Characterization of *Pyrus* Species and Cultivars Using Gradient Polyacrylamide Gel Electrophoresis[1]

R.A. Menendez[2] and L.S. Daley[3]
Department of Horticulture
Oregon State University
Corvallis, OR 97331

Abstract

A chemical identification procedure previously used to identify apple clones was tried with pear species and clones. Following electrophoresis, the peroxidase, esterase, and acid phosphatase isozyme patterns on anionic polyacrylamide gradient gels were determined. These patterns were found to vary with the species and clone, but not to change, within a clone during the growing season. Thus, these patterns were considered to represent genetic characteristics. The patterns were used to identify 37 selected *Pyrus* accessions at the National Clonal Germplasm Repository, Corvallis, Oregon. All species tested were distinguishable using this system. All the accessions of *P. calleryana* selected from the NCGR collection were distinct: however, one clone from outside the collection had an identical pattern to one inside the collection. Among the Chinese pear clones (complex hybrids of *P. ussuriensis* x *P. pyrifolia*) tested, three pairs of clones had the same combination. This technique appears to have the potential to readily identify pear specimens, and could be an important aid in the characterization of germplasm material.

Index words: Pear, *Pyrus,* Chinese-pear, electrophoresis, peroxidase, esterase, acid phosphatase, chemotaxonomy, plant fingerprinting

Introduction

Chemical identification (fingerprinting) of plant species and cultivars has received increased attention (3, 5, 6, 7, 14) from plant breeders, the nursery industry, growers, and U.S. Trade (1) because of the increased recognition of germplasm reserves and the importance of exact clonal identification. Genetic markers have been reported to be useful in identifying clonally propagated material in many crops (2, 9, 11, 15). The National Clonal Germplasm Repository (NCGR) system collects, maintains, identifies and characterizes clones of selected crop genera (8). The NCGR at Corvallis, Oregon is responsible for seven genera, including *Pyrus* (pears). This world collection of pears (13) comprises thousands of individual clones. Thus, any combination of methods chosen for identifying these clones must be able to separate an individual clone from among these thousands. In addition, it is preferable that the methods chosen can be used in any season of the year, and be able to utilize material from plants as young as one year old.

An appropriate method has been developed for apple (*Malus*) clones (10). This method showed that shoot extracts of apple clones yielded identifying electrophoretic isozymic patterns. The particular patterns that identified each clone remained constant throughout the year and were not affected by the age of the plant from which the shoots were collected. This technique can be used with very young specimens, in many cases before phenotypic morphological characteristics become apparent. This paper reports the first application of this method to the genus *Pyrus.*

The gene pool for cultivated plants resides, largely, in the wild or uncultivated species of the same genus, and closely related genera (13). In this first study we have examined ten representative species of genus *Pyrus.* This was done to explore the isozymic diversity of the genus. Then, to determine how this diversity is allotted among individual clones within a species, one species *P. calleryana* and a group of Chinese pear clones (complex hybrids of *P. ussuriensis* x *P. pyrifolia*) were examined in more depth.

Materials and Methods

Plant material was obtained from the NCGR, Corvallis, OR world *Pyrus* Collection. Shoots were collected, for extraction, every month from June through October 1985. The technique used for *Pyrus* was modified slightly from that used in the apple characterization work (10). To reduce effort protein was extracted from the whole shoot. The extraction buffer component beta-mercaptoethanol was replaced by 8mM glutathione. In addition, mixed-bed resins (4) were added during extraction to minimize browning reactions. The electrophoretic conditions previously reported were used (10). Either currently growing shoots or, in winter, one-year-old shoots were chosen. Whole shoots, 5 to 10 mm (0.2 to 0.4 in) in diameter and 10 to 15 cm (4-6 in) in length, were ground in a pencil sharpener. Six grams of this tissue were homogenized in a Tissumizer (Tekman Co., Cincinnati, OH) in 24 ml of a 50mM Tris-HCl, pH 7.6 buffer containing: 1mM sodium EDTA, 3mM dithioerythritol, 5mM ascorbic acid, 3mM sodium metabi-

[1]Received for publication November 22, 1985; in revised form, March 10, 1986. Published as Oregon Agricultural Experiment Station Technical Paper No. 7712. The authors wish to acknowledge the contributions of: N. Daley for editorial assistance; P. Breen and J. Baggett for their prompt and careful reviews, and M. Westwood for his help and guidance in taxonomic matters.

