

RESISTANCE OF PIN OAK TO IRON CHLOROSIS:
A TECHNIQUE FOR DETECTING GENETIC VARIATION'

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INTRODUCTION

A much-critized aspect of the "Green Revolution" breeding programs sponsored by the Rockefeller Foundation is that they have emphasized too strongly the improvement of crop plants for **use** only with intensive fertilization. The criticism may or may not be justified in terms of the particular goals of this most famous of plant breeding endeavors. But it is becoming increasingly obvious that geneticists have paid too little attention to traits that confer adaptation to the edaphic environment. In the words of one proponent of the subject (Foy 1975), "Crop varieties have been directly selected for practically everything except adaptation to the soil, the most basic resource of all." Opportunities for breeding plants for resistance to mineral deficiencies or excesses in the soil have not been widely perceived, let alone explored in actual breeding programs (Epstein 1972). It has been too easy and inexpensive to modify the soil (usually with fertilizers) to fit the plant, rather than genetically modify the plant to fit the soil.

This situation is changing, however, with the increasing cost of fertilizers. Furthermore, it has never been the case with some low-value crops and some soils in which conditions differ radically from the optimum. According to Foy (1975) some soil fertility situations in which the genetic approach looks promising are: acid soils with soluble aluminum in toxic concentrations, soils polluted with heavy metals, and calcareous soils with unavailable iron. There is ample evidence that plant species can contain substantial genetic variation in adaptation to soil conditions (Epstein 1972, Foy 1975). Reports cited by these authors concern agronomic crops and some non-crop species, but some studies have dealt with forest trees. In some instances, intraspecific variation in actual performance on different kinds of soil has been

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found (Jenkinson 1974, Teich and Holst 1974). In others, leaf tissue analyses have shown clones or provenances to differ in the accumulation of certain nutrients (Baker and Randall 1974, Gerhold 1959, Steinbeck 1965). When, as in the cases cited, trees differ in the accumulation of some nutrients but not others, there is good reason to believe that the differences are due to genetically controlled mechanisms of *mineral absorption or distribution (Epstein 1972).

In light of this, we considered the possibility of selecting within pin oak (Quercus palustris) for resistance to iron-deficiency chlorosis. In spite of the fact that the number of pin oaks sold in the United States surpasses the number of all other oak species combined (Flemer 1971), pin oak is often avoided because of its tendency to develop chlorosis on calcareous soils. On such soils, iron in an available form is frequently not present in the concentration needed by plants. Chlorosis in pin oak is characterized by smaller leaves and a yellowing of the leaves between veins. In severe cases, chlorosis causes stunted growth, necrosis and abscission of leaves, twig and branch dieback, and even death of the tree (Neely 1976). Various methods of soil modification and trunk implantation of iron have been used to correct the symptoms (HacsKaylo and Struthers 1959, Neely 1973 and 1976, Schoeneweiss 1973, Smith 1973), but the treatments are expensive and only temporarily effective. A cultivar resistant to iron chlorosis would remove a major objection to planting pin oak and reduce costs associated with its maintenance and replacement. Such cultivars have been developed in some agronomic crops and fruit and nut species (Brown 1961, Foy 1975, Wallace and Lunt 1960), and there is some evidence that sweetgum (Liquidambar styraciflua) varies in susceptibility to iron chlorosis (Wallace and Lunt 1960).

In this report we present the results of an experiment to determine an appropriate nutrient solution technique for screening seedlings for resistance to chlorosis. Hydroponics was selected as a basis for the technique because it affords the possibility of space-efficient, early screening of progeny for actual testing on calcareous soils, and because it enables one to control all aspects of the root environment. Many edaphic factors besides iron concentration may influence the development of chlorosis (Brown 1956, Brown 1961, Neely and Schoeneweiss 1974, Wallace and Lunt 1960), and the uncontrolled effects of these variables in one or a few field tests could hamper the selection of a clone of general, rather than specific, utility. Furthermore, sequentially growing a seedling in two entirely different environments is one means of replication and could enable

the detection of individual tree differences in susceptibility without cloning. This is especially true of a trait like chlorosis, which may be "turned off" by correction of the iron deficiency to eliminate carry-over effects from one environment to the next.

