PRECOCIOUS FLOWERING and REPRODUCTIVE BIOLOGY of *TRIPLOCHITON SCLEROXYLON* K. Schum

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SUMMARY

Whereas *Triplochiton scleroxylon* rarely flowers in its native West Africa until at least 15 years old, 15 plants representing eight different clones flowered in successive years between February and August in tropicalised glasshouses near Edinburgh, within 26 to 82 months of germination. Cuttings originating from one mature tree also flowered. Freshly collected pollen remained viable for at least 16 wk when stored dry at -25° C, and germinated best in 20% sucrose + 0.005% boric acid. Pollen stored at room temperature lost viability within 1 wk. Cross-pollinations with fresh and stored pollen were successful, with 8% of the resulting fruits reaching maturity; 16% of the seeds from these fruits subsequently germinated, forming normal seedlings.

Introduction

Supplies of *Triplochiton scleroxylon* K. Schum. (Sterculiaceae) in the semi-deciduous forests of West Africa (Hall and Bada, 1979) have been intensively exploited (Leakey *et al*, in press *b*). To conserve what remains of this fast growing hardwood (Obeche, Wawa or Samba), and to maintain examples from the full existing range of genetic variation for future use, steps have been taken to develop techniques of vegetative propagation (Howland, 1975; Leakey *et al*, in press *a*; Leakey, *in litt*). These have been applied to seedling material originating from Sierra Leone along the West Coast to Cameroon (Bowen *et al*, 1977).

Field trials with rooted cuttings in Nigeria are indicating criteria on which to select clones for commercial forestry (Longman *et al*, 1979; Ladipo *et al*, in press). Thus it is already possible to be discriminating when selecting clonal planting stock, but for the future the choice would be greatly extended if controlled crosses could easily be made (Longman, 1976*a*). Successful tree breeding programmes depend upon regular stimulation of flowers at a manageable height, but in *T. scleroxylon* little is known about the factors controlling the highly irregular incidence of natural flowering. However, phenological observations made in Nigeria and Ghana indicate that flowering normally occurs during the long dry season between November and March, when mature trees usually have a leafless period. Furthermore, these records, kept since the 1920s, suggest that the extent of flowering and seed production might be related to the severity of the short dry season in the previous July/August (MacGregor, 1934; Mackenzie, 1959; Taylor, 1960; Jones, 1974; Howland and Bowen, 1977). Before these relationships can be elucidated, the timing of floral initiation, and the effects of experimental treatments, need to be investigated.

The hemaphrodite flowers of *T. scleroxylon* occur on irregular paniculate cymes, generally arising from axillary buds on the previous season's wood (Gbile, 1975), and have white to pinkish-white petals with deep mauve bases. The flowers, which are insect-pollinated, usually open late in the day, and wither within 18 h. The 5 carpels develop into winged samaras, each of which generally contains a

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singel embryo (Jones, 1974; 1975*a*). Fruit development extends into the start of the main rainy season, and is frequently impaired by pests, such as the fruit-boring weevil *Apion ghanaense* (Ashiru, 1975; Jones, 1975*b*; 1976) and pathogens, including the smut fungus *Mycosyrinx* spp. (Odeyinde, 1975).

T. scleroxylon rarely flowers before it is 15 to 30 years old, and even then flowering is erratic, although as many as 1.5×10^6 floral buds may be initiated per tree (Howland and Bowen, 1977). Controlled crosses are very difficult to make *in situ* because the short-lived flowers in the canopy tend to be inaccessible, especially in the absence of lower branches. By contrast, flowers produced by mature scions grafted on seedling rootstocks have been easily pollinated, but flowering is still unreliable and irregular. Such flowers have however formed fruits and viable seeds when cross-pollinated; self-pollinations were unsuccessful (Howland and Bowen, 1977).

Flowering in woody plants usually only occurs following transition from the juvenile to mature stage of development, this process of phase-change probably involving more than one physiological step (Borchert, 1976). Experimentally, however, "juvenile" plants of several woody species have been induced to form flowers or cones within the first few years by complete girdling and (in certain conifers) by applying gibberellic acid (Longman, 1976*b*; Pharis and Kuo, 1977). The attractions of induced precocious reproduction to forest tree breeders are clearly evident: shortening the interval between generations and ensuring accessibility of flowers, probably without the dangers of selecting genotypes with a propensity to flower rather than produce timber (Longman, 1978; Linhart *et al*, 1979).

Materials and Methods

Single-node cuttings, taken from seedlings originating from Nigeria (Table 1), were rooted using the methods described by Leakey *et al* (in press *a*). After appropriate "weaning", potted plants were grown with daily or twice-daily watering in tropicalized glasshouses at the Institute of Terrestrial Ecology, near Edinburgh, Scotland. These were maintained continuously at temperatures between 20° and 30° C (Table 2), with fluorescent and mercury-fluorescent lighting extending day-lengths to 19.5 h throughout the year, and maintaining light intensities above 1500 $\mu Em^{-2}s^{-1}$. As a result, seasonal variations were minimized, but nonetheless light intensities were greater in summer than winter. Other glasshouse regimes sometimes used were 20° C without supplementing light intensity.

TABLE 1

Nigerian origins of rooted *T. scleroxylon* cuttings flowering precociously in tropicalised glasshouses near Edinburgh.