[2]Research Associate, National Clonal Germplasm Repository, Corvallis, OR 97333.

[3]Assistant Professor of Horticulture, National Clonal Germplasm Repository, Corvallis, OR 97333

sulfite, 6mM diethyldithiocarbamate, 5mM phenylmethyl sulfonyl fluoride, 8mM glutathione, 2.4 g mixed-bed resin water demineralizing resin (type HN 8901 Barnstead and Co., Boston, MA), 0.1% Triton X-100 and 12g of hydrated polyvinylpolypirrolidone. The homogenate was filtered through 'miracloth' (Calbiochem, San Diego, CA). The resulting filtrate was either used immediately for electrophoresis or stored at -70 °C (-94 °F).

Electrophoresis was performed in a 16 cm (6.3 in) dual vertical slab electrophoresis cell (Model SE 600, Hoefer, San Francisco, CA) which was placed in a cold room at -1 °C (30 °F). The anionic discontinuous system consisted of a 5 to 15% bis-polyacrylamide linear gradient (5% cross-linking) separating gel below a stacking gel. The separating gel was prepared with pH 8.3 300 mM Tris-HCl buffer. The stacking gel was a 5% bis-polyacrylamide (20% cross-linking), containing a pH 6.5 100 mM Tris-sulfate buffer. The upper chamber electrode was connected to the negative pole of the power supply. The upper chamber buffer was 40mM glycine titrated to pH 8.9 with Tris-base. The lower chamber contained a pH 7.5 63mM Tris-HCl anionic buffer. All buffers were prepared at room temperature. Samples were mixed with 60% glycerol and 0.1% bromophenol blue, and placed in the sample wells after the buffer was added using a microliter pipettor. The unit was run at a constant 20 watts, until the tracking dye reached 0.5 cm from the bottom of the gradient gel. The enzymic stains for peroxidase, esterase and acid phosphatase were done according to Shaw and Prasad (12); 3-amino-9-ethylcarbazole was used as a substrate for peroxidase, alpha-naphthyl acetate for esterase, and beta-naphtyl acid phosphate for acid phosphatase. Isozyme patterns were visually identified and the gels were photographed with a 35 mm camera using Kodak Panatomic-X B/W ASA 32 film.

Results and Discussion

The photographs in Fig. 1 are representative of the peroxidase and esterase isozyme patterns observed in this study. A diagramatic representation of all the isozymic patterns for the three enzymes studied is presented in Fig. 2. The numeric code assigned to each of these patterns is presented in Fig. 2, and listed with the appropriate species or clone in Table 1 for each of these patterns. Table 1 reports the assignment of these isozymic patterns, utilizing this code, to the individual clones tested in this paper.

The patterns of isozyme separations suggest a rather wide variety of genotypes among the species tested. This method permits differentiation among all species tested. All NCGR clonal accessions of *P. calleryana* that were tested were distinct; however, one clone (referred to as PC-OB, for *P. calleryana* cross from the 'old block') from outside of the collection had the same patterns as code number CPYR 661 of the NCGR collection. The number of carpels in the fruit of PC-OB was too great to be 'true' *P. calleryana* and for this reason, plus other morphological traits, this plant must be considered to result from interspecific crossing (Westwood, pers. communic.).

Since the clone labelled CPYR 661 in the new NCGR planting has not reached an age where its morphological characters could be compared with PC-OB, the resolution of this point is deferred. Three pairs of clones of 'Chinese pears,' which are complex hybrids of *P. ussuriensis* x *P. pyrifolia* were not resolved. These pairs were 'Lo Suan Li'/'Ta Mo Pan,' 'Nan Li'/'Ta Suan Li' and 'Huang Hsing Sui Li'/'Ping Li.' While these three pairs of clones were undistinguishable using the three enzymes tested, these pairs of clones may be resolved with the aid of additional enzymic staining systems.

The isozymic patterns produced by the three enzyme staining systems remained constant in the clones tested during the sample period. The stability of isozyme patterns throughout the year was similar to that reported in *Malus* (10).