MATERIALS AND METHODS

Pin oak seeds were collected in fall 1975 from 5 local street trees, bulked into one seedlot, and stratified. In spring 1976 the seeds were germinated in a mixture of peat, perlite, and soil. After germination the seedling shoots were cut back once in the germination flat to control size and insure that most shoot growth would occur after the treatments had begun. Root systems were washed thoroughly upon transfer to the nutrient solution treatments. At that time, regrowth of the shoots had not yet occurred, so cotyledons were temporarily left attached to the plants until leaves could supply photosynthate.

The apparatus for the hydroponics system consisted of 18 glass jars, each containing 900 ml. of nutrient solution supplied with air forced through microtubules. Jars were capped with Styrofoam plugs containing slits for supporting the seedlings and were covered with aluminum foil to prevent heat accumulation and algal growth. A mechanism was devised to maintain water levels in the containers as transpiration occurred, but transpiration was not great enough to warrant its use.

On April 30, two seedlings were transferred to each container, and each pair of seedlings was given one of the following treatments in the stock solution shown in Table 1:

<u>Treatment number</u>	Iron concentration (p.p.m.)	pH
1	0.1	6.0
2	0.1	7.0
3	1.0	6.0
4	1.0	7.0
5	10.0	6.0
6	10.0	7.0

Iron was supplied in the form of an EDTA chelate and pH was adjusted with HCl or KOH. The experiment was set up on a greenhouse bench in a completely randomized design with 3 replications. No supplementary lighting was given. After April 30, pH's of the solutions were read and adjusted every 7 days, and the solutions were replaced every 14 days for the duration of the treatments.

After 10 weeks the treatments were terminated and the seedlings harvested for measurement. By that time most seedlings had made two, and in some cases, three, flushes of growth. Since chlorosis tended to become progressively more severe as the seedlings grew, we measured that trait on only the last set of leaves of those seedlings that had more than one growth flush. There were enough seedlings in this category to give us container averages for all treatment-replicate combinations except one in which both seedlings had died. Chlorosis was rated in two ways: visually scoring the seedlings on a 1-5-point scale and determining actual chlorophyll content of the leaves. To obtain the latter measurement, we extracted the chlorophyll from two 1.3 mm. leaf disks per jar in boiling 80% ethanol, cooled the solutions to room temperature, brought each to 10 ml. of volume, and read optical density on a Beckman-B spectrophotometer. We also measured shoot height above the cotyledonary node and length and oven-dry weight of the root system below the cotyledonary node.

RESULTS

According to both measures of chlorosis, visual scoring and chlorophyll content, iron concentration had a significant effect on the development of chlorosis (Table 2,3). Particularly when measured by chlorophyll content of the foliage, chlorosis declined as iron concentration in the solution increased, but the effect of raising iron from 0.1 to 1.0 p.p.m. was much greater than the effect of raising it from 1.0 to 10.0 p.p.m. The effect of pH treatment, on the other hand, was significant only when chlorosis was visually rated--chlorosis was greater at the higher pH. In both cases the variance component for iron treatment was much larger than that for pH treatment (Table 4). Iron x pH interaction was not significant with either variable.

Shoot height was significantly affected by both iron and pH treatments. The lowest iron concentration resulted in seedlings that were shorter than those in either of the two other treatments, and seedlings were shorter when grown in solutions adjusted to the higher pH. Again, the effect of iron treatment on variation in height growth was greater than that of pH treatment, and iron x pH interaction was not significant.

Neither root length nor weight was significantly affected by iron or pH treatments. However, this result contrasts with the fact that we observed obvious differences in root development associated with iron concentrations. Plants grown at progressively higher concentrations had darker

(almost black at 10.0 p.p.m.) and more fibrous root systems. The pronounced proliferation of very small roots, particularly at 10.0 p.p.m. iron, presumably had little effect on the total weight of the roots. In addition, there were apparently physiological differences in effect on the edaphic environment among the root systems grown at different iron concentrations. When pH of the solutions at 7 days after adjustment was averaged for the duration of the experiment, it was found that those adjusted to **6.0** had tended to rise to 6.3 and those adjusted to 7.0 to fall to 6.7. However, the change at the two lower concentrations of iron was significantly greater than the change at the higher concentration.

