STUDIES OF WESTERN GALL RUST RESISTANCE IN PONDEROSA PINE

J. A. Walla and G. A. Tuskan 1/

ABSTRACT

Western gall rust (*Endocronartium harknessii*) with its' broad host range and potential for damage to pines is a prime candidate for the expansion of *in vitro* selection systems in forest species. Ponderosa pine was selected as the test host species because of its importance as a windbreak species and genetic history (GP-13 provenance study). Initially, 5 defined media and 8 BAP/NAA phytohormone combinations were tested for growth and differentiation of ponderosa pine tissue. LP medium with 1.0 mg/l BAP and 1.0 mg/l NAA was selected for callus production. Best shoot development occurred on GD medium with 10 mg/l BAP and 0.01 mg/l NAA. Rooting occurred with a 100 ppm NAA dip on a hormone-free GD medium. Inoculations of seedlings, regenerated shoots and callus were attempted, with infection expressed only in the seedlings. Apparent susceptible and possible resistant reactions were observed. Finally, starch gel electrophoresis was used to characterize variability in *E. harknessii*. Contingency chi-square indicated significant variability among sites but not between host species. Isozyme variability will be used to determine inoculum source for future testing. Application of these results and future research are discussed.

INTRODUCTION

Trees are planted in rural areas of the Great Plains region of the United States for protection, profit and beautification. Tree windbreaks in fields are a primary soil conservation tool, protecting 15 million acres of agricultural land from soil erosion. Trees are also planted to modify the harsh environment of the Great Plains through snow management and wind protection. Pines are among the trees commonly utilized in this region for field protection, farmstead protection, livestock protection, wildlife habitat, land reclamation, Christmas trees and wood products plantings. Monetary value of most of these types of plantings cannot be established due to the intrinsic nature of their benefits (Rensema and Tuskan 1986).

1/ Instructor, Dept. of Plant Pathology, and Assistant Professor, Dept. of Horticulture and Forestry, North Dakota State Univ., Fargo, ND 58105.

Western gall rust caused by Endocronartium harknessii (Moore) Hiratsuka (Peridermium harknessii Moore) threatens ponderosa pine (Pinus ponderosa Law.) and Scots pine (P. sylvestris L.) in Great Plains tree plantings through seedling mortality, reduced growth rate, branch or stem breakage, shape deformation and apical leader death. E. harknessii has been described as a short cycle, autoecious rust, with aecioid teliospores being the only consistently present spore state (Hiratsuka 1973). As an autoecious rust, it infects directly from pine to pine.

In the Great Plains, western gall rust represents a potential loss in the performance of pine plantings. Incidence of galls can be on as high as 75% of all pines in a windbreak (Walla and Stack 1979a). Western gall rust also occurs in and damages Christmas tree and wood products plantings in North Dakota (Walla and Stack 1979b).

Western gall rust problems are not unique to pine plantings in the Great Plains. Nelson (1971) and Allen (1984) list thirteen susceptible native pines and seven susceptible exotic pines. Damage to pines in natural stands is considered significant (Peterson and Jewell 1968), but is usually difficult to measure. Infection and damage in pine plantations occurs, but is also difficult to measure. Merrill and Kistler (1976) report up to 75% of the Scots pine in some plantations in Pennsylvania were so severely galled that they had to be destroyed. Merrill and Wenner (1985) report the total length of new shoots on infected ponderosa pine in Pennsylvania was about 25% of that of non-infected trees. Allen (1984) reports the greatest potential for damage due to western gall rust is in plantations as a result of production and outplanting of susceptible seedlings.

There is a large and increasing worldwide use of pine species susceptible to western gall rust and a concomitant potential for damage due to this disease. Resistance could reduce this potential for damage. There is a paucity of information on host/pathogen interactions that involve western gall rust, and time is required to understand these interactions. This paper reports on investigations that attempt to provide a better understanding of interactions between ponderosa pine and western all rust and provide a system to identify resistant trees. The host/pathogen interaction is being examined through 1) variation in resistance of the pine and 2) variation in virulence of the fungus.

