Isoenzyme Variation in Picea glauca (Moench) Voss Seedlings

By Peter P. Ferret

Introduction

The analysis of genetic variation in forest trees has traditionally been approached by studying quantitative aspects of inheritance and natural variation for a wide variety of morphological, physiological, cytological and biochemical characteristics. The technique of disc gel enzyme electrophoresis (Davis, 1964) allows the analysis of the end product, the intracellular enzyme patterns, of living, immature organs. The electrophoretic mobilities of several isoenzymes in individual seedlings of Picea glauca (Moench) Voss, and 2) to investigate the usefulness of the electrophoretic technique to determine the genetic control of isoenzyme variation in P. glauca.

Literature Review

The technique of gel electrophoresis has been utilized to study seed-proteins in several forest-tree species. Lewis and Ceci (1969) reported protein electrophoretic variation in Pinus serotina Ehnl. seeds collected from a single stand in West Virginia. Hare and Switzer (1969) analyzed seed-protein extracts of Pinus echinata Mal. and P. taeda L. and Durzan (1966, 1968) compared disc electrophoretic patterns

Research submitted as partial fulfillment of the Ph. D. degree in Plant Breeding and Plant Genetics at the University of Wisconsin, Madison, Wis. The author wishes to acknowledge Dr. James P. King of the U. S. Forest Service, North Central Region, for providing the P. glauca seedlings, and Dr. Gerald R. Grans for his guidance and suggestions during the course of study.

1) Assistant Professor of Forest Genetics, Division of Forestry and Wildlife, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

of embryo and female gametophyte tissue proteins from Picea glauca (MOENCH) Voss, Pinus banksiana Lamb., and P. strobus L. In all cases, protein patterns differed between embryo and gametophyte tissue. Pickering and Fairbrothers (1967) studied soluble seed-proteins from four Pinus glauca taxa to determine the genetic control of isoenzyme variation in P. glauca.

Material and Methods

Peroxidase and esterase enzyme variation in P. glauca was investigated in four open-pollinated (OP) half-sib families three families of selfed origin (S4) and twelve full-sib (F4) families. The families were represented by a total of 572 P. glauca three-year-old seedlings derived from four female parents (A–D) and six male parents (E–J) selected for height growth performance on the Chequamegon National Forest, Wisconsin. Seedlings were grown at the Hugo Sauer Nursery at Rhinelander, Wisconsin. The number of seedlings in the F, and open-pollinated progenies varied from 25 to 39; the S4 progeny numbers varied from 13 to 17. P. glauca parent and progeny needle tissue was collected July 17 and 18, 1969. Depending on the seedling and needle size, three to five laterals per seedling were sampled, placed in a polyethylene bag and stored, within one hour of collection, below 0\(^{\circ}\)C. Samples were placed at –20\(^{\circ}\)C within 36 hours after collection. A second collection of family

of embryo and female gametophyte tissue proteins from Picea glauca (MOENCH) Voss, Pinus banksiana Lamb., and P. strobus L. In all cases, protein patterns differed between embryo and gametophyte tissue. Pickering and Fairbrothers (1967) studied soluble seed-proteins from four Pinus glauca taxa to determine the genetic control of isoenzyme variation in P. glauca.

Material and Methods

Peroxidase and esterase enzyme variation in P. glauca was investigated in four open-pollinated (OP) half-sib families three families of selfed origin (S4) and twelve full-sib (F4) families. The families were represented by a total of 572 P. glauca three-year-old seedlings derived from four female parents (A–D) and six male parents (E–J) selected for height growth performance on the Chequamegon National Forest, Wisconsin. Seedlings were grown at the Hugo Sauer Nursery at Rhinelander, Wisconsin. The number of seedlings in the F, and open-pollinated progenies varied from 25 to 39; the S4 progeny numbers varied from 13 to 17. P. glauca parent and progeny needle tissue was collected July 17 and 18, 1969. Depending on the seedling and needle size, three to five laterals per seedling were sampled, placed in a polyethylene bag and stored, within one hour of collection, below 0\(^{\circ}\)C. Samples were placed at –20\(^{\circ}\)C within 36 hours after collection. A second collection of family

Isoenzyme Variation in Picea glauca (Moench) Voss Seedlings'
A × E was made November 1, 1969, to investigate peroxidase enzyme variation in dormant trees. All tissue samples were extracted within ten weeks of collection.