DISCUSSION

The effect of decreasing iron concentration on chlorosis development was, of course, expected. However, it is somewhat surprising that some chlorosis was observed at 1.0 and even 10.0 p.p.m. iron (Table 2). According to Lindsay (1974), most crops require less than 0.5 p.p.m. iron. A commonly used modification of Hoagland's solution (Epstein 1972) only provides 1.12 p.p.m., and most nutrient solutions are formulated to provide nutrients in higher concentrations than are needed by most plants. Carpenter (1952) also observed chlorosis in pin oak growing in a solution containing 1 p.p.m. iron at pH 7.0. Hence, pin oak may require more iron than other plants, regardless of pH or the presence of calcareous soil. If this is true, it removes some potential sources of genotype x environment interaction in genetic studies on this trait in pin oak. The parallel variation between lack of chlorosis and shoot height ($r = + .80$, with 15 d.f.) is as expected since chlorosis impairs the production of photosynthate.

High soil pH on calcareous sites is frequently associated in the literature with chlorosis in pin oak (Neely 1973, Neely and Schoeneweiss 1974, Smith 1973). The critical pH is usually assumed to be 6.7-7.0. The effect of pH is to change the solubility of ions and the relative concentrations of specific ionic forms--and plant absorption depends on ionic form as well as concentration (Moore 1974). In the case of iron, the effect of increasing pH is to increase the oxidation of ferrous to the much less soluble ferric ionic form, and to decrease the solubility of both forms (Lindsay 1974, Wallace and Lunt 1960). These effects of pH are counteracted, however, when iron is supplied as a chelate, the extent of counteraction depending on the particular chelate used (Lindsay 1974).

This may explain the fact that pH treatment had a smaller effect on chlorosis than iron treatment in this experiment in which Fe-EDTA was used as the iron source. Schoeneweiss

(1963) observed only slight chlorosis on pin oak at pH 7.0 when iron was supplied to the nutrient solution in chelated form, but severe chlorosis at the same pH when the iron source was FeSO_4 . Similarly, Carpenter (1952) observed "acute" chlorosis on pin oak growing in a solution containing 1 p.p.m. iron at pH 7.0 while we observed only slight chlorosis for the same treatment. Though the source of iron was not stated for that study, its date indicates that a chelate probably was not used. If so, this would explain the discrepancy between our results.

Schoeneweiss and Carpenter used treatments of pH 5.0 and 7.0, and both found differences in chlorosis development between the two levels. Our results for pH were inconsistent -- one measurement method showed a significant effect of pH on chlorosis and the other showed virtually no effect. However, considering the fact that pH treatment had less effect than iron treatment on chlorosis when measured by both methods, it appears that resistance to chlorosis could be effectively screened by monitoring only an appropriate iron concentration, one in the range of 0.1 to 1.0 p.p.m., and letting pH stabilize. This conclusion is strengthened by the fact that the pin oak roots tended to change pH of the solutions toward the midpoint of the pH 6.0-7.0 range used in the experiment.

It is surprising that we found a significant effect of pH on chlorosis when measured by the visual scoring system, but not when measured by the presumably more accurate method of chlorophyll content. However, more weight should be placed on the former result, especially since shoot growth was also significantly affected by pH. On the other hand, measuring chlorophyll content was the more useful method at the two higher iron (and chlorophyll) levels, where the visual method was apparently inadequate for detecting real differences (Table 2).

The significantly greater buffering effect on nutrient solution pH of the plants growing at low iron concentrations is similar to one of the known mechanisms of chlorosis resistance. Though the difference was not significant, the effect was slightly greater at pH 7.0 than at pH 6.0, particularly at the two lower iron concentrations and in the later weeks of the experiment when the root systems were larger. It is well known that roots can bring about significant changes in the pH of the root environment, and these changes in pH can have profound effects on the roots themselves (Moore 1974). Foy (1975) has observed that the ability of plants to alter soil chemical properties within the microzones of their roots may be one trait complex that is susceptible of genetic manipulation. He has found,

for example, that one iron-efficient strain of weeping love-grass that does not develop chlorosis in low-iron nutrient solutions also maintains a lower pH in solution than does a strain susceptible to chlorosis.

