Cronartium flaccidum

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Scientific Name

Cronartium flaccidum (Alb. & Schwein) Winter

Synonyms:
Endocronartium pini (autoecious form), *Peridermium pini* (autoecious form), *Aecidium asclepiadeum*, *Aecidium paeoniae*, *Aecidium pini*, *Caeoma pineum*, *Cronartium asclepiadeum*, *Cronartium flaccidum* f.sp. *gentianeum*, *Cronartium flaccidum* f.sp. *ruelliae*, *Cronartium flaccidum* f.sp. *typica*, *Cronatrium nemesiae*, *Cronartium paeoniae*, *Cronartium pedicularis*, *Cronartium pini*, *Cronatrium vincetoxici*, *Erineum asclepiadeum*, *Lycoperdon pini*, *Peridermium cornui*, *Sphaeria flaccida*, and *Uredo pedicularis*.

Common Names

Scots pine blister rust, *Cronartium* rust, blister rust, pine-stem rust, resin canker, resin top disease, two-needle pine blister rust

Type of Pest

Fungal pathogen

Taxonomic Position

Kingdom: Fungi, **Phylum:** Basidiomycota, **Class:** Urediniomycetes, **Order:** Uredinales, **Family:** Cronartiaceae

Reason for Inclusion in Manual

CAPS Priority Pest (FY 2013)

Pest Description

Cronartium flaccidum is a rust fungus that affects several two-needle pine species in Europe and Asia. A rust fungus may produce as many as five distinct fruiting structures with five different spore stages in its life cycle in a definite sequence (Table 1). *Cronartium flaccidum* is macrocyclic and is known to produce all five spore stages. Like all rust fungi, *C. flaccidum* is an obligate parasite that requires living host cells to complete its life cycle.

This fungus is genetically identical to the autoecious rust *Peridermium pini* (*Endocronartium pini*), but is heteroecious (Hantula et al., 2002). Autoecious refers to rust fungi that produce all spore forms on one species of host plant (in this case, pine); while heteroecious refers to rust fungi that require two unrelated host plants for completion of its life cycle (in this case, pine and another host).

Moricca et al. (1996) and Hantula et al. (1998) showed that *C. flaccidum* was very closely related to *P. pini* by examining internal transcribed spacer (ITS) sequences and random amplified microsatellite (RAMS) markers, respectively. Vogler and Bruns (1998) determined that there was a close phylogenetic relationship between *C. flaccidum* and *P. pini*. The aeciospores of *P. pini* and *C. flaccidum* are also morphologically indistinguishable (Kasanen, 1997). Kaitera et al. (1999b) showed that *Peridermium pini* and *Cronartium flaccidum* could not be distinguished based upon germ tube morphology as previously suggested by Hiratsuka (1969). Based on molecular and morphological data, authors now consider the two fungi to be synonymous. *P. pini* was shown to be clonal and it was believed to have its origin as a haploid life cycle mutant of *C. flaccidum*, which has a sexual life cycle (Kasanen et al., 2000; Kasanen, 2001). The two fungi are considered synonymous in this datasheet.

Table 1. The five spore stages of a *Cronartium flaccidum*.

STAGE	DESCRIPTION	ROLE
0	Spermagonia* bearing spermatia (n) and receptive hyphae (n)	Formed on pine; Sexual cycle of rust
I	Aecia bearing aeciospores (n+n)	Formed on pine; Infect alternative hosts
II	Uredinia (uredia) bearing urediniospores (uredospores) (n+n)	Formed on alternate host; Reinfects alternate hosts (cycling stage)
III	Telia bearing teliospores (n+n → 2n)	Formed on alternate hosts
IV	Basidia bearing basidiospores (n)	Formed on alternate host; Cause of initial infections on pine

*Note: Spermagonia were formally known as pycnia and spermatia were formally known as pycniospores, and some references use the older nomenclature.

From Mordue and Gibson (1978):
Spermagonia and aecia caulicolous, on slightly to moderately swollen fusiform cankers.

Spermagonia: Spreading beneath the periderm, flat, about 40-50 µm deep and 0.5-3mm diameter, at first yellowish, exuding spermatia 1-2 µm diam. in orange droplets, later darkening, gradually disrupted by enlarging aecia.

