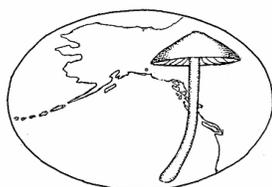


# Pacific Northwest Fungi



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## ***Alternaria malorum*: A Mini-Review with New Records for Hosts and Pathogenicity**

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**Abstract:** Modern host-fungus indices and databases contain deceptively few entries for *Alternaria malorum* or its synonym, *Cladosporium malorum*. Close inspection of literature from the 1930s through the 1960s indicates more hosts and wider prevalence than more modern indices and databases indicate. Reports from 2002 to the present document diverse additional hosts in the Pacific Northwest, including this report from roots of *Pinus ponderosa* and *Pseudotsuga menziesii*, the first reports from gymnosperms. Cherry tomato and grape tomato (*Lycopersicon esculentum*) were found to be hosts for *A. malorum* by artificial inoculation. Rarely documented in synoptic indices or databases in the last 20 years, the Cladosporium-like *C. malorum* is neither rare nor a true *Cladosporium*.

Key words: *Alternaria malorum*, *Cladosporium*, cherry tomato, grape tomato, *Lycopersicon esculentum*, *Pinus ponderosa*, *Pseudotsuga menziesii*.

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### **Introduction**

For good reason, *Alternaria malorum* (Ruehle) U. Braun, Crous & Dugan spent most of its nomenclatural history as

*Cladosporium malorum* Ruehle. Ruehle, then F.D. Heald's graduate student at the State College of Washington (now Washington State University, WSU) was the

first person to receive a doctoral degree from WSU (Bruehl n.d.). Ruehle isolated the species from cold-stored apple fruit, illustrated and described the fungus and, with Heald, illustrated and described the rot it caused (Heald and Ruehle 1930, 1931; Ruehle 1930, 1931). The species fit well within the broadly applied concept for the genus *Cladosporium* during Ruehle's time, and much of the time thereafter. Long, occasionally branched chains of olive-brown, mostly 0-septate, blastic conidia arise from conidiophores of varying lengths (Figs. 1-2). Later, other mycologists isolated this species and applied different names. Matsushima (1975) isolated a fungus from radish seed in Japan, and applied the name *Cladosporium porophorum* Matsushima. In South Africa, Marasas and Bredell (1974) isolated a fungus from lucerne seed, plus oat and wheat straw, and named their isolates *Phaeoramularia kellermaniana* Marasas & Bredell. Their fungus was transferred first to *Cladophialophora*, then to *Pseudocladosporium* (Braun and Feiler 1995; Braun 1998). *C. malorum* was transferred to *Alternaria* by Braun et al. (2003). A comprehensive nomenclatural synopsis can be found in Dugan et al. (2004). In addition to the works cited above, illustrations were provided in Ho et al. (1999), and in Dugan et al. (1995), who included a drawing of material deposited at Centraalbureau voor Schimmelcultures (CBS) by F.D. Heald in 1931. The fungus illustrated under the name *Cladosporium malorum* by Zhang et al. (2000, Fig. 78) does not closely resemble *Alternaria malorum*, but appears to be a species of *Cladosporium* sensu stricto.

Shaw (1973) reported *C. malorum* on seed of *Beta vulgaris* and *Daucus carota* from British Columbia (citing Conners 1967), and on fruit of *Malus sylvestris* (citing Ruehle 1931). Conners (1967), citing an unpublished manuscript by J.W. Groves, also reported *C. malorum* on seed of *Agropyron cristatum*, *Bromis inermis*, *Linum usitatissimum*, *Medicago sativa*, *Pastinacia sativa*, *Pisum sativum*, *Spinacia oleracea*

and *Zea mays*. Farr et al. (1989) listed only apple fruit (*Malus sylvestris*) as a host, citing Ruehle (1931). Ginns (1986) did not document any reports from Canada. Presently Farr et al. (n.d.) list few hosts or substrata: *Malus* and *Prunus* species (from China, citing Zhang et al. 2000, and from Washington State), smooth brome (*Bromus inermis*, Canada), grass litter (Canada), wheat (South Africa) and soil (Lebanon). Thus, recently published host-fungus indices and databases may give the impression that the fungus, although cosmopolitan, is rare.