The extraction procedure utilized 0.7 grams of spruce needle tissue homogenized in 4 ml buffer. The buffer system selected was 0.1 M Tris (2 amino 2-hydroxymethyl-1, 3 propanediol) — HCL (pH 8.0) containing the following additive concentrations: 0.5 M sucrose; 6 mM cysteine; 6 mM ascorbic acid; and 1% (v/v) Tween 80. Addition of Tween 80 (FRENDZEL, et al., 1968) was essential for the maintenance of esterase activity in spruce, and it also provided increased clarity in spruce zymograms.

Tissue homogenates were centrifuged at 0°C to remove cell debris. The tissue and buffer characteristics required two centrifugations; ten minutes at 12,000 × G followed by centrifugation of the 12,000 × G supernatant at 40,000 × G for one hour. The 40,000 × G supernatant was decanted into a 5 × 75 mm test tube and stored at -20°C until used. Each tissue homogenate was number coded. Homogenates were then electrophoresed at random to avoid bias in the recording of electrophoretic results. The extracts contained protein concentrations of 8 mg/ml as determined by the Folin reaction (Lowry et al., 1951). Bovine serum albumin dissolved in extract buffer was used as the protein standard. 250 µg of protein was applied to the polyacrylamide gels for electrophoresis.

Discontinuous disc gel electrophoresis was performed at pH 9.5 according to DAVIS (1964) using a 7.5% running gel and 3.7% spacer gel.¹ Electrophoresis was performed at 4°C for 95 minutes using 3 ma. per gel. The voltage gradient across the reservoirs was 95 V.D.C. at initiation. Following ¹ Detailed descriptions of equipment, gel formulation procedures and electrophoretic analysis are available from the author upon request.

Table 1. — Percentage of F1, S1, and open-pollinated progeny having each isoenzyme in relation to presence (+) or absence (−) of the isoenzyme in the parent. Isozymes 20 and 29 were present in 100% of the parents and offspring. The F1 and open-pollinated progenies varied from 25−59 trees; the S1 progeny from 13−17 trees. Parents A to D were used as females; parents E to J were used as males.

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>SELF</th>
<th>OP</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(+)</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>85</td>
<td>100</td>
<td>−</td>
<td>50</td>
</tr>
<tr>
<td>B(+)</td>
<td>30</td>
<td>60</td>
<td>25</td>
<td>55</td>
<td>45</td>
<td>65</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>C(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>D(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>33</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>A(−)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>B(−)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>C(−)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D(−)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>A(−)</td>
<td>25</td>
<td>65</td>
<td>65</td>
<td>50</td>
<td>55</td>
<td>55</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>B(−)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>70</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>C(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>D(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>A(−)</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>60</td>
<td>100</td>
<td>100</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>B(−)</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>C(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>A(−)</td>
<td>35</td>
<td>35</td>
<td>0</td>
<td>55</td>
<td>0</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(−)</td>
<td>35</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>C(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>A(−)</td>
<td>5</td>
<td>45</td>
<td>20</td>
<td>30</td>
<td>70</td>
<td>30</td>
<td>−</td>
<td>35</td>
</tr>
<tr>
<td>B(−)</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>60</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>C(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>75</td>
<td>45</td>
</tr>
<tr>
<td>D(−)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>75</td>
<td>45</td>
</tr>
</tbody>
</table>

Enzyme Staining and Recording Procedures

Peroxidase isoenzyme activity was located in the acrylamide gels using techniques adapted from MACRO et al. (1967). Hydrogen peroxide (0.15% v/v) was selected as the enzyme substrate and benzidine dihydrochloride (0.02 M in 7% acetic acid) as the hydrogen donor and color indicator. Peroxidase activity was indicated by the blue color of oxidized benzidine.