Aecia: Peridermioid, about 2-7 mm diam., dehiscence circumscissile or irregular. Peridium several cells thick, the cells rhomboid ellipsoid, elongated up to 80 µm long by 38 µm wide, the walls 4-8 µm thick, strongly verrucose (wart-like); rigid hair like peridial filaments are frequently present. Aeciospores are globose to ovoid-ellipsoid, 21-36 x 14-24 µm (mean 26 x 19 µm) with hyaline walls 2-4 µm thick; walls verrucose except for smooth area at base or side, the warts approx. 1 µm diam. and 1-2 µm high.

Uredinia: Hypophyllous (growing on underside of leaves), in groups or scattered, bullate (appearing puckered, blistered), 0.1-0.3 mm diam., peridiate (with protective layer enclosing spores), dehiscing (splitting open) by a central pore. Urediniospores broadly ellipsoid to obovoid, 18-30 x 11-20 µm (mean 24 x 15 µm), wall hyaline, 1.5-2.5 µm thick, echinulate (spiny) with the spines 2-4 µm apart and about 1 µm high, though some spores show almost smooth areas; germ pores inconspicuous.

Telia: Develop in the uredinia or separately, producing basally peridiate teliospore columns up to 2 mm long and 0.1-0.2 mm wide, pale orange to cinnamon brown, sometimes closely grouped on clearly defined spots, sometimes more scattered. Teliospores catenate (arranged in chains), firmly adherent, fairly short ellipsoid at apex of telial columns, longer and more cylindrical below, ends rounded or truncate, 20-64 x 10-16 µm (commonly about 55 x 12 µm), wall hyaline, yellowish to golden, about 1 µm thick, often thickened at ends or corners (particularly at apex of spore) to 2-3 µm, smooth. The teliospores germinate without dormancy and the upper part of the telial columns usually has a whitish powdery appearance due to the presence of basidia and basidiospores.

Basidia: Mature basidium septate with four cells, 33-40 µm long; each with a conical protuberance called sterigma, about 4 µm in length. Each sterigma has a basidiospore at the apex. In total there are four basidiospores for each basidium. Basidiospores rounded, smooth-surfaced, hyaline, 3-4 µm in diameter (Ragazzi et al., 1987). Basidiospores produce germ tubes that are often ramified. They vary in length (some more than 200 µm after 4 days of incubation) with a diameter of 2-3, 5 µm (Ragazzi et al., 1987).

Biology and Ecology

Table 1 provides a summary of each spore stage of *Cronartium flaccidum* and its role in the lifecycle of the pathogen. *C. flaccidum* infects hosts (*Pinus* spp.) by basidiospores (Stage IV) that are formed on leaves of alternate hosts and aerially dispersed (Ragazzi and Dellavalle Fedi, 1992). The basidia directly penetrate into the stomata to cause the initial infections on pine (Ragazzi and Dellavalle Fedi, 1992). Symptoms, however, only become apparent later in development in the branch and main stem (Geils et al., 2009). On pine shoots, spermagonia (Stage 0) and aecia (Stage 1) are developed, spreading the rust aerially among alternate hosts by aeciospores (Ragazzi et al., 1986a). A period of several years (2-4 years for the autoecious form but longer for heteroecious form) may elapse between infection and the appearance of the aecial state on infected tissue (Mordue and Gibson, 1978; Ragazzi and Moriondo, 1980; Kaitera, 2000). After successful disease establishment, uredinia (Stage III) are formed on alternate hosts, followed by telia (Stage IV) formation from uredinia or directly through the leaf epidermis (Ragazzi et al., 1987; Kaitera and Nuorteva, 2003a). After germination, basidia are formed on telia followed by basidiospore formation. The cycle then repeats. The pathogen survives as mycelium within host tissues.

Several environmental factors influence the development of the disease and the life cycle of *C. flaccidum*. Ragazzi et al. (1989) evaluated temperature, spore type, and host leaf age as variables in the production of uredia and telia of *C.*

flaccidum on the alternate host *Vincetoxicum hirundinaria*. The authors found that 20°C (68°F) was optimal for the production of uredia and telia on host leaves (5-10 days old). The production of uredia was best, however, when urediniospores rather than aeciospores were used as inoculum. Ragazzi (1983) reported that the optimum temperature for formation of uredinia and telial columns was 20-22°C (68-72°F), and temperatures less than 18°C (64°F) or greater than 22°C (72°F) were detrimental to rust fructification.