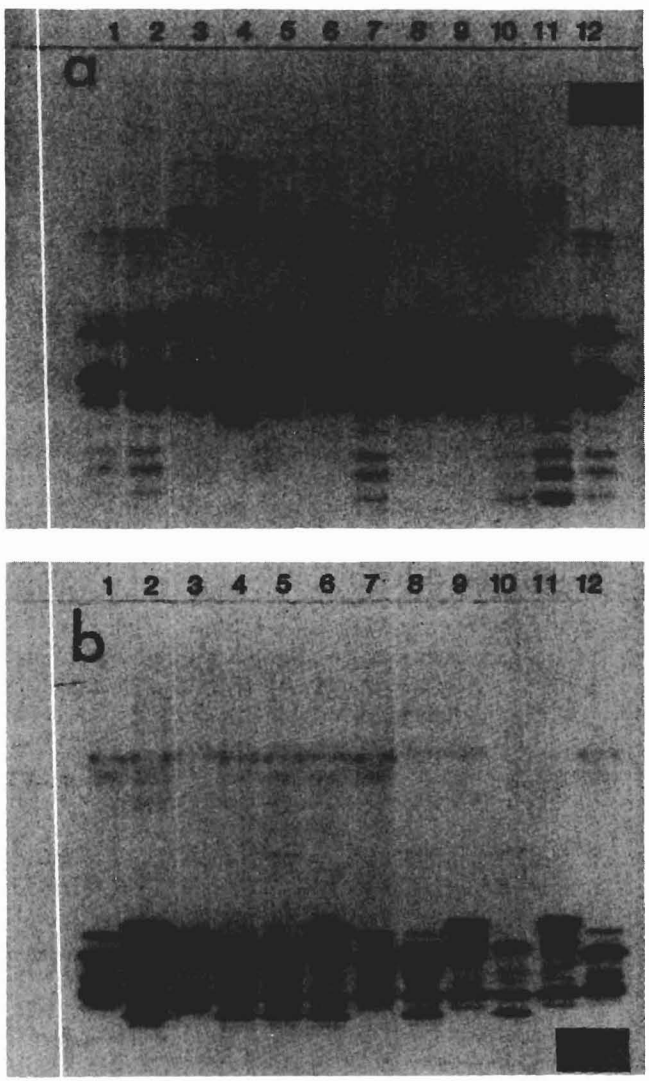

Fig. 1. Photographs of polyacrylamide electrophoresis gels showing isozyme patterns of peroxidase and esterase. Figure 1a shows peroxidase staining patterns. The samples from left to right (1 to 12) represent extracts from: *P. calleryana* cross (PC-OB), and accessions (CPYR prefix omitted): 661(2), 738(3), 907(4), 680(5), 96(6), PC-OB standard (7), 28(8), 146(9), 503(10), 256(11), and PC-OB standard (12). Figure 1b shows esterase staining patterns. The samples from left to right (1 to 12) represent extracts from: PC-OB standard (1), 378(2), 562(3), 409(4), 561(5), 133(6), PC-OB standard (7), 355(8), 288(9), 560(10), 454(11), and PC-OB standard (12). The species and cultivars corresponding to these accession numbers can be obtained from Table 1.