There remains the question of whether any pin oaks selected for resistance to chlorosis with this method would also manifest resistance in field tests. An affirmative answer is supported by two observations. The first is that a closely related species, red oak (Quercus rubra), does not develop chlorosis in some situations in which pin oak does, and this is true either in the field or in nutrient solutions in which iron source and pH are varied (Schoeneweiss 1963). If some pin oaks have a similar mechanism of resistance, they could be effectively selected in a hydroponics system. The second concerns a series of studies reviewed by Brown (1961) on chlorosis-susceptibility in two varieties of soybean. One variety develops chlorosis when grown on three kinds of calcareous soil, and it is also chlorotic when grown in nutrient solutions containing less than 5 p.p.m. iron. The other does not develop chlorosis on any of the three soils, and it does not become chlorotic in solutions containing as little as 2 p.p.m. iron.

SUMMARY

Nutrient culture techniques would be effective in screening for efficiency of iron utilization in pin oak, and the results would probably be applicable to the problem of iron chlorosis on calcareous soils. The method outlined here could be expanded to aggregate culture (where roots provide support as well as nutrient absorption) in large containers for use in screening large numbers of seedlings. Such a technique, as opposed to testing on calcareous soil, would be space- and time-efficient and would insure that selection is for response to only one environmental variable. Chlorosis in response to variables other than iron concentration would be eliminated as a possibility. In addition, if the seedlings were later outplanted to a calcareous site, the replication obtained would permit measurement of individual tree genetic variation without cloning. The possibilities of obtaining genetic superiority in chlorosis-resistance in this species are unknown, but we may be encouraged by the successes achieved in other species.

LITERATURE CITED

- Baker, J. B and W. K. Randall.
1974. FOLIAR NITROGEN AND POTASSIUM VARIATION IN COTTONWOOD AS AFFECTED BY GENETIC AND SITE FACTORS. Proc. Cent. States For. Tree Imp. Conf. 9: 106-111.
- Brown, J. C.
1956. IRON CHLOROSIS. Ann. Rev. Plant Physiol. 7: 171-190.
- Brown, J. C.
1961. IRON CHLOROSIS IN PLANTS. Adv. in Agron. 13: 329-369.
- Carpenter, I. W.
1952. IRON DEFICIENCY IN PI OAK. Abstract in Proc. Indiana Acad. Sci. 61: 67.
- Epstein, E.
1972. MINERAL NUTRITION OF PLANTS: PRINCIPLES AND PERSPECTIVES. John Wiley and Sons, Inc., New York. 412 p.
- Flemer, W.
1971. RECENT PROGRESS IN TREE BREEDING AND PRODUCTION. Proc. Intern. Shade Tree Conf. 47: 38a-44a.
- Foy, C. D.
1975' PLANT ADAPTATION TO MINERAL STRESSES IN PROBLEM SOILS. Presented to Ann. Northeast. Region. Grassl. Council Meet. (Blackwater Falls, WV), Sept. 18-19, 1975. 15 p*
- Gerhold, H. D.
1959' SEASONAL VARIATION OF CHLOROPLAST PIGMENTS AND NUTRIENT ELEMENTS IN THE NEEDLES OF GEOGRAPHIC RACES OF SCOTCH PINE. Silv. Genet. 8: 116-123.
- Hacskeylo, J. and P. Struthers.
1959. CORRECTION OF LIME-INDUCED CHLOROSIS IN PIN OAK. Ohio Agric. Exper. Sta. Res. Circ. No. 71, 5 p.
- Jenkinson, J. L.
1974. PONDEROSA PINE PROGENIES: DIFFERENTIAL RESPONSE TO ULTRAMAFIC AND GRANITIC SOILS. USDA Forest Service Res. Pap. PSW-101, 14 p.
- Lindsay, W. L.
1974. ROLE OF CHELATION IN MICRONUTRIENT AVAILABILITY. In E. W. Carson (ed), The plant root and its environment. Univeristy Press of Virginia, Charlottesville, pp. 507-524.

