Peroxidases in Developing Acrons and Seedlings of Quercus alba L.[1])

By J. S. Mayberry[2]) and P. P. Feret[3])

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Introduction and Literature Review

The genotype of a plant is usually identified by noting morphological or anatomical characters. An aid to genetic studies in trees has been the use of a variety of biochemical genetic markers and one tool that has gained importance in genetic analysis has been electrophoresis of isoenzymes (Feret and Bergmann, 1976). This study was conducted to describe peroxidase (donor: H_2O_2 oxidoreductase E.C. 1.11.1.7) isoenzyme variability in developing and germinating acorns of white oak *(Quercus alba* L.) and to define tissues suitable for use in genetic analysis of white oak.

Early electrophoretic studies established the electrophoretic technique as a valuable tool for monitoring isoenzymes. Shannon (1968), Scandalios (1974), and Feret and Bergmann (1976) have reviewed literature describing isoenzyme ontogenetics in plants. In agronomic crop species Brim *et al.* (1969), Scandalios (1969), Gupta and Pawar (1974), and Kadam *et al.* (1973) demonstrated quantitative and qualitative isoenzyme variation in seeds and young seedlings of soybeans, maize, rice and maize. Racusen and Foote (1966), Siegal and Galston (1967), Upadya and Yee (1968), Hamill and Brewbaker (1969), and Thomas and Nuecere (1974) hove shown peroxidase isoenzyme pattern differences over time among and between organs of bean, pea, barley, maize, and peanut.

Isoenzyme variation through stages of growth and development in forest trees has been little sudied. Several studies are available that show the changes in isoenzymes between tissues and over time in gymnosperms. Durzan (1966), using disc electrophoresis, found intraspecific as well as interspecific isoenzyme variability in white spruce, jack pine, and white pine embryos and female gametophytes. Conkle (1971) demonstrated in knobcone pine the presence of peroxidases linked with the developmental stage of the plant and tissue extracted. Other authors have followed isoenzyme changes in forest tree angiosperms. Perry (1971) showed seasonal variation of fats, phenols, isoenzymes and pigments in red maple. Gordon (1971) found quantitative and qualitative changes in leaf peroxidase of eastern cottonwood just prior to a constant nitrogen to tissue ratio and demonstrated that the appearance of photosynthetic acitivity roughly coincided with the appearance of a major leaf peroxidase isoenzyme. Jennings and Street (1974) found peroxidase isoenzyme patterns complex in batch cultured cells of sycamore. Wolter and Gordon (1975) illustrated differences in peroxidase patterns in aspen callus cells under various auxin and cytokinen treatments.

Materials and Methods

Young seedlings of three white oak trees were selected for characterization of isoperoxidase variation during acorn germination. Two of the trees (referred to as I and II) were located in Mercer County, West Virginia. They were separated by 32 kilometers of rugged mountainous terrain. The third tree was located at the Reynolds Homestead Research Center near Critz, Virginia (referred to as III).

Twenty acorns or seedlings were drawn at each sampling date. This sample size corresponded to the number of samples needed to separate any given isoenzyme occurring with a frequency greater than or equal to 0.15 with 95 percent confidence using the expression $\log_{10}(a) = N(1 -$ frequency), where N equals sample size. Dates of acorn collection and relative size of acorns at each collection date are described in ***Table*** 1.

Table 1. — Collection dates and mean acorn lengths (cm.)[1]) of acorns used for peroxidase extractions.

Collection Dates	Tree I	Tree II	Tree III
8-14-75	1.08 (.48)[2]/	1.34 (.59)	
8-21-75			1.15 (.55)
8-28-75	1.52 (.68)	1.94 (.86)	
9-4-75			1.44 (.69)
9-11-75	2.18 (.97)	1.99 (.88)	
9-18-75			1.89 (.91)
9-25-75	2.24 (1.0)	2.26 (1.0)	
10-2-75			2.08 (1.0)

[1]) Sample Size equals 20 acorns per tree per collection.
[2]) Value in parentheses expresses length as a percent of final length at date of maturity.

