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Natural Control of White Pine Blister Rust by *Tuberculina maxima*

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Tuberculina maxima Rostr. is classified in the Deuteromycetes, family Tuberculariaceae. It was described by Rostrup (16) from white pine blister rust cankers (*Cronartium ribicola* J. C. Fischer) on strobe pine (*Pinus strobus* L.). The fungus is commonly called the purple mold or lilac fungus and, until recently, was believed to be a parasite of the associated rust fungus. Early reports from Europe (15,18–20) reflected considerable optimism for the capability of the purple mold to control white pine blister rust. Interest in the purple mold as a biocontrol agent was renewed in 1964 when it was realized that the current control programs were not protecting western white pine (*Pinus monticola* Dougl.) from white pine blister rust (6).

History of *Tuberculina maxima*. The purple mold was one of the first fungi reported to be associated with pine stem rusts (16). Tubeuf (18) reviewed the taxonomy and host range of the genus *Tuberculina* and discussed the potential for *T. maxima* to destroy the pathogen that causes blister rust of strobe pine. Later observations by Tubeuf (19,20) did not substantiate earlier

expectations of disease control. However, Tubeuf (21) and Rohmeder (15) reported that some benefits are derived by using the purple mold to combat blister rust.

Lechmere (9) clarified the life history of *T. maxima* and reported on his study of the relationships of purple mold and the pine stem rusts. He believed the association was a typical case of double parasitism. He reported, mainly from observations, that pycnia and aecia only were attacked and that the parasite did not invade the pine tissues and destroy the rust mycelium. He concluded that *T. maxima* could not be expected to control the rust.

The purple mold was first reported in the United States by Weir and Hubert (22) on native rust cankers of hard pines in Montana. In 1926, it was first found associated with blister rust cankers on western white pines at Daisy Lake, B.C. in Canada (11). It was first found on western white pine blister rust cankers in the United States at Newman Lake, near Spokane, WA, in 1932 (11).

Early observations on the ability of *T. maxima* to arrest blister rust canker development on western white pines were not encouraging (2,4,11). Most of these early workers expressed hope that the purple mold would develop more virulent strains that would effectively arrest canker growth. In 1931, Hubert (5) obtained *T. maxima* isolates from Germany to test whether they would control the rust in western North America as was claimed in parts of Germany. These cultures, along with others obtained in British Columbia in 1931 and 1932, were used to inoculate blister rust cankers on western white pines at two locations in northern Idaho. Satisfactory control of inoculated cankers was not achieved (5).

The observations of *T. maxima* on western white pine blister rust cankers from western North America, together with those reported from Europe, document the ability of the purple mold to parasitize rust cankers. In the Pacific Northwest, Goodding (2) reported that the purple mold often completely eliminates the production of aecia. Hubert (4) reported that production of aecia ceased in every canker supporting the purple mold. He noted the severe effects of the parasite upon the canker; his concern was with the inability of *T. maxima* to spread to enough cankers to reduce the losses being inflicted by the rust. These observations were supported by reports from British Columbia and Germany that associated reductions in aecia production with healing of the aecial stroma following attack by the purple mold (11,19).

Mielke (11) observed that pycnia may be attacked by *T. maxima* anytime during their seasonal development. He reported a reduction in pycnia production upon invasion by the purple mold and that a pycnial zone attacked one season fails to produce aecia the following year. In a study conducted at Oregon State University, Mielke (11) obtained good germination of *T. maxima* spores in aqueous solutions of pycnial fluid. Because of the observed frequency of the purple mold on pycnia, he suspected that its effect on aecia was indirect. He removed the outermost tissues in the sporulating zones of parasitized stem cankers and 1 mo later found *T. maxima* sporulating on the cut surface. He concluded that the mycelium of the purple mold invades the bark beneath the pycnia and aecia and that this explained why *T. maxima* hastens the death of rust-invaded bark.

In Europe, Spaulding (17) observed that pycniospore production was reduced on cankers inhabited by *T. maxima*.