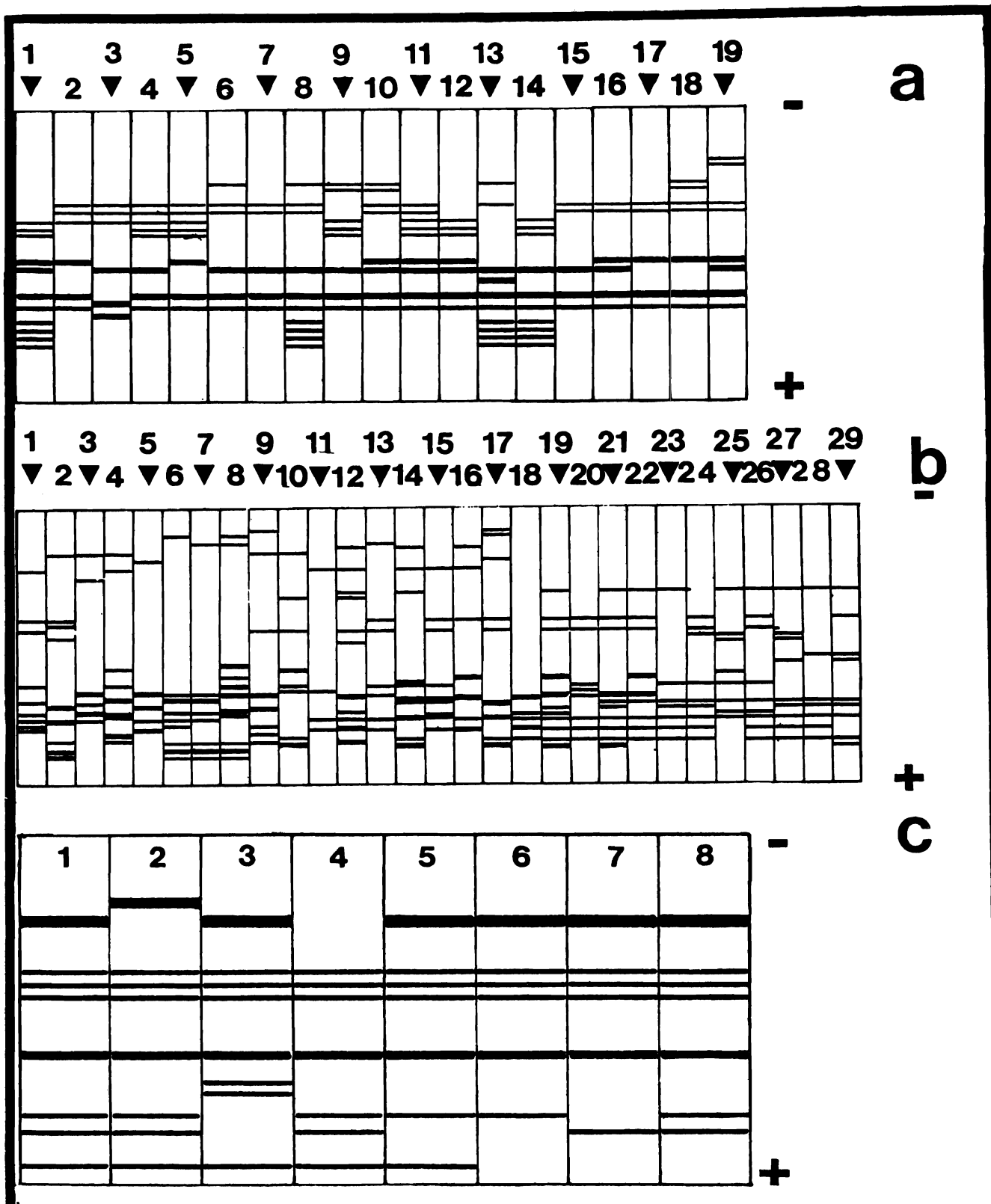

Fig. 2. Diagramatic representation of isozyme patterns for 37 pear species and clones. The pattern code is indicated by adjacent numbers. Figure 2a shows peroxidase patterns; Fig. 2b esterase patterns; Fig. 2c acid phosphatase patterns. The correspondence between pattern numbers and species or cultivars can be obtained from Table 1.

Table 1. Isozyme banding patterns of peroxidase, esterase and acid phosphatase found in 37 pear species and clones. Any two accessions having the same pattern numbers for all three enzymes are not distinguishable. Four pairs of clones, in this table, denoted respectively by: *, **, *** and ****, are not resolved. Examples of patterns can be found in Fig. 1, and diagrams of the patterns in Fig. 2. NCGR numbers (CPYR prefix omitted) are included for clone identification and to facilitate requests.