The acorn organs assayed included embryos and cotyledons. The first samples were taken approximately 85 days after pollination when sufficient material was present for enzyme extraction. Samples were collected at two week intervals thereafter. Immediately after collection the acorns were stored at —10 C until extracted. Due to poor acorn production the acorn samples from trees I and III were insufficient to carry out germination and seedling analyses.

At the time of acorn maturation, collections were made from trees I and II. Mature acorns were stored at 4 C until planting. From each tree 200 acorns were planted one centimeter deep in 100, 6-inch pots. The potting medium was a 2 : 1 : 1 : 2 mix of sand, perlite, forest soil and peat. The forest soil fraction used was the A horizon soil from a natural stand of white oak.

The acorns were placed in the greenhouse after potting and allowed to germinate. Germination was defined as the appearance of the epicotyl tip at the soil surface. Subsequent dates of collections were based on the date of germination of each acorn. Seedling collections were begun on tree II one week after epicotyl appearance. Collections were first made on tree I two weeks after epicotyl appearance. This delay was due to slow germination rates of tree I seedlings. Collections continued on a weekly basis for each tree for five weeks.

The seedlings were washed in cold water and chilled on ice after removal from the pot. Within forty minutes all collected seedlings were frozen at —10 C until extracted.

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[2]) Research Assitant, Department of Forestry and Forest Products, VPI & SU, Blacksburg, VA.
[3]) Associate Professor, Department of Forestry and Forest Products, VPI & SU, Blacksburg, VA.

Organs extracted for peroxidase included hypocotyls, cotyledons, epicotyls and first leaves.

Extraction of peroxidase from all materials was carried out at 1—4 C. Organs to be assayed were finely chopped and mechanically homogenized in 0.1M Tris-HCl (pH 8.0) extract buffer containing ascorbate (6mM), cysteine (6mM), 0.5M sucrose and 1 percent (v/v) Tween 80 (polyoxyethylene sorbitan monooleate). The homogenates were centrifuged at 27,750 × g for twenty minutes. The supernatant was then stored at —10 C.

The system used for electrophoretic analysis was discontinuous polyacrylamide gel electrophoresis. The method of polyacrylamide gel preparation was that of Davis (1964) with some modification. The running gel buffer was Tris-HCl (pH 8.0) buffer. A 7.5 percent polyacrylamide solution with 1 ml. TEMED (N,N,N',N'-tetramethylethylenediamine) and 0.1 ml. Tween 80 per liter was used for gel formulation. All electrophoretic runs were anodal. Each sample was run for two hours at an initial voltage of 200 volts (approximately 3 ma./tube) across 20 6mm × 82mm tubes. A total of 110 microliters of protein extract was used for each analysis.

The gels were removed from the tubes and stained in a solution of 0.5 percent benzidine containing 1.5 percent H_2O_2 (Scandalios, 1969). Immediately after staining, the gels were fixed in tubes containing 7 percent acetic acid. Once fixed, each zymogram was recorded by sketching the number, position, and intensity of individual isoenzymes and the marker front. The zymogram data were quantified by calculating relative mobility (Rm) ratios of the isoenzyme migration distance using the marker front (bromophenol blue) migrating distance as the divisor. Rm values were repeatable to within ±.03 Rm units.

Results

Isoenzymes were named according to the organ in which the isoenzymes were analyzed and their relative mobility