VARIATION IN RESISTANCE OF THE PINE

Control of other diseases caused by rust fungi has often utilized genetic variation in the host. Breeding for resistance has received major emphasis for control of white pine blister rust and fusiform rust (Bingham 1983, Powers 1984). These programs have identified resistant germplasm and successfully used seedlings to screen for resistance. There appears to be a genetic basis for resistance to western gall rust. Martinsson (1980), Hoff (1985) and Old et al. (1985) associated variation in susceptibility with seed source origin in several pine species. Resistance of Monterey pine appears to be horizontal (Old et al. 1985) and age related (Zagory and Libby 1985). Kinloch (1972) notes

that tree-to-tree variability in western gall rust occurrence is very high. Tree-to-tree variability in western gall rust is present in ponderosa pine plantings in North Dakota (Walla unpublished). A provenance study of ponderosa pine replicated at 17 locations and containing 79 seed sources was established between 1968 and 1970 (Read 1983). Peterson and Riffle (1984), Thomas et al. (1984) and Merrill et al. (1986) have examined western gall rust resistance in this ponderosa pine provenance study. Using two plantings of this ponderosa pine provenance study, Thomas et al. (1984) found variation in susceptibility among subspecies within species and among seed sources within subspecies.

Several seed sources of the above ponderosa pine provenance study will be used in our investigations. Resistance will be examined on seedlings, excised embryos, regenerated shoots, and callus derived from open-pollinated and control-pollinated crosses between and within field resistant and field susceptible trees. These systems will attempt to relate in vitro response to field response and provide information as to the type of material that might best be utilized in screening for western gall rust resistance.

A large number of conditions must be satisfied before actual testing can begin. Plant materials must be able to be manipulated so large numbers of ontogenetically identical material can be produced. For the cell culture material, this involves developing new techniques because ponderosa pine has not previously been grown as callus, regenerated shoots or excised embryos on a defined medium. Various techniques for inoculation and characterization of symptom development for each type of host material must be examined. A number of these conditions have been satisfied.

In vitro growth, development and inoculation of ponderosa pine.

Five mineral salt formulas were initially tested for suitability in culturing ponderosa pine. They were Schenk Hildebrandt, Campbell Duncan, Lloyd McCown, LePoivre, and Gresshoff Doy (Skirvin 1981). Of these, LePoivre and Gresshoff Doy performed the best and were tested further (Table 1). LePoivre's medium was selected for callus culturing based on callus growth rate, color and consistency. A wide range of growth rates occurred on the various media. Callus growth rates varied by host genotype (Table 2). Eight different ratios of 6-benzyl-amino purine (BAP) and α -aphthaleneacetic acid (NAA) were also tested. Callus production occurred with 10/1.0, 1.0/1.0 and 0.01/1.0 mg/l ratios of BAP/NAA (Table 1). Callus is now grown on LePoivre's medium modified with a 1.0/1.0 mg/l BAP/NAA ratio for ease and economy in medium preparation.

Initiation of shoots and roots of ponderosa pine in culture on defined media has not previously been reported. The same five mineral salt formulas tested for callus growth were tested for suitability in initiating ponderosa pine shoots. Gresshoff Doy medium resulted in the best shoot initiation. Using the above BAP/NAA ratios, it was determined that shoot initiation and growth were best with 10 mg/l BAP and 0.01 mg/l NAA (Table 3). Complete plantlets have been produced. This procedure follows a modification of Amerson and Mott (1978).

Table 1. Callus score differences at 60 days for cotyledon explants of ponderosa pine grown on 8 phytohormone combinations and 2 mineral salt formulas.

Mineral salt formula[2/]	Callus Score[1/] 0	10	20	30	40	50
	% of all cultures					
LePoivre	30	3	8	7	29	23
Gresshoff Doy	24	8	13	9	29	17
BAP-NAA ratio[3/] (mg/l)						
25 - .01	79	5	9	2	0	5
10 - .01	10	6	26	24	9	25
10 - 1.0	0	0	2	0	52	46
1.0 - .01	1	31	38	31	0	0
1.0 - 1.0	2	0	0	0	54	44
1.0 - 10	59	1	0	0	38	2
.01 - 1.0	25	0	0	0	52	22
0.0 - 0.0	100	0	0	0	0	0

[1/] 0 - death of the explant.
10 - no growth.
20 - stressed appearance, callus at the tip of the explant.
30 - healthy appearance, callus at the tip of the explant.
40 - yellowish brown callus along the explant.
50 - green callus along the explant.

[2/] Callus scores are not significantly different between mineral salt formulae based on a contingency X^2 at $\alpha \leq 0.05$.

[3/] Callus scores are significantly different among phytohormone combinations based on a contingency X^2 at $\alpha \leq 0.05$.

Table 2. Genotypic differences in callus growth for ponderosa pine cortex grown on LePoivre medium with 0.01 mg/l BAP and 1.0 mg/l NAA.