The purple mold has not been reported in association with uredial or telial sori of *C. ribicola* on *Ribes* spp. (wild currants and gooseberries). Tubeuf (18) reported collections by several European workers (Gobi, Frank, Mangus, Schroter, and Rostrup) of *Tuberculina* spp. associated with uredia and telia as well as pycnia and aecia of several rusts. In all cases, when enough information was provided to determine the nature of the life cycle of the specific rust, the collections of *Tuberculina* spp. reported in association with uredia and telia were on autoecious rusts, regardless of whether they were macrocyclic, demicyclic, or microcyclic. In such a case, infection by *Tuberculina* spp. may have occurred during pycnia and/or aecia production. One collection listed by Tubeuf (18) reports a *Tuberculina* sp. on *Darluca filum* (Biv.-Bern ex Fr.) Cast., another imperfect fungus associated exclusively with members of the Uredinales. Gobi (1) reported a *Tuberculina* sp. on *Paris quadrifolia* L. in the absence of a rust pathogen.

Recent surveys of the purple mold in western North America. With the renewed interest in *T. maxima* as a biocontrol for western white pine blister rust more information on its distribution and occurrence was required. Results from a survey in 1965 (7) showed the purple mold to be distributed throughout the range of the western white pine type in northern Idaho. At the time of examination, the fungus was fruiting on 24.3% of active, lethal-type cankers. This figure is conservative because *T. maxima* fruits only on active cankers and it is not possible to accurately determine its past occurrence on cankers. Aecia production was reduced to 14.2% for the active lethal-type canker population. This was not entirely the result of *T. maxima*, but a large percentage of the reduction was undoubtedly related to canker invasion by the purple mold because the fungus was fruiting on 1.4% of the cankers producing normal aecia, 39.7% of those producing partial aecia, and 25.2% of those producing no aecia. Our inability to determine the presence of nonsporulating *T. maxima* certainly prevented evaluation of the relationship between this fungus and aecia production by the rust pathogen.

More recent surveys and collection reports (12–14) indicate that *T. maxima* is distributed throughout the forests of western Canada where it is associated with the introduced *C. ribicola* and with native rust fungi (ie, *Cronartium comptoniae* Arth., *C. coleosporioides* Arth., *C. commandrae* Pk., and *Peridermium harknessii* J. P. Moore). In southern Alberta (13), during a 4-yr period, the purple mold was found at 19 of 23 locations where *C. commandrae* was infecting *Pinus contorta* Dougl. The fungus occurred on 20–41% of the active cankers, depending on the year. At some locations, 70% of the active cankers were inhabited by *T. maxima*. Aecia production was reduced by 10–15%.

T. maxima has been observed infrequently in eastern North America (3,5). In recent years, the frequency of the purple mold is reported to be increasing in Wisconsin and Minnesota on conifer stem rust cankers and on fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* Burdsall and Snow) cankers in the southeastern United States (8,26).

The purple mold is known to inhabit cankers of *C. ribicola* on white pines in Japan (S. Yokota, *personal communication*), but I did not observe the fungus in Hokkaido in 1974 (34).

Recent research on the biology and ecology of *T. maxima*. The purple mold has been isolated from mycelium in the discolored margins and from spores and sporodochia of "dormant" cankers of *C. ribicola* on western white pine collected from the field during the winter (30). Continuous freezing for periods up to 19 mo does not completely destroy spore viability. Thus, *T. maxima* is well adapted to overwintering in the imperfect state and annual canker reinfection is not necessary. Viable inoculum is available, under natural conditions, for invasion of rust cankers when they are susceptible and the enivronment is favorable. The mycelium of *T. maxima* ramifies throughout the cankered pine tissues and is not restricted to the aecia and pycnia as reported by others. The mycelium of the purple mold does not invade pine bark tissues beyond those invaded by the rust pathogen and, therefore, does not constitute a threat to the tree as a girdling canker.

Western white pine blister rust cankers are infected by *T. maxima* only during pycnia and aecia production (27). Nonsporulating cankers could not be infected even following scarification of the discolored canker tissues with carborundum.