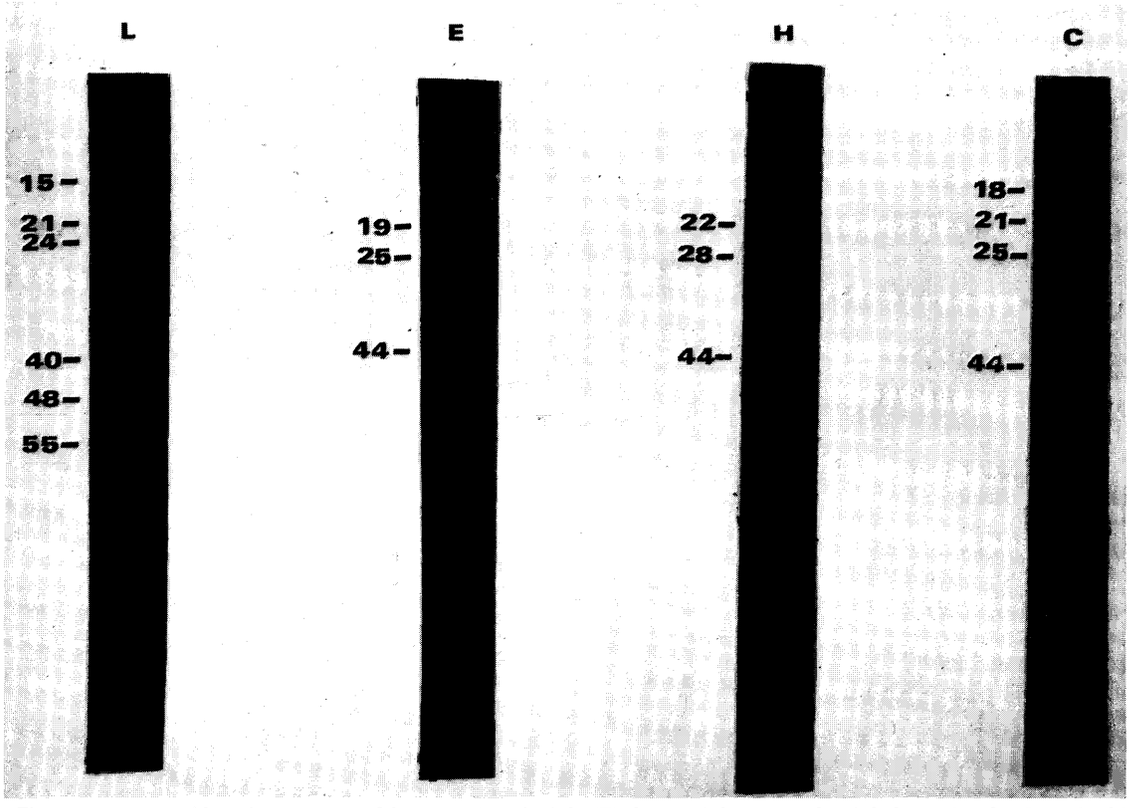

Figure 1. — Peroxidase isoenzymes of leaves (L), epicotyls (E), hypocotyls (H), and cotyledons (C) of a 5-week-old Tree I seedling. Isoenzyme Rm values are to the left of each isoenzyme. Isoenzymes C18 and C21 are faint in staining intensity. Isoenzyme E19 is weak in intensity, L15 moderate and H44 strong in staining intensity.

Table 2. — Acorn cotyledon isoperoxidase frequencies.

Date of Collection[2]	Sample Size[1]			Isoperoxidase[1]															
	I	II	III	IIC15	IIC18	IIC21	IIIC22	IC23	IIC25	IIIC25	IC28	IIC31	IIIC31	IIC35	IIIC41	IIC49	IIIC51	IIC54	IIC60	IIC68
A	0	20	8	NP[3]	NP	0.70	0.44	----	0.75	0.78	----	NP	NP	NP	0.56	NP	NP	0.56	NP	NP
B	20	20	20	NP	NP	0.35	NP	0.15	0.25	NP	0.30	NP	NP	NP	NP	NP	NP	NP	0.25	NP
C	12	20	20	NP	NP	NP	NP	0.50	0.55	0.20	0.83	0.50	0.40	NP	NP	NP	0.55	NP	NP	0.30
D	18	20	20	0.15	0.25	0.21	0.20	0.72	NP	0.12	0.72	0.60	NP	0.65	0.60	0.75	NP	0.25	NP	NP

[1] Designations symbolize the following: I, II, III refer to parent tree number; C15, C18 . . . C68 refer to cotyledon isoperoxidases.

[2] Collection dates were as follows: For Tree I and Tree II: A = 8/14/75; B = 8/22/75; C = 9/11/75; D = 9/25/75. For Tree III: A = 8/21/75; B = 9/4/75; C = 9/18/75; D = 10/2/75.

[3] NP indicates "not present".

Table 3. — Acorn embryo isoperoxidase frequencies.