Genotype	Callus Frequency	Final[1/] Weight (mg)
1	100	1633
2	44	367
3	30	545
4	100	2248
5	71	1611
6	77	1360
7	44	578
8	11	321
9	100	1325
10	88	3002

[1/] Final weight is based on the average of all cultures at the end of 90 days. Initial cultures wre subcultured every 30 days.

Table 3. Number of shoots derived from ponderosa pine cotyledon explants. 1/

Media Type	Time (Days) 30	60	90
	# of shoots		
LePoivre	6	5	2
Gresshoff Doy	20	34	31
BAP-NAA ratio (mg/l)			
25 - 0.1	8	11	11
10 - .01	18	28	22
10 - 1.0	0	0	0
1.0 - .01	0	0	0
1.0 - 1.0	0	0	0
1.0 - 10	0	0	0
.01 - 1.0	0	0	0
0.0 - 0.0	0	0	0

1/ A shoot was considered as a differentiated meristem producing a mass of needles $\geq$ 0.5 cm.

Callus tissue has been inoculated with spores of E. harknessii. To date, no infections have been detected. Initial inoculations had severe problems from fungal and bacterial contaminants. Streptomycin at 100 ppm was found to control bacterial contamination without affecting germination rate or germ tube growth rate of E. harknessii. Benomyl 50 Wp (chemical = methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) was tested at 1, 5 and 10 ppm active ingredient incorporated into 2% water agar and mixed in water for its affect on contaminants and on E. harknessii germination. Contaminants did not develop on water agar containing 5 ppm benlate or in water containing 1, 5, or 10 ppm benlate. Growth of E. harknessii germ tubes was profoundly affected by presence of benomyl at each of the tested concentrations. Germ tubes were shorter and more spores produced multiple germ tubes with increased benomyl concentrations. Therefore benomyl cannot be used to control contaminants while retaining normal development of E. harknessii. Currently, contaminants are controlled by using relatively clean spores and diluting the inoculum below the point where contaminants are a problem.

Initiated shoots have been inoculated with spores of E. harknessii. Inoculated shoots (ca. 1 cm long with juvenile needles) have displayed no signs of infection. Because of the size of the shoots and compactness of the needles, spores were applied to the needles, a tissue which is not known to be normally infected in the field.

Inoculation and incubation of seedlings

Ninety-day-old nursery-run ponderosa pine seedlings were inoculated with western gall rust. Seedlings were about 10 cm tall and had primary and secondary needles. Some individual mature needles were just emerging from needle sheaths.

Inoculum was a bulk sample of spores collected from a native stand of ponderosa pine in southwestern North Dakota approximately 9 months before inoculation. Three inoculation methods and two spore rates were tested. The first inoculation method applied spores suspended in "Soltrol 170" oil to dry seedlings, which is a method routinely used at North Dakota State University for inoculation of wheat with various rust fungi. Two rates (high and low) of inoculum were applied. The second inoculation method involved application of two rates (high and low) of dry spores with a puffer onto seedlings that had previously been misted with water. Spore application rates were monitored in methods one and two by counting the number of spores that fell onto a 100 mm^2 area on glass slides placed between seedlings during inoculation. The third inoculation method involved application of dry spores with a brush onto stems of seedlings that had previously been misted with water.

All seedlings were incubated in the dark at 68° F at near 100% R.H. for about 30 hr. Seedlings were then taken to a greenhouse room where symptom development was monitored periodically. Small discolored lesions of various colors and shapes developed on stems of 26% of the 50 inoculated seedlings between 4 and 25 days after inoculation. None of the lesions increased in size after 25 days following inoculation and no further symptoms developed in their immediate vicinity. Some of these lesions remained visible for 105 days after inoculation.

Stem swelling and cracking was noted on some seedlings beginning 60 days after inoculation. When these symptoms occurred on seedlings that developed lesions 4-25 days after inoculations, the two symptom types (lesions, swelling) were not in proximity to each other. By 90 days, swellings had developed into obvious galls. No new galls developed through 500 days after inoculation. The three methods of inoculation (oil-suspended spores sprayed onto dry seedlings, dry spores puffed onto wet seedlings, and dry spores brushed onto wet seedlings) resulted in 55%, 90%, and 90% galled seedlings, respectively (Table 4). No control seedlings developed galls. The high spore rate resulted in slightly fewer galls in the two methods where different spore rates were applied.

With these tests, we have observed that there are apparent susceptible and possible resistance reactions on seedlings. Galled seedlings are currently being observed for further resistance reactions as the fungus goes through its two year life cycle.

