and a thin ventral wall, six scattered germ pores in the urediniospore and a kidney-shaped basidiospore, by which characters it can be distinguished from the morphologically similar *P. ampelopsidis* on *Ampelopsis* and *P. vitis* on *Parthenocissus*.

Nevertheless, except for characters of the uredinial paraphrases, the differences in morphology of the species on Vitaceae are subtle, and not all of the spore forms will be available for examination at one time. Sequences of the D1D2 and ITS2 regions of ribosomal DNA for four species (Chatasiri and Ono, 2008) are available in GenBank for comparison with those of isolates (NCBI, 2009).

**NOTES ON CROPS/OTHER PLANTS AFFECTED**
Cross inoculation experiments with Japanese isolates showed that the fungus parasitizes *Vitis amurensis*, *V. coignetiae*, *V. ficifolia*, *V. flexuosa*, and *V. labrusca* varieties Campbell Early, Delaware and Kyoho, producing uredinia and telia on the leaves (Ono, 2000). The other species of *Vitis* listed are inferred as hosts of this fungus based on morphological similarity and taxonomic relationship of their reported rust pathogens. All fungal isolates on the Japanese *Vitis* species and grape cultivars were shown to form spermogonia and aecia on *Meliosma myriantha* Siebold & Zucc. (Meliosmaceae).

**SYMPTOMS**

Spermogonial-aecial infection causes pale yellowish, circular or orbicular lesions on the leaves of the alternate host, *Meliosma myriantha*. Tiny orange-brown dots (spermogonia) appear on the adaxial surface of the lesion and become blackish. Dome-shaped, pale yellowish orange-coloured aecia appear on the abaxial surface of the lesion, usually opposite the spermogonia. The aecia grow into a long columnar shape, the tip of which opens to release aeciospores.

Uredinial-telial infection on *Vitis* causes yellowish to brownish lesions of various shapes and sizes. Sometimes no appreciable lesion is formed on the upper leaf surface even though fungal sporulation takes place on the lower surface. Yellowish-orange masses of urediniospores are produced in uredinia formed on the abaxial surface of the lesion. Telium formation follows the uredinal stage; the telia are formed around the uredinia or separately. The telia are crust-like and orange-brown initially, but become dark brown or almost black. Heavy infection is not rare, causing entire leaves to become yellow or brown with dense uredinium and/or telium production. Heavy infection causes early senescence and dropping of the leaves.

**BIOLOGY AND ECOLOGY**

Where the alternate hosts are present, teliospores on fallen or persistent leaves of the grapevine germinate in the spring and disperse thin-walled basidiospores that are wind-borne to the *Meliosma* trees. Ono (2000) obtained teliospore germination at 18-20°C in the dark. Infected alternate host leaves produced subcuticular spermogonia within 7-15 days. Production of asexual spores in aecia on the lower surfaces of leaves on inoculated plants occurred after another 7 to 14 days (Ono, 2000).

In nature, aeciospores are wind-borne to *Vitis* leaves. In experiments, inoculation with aeciospores succeeded when plants were sprayed with distilled water and incubated in a mist chamber at 20°C for 48 hr (Ono, 1994, 2000). Infected leaves bear uredinia and dispersal of urediniospores, primarily by wind, can result in repeated cycles of infection on *Vitis*, as environmental conditions permit, through the growing season. Leu and Wu (1983) found the optimal temperature for germination to be 24°C with a minimum of 8°C and a maximum of 32°C. Inoculation of wet leaves with urediniospores produced new uredinia on *Vitis* within 5 to 19 (rarely 25) days (Ono, 2000). Because the fungus penetrates the leaf through stomata, the youngest leaves that lack mature stomata, are not infected (Leu and Wu, 1983). In Brazil, both young and mature grape leaves can be infected (Angelotti et al., 2008).

Telia develop in autumn on the lower sides of the leaves near uredinia. The fungus may overwinter as telia but, in tropical and subtropical climates, may persist solely in the uredinial state without need for an alternate host (Leu, 1988). Uredinial mycelium may survive unfavorable conditions in dormant buds (Weinert et al., 2003).

This rust appears to be limited in host range to the genera of its known hosts. In test inoculations, basidiospores of *P. euvitis* from different *Vitis* species infected only *Meliosma myriantha*, not *M. tenuis* (Ono, 2000). Aeciospores produced on the alternate host from any of the basidiospore infections were not specific in their pathogenicity, infecting wild and cultivated *Vitis* species. Urediniospores from different *Vitis* species also showed no specificity of infection on those species in Japan (Ono, 2000). On the other hand, neither aeciospores nor urediniospores successfully infected plants of the species tested in the related genera of *Ampelopsis*, *Cayratia* or *Parthenocissus* (Ono, 2000).

**MOVEMENT AND DISPERSAL**

**Natural Dispersal:** Urediniospores and basidiospores of rusts are distributed by wind (Alexopoulos et al., 1996). Long-distance dispersal of urediniospores may be prevented by desiccation through the thin spore walls (Weinert et al., 2003).

**Accidental introduction:** Weinert et al. (2003) suggested that grape leaf rust could have entered northern Australia from islands to the north on wind currents, on clothing, or as the result of illegal plant introductions.