Date of Collection[2]	Sample Size[1] I	II	III	Isoperoxidase[1] III EM14	III EM17	I EM22	II EM23	I EM24	III EM24	II EM27	I EM29	III EM29	II EM38	I EM42	II EM42	III EM42	II EM46	III EM47	I EM48	III EM49	III EM51	II EM52
A	4	7	10	NP[3]	NP	+[4]	1.00	+	1.00	1.00	NP	0.60	NP	+	1.00	1.00	1.00	NP	NP	NP	NP	0.86
B	20	20	19	NP	NP	NP	1.00	0.70	0.68	1.00	0.75	0.53	NP	0.75	0.90	NP	0.75	0.17	0.48	0.89	NP	NP
C	13	19	20	NP	0.70	NP	0.89	0.92	1.00	0.95	0.92	0.95	NP	0.46	NP	NP	0.32	NP	0.77	0.48	0.90	0.68
D	20	20	20	0.55	0.80	0.70	0.30	0.65	0.75	1.00	0.35	NP	0.35	NP	0.35	0.80	NP	0.65	1.00	NP	NP	0.90

[1] Designation symbolize the following: I, II, III refer to parent tree number; EM14, EM17 . . . EM52 refer to embryo isoperoxidases.
[2] Collection dates were as follows: For Tree I and Tree II: A = 8/14/75; B = 8/22/75; C = 9/11/75; D = 9/25/75. For Tree III: A = 8/21/75; B = 9/4/75; C = 9/18/75; D = 10/2/75.
[3] NP indicates "not present".
[4] Indicates presence in at least on sample.

Table 4. — Tree II gel region 1 (Rm 0—29) seedling peroxidase frequencies.

Collection	Sample Size[1] Leaf	Epicotyl	Hypocotyl	Cotyledon	Isoperoxidase[1] L16	L18	L22	E22	H22	C22	E24	H24	L25	C26	H27	L29	E29	C29
Week 1	16	20	20	19	NP[2]	0.38	0.94	0.95	0.85	0.74	0.55	0.90	1.00	0.84	NP	NP	NP	NP
Week 2	19	20	20	20	NP	0.58	0.63	1.00	0.95	0.89	0.60	0.75	0.42	0.95	NP	NP	NP	NP
Week 3	20	20	19	20	NP	0.60	0.95	0.05	0.89	0.79	1.00	NP	1.00	1.00	0.79	0.35	1.00	NP
Week 4	17	17	17	17	NP	1.00	1.00	0.45	0.84	0.28	0.70	NP	1.00	0.56	0.74	NP	0.55	0.39
Week 5	19	10	17	17	0.47	0.68	0.95	NP	NP	NP	0.90	1.00	0.79	0.59	0.65	NP	0.90	0.94

[1] Designations symbolize the following: Letters L, E, H, C, designate, leaves epicotyls, hypocotyls, and cotyledons respectively; 16, 18, . . . 29 designate organ isoperoxidases.
[2] NP indicates "not present".

Table 4 (continued). — Tree II gel regions 2 and 3 (Rm 30—100) seedling peroxidase frequencies.

Collection	Sample Size[1] Leaf	Epicotyl	Hypocotyl	Cotyledon	Isoperoxidase[1] L39	C40	E41	H42	L43	C43	E45	C45	H46	L48	L55	L58
Week 1	16	20	20	19	0.75	0.79	0.60	1.00	0.69	0.42	0.65	NP[2]	0.20	0.25	NP	NP
Week 2	19	20	20	20	0.58	0.79	1.00	0.95	0.42	0.37	0.05	NP	0.35	NP	NP	NP
Week 3	20	20	19	20	0.30	NP	0.56	0.68	0.80	0.47	1.00	NP	0.79	0.30	NP	NP
Week 4	17	17	17	17	NP	NP	NP	0.16	0.30	0.11	1.00	0.22	1.00	NP	0.45	NP
Week 5	19	10	17	17	0.47	NP	NP	NP	0.63	NP	1.00	NP	0.74	NP	0.37	NP

[1] Designations symbolize the following: Letters L, E, H, C designate leaves, epicotyls, hypocotyls and cotyledons respectively; 39, 40, . . . 58 designate organ isoperoxidases.
[2] NP indicates "not present".

value. Organs included leaves (L), epicotyls (EP), hypocotyls (H), cotyledons (C) and embryos (EM). All zymograms were divided into three regions. Regions 1, 2 and 3 encompass Rm values of from 0.00 to .30, .31 to .40, and .41 to .60 respectively.