SPECIES OR CULTIVAR (NCGR Number)	ENZYME STAINING PATTERN			
	Peroxidase	Esterase	Acid phosphatase	
'Anjou Russet' (503)	8	8	6	
'Bartlett Bagley Russet' (28)	6	6	6	
'Bosc' (OP-5) (1165)	13	17	8	
'Ba Li Hsiang' (27)	4	15	8	
'Cheih Li' (133)	15	19	1	
'Comice Spur' (146)	7	7	6	
'Huang Hsing Sui Li' (288)	14	22	8	*
'Lo Suan Li' (355)	16	21	8	**
'Man Yuang Hsing' (378)	4	19	8	
'Nan Li' (409)	11	21	1	***
'Pa Li' (1176)	12	16	8	
'Ping Li' (454)	14	22	8	*
'Ta Mo Pan' (560)	16	21	8	**
'Ta Suan Li' (561)	11	21	1	***
'Ta Tau Huang' (562)	11	20	8	
'Ya Li' (96)	5	5	5	
P. calleryana cross (PC-OC)	1	1	1	****
P. calleryana (661)	1	1	1	****
P. calleryana (663)	12	28	1	
P. calleryana (666)	12	23	8	
P. calleryana (668)	19	23	8	
P. calleryana (669)	17	27	8	
P. calleryana (670)	17	24	1	
P. calleryana (671)	14	26	1	
P. calleryana (672)	18	29	1	
P. calleryana (673)	12	25	1	
P. calleryana (675)	12	25	8	
P. caucasica (680)	4	4	4	
P. cordata (738)	2	2	2	
P. koehnei (815)	11	13	8	
P. mamorensis (834)	9	10	7	
P. nivalis (256)	8	9	6	
P. pyraster (1288)	14	18	1	
P. pyrifolia (1290)	10	11	8	
P. syriaca (907)	3	3	3	
P. ussuriensis. (Manchurian) (379)	4	12	1	
P. ussuriensis (924)	4	14	8	

Acid phosphatase isozyme patterns showed relatively less diversity in the species and cultivars tested than those produced by esterases and peroxidase activities. All distinguishable clonal accessions tested could be identified by their peroxidase and esterase patterns alone. However, acid phosphatase patterns are included, because of their potential usefulness in future work with *Pyrus* germplasm.

The gradient polyacrylamide electrophoretic technique and subsequent isozymic staining appears to be useful for characterizing species and clones of the genus *Pyrus*.

Significance to the Nursery Industry

The identification of pear species and cultivars constitutes an integral part of the program of conservation, characterization and study at the National Clonal Germplasm Repository (NCGR), Corvallis, Oregon. The nursery industry, institutional breeding programs, and independent breeders have the NCGR germplasm collections at their disposal. This collection is a source of clonal plant material which contains many desirable characteristics including disease resistance and cold hardiness. This paper describes a dependable, simple procedure for identification that can be used year-round, regardless of plant age. This method should help prevent the costly consequences of mis-labeling of clones.

Literature Cited

1. Brady, N.C. 1985. Agricultural research and U.S. trade. Science 230:499.

2. Bringhurst, R.S., S. Arulsekar, J.F. Hancock, and V. Voth. 1981. Electrophoretic characterization of strawberry cultivars. J. Amer. Soc. Hort. Sci. 106:684-687.

3. Carter, G.E., Jr. and M.M. Brock. 1980. Identification of peach cultivars through protein analysis. HortScience 15:292-293.

4. Daley, L.S., F. Dailey, and R.C. Criddle. 1978. Light activation of RuBPCase: properties of the enzyme from *Nicotianum tabacum*. Plant Physiol. 62:718-722.

5. Challice, J.S. and M.N. Westwood. 1973. Numerical taxonomic studies of the genus *Pyrus* using both chemical and botanical characters. Bot. J. Linn. Soc. 67:121-148.

6. Desborough, S.T. and S.J. Peloquin. 1968. Potato variety identification by use of electrophoretic patterns of tuber proteins and enzymes. Amer. Pot. J. 45:220-229.

7. Fretz, T.A. 1977. Identification of *Juniperus horizontalis* Moench. cultivars by foliar monoterpenes. Sci. Hort. 6:143-148.

8. Jahn, O.L. and M.N. Westwood. 1982. Maintenance of clonal plant germplasm. HortScience 17:122.

9. Kuhns, L.J. and T.A. Fretz. 1978. Distinguishing rose cultivars by polyacrylamide gel electrophoresis. II. Isoenzyme variation among cultivars. J. Amer. Soc. Hort. Sci. 103:509-516.

10. Menendez, R.A. 1985. Identification of apple (*Malus domestica* Borkh.) clones based on isozymic diversity. Ph.D. dissertation. Washington State University, Pullman, WA.

11. Santamour, F.S., Jr. and P. Demuth. 1980. Identification of Callery pear cultivars by peroxidase isozyme patterns. J. Hered. 71:447-449.

12. Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. 4:297-320.