**SEEDBORNE ASPECTS OF DISEASE**

**Economic impact:** Heavy infection near harvest time, which occurs often in warm temperate and subtropical regions, would cause considerable fruit-quality reduction and yield loss. Heavy infection and resultant premature defoliation during the growing season also causes poor growth of the shoot, which
results in retarded growth of the grapevines in the next spring and subsequent reduction in fruit setting and enrichment of fruit during the growing season. According to Ozoe and Kadowaki (1971), heavily diseased vines sprouted six days later than slightly diseased vines, and the diameter of new shoots formed from the heavily diseased vines was narrower than those of the slightly diseased vines (0.56 vs. 0.63 cm). The flowering time was three days later, the number of flower/fruit clusters per shoot was lower (1.3 vs. 2.2) and the number of flowers per cluster was reduced (38.4 vs. 62.0) for heavily diseased vines. The number of mature fruits per cluster was considerably reduced (12.5 vs. 52.4); thus, weight of a fruit cluster was significantly lower (9.1 vs. 30.3 g) than for the slightly diseased vines.

MANAGEMENT

Due to the danger of introduction into its Northern Territory, Australia has placed *P. euvitis* on its NAQS listed of targeted plant pathogens (AQIS, 2009). A public awareness campaign in Northern Australia resulted in additional reports on the extent of grape leaf rust spread (Weinert et al., 2003). A National Grapevine Leaf Rust Eradication Program was begun in 2001 to eradicate the fungus from the Northern Territory of Australia (DAFF, 2009).

CHEMICAL CONTROL

Field trials of chemical protectants and eradicants have been carried out to control the rust disease in conjunction with other foliar diseases. However, most of the results have not been published. Ozoe and Kadowaki (1971) reported that application of Bordeaux mixture, Captan, difolatan, prochloraz and polycarbamate significantly reduced the disease incidence in a 1969 field trial; benomyl and BDC were also reported to be effective. Of eight fungicides tested in Brazil, propiconazole, tebuconazole and azoxyxystrobin provided the best control (Naruzawa et al., 2006).

HOST RESISTANCE

The use of resistant species and cultivars is most promising in preventing rust epidemics in grapevine cultivation. In a field study conducted in Maharashtra, India, *Vitis berlandieri*, *V. candicans*, *V. champini*, *V. palmata*, *V. parviflora* and *V. tiliifolia* were highly resistant to a local isolate of *P. euvitis* (reported as *P. ampelopsisidis*) (Patil et al., 1998). In contrast, the cultivars Thomson Seedless and Tas-A-Ganesh, widely grown in Maharashtra, appeared susceptible.

Leu (1988) observed that cultivars derived from tropical *Vitis* species, such as *V. tiliifolia* and *V. simpsonii*, are almost immune, while those derived from temperate zone species are susceptible. In Japan, all cultivars including Delaware, Campbell Early and Kyoho, which are widely cultivated and derived from *V. labrusca* and *V. vinifera*, were highly susceptible to local isolates of the rust fungus; among the wild species, only *V. flexuosa* is highly resistant, while *V. amurensis*, *V. coignetiae*, and *V. ficifolia* are highly susceptible (Y. Ono, Ibaraki University, Ibaraki, Japan, unpublished data). In a field survey in Sukothai, Thailand, in December, 1985, cultivars Sehwarse, Ramsey and Carolina were found to be highly susceptible (Y. Ono, Ibaraki University, Ibaraki, Japan, unpublished data).

Field observations in vineyards in Orlando Florida, USA in November, 1923 (Shear, 1924) demonstrated that most of the cultivars were defoliated prematurely due to heavy infection of the rust fungus. However, cultivars Fern, Blondin, Wonder, Royal and Carmen showed considerable resistance; the last cultivar proved to be the most successful at resisting the rust infection in Florida (Shear, 1924).

Compared with cultivars derived from *V. labrusca* and *V. vinifera*, most of the cultivars derived from *V. rotundifolia* seemed to be highly resistant or even immune to the rust fungus (Clayton and Ridings, 1970). The field survey conducted in 1967 at the Central Crops Research Station at Clayton, North Carolina, USA, revealed that most of the 742 cultivars surveyed, only 3% were highly susceptible while 82% showed no rust (Clayton and Ridings, 1970). Following inoculation with naturally formed urediniospores under greenhouse conditions, NC57-56 and Howard showed high susceptibility; Higgins and Hunt were slightly susceptible; and NC17-123, NC20-30, NC11-137, Magnolia, Pamlico, Topsail, Scuppernong, Roanoke, Tarheel, Albemarle, Chowan and Thomas showed no sign of infection. Of the *V. labrusca* cultivars inoculated, Portland and Fredonia were slightly susceptible while Niagara and Concord were immune (Clayton and Ridings, 1970).

Rootstock cultivars were the most resistant of 15 genotypes tested in Brazil, exhibiting a hypersensitive response to infection and yielding low numbers of small uredinia (Angelotti et al., 2008). Evidence of both qualitative and quantitative resistance to leaf rust was observed.

GAPS IN KNOWLEDGE/RESEARCH NEEDS

The identities and relationships of the rust fungi in *Phakopsora* on grapes in the Americas should be clarified to determine the actual distribution of these pathogens and the threat of their introduction to other areas. Efforts to select and breed for resistant tropical cultivars should continue.