Isoenzyme staining intensities were visually recorded. Four classifications were used: faint, weak, moderate and strong. *Figure 1* illustrates staining intensity classifications.

Acorn Analysis

Cotyledonary peroxidases: Cotyledonary peroxidases were the most variable group of isoenzymes in maturing acorns *(Table 2)*. The staining intensities generally increased with increasing acorn age.

Embryo peroxidases: Qualitative isoenzyme differences were present between the three trees sampled *(Table 3)*. The amount of qualitative variation among collection dates was less distinct for trees I and II while tree III isoenzymes differed among all four collection periods. Staining intensity and isoenzyme resolution of embryo peroxidases was in general far superior to that found using cotyledon tissue.

Isoenzymes assayed in tree I ranged from faint to strong in activity during all collections except collection C which was uniformly strong. Isoenzyme clarity and resolution were excellent.

For tree II, staining intensities of the A collection were variable from sample to sample with EM23 showing strong activity. One wide isoenzyme of strong intensity replaced EM42 and EM46 for the C collection date.

Analysis of embryos collected from tree III revealed a strong staining EM42 isoenzyme. Staining intensities of the five isoenzymes assayed in collection D varied from weak to strong with strong activity present in gel region 3 only. Resolution of isoenzymes in tree III embryos was less distinct than from trees I and II.

Seedling Analysis

Leaf peroxidases — tree II: Expanding leaves produced complex zymograms with up to nine different isoenzymes recorded in any one sample. The results of the leaf peroxidase analyses are displayed in *Table 4*.

The three persistent isoenzymes of gel region 1 (L16, 18, 22 and 25) varied in frequency from week to week showing no pattern of either increasing or decreasing in fre-

quency. The staining intensity of L18, L22 and L25 was weak to moderate the second week, moderate to strong the third and fourth weeks and weak to moderate the fifth week.

Isoenzymes were commonly recorded in gel region 3 at Rm 0.39 and 0.43. No uniform pattern of increasing or decreasing frequency was recorded. Staining intensities were moderate to strong in the first two weeks of sampling; third week staining intensities were faint to weak for L39 while L43 remained moderate in activity. L39 was absent and L43 was faint in staining intensity by the fourth week of sampling. Both L39 and L43 were present the fifth week but activity was faint to weak.

Epicotyl peroxidases — tree II: The epicotyl tissues contained less isoperoxidase variability than leaf tissue *(Table 4).* EP41 and EP45 stained intensely while the other isoenzymes were weak to moderate in staining intensity.

Hypocotyl peroxidase — tree II: Hypocotyls like epicotyls, possessed five isoenzymes *(Table 4).* Staining intensities in gel regions 1 and 3 were weak to moderate with H22 activity intense during the fourth week. Isoenzymes H42 and H46 also assayed with high activity during the final weeks of collection.

Cotyledon peroxidases — tree II: Seedling cotyledon isoenzyme data are summarized in *Table 4.* The cotyledons by the third week of collection were shriveled and beginning to discolor and at the fifth week the cotyledons were either disintegrated or diminutive in size and black in color. Among weeks peroxidase activity differed. Isoenzyme staining intensities shifted from faint to weak to moderate. The presence of faster migrating isoenzymes in older samples was noticeable.

The seedlings study of tree I, though identical to the tree II study, was largely inconclusive because insect and fungal damage was present on the majority of the acorns which displayed deterioration and related poor germination and, therefore, results of the tree I study are not presented.

Discussion

The results of this study demonstrate a large amount of variation in the peroxidase isoenzymes of *Quercus alba.* Variation can be attributed to individuals, organs and stage of development. There is notable variation in any one organ sampled over time with some organs being more constant in their peroxidase isoenzyme composition than others.