Table 4. Results of Endocronartium harknessii inoculations of ponderosa pine seedlings after 105 days.

Inoculation Method	Seedlings Inoculated	Spores /mm^2	Percent with galls
Oil suspended spores			
-high rate	10	15	50
-low rate	10	5	60
-no spores	5	0	0
Dry puffed spores			
-high rate	10	91	80
-low rate	10	28	100
-no spores	9	0	0
Dry brushed spores	10	-	90

VARIATION IN VIRULENCE OF E. HARKNESSII

Development of useful resistance in pines to western gall rust requires that not only variation in resistance of pines be examined but also that variation in virulence of the pathogen be examined. Rusts that have been examined closely are known to vary in virulence. Notable examples include flax rust and wheat stem rust. Resistance generally soon breaks down in hosts selected for resistance to rust diseases without consideration of pathogen variation. Pine rusts such as fusiform rust and white pine blister rust have also been found to vary in virulence since the time hosts with some resistance have been identified (Powers 1982). It can be expected that virulence will vary in E. harknessii.

For diseases such as flax rust and wheat stem rust, systems have been worked out to identify the presence of virulence genes in the pathogens utilizing host differentials. Host differentials are supposedly genetically pure cultivars of the host that have been found to contain certain known resistance genes by using pathogen differentials. By finding the reaction to infection by a pathogen on a number of host differentials, it can be determined which virulence genes are present in a pathogen and which are not.

Such a system is not known for E. harknessii or any other pine rust. Domestication has only recently begun in pines, so extensive collections of genetically pure lines are not available. The long life cycle of the hosts exclude the possibility of rapid testing and development of host differentials. The long life cycle of the pathogens makes availability of adequate inoculum of pure pathogen lines for testing differentials a considerable obstacle.

Given these obstacles to examining variability in virulence of E. harknessii in a traditional way, an alternate approach was examined. Variation in an organism is a result of its genetic makeup. Enzymes present in an organism are gene products that contribute to variation in that organism. Variation in enzymes in an organism provides an indication of the amount of variability in one organism compared to another. Variation in enzymes in collections of E. harknessii would provide insight into the degree of variability in the population of E. harknessii.

Horizontal starch gel electrophoresis was used to study enzyme variability in bulk samples of E. harknessii spores from two geographic sources collected in 1985 in North Dakota (Tuskan and Walla 1986). Samples were electrophoresed and screened for 33 enzyme systems. Twenty-one enzymes displayed activity. Differences were found between the geographic sources of western gall rust spores. Two unique bands were found in the sample from a native pine stand in southwestern North Dakota. Three unique bands were found in the sample from a pine plantation in northeastern North Dakota. Enzyme variability among western gall rust isolates was found within the region and enzymes were found to be sufficient to separate geographic sources.

Horizontal starch gel electrophoresis was also used to study enzyme variability among collections of spores from individual western gall rust galls collected in 1986 from several trees within three widely separate sites (Tuskan and Walla 1987). The objectives of this study were to determine if enzymes in E. harknessii varied among isolates from distinct stands, within stands, and within host genotypes. Thirty-three putative loci were represented in the twenty-one examined enzymes, though only 12 produced consistently interpretable results. Of the 12 enzymes, 7 were polymorphic across sites, with no heterozygosity present. Genetic crosses have not yet been possible in E. harknessii to confirm inheritance but banding patterns have followed recognizable patterns reported in other species (Micales et al. 1986). Allelic frequencies did vary among sites. Pairwise contingency chi-square indicated significant differences between the natural stand and either the shelterbelt planting or the Christmas tree planting (Table 6). There

Table 5. Enzyme identification and buffer systems for 37 enzymes used to evaluate western gall rust isozyme variability.