T. maxima completes its life cycle (spore to spore) within a minimum of 2 wk. Data from artificial inoculations of blister rust cankers with aqueous suspensions of *T. maxima* spores, both in the field and in controlled growth chambers, show that the life cycle is completed within 2–7 wk for inoculations of either aecia or pycnia (31). A higher percentage of canker infection is obtained by inoculation of pycnia than of aecia. Sporodochia produce a dry mass of globose, lilac-colored spores that are readily disseminated by wind. The sticky pycnia probably function better as a trap for such spores than do the dry aecia.

A variety of artificial media can be used to grow the purple mold. My investigations (*unpublished*) show that the fungus can utilize

many carbon and nitrogen sources. It cannot use a few of these sources. No vitamins were identified as essential for growth. The purple mold grows very well in pycnial fluid of *C. ribicola*. Pycnial fluid is known to contain ammonia, 21 amino acids, inorganic phosphate, fructose, three acyclic sugar alcohols and inositol (28). Growth of the fungus is favored by high C/N ratio (20:1 or 25:1).

My investigations of the ecology of *T. maxima* (*unpublished*) have defined the temperature and pH ranges. Spores germinate at 5 C with little or no additional growth. The optimum temperature range for growth is 20–25 C and it varies slightly depending upon the medium used. The thermal death point is between 25 and 30 C. The fungus will grow on media ranging from pH 3.0 to 8.0; the optimum is pH 5.2.

Histological study of the pine-rust/purple-mold association (32,33) shows that the effects of *T. maxima* on the rust-infected tissues of pine seedlings and tissue cultures are rapid, lethal, and result in structural degradation. Walls, cytoplasm, and nuclei of rust-infected pine cells are destroyed as the mycelium of the purple mold invades them. Haustoria, hyphae, and sporogenous cells of the rust pathogen lyse when the pine cells are destroyed. *T. maxima* does not attack the rustfree pine tissues in vivo. However, it can establish parasitic and pathogenic relationships with rustfree pine tissues growing in vitro.

Dual culture in vitro experiments (25) produced no evidence of antibiosis, lysis, or direct parasitism between *T. maxima* and *C. ribicola*.

Efficacy of *T. maxima* for the biocontrol of white pine blister rust. The published literature and numerous unpublished observations suggest that *T. maxima* is the most active biological agent associated with blister rust of white pines. This fungus reduces the inoculum potential of the target rust organism by suppressing production of both pycnia and aecia. It inhibits growth and development of the cankers, which results in partial or, more rarely, complete inactivation of cankers.

The manner in which biological agents affect a disease may be through direct association with the causal agent or indirect association with the suscept. The most common modes of action are direct parasitism, competition for an essential environmental factor, and production of antibiotics.

Traditionally, *T. maxima* is considered a hyperparasite of rust fungi, which implies a direct food relationship between the purple mold and the rust (24). This concept has evolved strictly on the basis of empirical evidence derived from field observations. No experimental data exist that show a direct food relationship between *T. maxima* and its rust fungus associate.

Histology of western white pine tissues infected by *C. ribicola* shows that structural degradation of the pine cells does not occur. Rust haustoria penetrate cell walls, but such penetration is not known to be the result of enzymatic activity. If it is, it is very localized. There is evidence (10,32) that the middle lamellae of pine cells are degraded during intercellular invasion by the rust pathogen, which implicates pectinase activity (23). Although a variety of pectic materials are being degraded (23) such degradation is not so intense as to cause maceration of the pine tissues. If extensive cellular degradation did occur, it would be terminal for the rust pathogen because of its obligate requirements.

In contrast, histology shows that invasion of rust-infected pine tissues by *T. maxima* causes degradation of pine cells. Such degradation is likely to result from the controlled and systematic actions of several enzyme systems produced by the purple mold. This action affects the rust-parasitized pine cells rather than those of the rust fungus. Thus, *T. maxima* suppresses the rust by enzymatically destroying the food source vital to the survival of the obligate pathogen.