The temperatures reported for germination of the different spore types are 5-30°C (41-86°F) for aeciospores, 5-30°C (41-86°F) for urediniospores, and 10-25°C (50-77°F) for basidiospores (Mordue and Gibson, 1978; Ragazzi et al., 1986b). The optimum temperature for germination of aeciospores, urediniospores, and basidiospores was reported as 15°C (59°F), 20°C (68°F), and 20°C, respectively (Ragazzi et al., 1986b). High moisture levels and precipitation increase the incidence of disease (CABI, 2005).

Fig. 51. Top: Aecia of *Cronartium flaccidum* on pine. Bottom: Close-up of aecia. Photos courtesy of Ondrej Zincha. www.biolib.cz/en

In addition, pathogenic variability of *C. flaccidum* strains has been observed. Differences in pathogenicity was correlated to different hosts and habitats with significant differences dependent on the *Pinus* spp. inoculated and the elevation from which *C. flaccidum* strains were obtained (Mittempergher and Raddi, 1977).

Cladosporium tenuissimum has been reported as a hyperparasite of *Cronartium flaccidum*. *Cladosporium tenuissimum* and has been isolated from the aeciospores of *Cronartium flaccidum* and it autoecious form *Peridermium pini* (Moricca et al., 1999; Moricca et al., 2001; Nasini et al., 2004). Based on its ability to reduce aeciospore germination, reduce viability of aeciospores, reduce rust development under greenhouse conditions over 2 years, and survive and multiply in forest ecosystems without rusts being present, *C. tenuissimum* appears to be a promising agent for the biological control of pine stem rusts in Europe (Moricca et al., 2001).

Raddi et al. (1979), Raddi and Ragazzi (1980), and Raddi et al. (1980) discuss current progress and issues with breeding for resistance to *C. flaccidum* in pines.

Pest Importance

Blister rust caused by *C. flaccidum* has been described as 'severe', 'rapidly advancing', and 'dangerous' (Ragazzi and Dellavalle Fedi 1983, Hantula et al. 2002). Blister rust has been a major factor in reducing forest productivity for centuries (Hantula et al., 2002). In the 1960s and 1970s the heteroecious form (*C. flaccidum*) spread epidemically in Mediterranean countries and decimated forests of two-needle pines. The disease is severe on Scots pine (*Pinus sylvestris*). The high numbers of coniferous hosts and the very widespread distribution of one of the main alternate hosts (*Vincetoxicum hirundinaria*), led to great losses in Italy, especially in young pine stands (Hantula et al., 2002).

In Britain, the disease rate on Scots pines caused by the autoecious form (*Peridermium pini*) increased from the 1960s to the 1980s (Greig, 1987) causing considerable volume losses on trees with stem lesions and crown symptoms (Gibbs et al., 1987). In Finland, more than 60% and 20% of Scots pines in single stands may be affected by the heteroecious (Kaitera, 2000) or the autoecious rust forms (Kaitera et al., 1994), respectively. In Greece, in a six year period *C. flaccidum* had infected/killed over 5000 m^3 in a forest of approximately 1000 ha (Diamandis and De Kam, 1986). In Sweden, radial stem increment of Scots pine was reduced 40-70% by severe attacks of *C. flaccidum* and 20-40% by minor attacks (Martinsson and Nilsson, 1987).

Signs of *Cronartium flaccidum* and symptoms of the disease may be latent (inactive, hidden, or dormant) for 2 or more years in infected pine host material and up to a month in leafy hosts. The chance of introduction into the United States is high because visual survey of propagative material may not be effective due to this latency (Geils et al., 2009). According to Geils et al. (2009), Japanese black pine (*Pinus thunbergii*), mugo pine (*Pinus mugo*) or other 2 or 3-needled pines, commonly used for bonsai, pose a significant risk for the introduction of *C. flaccidum* if imported as whole plants.

Fig. 52. Left: Telia of *Cronartium flaccidum* on alternate host. Right: Close-up of telial columns. Photos courtesy of Miroslav Demi. www.biolib.cz/en

Symptoms

Cronartium flaccidum causes blister rust in pines. The first symptoms of disease are yellowish, necrotic spots on the pine needles. Chlorosis and necrosis of the infected sites, yellowing and premature defoliation of leaves/needles, branch death, bark discoloration, cankers (lesions) and deformed growth are also commonly observed symptoms of the disease (CABI, 2005). Resinosis (excessive resin exudation) can be seen in the lesions.