Esterase isoenzyme activity was located using the method suggested by BREWBAKER et al. (1968). Gels were developed in 100 ml 0.2 M phosphate buffer (pH 7.0) containing 0.1% (w/v) Fast Blue RR diazotized salt and 2 ml of 1% (w/v) alphanaphthyl acetate substrate dissolved in 70% (v/v) acetone. Esterase activity was indicated by the black color of the reduced diazonium salt.

Enzyme patterns in the gels were diagrammatically sketched and electrophoretic mobilities relative to the front (Rf) for each isoenzyme were calculated for enzyme identification. Pigment compounds in the enzyme extract exhibited a constant electrophoretic mobility for all samples tested. This was established using bromophenol blue marker dye, coelectrophoresis of mixed homogenates and ammonium persulfate oxidation of benzidine. Rf (×100) values were calculated from the ratio of the distance between leading edge of the enzyme band and the top of the small pore gel to the distance of pigment band migration. Rf values for multiple samples of single extracts and for multiple extracts of single trees were repeatable to within 1.0 Rf ×100 units (std. dev.). The reproducibility obtained corresponded well with the results of McCOWN et al. (1968).
and Hall et al. (1969). Coelectrophoresis of homogenates possessing dissimilar esterase profiles was routinely used to verify enzyme identification. For peroxidases, coelectrophoresis commonly resulted in overstained zymograms. Thus, for these samples, multiple analyses were used to verify Rf values.

**Results and Discussion**

Spruce peroxidase zymograms were characterized by non-repeatable weak banding in the gel regions bordered by Rf 0—20 and 50—75. Because of non-reproducibility, these isoenzymes were not considered in the analysis. Tannin-protein complexing was the probable cause of non-reproducibility and has been discussed (Pearse and Stains, 1970; Loogis, 1969). All extracts included, a total of ten peroxidase isoenzymes were recorded in the gel region bordered by Rf 20 to 50. Typical spruce peroxidase zymograms are shown in Fig. 1A.

Table 1 shows the peroxidase profiles obtained from the trees utilized as parents of the OP, F1, and S1 families. Parent-tree zymograms exhibited from four to seven peroxidase isoenzymes, but only two samples contained identical peroxidase profiles (F1 and I). Isoenzymes 20 and 29 were common to all parent tree extracts; while isoenzyme 25 was also present in each extract except that of parent tree D. Isoenzymes 36, 38, 40, and 44 occurred frequently in extracts of the parental material; however, isoenzymes 33 and 46 were present only in zymograms of trees G and C.

Within each open-pollinated family, from 18 to 23 different peroxidase patterns were observed. The data in Table 1 indicates that each open-pollinated family was characterized by the absence of one or two isoenzymes. Isoenzymes 33 and 38 were absent from samples of family C-OP while isoenzymes 46 and 48 were not found in samples of Family A-OP. Extracts of progeny from family D-OP lacked only isoenzyme 38 hence distinguishing this family from the others. Only isoenzyme 46 was absent in zymograms of family B-OP.

In contrast to the open-pollinated families, extracts of F1 and S1 progeny within each family exhibited relatively few (4 to 18) peroxidase isoenzyme combinations. Zymograms from the F1 progeny usually contained isoenzyme profiles intermediate between the profiles of the seed and pollen parent tree extracts. Extracts of individual seedlings derived from self-pollination yielded only 1—8 peroxidase isoenzyme combinations per family. One family B × B exhibited no within family peroxidase variation.

The data summary provided in Table 1 demonstrates that in some F1 and S1 families the peroxidase phenotypes of the progeny did not correspond directly to the parent tree peroxidase phenotypes. Several families included progeny whose extracts contained non-parental isoenzymes or a total absence of parental isoenzymes. The former condition occurred in less than 5% of the S1 and F1 progeny analyzed. For example, isoenzyme 35 did not appear in zymograms of tree D but did occur in 70% of the progeny derived from self-pollination of this tree. In contrast, isoenzyme 25 was observed in zymograms from tree B but was not found in extracts of its S1 progeny.