One noticeable trend throughout the study was a shift in activity in some organs during the later collection dates. This trend was present in both the acorn and seedling studies. The shift, though not evident in all organs, was towards isoenzymes with higher electrophoretic mobility. The shifts were generally concentrated in gel region 3 (Rm .41 to .60). Activity shifts were common in leaves and cotyledons. *Tables 2, 3 and 4* illustrate these changes. Several authors have shown this activity shift in other species. Hamill and Brewbaker (1969) and Scandalios (1969) both reported shifts in activity in maize. Felder (1976) reported shifting of two isoenzymes in barley. Scandalios speculated the shifts may reflect changes in relative activities of different genes. The lack of genetic control in materials used here limits any speculation as to possible reasons for shifting activity.

An overview of *Tables 2 through 4* reveals a similarity of Rm values between organs from the same sample tree and different sample trees. For example, tree II has an average Rm value of .22 in leaves, epicotyls, hypocotyls and cotyledons. Other isoenzymes among these organs vary in average value by only 0.01 Rm unit. Similarly, embryos contained one common isoenzyme among all three trees at Rm .42. Other isoenzymes differed in value by 0.01. Certainly, experimental error may be one reason Rm values are not exactly duplicated in sample sizes of twenty or less. A repeatability of Rm values between organs does not necessarily mean that the same isoenzyme occurs in the organs or that the isoenzyme has the same metabolic function. However, the repeatibility of Rm values strongly suggests that minor variation in mobility within the same gel regions between organs and trees is probably due to measurement error rather than a meaningful biological difference. The three trees used in the present study are widely separated. Thus, it is unlikely that maternal sources of variation are duplicated between trees or the pollen source was the same for any two trees. The probable lack of common parental genomes in acorns of the three trees implies that isoenzymes with common Rm values between organs and trees are present because of biological importance rather than common parentage. Lewis and Cech (1969) have also shown similar peroxidase isoenzymes in seeds of black cherry collected from different areas.

Isoenzymes that are common between organs are usually the same isoenzymes that are the most constant throughout the collection periods. Embryos, epicotyls, and hypocotyls show the isoenzymes of highest constancy.

Acorn Analysis

Cotyledonary peroxidases: The cotyledonary peroxidases were variable in both number of isoenzymes and staining intensity. Between trees, isoenzyme numbers ranged from two in tree I to ten in tree II. Several cotyledonary isoenzymes (C22, C23, C25, C31, C42 and C51) had identical or nearly identical Rm values among the three trees and among organs. This is in contrast to results published by Brim *et al.* (1969) who reported radically different isoenzymes in soybean cotyledons as opposed to other tissues.

Both tree I and tree II showed very weak peroxidase activity in cotyledons. Siegal and Galston (1967) reported little acivity in the early stages of cotyledon development in pea. *Table 2* reveals a difference of eight isoenzymes between tree I and tree II. Six isoenzymes in tree II were present in only one collection period.

The amount of isoenzyme variation in tree III cotyledons may be due in part to abnormal acorn development. The lack of peroxidase activity during the second week of collection for tree III probably coincides with a cessation of growth. Isoenzymes in subsequent weeks are perhaps indicators of senescent tissue (Racusen and Foote, 1966; Hamill and Brewbaker, 1969).

In conclusion, cotyledons are not desirable as model organs for use in further genetic studies of white oak. The high amount of variability coupled with a low intensity of staining and nonrepeatability of isoenzymes between samples limit the value of cotyledon peroxidases for genetic studies.

Embryo peroxidases: Embryo tissues yielded the most consistent data. At the first acorn collection period embryos were approximately one half the size they were at maturity (8mm × 4mm). The embryos represent the early stages of organogenesis in the new sporophyte. Since the role of peroxidase has been connected with the growth hormone indole-3-acetic acid (IAA) (Ockerase *et al.,* 1966) embryos should be a center of much peroxidase activity. However, reports are conflicting as to whether the interaction is positive or negative. Ramaiah *et al.* (1971) and Wolter and Gordon (1975) reported peroxidase activity was closely and

positively correlated with tissue growth. GORDON (1971) indicated high peroxidase activity was concomitant with slow growth. The analysis of embryo tissue here neither refutes nor confirms the above hypotheses.

The data in *Table 3*, while showing variation in isoenzymes, fails to show significant changes in the number of isoenzymes per week. Furthermore, dramatic shifts in activity were not noted. This implies that embryo tissue would be useful for genetic studies of *Quercus alba*.