Reports of *C. malorum* from barley straw and stored grain (Pakistan and Turkey), and *Persica vulgaris* (Libya) are mentioned by Braun and Feiler (1995). Our laboratories in the Pacific Northwest (PNW) have in a relatively short period of time isolated the fungus from dormant buds of *Vitis vinifera* (Dugan et al. 2002), from culm nodes of *Aegilops cylindrica*, *Festuca idahoensis*, and *Leymus cinereus* (Dugan and Lupien 2002), from seed of *Aegilops cylindrica*, *Bromus tectorum*, *Pseudoroegneria spicata*, and *Triticum aestivum* (Dugan and Lupien 2002), and from chickpea (*Cicer arietinum*) debris (Dugan et al. 2005). Most recently, one of us (Goetz) has repeatedly isolated *A. malorum* from conifer roots. We augment this review by formally reporting the isolation from conifer roots and describing pathogenicity tests on miscellaneous fruits.

## Materials and methods

**New report:** *A. malorum* isolates CROW3 C10-4 OUT and CROW6 G14-2 IN were recovered from root cores of *Pinus ponderosa* collected 7 July and 14 July 2004 respectively at Mission Creek, Okanogan-Wenatchee National Forest, Washington state in habitat described by Agee et al. (2001) and Hoff et al. (2004). Isolate TRIPP Q9-1 OUT was recovered from a root core of *Pseudotsuga menzeisii* on 31 August at the same location. Cores were extracted from asymptomatic roots with a sterile increment borer and stored in plastic straws on dry ice. Xylem tissues

were excised from each core, divided into paired samples from each core, dipped in 70% ethanol, flame-disinfested, transferred to benomyl-dichloran-streptomycin agar (Worrall and Harrington 1993) or 2% malt-extract agar (15 g agar, 20 g malt extract, and 100 mg streptomycin sulfate per L) and incubated in the dark at 22° C for  $\geq$  8 weeks. Isolates are stored on agar media at 4° C at the USDA Rocky Mountain Research Station, Moscow, ID. Identification on morphological criteria was according to Ho et al. (1999). Mycelium from young, actively growing cultures was used to extract template DNA for amplification of ITS1, ITS2 and 5.8S sequences of nuclear ribosomal DNA (rDNA) as described previously (Hoff et al. 2004). Amplified DNA was sent to the University of Wisconsin Biotechnology Center (Madison, WI) for sequencing. Sequences were edited using BioEdit (Hall 1997-2005). BLAST searches were conducted to compare ca. 600 bp (partial ITS1, 5.8S and partial ITS2) with sequences in GenBank.

**Pathogenicity tests with isolates of *A. malorum*:** A single-spore isolate of V5#19 (CBS 112048, ex-type for *A. malorum* var. *polymorpha*) from dormant bud of grape, *Vitis vinifera* (Braun et al. 2003; Dugan et al. 2002), was grown on half-strength V8 agar (Stevens 1981) under 12 hr / 12 hr fluorescent + near ultraviolet lights / darkness at 22-25° C for 14 days. Conidia were harvested into sterile distilled water, quantified with a hemacytometer, and the resultant suspension adjusted to 10<sup>6</sup> conidia/ml. Fruits of seedless green table grape (*V. vinifera*) and cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*), purchased at local retail outlets, were disinfested by immersion for 5 min in 0.5% NaOCl, rinsed in sterile water and individually wounded by penetration to a depth of 7 mm with a flame-disinfested needle. Five wounded fruits of each host species were inoculated by placing a drop of the conidial suspension directly onto the wound, and five corresponding control fruit of each species received only drops of

sterile distilled water. The procedure was twice repeated, once using isolate SB99-28 (Dugan and Lupien 2002) from seed of wheat (*Triticum aestivum*) and once with isolate CP-96A (Dugan et al. 2005) from stem of chickpea (*Cicer arietinum*). Fruits were incubated at ambient laboratory conditions and lesion diameters were measured at 30 days post-inoculation.