Enzyme (abbreviations)	Enzyme Commission Number	Buffer[1/] System	Putative No. of Loci
Acid Phosphatase (ACP)	3.1.3.2	M/L	2
Aconitase (ACOL)	4.2.1.3	M	-
Adenylate Kinase (ALK)	2.7.4.3	H	-
Alcohol Dehydrogenase (ADH)	1.1.1.1	L	-
Alanine Aminopeptidase (AAP)	3.4.11.1	L	-
Aldolase (ALD)	4.1.2.13	H	-
Alkaline Phosphatase (ALP)	3.1.3.1	H	-
Catalase (CAT)	1.11.1.6	L	1
Diaphorase (DIA)	1.6.4.3	M	1
α/β Esterase (EST)	3.1.1.1	H	4
Fructose 1-6 Diphosphatase (FDP)	3.1.3.11	M	-
Fumerase (FUM)	4.2.1.2	H	-
General Protein (Gen Pro)	- - - -	H	-
Glucose-6-P-Dehydrogenase (G6P)	1.1.1.49	M	3
Glucose Dehydrogenase (GLD)	1.1.1.47	H	-
Glutamic Dehydrogenase (GDH)	1.4.1.3	L	-
Glytamic Oxaloacetic Transmutase (GOT)	2.6.1.1	L	2
Glycerate Dehydrogenase (GyD)	1.1.1.29	H	-
Glyceraldehyde-3-phosphate Dehydrogenase (G3P)	1.2.1.12	H	-
Glutathione Reductase (GRT)	1.6.4.2	L	-
Hexokinase (HXK)	2.7.1.1	H	-
Isocitric Dehydrogenase (IDH)	1.1.1.42	M	-
Leucine Aminopeptidase (LAP)	3.4.11.1	L	-
Malate Dehydrogenase (MDH)	1.1.1.37	H/L	-
Malic Enzyme (MAE)	1.1.1.40	M	3
Menadione Reductase (MNR)	1.6.99.2	H/L	1
NADH Dehydrogenase (NDH)	1.6.99.3	L	-
Phosphoenolpyruvate-Carboxylase (PPC)	4.1.1.31	L	-
6-Phosphogluconate Dehydrogenase (6PG)	1.1.1.44	M	1
Phosphoglucomutase (PGM)	2.7.5.1	H	3
Phosphoglucose Isomerase (PGI)	5.3.1.9	L	1
Phosphormannose Isomerase (PMI)	5.3.1.8	L	1
Shikimate Dehydrogenase (SKD)	1.1.1.25	H	-
Sorbitol Dehydrogenase (SDH)	1.1.1.14	L	-
Succinate Dehydrogenase (SuD)	1.3.99.1	H	-
Uridine Diphosphoglucose Pyrophosphorylase (UGP)	2.7.7.9	M	-
Xanthine Dehydrogenase (XDH)	1.1.1.37	L	-

[1/] M - Morpholine-Citrate, pH = 6.1
H - Histidine-Citrate, pH = 7.0
L - Lithium-Borate, pH = 8.3

was minimal difference in the allelic frequencies among the host species of ponderosa pine and Scots pine, indicating the rust can freely infect either species. Enzyme variability may not be directly responsible for virulence, but individual isozyme patterns may be correlated with virulence, as was found with Puccinia graminis f. sp. tritici (Burdon and Roelfs 1985). Because enzyme variability and pathogen virulence are gene products, and there are as yet no means of assessing differences in virulence, initial inoculations will rely on those isolates that are most different with regard to isozyme phenotype.

Table 6. Contingency chi-square values for allelic frequency differences between pairwise combinations of three host environments.

Putative Locus	Natural Stand/ Shelterbelt	Natural Stand/ Christmas Tree Planting	Shelterbelt/ Christmas Tree Planting
ACP	6.42*	16.27**	1.62
CAT	58.13**	51.74**	9.04**
EST	47.50**	43.29**	7.05*
G6P	74.06**	3.91*	19.20**
GOT	45.69**	69.67**	0.56
MAE	5.53*	4.83*	2.07
PGM	35.60**	11.04**	18.78**

* - significant at $\alpha \leq 0.05$.
** - significant at $\alpha \leq 0.01$.

CONCLUSIONS

The primary goal of this research is to bring together information and techniques available in our laboratory, and in diverse fields, and test them in a single system for understanding host/pathogen interactions, specifically those between ponderosa pine and western gall rust. Our recent results show that genetic variation among western gall rust isolates does exist. We plan to characterize the degree of genetic variation among rust biotypes, and in the process contribute to the understanding of the pathogen's reproductive biology. We will manipulate in vitro inoculation techniques to develop a system whereby genetically identical host material can be studied for mechanisms of resistance. We plan to examine cellular reactions to infection in callus, regenerated shoots, excised embryos and intact seedlings derived from field resistant and susceptible seed sources, and determine the degree of inheritance for each reaction. Finally, we plan to determine if field resistance is genetically or phenologically controlled.

REFERENCES

Allen, E. A. 1984. Infection of juvenile lodgepole pine by Endocronartium harknessii. M.S. Thesis, University of Alberta. 78 p.

Amerson, H. V. and R. L. Mott. 1978. Technique for axenic production and application of Cronartium fusiforme basidiospores. Phytopathology 68:673-675.