Wicker and Shaw (29) identified six attributes they consider essential to the success of a biological control agent: distribution coincident with that of the target pathogen; ecologic amplitude sufficient to ensure persistence within the suscept's environment; production of abundant inoculum; high infectivity; high virulence; and an efficient mode of action for curtailing development of target disease. When using these attributes to evaluate *T. maxima*, we saw some serious weaknesses for the latter three. The purple mold has delayed blister rust damage and prolonged the life of infected trees, but it has not controlled the disease.

DISCUSSION AND CONCLUSIONS

The potential of biological agents for the tactical control of plant disease has not received detailed study. Thorough exploration of the potential of biological agents in combating plant disease is necessary to the development of integrated strategies and systems for manipulating pest populations. The use of biological agents against disease is very appealing from the standpoint of procedures, costs, and the absence of residue problems. These agents are an integral part of the suscept-pathogen's environment and seldom do they cause environmental or human health hazards.

Plant pathologists and foresters have not been attracted to biological control because such measures are often difficult to appraise; slow and erratic in effect; less than spectacular in achieving control; biologically complex and, therefore, more difficult to understand and manipulate; not economically effective unless actively manipulated; and less likely to be complementary to other control measures in an integrated control program than are cultural, chemical, or genetic control measures. Furthermore, control is relative.

Prior to 1960 most of the knowledge of biological control agents was the result of mycological curiosity and motivation. Thus, the majority of the available information concerns structure of the active agents. Data on function are necessary to evaluate their potential. A detailed understanding of their mode of action is imperative.

Within the past decade, the rates of utilization and exploitation of renewable natural resources, particularly goods and services from forests, have become a worldwide interest and concern. Social, economic, and political pressures for maximum and efficient use and conservation of these goods and services are increasing at an alarming rate. These pressures place greater demands on our forest production system. Reduction of losses due to disease is a viable option for ameliorating these demands by increasing productivity. However, the application of single, specific control tactics through isolated, independent, single-purpose actions is seldom feasible. Also, effectiveness is usually of very short duration. Biological control agents are no exception. They should not be expected to provide effective and feasible disease control when used as a single tactic. To achieve the desired reduction of losses caused by forest diseases we need many preventive and suppressive strategies and systems integrated into our production management and planning which must be based on sound ecological principles and ecosystems management concepts. It is within these concepts that biological agents will harmonize with other tactics and fulfill their potential.

LITERATURE CITED

1. Gobi, C. Von. 1885. Über dem *Tubercularia persicina*, Ditm. genannten Pilz. Mém. Acad. Imp. Sci., St. Pétersbourg, Sér, 7, 32:1-26.
2. Goodding, L. M. 1932. Notes on biological control of blister rust. U.S. Dep. Agric. Western Blister Rust News Lett. 7:51-53.
3. Hedgecock, G. G. 1935. Notes on the occurrence of *Tuberculina maxima* on the aecia of *Cronartium cerebrum*. Phytopathology 25:1117-1118.
4. Hubert, E. E. 1932. Biological control by means of the purple mold. U.S. Dep. Agric. Western Blister Rust News Lett. 7:96-98.
5. Hubert, E. E. 1935. Observations on *Tuberculina maxima*, a parasite of *Cronartium ribicola*. Phytopathology 25:253-261.
6. Ketcham, D. E., Wellner, C. A., and Evans, S. S., Jr. 1968. Western white pine management programs realigned on northern Rocky Mountain forests. J. For. 66:329-332.
7. Kimmey, J. W. 1969. Inactivation of lethal-type blister rust cankers on western white pine. J. For. 67:296-299.
8. Kuhlman, E. G., and Miller, T. 1976. Occurrence of *Tuberculina maxima* on fusiform rust galls in the southeastern United States. Plant Dis. Rep. 60:627-629.
9. Lechmere, E. Von. 1914. *Tuberculina maxima*, Rost. Ein Parasit auf dem Blasenrost der Weymouthskiefer. Naturwiss. Zeitschr. Forst Landwirtsch. 12:491-498.
10. Martin, N. E. 1967. Histochemical analysis of the blister rust fungus,