The above trial was followed by an additional trial using CROW6 G14-2 IN (from root of ponderosa pine), with SB99-28 as a positive control, to inoculate fruits of grape tomato (*L. esculentum*). Twenty fruits were used for each treatment (CROW6, positive control, negative control inoculated with distilled water only) with growth of isolates, disinfestation and inoculation protocols as described above. Fruits were incubated 24 days under ambient lab conditions. In each trial, *A. malorum* was recovered into pure culture on half-strength V8 by transfer of lesions excised from inoculated fruits. Statistical analysis via ANOVA was with SYSTAT 9 (SPSS Science, Chicago, Illinois).

## Results

**New report:** On the basis of morphology and ITS sequence homology (99%), isolates from conifer roots were identified as *Alternaria malorum*. We here note that the GenBank sequences with which our results were compared were derived from research of Braun et al. (2003) or research performed on authentic or representative material at American Type Culture Collection. To our knowledge, these are the first reports of isolation of *A. malorum* from gymnosperms.

**Pathogenicity tests:** All three isolates of *A. malorum* used in the first trial proved pathogenic to cherry tomato, creating sunken, brown lesions. Diameters of lesions induced by V5#19 ranged from 12 to 20 mm; those for CP96A from 5 to 25 mm; and those for SB99-28 from 3 to 18 mm. Lesions were absent on two of five fruits inoculated with V5#19 or CP96A, and from one fruit inoculated with SB99-28. No

lesions developed on controls. Overall differences (LSD) were significant at  $P = 0.035$ .  $P$  values for each isolate relative to its control were 0.059 for CP96A, 0.035 for SB99-28, and 0.029 for V5#19B. When controls were combined (since lesion diameters were zero in all instances), overall  $P$  value was 0.006, and  $P$  values of each isolate relative to controls were 0.018 for CP96A, 0.008 for SB99-28, and 0.006 for V5#19B. No lesions developed on inoculated grapes or the control grapes. In no instances did isolates significantly differ from each other.

In the second trial, CROW6 and the positive control SB99-28 differed from the non-inoculated controls at  $P = 0.00$ , and CROW6 and the positive control did not differ from each other. Except for one fruit on which grew a colony of dictyoseptate *Alternaria* sp. (not *A. malorum*), no non-inoculated controls developed lesions, whereas lesions for CROW6 ranged from 1.8 to 6 mm, and those from SB99-28 from 1.3 to 10.1 mm. Two fruits in the CROW6 treatment and 5 fruits in the SB99-28 treatment did not develop lesions. In both trials, *A. malorum* was recovered back into culture from tissues of symptomatic fruits.

**Discussion:** We have presented in the introduction evidence that past reports of *Cladosporium malorum* were more common than modern compendia and current databases indicate. We use our discussion of this species to emphasize its high prevalence in the PNW, its proper classification in the genus *Alternaria*, and the range of hosts on which it is pathogenic.

Imposing but overlooked documentation has long been available for the potential importance of *A. malorum* in the PNW. Schnellhardt and Heald (1936) washed samples of market wheat from Washington State, and plated the wash water to agar media. They reported, "The prevalence of *Cladosporium malorum*, found on twenty of the twenty-four samples washed, is the outstanding feature of this study." The