Cronartium flaccidum affects plants by growing within the vascular system and impeding nutrient and water uptake. Mycelia grow on young shoots. As the pathogen spreads within the host, it interferes with normal tree growth by killing the cambium and damaging vascular tissue. This damage results in the loss of conductive ability, premature leaf loss, and eventual death of the tree. The pathogen can girdle the part of the tree located above the canker (Mordue and Gibson, 1978).

The disease may occur on pines of all ages. The development of disease is usually rapid and lethal to seedlings and young trees (Martinsson and Nilsson, 1987). Infection, which takes place primarily via needles, leads to swelling of young shoots and to production of blister-like structures in the cortex, which split to reveal masses of orange aeciospores (Fig. 51). The time from infection to visible aeciospores can take several years. In England, the aeciospores are usually observed in early summer (Greig, 1987). Spermogonia with spermatial fluid ('sweetish droplets') also occur on the infected bark. Uredinia and hair-like telia appear on the lower leaf surface of the alternate hosts in mid to late summer (Fig. 52).

Known Hosts

Cronartium flaccidum is known to have many pine hosts, with different levels of susceptibility. *Pinus sylvestris* (Scots pine) is considered a common (although moderately resistant) host, but the pathogen has been shown to cause disease on over 15 pine species. Species in bold are reported by multiple authors as being important hosts of *C. flaccidum*.

Major Pine Hosts:

Pinus brutia (brutian pine), ***Pinus densiflora*** (Japanese red pine), ***Pinus halepensis*** (aleppo pine), ***Pinus koraienis*** (fruit pine), *Pinus laricio* (black pine), ***Pinus massoniana*** (masson pine), *Pinus montana* (dwarf mountain pine), *Pinus mugo* (mountain, mugo pine), ***Pinus nigra*** (black, Austrian pine), *Pinus pallasiana*, ***Pinus pinaster*** (maritime pine), ***Pinus pinea*** (stone pine), *Pinus ponderosa* (ponderosa pine), ***Pinus pumila*** (dwarf Siberian pine), *Pinus rotunda*, ***Pinus sylvestris*** (Scots pine), ***Pinus tabuliformis*** (Chinese pine), *Pinus taiwanensis* (Taiwan red pine), *Pinus takahasii*, ***Pinus uncinata*** (mountain pine), ***Pinus wallichiana*** (blue pine), and ***Pinus yunnanensis*** (Yunnan pine) (Mordue and Gibson, 1978; Ragazzi and Dellavalle Fedi, 1982; Moricca et al., 1996; CABI, 2005).

Alternate hosts:

Asclepias spp. (milkweeds), *Asclepias cornuti* (milkweed), *Asclepias purpurascens* (purple milkweed), *Delphinium delavayi* (Delavayi larkspur), *Euphrasia brevipila* (drug eyebright), *Euphrasia maximowiczkii* (an-jeun-jop-ssal-pul), *Gentiana asclepiadea* (willow gentian), *Grammatocarpus* spp. (twining grammatocarpus), *Impatiens* spp. (impatiens, touch-me-knots), *Loasa* spp. (loasa), ***Melampyrum* spp.** (cow-wheats), *Melampyrum arvense* (field cow-wheat), *Melampyrum cristatum* (crested cow-wheat), *Melampyrum nemorusum* (wood cow-wheat), *Melampyrum pratense* (common cow-wheat) ***Melampyrum sylvaticum*** (small cow-wheat), *Nemesia* spp. (nemesia), ***Paeonia* spp.** (peony), *Paeonia albiflora* (white peony), *Paeonia anomala* (anomalous peony), *Paeonia arborea* (mu dan), *Paeonia broteri* (Brotero's peony), *Paeonia corallina* (peony), *Paeonia cultorum* (peony), *Peonia daurica* (peony), *Peonia edulis* (peony), *Paeonia japonica* (cao shao yao), *Paeonia lactiflora* (Chinese peony), *Paeonia mascula* (peony), *Paeonia moutan* (peony), *Paeonia obovata* (Chinese peony), *Paeonia officinalis* (common peony), *Paeonia peregrine* (peregrine peony), *Peonia suffruticosa* (Japanese tree peony), *Paeonia taurica* (peony), *Paeonia tenuifolia* (peony), *Paeonia triternata* (peony), ***Pedicularis* spp.** (louseworts), *Pedicularis lapponicum* (Lapland lousewort), *Pedicularis palustris* (marsh lousewort), *Pedicularis resupinata* (fan gu ma xian hao), *Pedicularis sceptrum-carolinum* (lousewort), *Phtheirospermum japonicum* (song hao), *Ruellia* spp. (wild petunia), *Schizanthus* spp. (butterfly flower, poor man's orchid), *Siphonostegia chinensis* (yin xing cao), *Tropaeolum* spp. (nasturtium), *Verbena* spp. (verbena), ***Vincetoxicum* spp.** (swallow wort), *Vincetoxicum albovianum* (swallow wort), *Vincetoxicum fuscatum* (swallow wort), ***Vincetoxicum hirundinaria*** (= *Cynanchum laxum, C. vincetoxicum*) (Louise's swallow wort), *Vincetoxicum mongolicum* (hua bei bai qian)*, Vincetoxicum nigrum* (black swallow wort)*, Vincetoxicum officinale* (white swallow wort), *Vincetoxicum rossicum* (European swallow wort), and *Vincetoxicum scandens* (Roll-Hansen, 1973; Mordue and Gibson, 1978; Kaitera and Hantula, 1998; Moricca and Ragazzi, 1998; Kaitera, 1999; Kaitera et al., 1999a; Kasanen, 2001; Kaitera and Nuorteva, 2003ab, Kaitera et al., 2005; Farr and Rossman, 2010).