Cronartium ribicola. (Abstr.) Phytopathology 57:820.
11. Mielke, J. L. 1933. *Tuberculina maxima* in western North America. Phytopathology 23:299-305.
12. Powell, J. M. 1971. Occurrence of *Tuberculina maxima* on pine stem rusts in western Canada. Can. Plant Dis. Surv. 51:83-85.
13. Powell, J. M. 1971. Incidence and effect of *Tuberculina maxima* on cankers of the pine stem rust *Cronartium commandrae*. Phytoprotection 52:104-111.
14. Powell, J. M. 1972. Additional collections of *Tuberculina maxima* on pine stem rusts in western Canada. Can. Plant Dis. Surv. 53:139.
15. Rohmeder, E. 1931. Anbäuflache und Gefährdungen der Strobe im Bayerischen Staatswald. Forstwiss. Centralbl. (Hamburg) 53:325-329.
16. Rostrup, E. 1890. Ustilagineae, Daniae. Dansk Botanisk Forening. April 1890:117-168.
17. Spaulding, P. 1929. White pine blister rust: A comparison of European with North American conditions. U.S. Dep. Agric., Tech. Bull. 87. 85 pp.
18. Tubeuf, C. Von. 1901. Über *Tuberculina maxima*, einen Parasiten des Weymouthskiefern-Blasenrostes. Arb. Biol. Abt. Land. Forstwirtsch. 2:169-173.
19. Tubeuf, C. Von. 1914. Biologische Bekämpfung von Pilzkrankheiten der Pflanzen. Naturwiss, Zeitschr. Forst. Landwirtsch. 12:11-19.
20. Tubeuf, C. Von. 1914. Neuere Versuche und Beobachtungen über den Blasenrost der Weymouthskiefer. Naturwiss. Zeitschr. Forst. Landwirtsch. 12:484-491.
21. Tubeuf, C. Von. 1930. Biologische Bekampfung des Blasenrostes der Weymouthskiefer. Z. Pflanzenkrankr. Pflanzenschutz 40:177-181.
22. Weir, J. R., and Hubert, E. E. 1917. Pycnial stages of important forest tree rusts. Phytopathology 7:135-139.
23. Welch, B. L., and Martin, N. E. 1974. Evidence of pectinase activity between *Cronartium ribicola* and *Pinus monticola*. Phytopathology 64:1287-1289.
24. Wetzel, H. H. 1929. The terminology of phytopathology. Int. Congr. Plant Sci. Proc. 2:1204-1215.
25. Wicker, E. F. 1979. *In vitro* dual culture of *Tuberculina maxima* and *Cronartium ribicola*. Phytopathol. Z. 96:185-189.
26. Wicker, E. F. 1980. Biocontrol of conifer stem rusts: The purple mold. Phytopathol. Mediterr. 19:21-26.
27. Wicker, E. F., and Kimmey, J. W. 1967. Mode and time of infection of western white pine blister rust cankers by *Tuberculina maxima*. (Abstr.) Phytopathology 57:1010.
28. Wicker, E. F., Mosher, D. P., and Wells, J. M. 1976. Organic constitutents of *Cronartium ribicola* pycnial fluid. Phytopathol. Z. 87:97-106.
29. Wicker, E. F., and Shaw, C. G. 1968. Fungal parasites of dwarf mistletoes. Mycologia 60:372-383.
30. Wicker, E. F., and Wells, J. M. 1968. Overwintering of *Tuberculina maxima* on white pine blister rust cankers. Phytopathology 58:391.
31. Wicker, E. F., and Wells, J. M. 1970. Incubation period for *Tuberculina maxima* infecting white pine blister rust cankers. Phytopathology 60:1693.
32. Wicker, E. F., and Woo, J. Y. 1969. Differential response of *Tuberculina maxima* to white pine tissues. (Abstr.) Phytopathology 59:16.
33. Wicker, E. F., and Woo, J. Y. 1973. Histology of blister rust cankers parasitized by *Tuberculina maxima*. Phytopathol. Z. 76:356-366.
34. Wicker, E. F., and Yokota, S. 1976. On the *Cronartium* stem rust(s) of five-needle pines in Japan. Ann. Phytopathol. Soc. Jpn. 42:187-191.