incidence of *A. malorum* in the twenty samples ranged from 8.3% to 100%, with fourteen samples showing an incidence of 70% or above. The authors noted that the pathogenicity of the fungus on wheat was not investigated. Although Sprague (1950) did not index *C. malorum*, his monographic work on fungal diseases of cereals and grasses briefly referenced Schnellhardt and Heald (1936) and mentioned the fungus. Unknown is the extent to which the reported dominance of *A. malorum* on market wheat represented an aberration. Even if highly exceptional, the extreme prevalence of the species in the survey of Schnellhardt and Heald (1936) argues that *A. malorum* must exist in well-established reservoirs in PNW regional ecology. There is also circumstantial evidence that the fungus was known on wheat by 1930. In a copy of Ruehle (1930) at Washington State University Libraries, there are miscellaneous marginal notes, in pencil, representing suggestions or minor corrections to the dissertation. One of these notes, immediately after '*Cladosporium malorum* n. sp.' (p. 99) reads "(also on wheat)".

Given the extreme prevalence of *A. malorum* in their results, it is somewhat surprising that neither Schnellhardt nor Heald pursued their findings. Heald, however, had many duties including department Chair (Bruehl n.d.), and Schnellhardt, like Ruehle, was a graduate student focused on apple decay (Schnellhardt 1935). After graduation, Ruehle worked on plant disease problems in Florida.

In 2001, an isolate of *Cladosporium malorum* from asymptomatic *V. vinifera* tissues transiently produced a small number of conidia highly reminiscent of *Alternaria* (Braun et al. 2003). These conidia were basal or intercalary in chains of *Cladosporium*-like conidia. This occasioned a re-examination of conidial morphology and conidiogenesis, and generation of phylogenetic data based on ITS and small

subunit rRNA gene sequences (Braun et al. 2003). Several isolates of *C. malorum* clustered with *Alternaria* species, and were well separated from *Cladosporium sensu stricto*. *Cladosporium malorum* was reassigned to *Alternaria* as *A. malorum*, and the isolate from grape was named *A. malorum* var. *polymorpha*. Braun et al. (2003, Figs.3-12) illustrated characters typical of *A. malorum*, as well as conidia diagnostic for *A. malorum* var. *polymorpha*. An isolate from Höller et al. (2002), identified by U. Braun as *A. malorum*, produced metabolites commonly associated with *Alternaria* spp. (Höller et al. 2002). This strain had been isolated from an unidentified resupinate polypore; so, *A. malorum* is apparently also capable of fungicolous habit. Readers should be aware that at least one other *Alternaria* species, *A. cetera*, produces predominantly cylindrical, aseptate conidia (Simmons 1996).

*Alternaria malorum* has proven pathogenic on ripe apple and cherry fruits (Dugan et al. 1995; Ruehle 1931). Cherry tomato and grape tomato are experimental hosts, as demonstrated above. Grape berries were resistant to infection under our experimental conditions. Tests with blueberry (*Vaccinium corymbosum*) were inconclusive because both inoculated and non-inoculated berries rapidly shriveled and/or molded with other fungi within a few days of inoculation (data not shown). Although *A. malorum* is now documented as a pathogen, it may not be as aggressive on its hosts as several other fungi. Lesions developed slowly on inoculated tomato fruits, and not all inoculated fruits progressed to decay, probably because the isolates failed to penetrate into the wound quickly enough to overcome host defenses.

In conclusion, we can confidently state that the seemingly rare fungus previously known as *Cladosporium malorum* is neither rare nor a *Cladosporium*. Based on isolations over the past decade (Dugan et al. 1995; Dugan and Lupien 2002; Dugan et al. 2002; Dugan et al. 2005, and the report from pine

roots above), and the report of Schnellhardt and Heald (1936), we predict that mycologists and plant pathologists of the PNW will see more of this fungus in the years ahead.

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Figure 1. Conidiophores of *A. malorum* SB99-28 range from small swellings (short arrow) to longer conidiophores with multiple conidiogenous loci (long arrow). DIC. Bar = 5  $\mu$ m.

Fig. 2. Detail of Fig. 1. Conidiogenous loci (arrow) are poroid. DIC. Bar = 5  $\mu$ m.