13. Westwood, M.N. 1986. Operations Manual for National Clonal Germplasm Repositories. National Clonal Germplasm Committee. Oregon State University Press, Corvallis.

14. Wehner, D.J., J.M. Duich and T.L. Watschke. 1976. Separation of Kentucky blue grass cultivars using peroxidase isozyme banding patterns. Crop. Sci. 16:475-480.

15. Wolfe, W.H. 1976. Identification of grape varieties by isozyme banding patterns. Amer. J. Enol. Viticult. 2:68-73.

Influence of Photoperiod on Winter Growth of Seven Species of Tropical Landscape Trees[1]

Timothy K. Broschat and Henry Donselman[2]
University of Florida, IFAS
Fort Lauderdale Research and Education Center
3205 College Avenue
Fort Lauderdale, FL 33314

Abstract

Dalbergia sissoo Roxb. ex DC. (sissoo), *Psidium littorale* Raddi. (cattley guava), *Lysiloma bahamensis* Benth. (false tamarind), *Callistemon rigidus* R.Br. (bottlebrush), *Calophyllum inophyllum* L. (mastwood), *Bucida buceras* L. (black olive), and *Koelreuteria elegans* (Seem.) A.C. Sm. (golden rain tree) seedlings were grown under long day (LD) and natural daylength (ND) photoperiods from September 7, 1982 to April 7, 1983 to determine if LD would prevent quiescence and thereby allow year round growth and reduced production time. *Koelreuteria* and *Dalbergia* under ND essentially ceased growth during these months, but plants grown under LD continued to grow at a rapid rate during this time. *Psidium, Calophyllum, Callistemon,* and *Lysiloma* grew at equivalent rates throughout the winter under both photoperiods, but in *Bucida* growth under ND was greater than under LD during this time.

Introduction

Although subtropical areas such as southern Florida have a climate mild enough to permit year round growth of most plants, many trees growing in these areas have distinct quiescent periods during which no growth takes place (5, 6). In temperate regions, most trees use photoperiod as an environmental cue signaling the onset of unfavorable growing conditions (3). At lower latitudes, however, fluctuations in daylength are reduced and plants must either become more sensitive to small changes in daylength or use other environmental cues such as temperature or water availability (3, 6).

Photoperiod has been shown to affect growth cycles in a small number of important edible tropical tree species such as *Coffea arabica* (8) and *Theobroma cacao* (9), some African forest trees (7), and some tropical landscape trees (1, 2, 4). For the vast majority of tropical trees, however, information concerning responsiveness to photoperiod is not yet known.

The purpose of this study was to determine how 7 commonly grown tropical landscape tree species respond to artificial LD conditions. Since temperature is generally not a limiting factor for many plant species grown in southern Florida, it may be possible to induce year round vegetative growth in responsive species by photoperiod manipulation.

Materials and Methods

Seedlings of 7 species of tropical and subtropical landscape trees (*Dalbergia sissoo* (sissoo), *Psidium littorale* (cattley guava), *Lysiloma bahamensis* (false tamarind), *Callistemon rigidus* (bottlebrush), *Calophyllum inophyllum* (mastwood), *Bucida buceras* (black olive), and *Koelreuteria elegans* (golden rain tree) were planted in 2.9 l (#1) plastic containers using a Canadian peat, perlite, sand, and cypress shavings (8:5:2:5 by vol) medium amended with 880 g/m^3 (1.5 lbs/yd^3) Micromax and 4.9 kg/m^3 (8.3 lbs/yd^3) dolomite. Each container was top dressed with 32 g (1.1 oz) Osmocote 18N-2.6P-10K (18-6-12) every 5 months and received 2 cm (0.8 in) of water from overhead irrigation daily. Plants were grown outdoors under 63% shadecloth at ambient temperatures (Fig. 1).

On September 7, 1982, 30 plants of each species were placed under an LD photoperiod provided by illuminating the plants nightly from 10:00 PM to 2:00 AM with a minimum of 1.5 μmol/sec/m^2 at a height of 1 m (39 in) from overhead 100 w incandescent bulbs. An additional

[1]Received for publication December 20, 1985; in revised form March 10, 1986. Florida Agricultural Experiment Stations Journal Series No. 6955.

[2]Associate Professors of Ornamental Horticulture.