Note: Considerable variation has been found in the susceptibility of alternate hosts from different locations and the virulence of *C. flaccidum* spore sources (Roll-Hansen, 1973; Kaitera, 1999; Kaitera et al., 1999a).

Raddi and Fagnani (1978) grew several pines from the United States in Europe, inoculated them with *C. flaccidum*, and found several species with no mycelium in needle tissue and no pycnia, aecia, or mycelium in the stem: *P. clausa* (sand pine), P*. contorta* (lodgepole pine), *P. echinata* (shortleaf pine), *P. elliottii* (slash pine), *P. glabra* (spruce pine), *P. radiata* (Monterey pine), *P. resinosa* (red pine), *P. serotina* (pond pine), *P. taeda* (loblolly pine), and *P. virginiana* (Virginia pine). They considered these pines to have a high degree of resistance to *C. flaccidum*, although some did display ‘spotted seedlings’. Kaitera and Nuoroteva (2008) showed no disease symptoms on artificially inoculated *P. contorta* (lodgepole pine), *P. peuce* (Balkan pine), *P. strobus* (eastern white pine), *P. resinosa* (red pine), *P. banksiana* (jack pine), and *P. cembra* (swiss, arolla pine).

Known Vectors

Insects may play a role in mating in *C. flaccidum* based on the similarity of its life cycle to that of *Cronartium ribicola* (Mordue and Gibson, 1978). Insects are attracted to sweet liquid produced from the spermogonia of *Cronartium ribicola* and appear to promote fertilization by carrying spermatia between them.

Outbreaks of Scots pine blister rust are often associated with insect infestations (*Myleophilus piniperda*, *Bupalus piniaria*, *Pissodes notatus*), which aggravate the damage caused. Egg laying of *P. notatus* is localized on pines attacked by *C. flaccidum* (Mordue and Gibson, 1978). Aeciospores have been shown to be artificially transmitted by *Pissodes piniphilus* (Pappinen and von Weissenberg, 1994). *Pissodes pini*, *Dioryctria splendidella*, *Laspeyresia coniferana*, *Lagria hirta*, and *Doryctria abietella* are reported as possible vectors for the rust on the basis of their occurrence and because they feed on *C. flaccidum* aecia (CABI, 2005).

Known Distribution

Asia: China, Japan, and Korea. **Europe:** Armenia, Austria, Azerbaijan, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech. Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Italy, Kosovo, Lithuania, Macedonia, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Russia, Scotland, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, and United Kingdom (Diamandis and De Kam, 1986; Gibbs et al., 1988; CABI, 2005; Geils et al., 2009).

A report from India is considered an invalid record (CABI, 2005). According to Farr and Rossman (2010) there is a record of a synonym of this pathogen (*Cronartium asclepiadeum*) from Vermont in 1898. The validity of this record is not known. All other sources indicate that *C. flaccidum* is exotic to the United States.