Bingham, R. J. 1983. Blister rust resistant western white pine for the Inland Empire: The story of the first 25 years of the research and development program. USDA For. Serv. Gen. Tech. Rep. INT-146. 45 p.

Burdon, J. J. and A. P. Roelfs. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of Puccinia graminis. Phytopathology 75:1068-1073.

Hiratsuka, Y. 1973. The nuclear cycle and the terminology of spore states in Uredinales. Mycologia 65:432-443.

Hoff, R. J. 1985. Resistance of lodgepole and ponderosa pine to western gall rust. Proc. IUFRO Rusts of Hard Pines Working Party, Athens, GA. pp. 307-319.

Kinloch, B. B. Jr. 1972. Genetic variation in resistance to Cronartium and Peridermium rusts in hard pines. Pages 445-463, In Proc. Biology of Rust Resistance in Forest Trees, USDA For. Serv. Misc. Pub. 1221. 681p.

Martinsson, 0. 1980. Stem rusts in lodgepole pine provenance trials. Silv. Genet. 29:23-26.

Merrill, W. and B. R. Kistler. 1976. Phenology and control of Endocronartium harknessii in Pennsylvania. Phytopathology 66:1246-1248.

Merrill, W. and N. Wenner. 1985. Pine-pine gall rust: history, distribution, and spread in the northeastern United States. Proc. IUFRO Rusts of Hard Pines Working Party, Athens, GA. pp. 271-283.

Merrill, W., N. Wenner and B. Towers. 1986. Resistance of Pinus ponderosa to Endocronartium harknessii in Pennsylvania. Plant Disease 70:317-320.

Micales, J. A., M. R. Bonde, and G. L. Peterson. 1986. The use of isozyme analysis in fungal taxonomy and genetics. Mycotaxon 27:405-449.

Nelson, D. L. 1971. The ecology and pathology of pine gall rust in California. Ph.D. dissertation, Univ. of California, Berkeley. 160 p.

Old, K. M., W. J. Libby and J. H. Russell. 1985. Patterns of susceptibility to western gall rust in Pinus radiata. Proc. IUFRO Rusts of Hard Pines Working Party, Athens, GA. pp. 285-305.

Peterson, G. W. and J. W. Riffle. 1984. Disease resistance investigations in relationship to tree improvement research in Nebraska. Proc. Forestry Committee, Great Plains Agricultural Council, Watertown, S.D. Great Plains Agricultural Council Publication No. 112. pp. 130-136.

Peterson, R. S. and F. F. Jewell. 1968. Status of American stem rusts of pine. Annu. Rev. Phytopathol. 6:23-40.

Powers, H. R. Jr. 1984. Control of fusiform rust of southern pines in the USA. Eur. J. For. Path. 14:426-431.

Powers, H. R., Jr. 1982. Pathogenic variability within the genus Cronartium. Proc. Resistance to Diseases and Pests in Forest Trees. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands. pp. 236-242.

Read, R. A. 1983. Ten-year performance of ponderosa pine provenances in the Great Plains of North America. USDA For. Serv. Res. Paper RM-250. 17p.

Rensema, T. and G. A. Tuskan. 1986. An economic analysis of wood utilization from field windbreaks of the Northern Great Plains. Proc. International Windbreak Symposium. p. 217-218.

Skirvin, R. M. 1981. Fruit crops. Chapter 3, In Conger, B. V. (ed.). Cloning Agricultural Plants via In Vitro Techniques. CRC Press Inc., Boca Raton, FL. Chapter 3.

Thomas, C. S., J. H. Hart and C. E. Cress. 1984. Severity of Endocronartium harknessii in two provenance stands of Pinus ponderosa in Michigan. Plant Disease 68:681-683.

Tuskan, G. A. and J. A. Walla. 1986. Isozyme differences between geographic sources of Endocronartium harknessii .(Abstr.) Phytopathology 76:958.

Tuskan, G. A. and J. A. Walla. 1987. In vitro screening of ponderosa pine for resistance to western gall rust. Proc. North Dakota Acad. Sci. 41:12.

Walla, J. A. and R. W. Stack. 1979a. Western gall rust in shelterbelts on the Northern Plains. (Abstr.) Phytopathology 69:543-544.

Walla, J. A. and R. W. Stack. 1979b. Western gall rust in North Dakota. Plant Disease Reporter 63:432-433.

Zagory, D. and W. J. Libby. 1985. Maturation-related resistance of Pinus radiata to western gall rust. Phytopathology 75:1443-1447.