- Moore, D. P.
 1974. PHYSIOLOGICAL EFFECTS OF pH ON ROOTS. In E. W. Carson (ed.), 'The plant root and its environment.' University Press of Virginia, Charlottesville. pp* 135-151.
- Neely, D.
 1973. PIN OAK CHLOROSIS -- TRUNK IMPLANTATIONS CORRECT IRON DEFICIENCY. J. Forest. 71: 34-o-342.
- Neely, D.
 1976. IRON DEFICIENCY CHLOROSIS OF SHADE TREES. J. Arboric. 2: 128-130.
- Neely, D. and D. F. Schoeneweiss.
 1974. CORRECTION OF PIN OAK CHLOROSIS. Arborist's News 39: 37-40.
- Schoeneweiss, D. F.
 1963. GRAFTING TO OVERCOME CHLOROSIS IN PIN OAKS. Proc. Intern. Plant Propag. Soc. 13: 138-140.
- Schoeneweiss, D. F.
 1973. CORRECTION OF LIME-INDUCED CHLOROSIS OF PIN OAK BY LIQUID SOIL INJECTION. HortScience 8: 333-334.
- Smith, E. M.
 1973. TREATMENTS TO CONTROL CHLOROSIS OF PIN OAK -- INTERIM REPORT. Ohio Agric. Res. and Develop. Center Res. Sum. No. 71: 41-42.
- Steinbeck, K.
 1965. FOLIAR MINERAL ACCUMULATION BY SEVERAL SCOTCH PINE (PINUS SYLVESTRIS L.) PROVENANCES. Ph.D. thesis, Michigan State University, East Lansing. 115 p*
- Teich, A. H. and M. J. Holst.
 1974. WHITE SPRUCE LIMESTONE ECOTYPES. Forestry Chronicle 50(3): 1-2.
- Wallace, A. and O. R. Lunt.
 1960. IRON CHLOROSIS IN HORTICULTURAL PLANTS, A REVIEW. Proc. Amer. Soc. Hart. Sci. 75: 819-841.

TABLE 1.--Composition (excluding iron) of nutrient solutions used in treatments.

Source	Concentration	Element	Concentration
	grams/liter		p.p.m.
KNO_3	5.055 ^a	N	210.2
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	11.808 ^a	P	31.0
KH_2PO_4	1.361 ["]	K	234.6
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	4.930 ^a	Ca	200.4
H_3BO_3	7.173 ^b	Mg	48.6
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.200 ^b	S	64.1
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	4.511 ^b	B	0.125
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.601 ^b	cu	0.005
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.110 ^b	Mn	0.125
		Cl	0.162
		Zn	0.014
		mo	0.004

$a_X 10^{-1}$

$b_X 10^{-4}$

TABLE 2. Average chlorosis score, index of chlorophyll content, and shoot height for three iron and two pH treatments.

Treatment	Chlorosis score	Index of chlorophyll content ^a	Shoot height
	1=extreme 7=none		
(ppm)	<u>Iron</u>		
0.1	1.50	8.53	12.05
1.0	4.33	16.57	18.83
10.0	<u>4.58</u>	<u>21.62</u>	<u>18.83</u>
Least Significant Difference: ^b	1.32	3.72	2.38
	<u>pH</u>		
6.0	4.06	15.61	<u>19.29</u>
7.0	<u>2.89</u>	<u>15.53</u>	<u>13.86</u>
Least Significant Difference: ^b	1.08	3.04	1.94

^aOptical Density Units on a Beckman-B spectrophotometer x 10; low values = low chlorophyll content.

^b5% level of significance.

TABLE 3 .--Analyses of variance for chlorosis score, index of chlorophyll content, and shoot height.

Source of variation	Degrees of freedom	Chlorosis score	Index of chlorophyll content	Shoot height
----- (mean squares) -----				
Fe	2	17.597***	261.211***	92.027***
pH	1	6.125*	0.027 ns	132.845***
Fe X pH	2	0.042 ns	16.734 ns	12.172 ns
Error	11	1.076	8.566	3.512

ns = Not significant
 * = Significant at the 5% level
 *** = Significant at the 0.1% level

TABLE 4 .--Variance components for chlorosis score, index of chlorophyll content, and shoot height.

Source of variation	Chlorosis score	Index of chlorophyll content	Shoot height
Fe	5.5071	84.2150	29.5050
pH	0.5610	0	14.3703
Fe X pH	0	5.4456	5.7731
Error	1.0758	8.5655	3.5121