Seedling Analysis

Leaf peroxidases: Expanding leaves, as a class, were the organs with the fastest rate of growth and contained the greatest number of isoenzymes per sample. The high number of isoenzymes recorded for leaves in this study is in contrast to the studies of UPADHYA and YEE (1968) and EVANS and ALLDRIDGE (1965). UPADYA and YEE (1968) recorded low peroxidase activity in young leaves of barley while EVANS and ALLDRIDGE (1965) has demonstrated the same effect in extreme dwarf and normal leaves of tomato.

Though isoenzyme variation was present in the leaves, four isoenzymes were found invariate over time (L18, 22, 25 and 43). The staining intensities for the isoenzymes were weak at early developmental stages, increased in activity during the third and fourth week from germination, and declined to moderate the fifth week. CHEN *et al.* (1970) demonstrated this same type of activity in developing cocklebur leaves. GORDON (1971) found similar changes in leaves of eastern cottonwood.

The use of leaves in genetic experiments is limited due to the high number of isoenzymes per zymogram. The high number makes data analysis cumbersome. Poor isoenzyme resolution would make difficult the recording of isoenzymes as possible allelic markers. High staining intensity in some samples tends to mask other isoenzymes. The system used in this study does not lend itself to assay of leaf material but leaves may prove of value using other assay systems.

Epicotyl and hypocotyl peroxidases: The epicotyl stem tissues used in this study consisted of internodal tissues. The reduction of the number of isoenzymes from leaves may reflect a reduced level of cell type complexity and differentiation in epicotyl stem tissues. Variation in isoenzyme frequency was the primary source of variation. Reductions in frequency of one isoenzyme may be accompanied by an increase in frequency of another isoenzyme. CHEN *et al.* (1970) have postulated that isoenzymes that are differentially affected by age are perhaps under independent genetic control. The epicotyl tissues are a good choice for material in future genetic studies of white oak. The low number of isoenzymes provides an acceptable spatial relationship for ease of isoenzyme visualization and recording. The staining intensities are moderate to strong for the prominent isoenzymes.

Hypocotyl peroxidases closely resemble epicotyl peroxidases. Hypocotyls represent suitable organs for genetic studies. Hypocotyls are intermediate organs between the cotyledons and the epicotyl. In such a position the hypocotyl is primarily an organ of transport from the cotyledons to the growing tissues. As the seedlings mature the importance of the hypocotyls diminishes. As the hypocotyls age a higher number of isoenzymes might be expected as was documented for maturing tree III acorn cotyledons. A higher number of isoenzymes during the last collection period was not demonstrated in this experiment. The staining activity was moderate to strong for the five isoenzymes. The strong activity was especially prominent during the later weeks of collection. The good isoenzyme resolution lessens the difficulty of visually recording isoenzymes.

Cotyledonary peroxidases: For tree II, the cotyledons were less variable in number of isoenzymes in the seedling study than in the acorn study. Only two isoenzymes at Rm .21—.22 and Rm .25—.26 were present in both studies. The physiological condition of the cotyledons, however, is reversed. In the acorn study the cotyledons were growing and functioning in the storage of metabolites. For the seedling study, the cotyledons were being depleted of their food reserves. This experiment demonstrates that for cotyledons undergoing growth (a metabolic state) and then depletion (a catabolic state) different isoenzymes are present. Cotyledonary peroxidase staining intensity, however, is no more uniform than in the acorn study. The staining intensities were predominantly faint to weak. The low amount of activity in the fifth week is probably due to the age of the tissues correlated with depletion of food reserves and tissue death.

Summary

Peroxidase isoenzymes have been shown to vary between organs and between trees in white oak. The isoenzymes differ both in total number of isoenzymes per organ and the relative mobilities of the isoenzymes. The amount of isoenzymes variation per organ is independent of isoenzyme patterns for other organs along a developmental sequence. Some organs (epicotyls and hypocotyls) have nearly identical isoenzyme patterns. Leaves and cotyledons showed the greatest amount of isoenzymes variation.