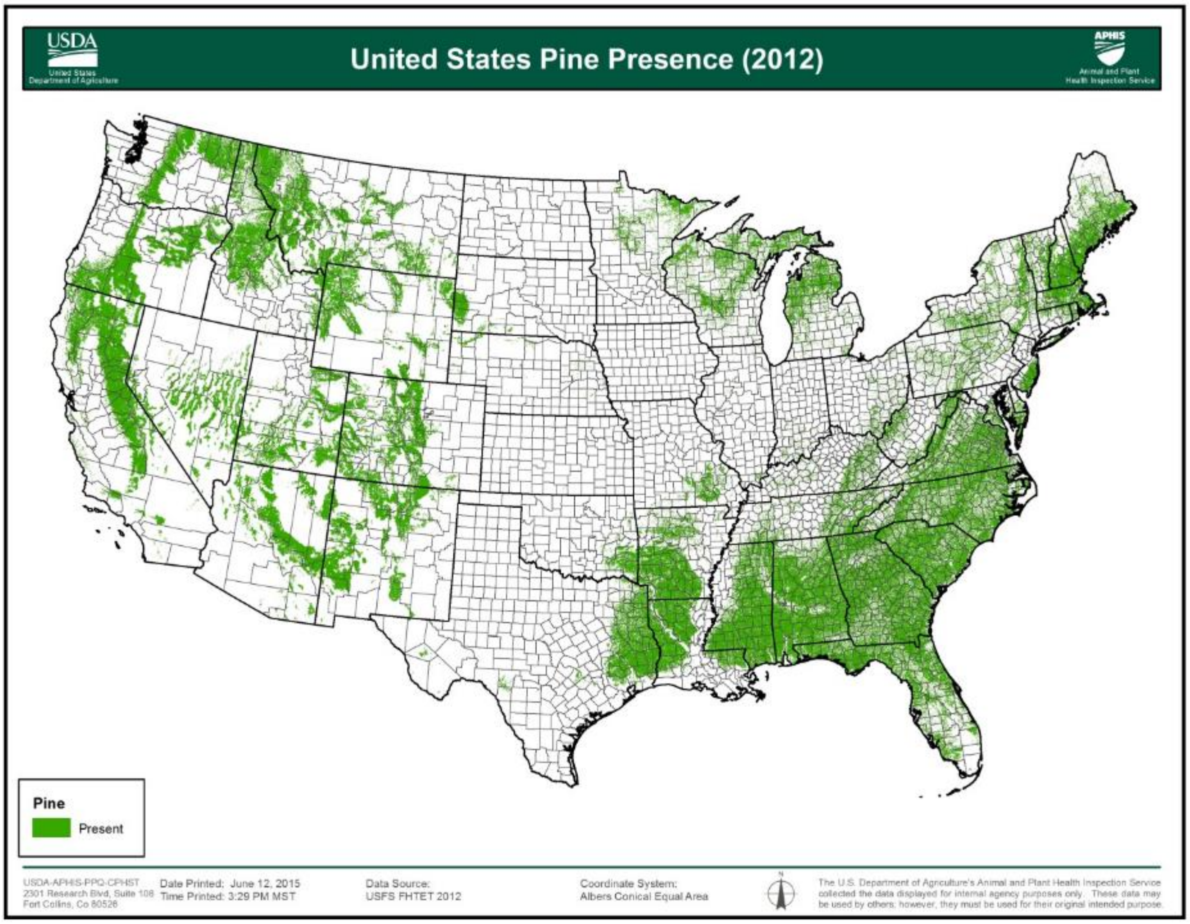

Figure 53. Tree species presence map for Pine (*Pinus* spp.) modeled in 2012 at a 240 meter resolution (USDA Forest Service, Forest Health Technology Enterprise Team). Map courtesy of USDA-APHIS-PPQ-CPHST.

Potential Distribution within the United States

With the exception of Ponderosa pine, most United States species were considered to have a high degree of resistance to *C. flaccidum* by Raddi and Fagnani (1978) by artificial and natural inoculation. If this rust has or gains the capacity to infect North American pines, the economic and ecological impact would be incalculable (Geils et al., 2009). For example, it has taken over 1 billion in current U.S. dollars to control white pine blister rust (caused by *C. ribicola*) since its introduction into North American in the 1900s, and this disease has caused much greater losses in forest productivity and ecological impacts.