Although segregation for peroxidase phenotypes was observed in all full-sib families and in two of the three families derived from self-pollination, a simple genetic model could not be formulated to account for the segregation ratios. The absence of simple segregation ratios in the progeny analyzed, the absence of parental isoenzymes in some families and the presence of some non-parental isoenzymes in other families suggests that regulatory elements may have confounded the genetic analysis. In view of the absence of simple Mendelian segregation ratios, the regulatory elements are probably not single genes. Consequently, one must consider the possibility of environmental or developmental factors directly controlling peroxidase gene activity, or that heritable genic regulators, possibly sensitive to environmental, developmental or epistatic interactions could exist. The presence of the non-parental isoenzymes in extracts of some F1 and S1 progeny indicated that the occurrence of non-parental isoenzymes is family specific. Thus, there is some evidence that heritable genic regulators are involved in peroxidase gene activity. However, Stutte and Todd (1969) and McCown et al. (1969a; 1969b) demonstrated that growth conditions can affect peroxidase zymograms in a variety of plant species. McCown et al. (1967) were able to demonstrate a correlation between peroxidase zymograms and early stages of development in T. aestivum. It, therefore, seems likely that a combination of factors may be responsible for the peroxidase variation observed in families of P. glauca.

To investigate the presence of environmental or developmental factors in spruce peroxidase isoenzyme expression, a second collection of family A × E was made November 1, 1969, after several frosts had occurred. The only difference observed was the complete absence of isoenzyme 36 in the dormant seedlings. This observation indicates that although expression of isoenzyme 36 is subject to environmental or developmental modification, the frequency of isoenzymes 20, 25, 33, 38 and 40 are influenced by more stable, presumably genetic factors.

Spruce esterase isoenzyme variation was analyzed in the same open-pollinated, F1, S1 families and parent trees described for spruce peroxidase analysis. In contrast with the complex zymograms of the peroxidases, the esterase zymograms contained only one to four isoenzymes. Typical spruce esterase zymograms are illustrated in Figure 1B where esterases are shown at Rf's 28, 34, 41 and 51. Isoenzyme 51 was characteristically detected as a broad band
with a trailing edge at Rf 44—46 while the other bands were well defined. The results of the spruce esterase analysis are summarized in Table 1.

Isoenzymes 34 and 41 were common to all parent tree samples while isoenzyme 28 was present in only three of the eleven parent samples (trees A, D, and H). Enzyme 51 was present in all extracts except those of trees E, F, and H.

In contrast with the results of the peroxidase isoenzyme analysis, most open-pollinated families could not be distinguished by the presence or absence of a particular esterase isoenzyme. However, isoenzyme combinations were family specific and could be used to characterize each family. Out of the total of seven esterase profiles observed, only the 34—41—51 profile was common to all half-sib families. Each F, and S, family also contained progeny samples exhibiting the 34—41—51 esterase phenotype.

Although progeny extracts exhibited isoenzymes common to both parental extracts, the segregation for esterase phenotypes was partially independent of the parent tree phenotypes. For example, families C x C and B x B were both derived from self-pollination of parent trees with identical esterase phenotypes. Extracts of trees C and B exhibitedzymograms with three esterases at Rf's 34, 41 and 51. The progeny in family C x C segregated only for isoenzyme 51, while the progeny in family B x B segregated only for isoenzyme 34 (see Table 1). A similar example is evident when comparing esterase profiles of progeny samples from families A x I and A x J. Both families were derived from a cross using female parent A, with a 28—34—41—51 phenotype, and a pollen parent tree with a 34—41—51 phenotype. The progeny of these full-sib crosses only segregated for isoenzyme 28, but in inverse ratios (see Table 1). Comparison of families A x E with A x F, B x E with B x F and B x I and B x J also illustrates the partial independence of segregation patterns and parental isoenzyme phenotypes.

The expression of isoenzyme 28 was family specific. Although esterase 28 was transmitted by parent tree A, parent trees H and D failed to produce progeny samples exhibiting isoenzyme 28. The absence of parental isoenzymes in progeny was previously discussed for spruce peroxidases.