Of the tissues assayed, three hold promise for use in future studies. In maturing acorns, embryos yield reproducible isoenzymes and clear resolution. Seedling epicotyls represent a choice organ with high isoenzyme reproducibility and resolution. Hypocotyls are similar to epicotyls with the disadvantage of being available only during a limited period.

Key words: Isoperoxidase, white oak, electrophoresis, acorns, seedlings.

Zusammenfassung

Es konnte gezeigt werden, daß Peroxidase-Isoenzyme zwischen Organen und zwischen Bäumen bei *Quercus alba* L. variieren. Die Isoenzyme sind unterschiedlich sowohl in der Anzahl pro Organ als auch der relativen Mobilität. Die Höhe der Isoenzymvariation pro Organ ist unabhängig von den Isoenzymmustern anderer Organe entlang einer Entwicklungsreihe. Einige Organe (Epikotyl und Hypokotyl) haben annähernd identische Muster. Blätter und Kotyledonen zeigten die größte Isoenzymvariation.

Von den untersuchten Zellgeweben versprechen 3 für künftige Untersuchungen aussichtsreich zu sein. In der reifenden Eichel erreicht der Embryo reproduzierbare Isoenzyme unter klarer Auftrennung. Das Epikotyl des Sämlings liefert als Alternative hierzu ebenso gute Reproduzierbarkeit und Auftrennung der Isoenzyme. Das Hypokotyl ist dem Epikotyl gleichwertig, jedoch mit dem Nachteil, daß es nur während einer begrenzten Zeit verfügbar ist.

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Short Note: Cross-fertilization in a conifer stand inferred from enzyme gene-markers in seeds

By G. Müller

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Introduction

Cross-fertilization will be described in terms of the probabilities with which the ovules of any given tree in a stand are fertilized by pollen originating from a single specified tree in the same stand as a function of the distance between the respective trees. Information about these probabilities as well as about the probabilities of self-fertilization are required in order to characterize the mating system of trees. It can be assumed that coniferous species such as Norway spruce and Scots pine do not mate at random, since at least two important conditions necessary for this mating system are not fulfilled: firstly, the probabilities of cross-fertilization have been proved to depend on the distance between the mating trees because of limited spatial pollen dispersal, and secondly, the probabilities of self-fertilization of individual trees are on the average higher than the reciprocal value of the population size (Koski and Malmivaara 1974; Müller 1976 a and 1977). This implies that other mating systems, such as preferential mating between relatives or assortative mating or any combination of both, can be accepted as being more realistic. More precise experimental data are required to characterize the actual mating system of the mentioned tree species and to estimate coefficients of inbreeding and kinship to avoid the well-known detrimental effects of these phenomena on the average expression of important characters in the future breeding populations.

[1]) Dr. Gerhard Müller
Lehrstuhl für Forstgenetik und Forstpflanzenzüchtung
Büsgenweg 2
D-3400 Göttingen-Weende
West Germany

The probabilities of cross-fertilization cannot be estimated by experiments on the spatial pollen dispersal, because the results would refer only to the probabilities of cross-pollination. It is necessary to identify the pollen contribution of an individual tree ("marker tree") in the viable seeds of other trees in the same stand by detecting its genes in the diploid embryo tissue of the respective seeds. This implies that the estimated probabilities of cross-fertilization comprise the effects of genotypic selection, if this type of selection occurs between formation of the zygote and the embryonic stage.

Identification of pollen can be performed precisely by applying enzyme analysis and using such gene-markers as criteria for identification which guarantee a one-to-one correspondence between enzyme phenotype and genotype. In this paper, an additional field of application of a method is presented which has already been proved to be suitable for the estimation of probabilities of self-fertilization of individual trees in conifer stands (Müller 1976 b and 1977).

Materials and Methods

In the winter of 1976/77, cones from each of 105 trees were collected separately from a continuous area of a 120-year-old Scots pine stand *(Pinus sylvestris)* in the forest district of Grebenau (Hessen), compartment 57 b. All trees had been cut down just before this collection. The former position of each tree in the stand before clear-cutting was marked in a map.

The pine stand continues only on the southern side of the experimental area; the other sides adjoin on either young plantations or farm land. In *figure 1* only those pine-trees are marked with numbers, the seed samples of which