In the United States, Scots pine (a known, common host) has been planted for erosion control and as an ornamental and also harvested for pulp and timber; however, its primary economic value is currently for Christmas trees (although other conifers are more recently favored). It has been widely planted in the colder regions of North America and is naturalized in the U.S. Northeast, Midwest, and Pacific Northwest (Geils et al., 2009). In 2002, Oregon, North Carolina, Michigan, Pennsylvania, Wisconsin, Washington, New York, and Virginia were the top Christmas tree producing states. The most Scots pine was grown primarily in the Lake States. Michigan was the top producer of Christmas trees in 1998 (Geils et al., 2009). These areas would be at high risk based on host availability.

A recent risk map developed by USDA-APHIS-PPQ-CPHST (Fig. 53) indicates that southeastern United States and portions of the western and northeastern United States have the greatest risk for *C. flaccidum* establishment based on host availability (all pine species), climate, and pathway within the continental United States.

Survey

CAPS-Approved Method: The CAPS-approved method is visual survey, spore trapping, or a combination of these methods to survey for *C. flaccidum*. For visual survey, collect twigs, bark, or leaves from symptomatic plants with signs (fruiting bodies) of the pathogen. Spore traps, similar to those used for soybean rust monitoring, can be used to detect spores.

Literature-Based Methods: Visually examine two-needle pines, especially Scots pine, for fruiting bodies (spermagonia and aecia) of the pathogen. Alternate hosts can also be examined for uredinia and telia of the pathogen.

Cronartium flaccidum can be detected in the tree most easily when fruiting. Spermogonia with spermatial fluid occur on the infected bark (next to the aecial scars of early summer) in late summer; aecia appear on the bark in the early summer, and uredinia and hair-like telia appear on the lower leaf surface of the alternate hosts in mid-to-late summer. The infected part of the shoot (lesion) is often swollen. The disease is also revealed by resinosis in the lesion. After the leader of the shoot carrying the lesion is killed, the top of the tree is dead, but green shoots below the lesion are visible. As an indication of infection in the shoot, the color of the needles above the lesion may turn light green to yellow (CABI, 2005).

Key Diagnostics

CAPS-Approved Method: Confirmation of *C. flaccidum* requires a morphological identification. Characteristics of pycnia, aecia, aeciospores, uredinia, urediniospores, telia, and teliospores can be used to distinguish from other rust fungi (Mordue and Gibson, 1978).

C. flaccidum can be cultured (axenically) by seeding aeciospores on modified Schenk and Hildebrandt's and Harvey and Grasham's media and incubating at

23-25°C (73-77°F) in the dark (Moricca and Ragazzi, 1994).

Further study is possible *in vitro* on *Pinus* spp. callus tissue (Ragazzi et al., 1995).

Literature-Based Methods:

The recovery plan for Scots pine blister rust suggests a morphological identification to genus and DNA sequencing to determine species (Geils et al., 2009).

Morphological: *C. flaccidum* can be cultured by seeding aeciospores on modified Schenk and Hildebrandt's (1972) and modified Harvey and Grasham's (1974) media incubated at 21-24°C (70-75°F) (Moricca and Ragazzi, 1994, 1996). Incubation in the dark is suggested since the germ tubes of *C. flaccidum* are light sensitive. For *C. flaccidum*, the optimal seeding rate was found to be 400-1200 aecispores/mm^2 (Moricca and Ragazzi, 1994). Growth is slow and may take weeks to months to develop colonies. High variation was observed in hyphal length and morphology, and in colony appearance, margin, and morphology (Moricca and Ragazzi 1994, 1996).

The modified Schenk and Hildebrandt's medium (SH1) contained the following ingredients per liter: 300 mg $NH_4H_2PO_4$; 5 mg H_3BO_3;151 mg $CaCl_2$; 0.100 mg $CoCl_2 \cdot 6H_20$; 0.200 mg $CuSO_4 \cdot 5H_20$; 20 mg $Na_2 \cdot EDTA \cdot 2H_20$; 15 mg $FeSO_4 \cdot 7H_20$;194.5 mg $MgSO_4$; 10 mg $MnSO_4 \cdot H_20$;1 mg KI; 2.5 g KNO_3; 0.100 mg $Na_2MoO_4 \cdot 2H_20$; 1 mg Zn $SO_4 \cdot 7H_20$; 8 g Difco Bacto agar; 3 g oxoid broth; l g malt extract; 30 g sucrose; 2 mg kinetin, and 0.5 mg 2,4 D (Moricca and Ragazzi, 1994, 1996).