The absence of predictable genetic segregation ratios for esterase phenotypes in the spruce F, and S, families, precluded the development of a simple genetic model to account for the esterase variation observed. Presumably, esterase isoenzyme expression is influenced by regulator elements. As with the spruce peroxidase isoenzymes, these regulators may be environmental or developmental factors directly controlling esterase gene activity, or they may be genetic regulators, possibly sensitive to environmental, developmental or epistatic interactions. The complex nature of the segregation patterns observed for the spruce esterases may also be due to interallelic interactions. Allen (1965) proposed that interallelic interactions accounted for the extreme segregation ratio distortion observed for multimeric esterases in Tetrahyymena pyriformis. She suggested that interallelic interactions could occur during mRNA synthesis, mRNA utilization or during quartenary association of the polypeptide monomers. She also suggested that development factors could modify the nature of the interactions. If spruce esterases are multimers, as has been postulated for other plant esterases (see Brown and Allard, 1969; Desbrough and Pelouquin, 1967; and Schwartz, 1968) interallelic interactions may have confounded the genetic analysis of esterasezymograms.

Conclusions

The techniques employed in this study were found to be acceptable for detailed analysis of P. glauca peroxidase and esterase isoenzymes. The presence of "tannins" in the needle extracts (Loosin, 1969) is thought to have been the cause of non-reproducibility in peroxidasezymograms between Rf's 0—20 and 50—75. Addition of polyvinylpyrroloidine (McCown et al, 1968) to the extract buffer has been suggested as a method for the removal of these substances.

Although the isoenzyme variation observed could not be explained by any simple genetic hypothesis, the results of this study indicated that the isoenzymes were under genetic control. The number of isoenzyme combinations was greatest in the open-pollinated families and least in the S, families. With few exceptions, the presence or absence of specific isoenzymes in the hybrids could be predicted from the isoenzyme complement of the parental trees. Finally, evidence from analysis of peroxidases in family A x E indicated that most of the isoenzymes were developmentally stable. However, screening of additional enzyme systems will be necessary to elucidate specific isoenzymes that are under simple genetic control.

Summary

Using techniques of polyacrylamide disc gel electrophoresis, peroxidase and esterase isoenzymes in P. glauca were studied in individual seedlings of half sib. F, and S, P. glauca families. A total of ten peroxidases and four esterases were analysed. Seeding isoenzyme patterns were correlated with parental isoenzyme patterns but no specific genetic hypothesis could be formulated to explain the isoenzyme variation observed. The existence of genetic or environmental regulator elements affecting the isoenzyme expression was discussed.

Literature Cited


Short Note

A Machine Storage and Retrieval System for Personal Files of Scientific Literature

By G. Namkoong and J. Graham

A computer storage and retrieval system for personal scientific reference files including program and codes, has been developed for use in genetics research and teaching. The program is intended for use of individual scientists, their students, or small groups who must maintain current literature files on books, articles, or other reference material pertinent to a particular research field. It may also be used for nonbibliographic diagnoses in taxonomy and pathology. A novel feature of the program allows one to identify several fields of data for each stored item with a common generic name, and conversely, to identify each field under several such names. Thus, one can store and retrieve items on the basis of multiple cross-indexing.

In the present application, the scientist who reads many articles in the areas of genetics, forestry, and statistics can simply and cheaply store information on each article of interest by an accession number, author, date, and journal location. He can classify the contents of each article by several subject matter code words and numeric codes for later retrieval. Several sets of 3-digit code numbers and 4-letter code words can be stored for each reference. Whenever the scientist wishes to search his files he must specify the kind of article he wishes retrieved according to any combination of data on any set of subject matter codes, author, year, journal, etc. The instructions may be inclusive or exclusive in selecting among all references in the file. The computer will then search for all references fitting the description and, for each, will print out the complete literature citation and any commentary, classification codes, etc. Included in the file.

This system is expected to be particularly useful for scientists who wish to maintain files of up to 10,000 references on items published in diverse journals and for which general abstracting service listings are inadequate. The system as currently used includes around 3,500 references. A typical search and retrieval operation on this number of records which may yield 100 references requires about 1 minute on an IBM-360 model 75 computer, and costs around $ 7.50. However, the system can conduct up to five independent search and retrieval operations at the same time with little additional time costs. In our experience, when running three or four concurrent operations, the average cost per operation is about $ 2.00. Further details on the capabilities and use of the program are available from the authors at:

Genetics Department
North Carolina State University
Box 5487
Raleigh, North Carolina 27607

Buchbesprechungen


F. W. Seitz