The modified Harvey and Grasham's medium (HG1) contained the following ingredients per liter: 500 mg $CaNO_3 \cdot 4H_20$; 281.73 mg $MgSO_4 \cdot 7H_20$; 25 mg $(NH_4)_2SO_4$; 250 mg $Fe_2(SO_4)_3 \cdot 7H_20$; 140 mg KH_2PO_4; 4.14 mg $MnSO_4 \cdot 3H_20$; 8 g

Difco Bacto agar; 4 g oxoid broth; and 30 g sucrose (Moricca and Ragazzi, 1994, 1996). The ph of both media was adjusted to 5.7-5.8 with 1N HCL and 1N NaOH before autoclaving at 121°C for 20 minutes (Moricca and Ragazzi, 1994). In general, isolates from Italy grew better at 21 than at 24°C and better on the HG1 medium than on the SH1 medium, but neither temperature nor medium significantly affected colony appearance and shape, sporulation, spore type, or hyphal type (Moricca and Ragazzi, 1996).

Moricca and Ragazzi (2001) developed a technique to grow mycelial clones axenically of *C. flaccidum* from basidiospores from single telia on HG1 medium containing 2 g/l of yeast extract, 0.5 g/l $CaCO_3$, and 10 g/l bovine serum albumin. Ragazzi et al. (1995) grew axenic cultures of *C. flaccidum* on pine callus tissue. The authors grew the pine calli on MS medium (Murashige and Skoog, 1962) supplemented 0.5 mg/l 2,4 D and used basidiospores to inoculate the callus tissue.

<u>Biochemical:</u> Cheng et al. (1995) were able to differentiate three *Cronartium* spp. (*C. ribicola*, *C. flaccidum*, and *C. quercum*) using isozyme analyses on the aeciospores.

<u>Molecular:</u> Kaitera and Hantula (1998) provide a protocol to compare restriction fragment length polymorphisms (RFLP) in ITS-region DNA based on digestion of PCR products with the restriction enzyme *Alu I*. This protocol was used to separate *C. fraxinea* and *C. ribicola* telia from ‘alternate hosts’ and to confirm aecia collected from Scots pine. *C. ribicola* showed two bands with apparent sizes of 220 bp and 450 bp, *C. fraxinea* showed three bands with apparent sizes of 130 bp, 230 bp, and 350 bp. The 220 and 230 bp bands appeared to be twice as intense as the other bands, and assuming these two represent double restriction fragments, the summed fragment sizes of the two patterns were 890 and 940 bp, indicating the digestions were complete.

Easily Confused Pests

At least eleven *Cronartium* species and six species of *Peridermium* occur in North America on pine (Chalkey, 2010). To a certain extent, these can be distinguished by the aeciospore and urediniospore morphology, as well as by symptomatology. While some cause stem cankers, other rusts produce galls or witches brooms in infected stems or branches. Others cause no symptoms at all (Chalkey, 2010). *C. flaccidum* belongs to a distinct group of *Cronartium* species distinguished by their aeciospores (in which an echinulate surface alternates with smooth areas) (Moricca and Ragazzi, 1996). *Cronartium comandrae*, a widespread North American pine stem rust that also infects two-needle species like *C. flaccidum*, produces unique tear-drop shaped aeciospores on pine (Chalkey, 2010)

Symptoms can be confused with those of *C. ribicola*, the causal agent of white pine blister rust. *C. ribicola* does not infect *Pinus sylvestris*, whereas *C. flaccidum* does not infect five-needle pines or *Ribes* species (Kaitera and Nuorteva, 2006b). Kaitera and Nuorteva (2006a) conducted inoculation studies with *C. ribicola* on the main alternate hosts of *C. flaccidum*. The authors found that neither uredinia nor telia developed on the leaves of *Vincetoxicum hirundinaria*, *V. nigrum*, *Melampyrum sylvaticum*, *M. pretense*, *M. nemorosum*, *M. arvense*, *M. cristatum*, or *M. polonicum*.

In Europe, other rust that can attack pines also have a heteroecious life cycle similar to *C. flaccidum*, but usually infect different alternate hosts. *Coleosporium tussilaginis*, the pine needle rust, shares a few telial hosts with blister rust, but produces its spermagonia and aecia on pine needles, not on the stems. Also, teliospores of this rust on species of *Melampyrum* are single to cylindrical, produced not in long columns but in waxy crusts. *Melampsora populnea* infects the shoots of two-needle pines, causing shoot bending and/or tip death. Its linear aecia lack a peridium and the aeciospores are significantly smaller than those of *C. flaccidum* (Chalkey, 2010).

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