Clone No.	Location	State	Latitude (°N)	Longitude (°E)
8001	Ilaro	Ogun	6°54′	3°06′
8002	Ilaro	Ogun	6°54′	3°06′
8019	Ilugun	Ogun	7°23′	3°40′
8020	Ilugun	Ogun	7°23′	3°40′
8037	Ilugun	Ogun	7°23′	3°40′
8045	Iwo-Ibadan Road	Oyo	7°38′	4°11′
8053	Iwo-Ibadan Road	Oyo	7°38′	4°11′
8051	Gbitigbiti	Oyo	8°30′	3°26′
8057	Ugueke	Bendel	6°42′	5°46′

In addition, the use of unheated water (>2°C) decreased soil temperatures especially in winter, until water heaters were installed in 1979. Thereafter water was used at about 20°C, except where otherwise stated. Since the same date, plants with flower buds have been kept in saucers of water to minimize stress. All plants received weekly applications of a 1% solution of "Solufeed" liquid fertilizer (23.0% N: 19.5% P: 16.0% K).

Pollen was collected from flowers before they withered in the morning, and was then generally dried in a dessicator over silica gel for 24 h before being stored at -25° in vials with silica gel in their caps. Pollination was done with a small brush in late afternoon, when flowers opened and stigmas appeared most receptive. Germinability of stored pollen was checked periodically in aqueous hanging drops containing various concentrations of sucrose and boric acid. Fruits were collected when brown, planted in a peat/sand/loam compost (7:3:1, supplemented with 4.2g kg^{-1} "Enmag", 2.6g kg^{-1} John Innes base and 0.3g kg^{-1} trace elements), and germinated in controlled-environment cabinets at 30° C day/20° night, with a 19.5 h photoperiod.

Observations and Results

(a) *Occurrence of Flowering*

Plants of *T. scleroxylon* flowered in the tropicalised glasshouse on 24 occasions from 1975 to 1980 (Table 2). The majority were rooted cuttings originating from young seedlings,with flower opening occurring 26 to 82 months after germination. The interval between rooting and flowering was sometimes as short as 18 months. Eight "juvenile" clones were involved, and two of the original seedlings also flowered, one of them on three separate occasions. Mature cuttings had also been rooted from physiologically older scionwood, previously grafted on seedling rootstocks. All cuttings of clone 8057 flowered, but the other five mature clones remained entirely vegetative, as did most seedlings and "juvenile" cuttings grown during this period.

More than 1000 floral buds were formed, with as many as 160 on a tree less than 0.7 m tall, where even the terminal shoot became reproductive. Flower buds emerged between February and August, and most of the flowering occurred on plants which had experienced a period of terminal bud dormancy and leaf loss, following transfer from warmer (30°C) to cooler (20°C) conditions. Interestingly, out of 11 clones in a batch of plants grown under this regime, the three which produced flowers were the slowest growing (Fig. 1). Flowering also occurred in a few plants kept under warmer conditions (30°C day/20°C night) but in all instances these had received cold water during the winter. In 1980, replicate plants of clone 8001 were watered with warm (20°C) and cold (2-10°C) water. Four out of 8 of the latter produced flower buds, but none of the former.

Thirty per cent of flower buds aborted before opening, and 6.5% of fruits were retained to maturity (Table 2). Detailed studies were made on a branch of the original seedling of clone 8002 which produced 305 floral buds on 20 spurs (Fig. 2). A week after being confirmed as an inflorescence, there were already 31 flower buds on spur 29, and several neighbouring spurs later formed similar numbers. The most proximal spur (No. 15) eventually produced most flower buds, while spurs 38-44 initiated least. Flower opening commenced on spur 29, (Fig. 3), but the sequence within and between spurs and sub-spurs did not appear to conform to a predictable pattern, and nor did flower bud abortion.

After cross-pollination, developing fruits were usually visible within 3 or 4 days. Abscission continued throughout the period of fruit development, but 117 fruits

TABLE 2

Numbers of flowers produced, opened and successfully pollinated on cuttings taken from:– (i) juvenile and (ii) mature material of *T. scleroxylon* grown in tropicalised glasshouses near Edinburgh from 1975-1980.

Clone No. (Cutting No.)	Age from Germination (months)	Temperature regimes (Day-Night) °C	No. of flower buds formed	No. of flowers opened	Self (S) or Cross (C) pollination	No. of fruits harvested (Potentially 5 per flower)	No. of seeds germinated
I. *Clones derived from young juvenile material*							
8051 (2998)	26	20°-20°	7	5	S†	0	0
" (2998)	33	20°-20°	22	20	C†	56	1
8045 (2880)	27	20°-20°	160	108	S†	4	0
" (2880)	33	20°-20°	4	1	C‡	0	0
" (2880)	60	30°-20°	53	38	C‡	0	0
8001 (seedling)	33	20°-20°	33	1	S‡	0	0
" (763)	72	20°-20°	12	10	C†	5	0
" (2350)	76	20°-20°	14	5	C‡	0	0
" (775)	78	30°-20°	20	9	C*	5	0
" (1169)	79	30°-20°	84	34	C*	18	3
" (786)	79	30°-20°	18	10	C*	9	3
" (778)	81	30°-20°	24	20	C*	8	1
8037 (29)	36	20°-20°	11	0	–	–	–
8053 (2923)	37	20°-20°	4	0	–	–	–
8019 (169)	38	30°-30°	3	0	–	–	–
8020 (2116)	64	20°-20°	4 spurs found after flowers opened			–	–
" (2712)	70	20°-20°	15	11	C‡	0	0
8002 (seedling)	72	20°-20°	13	11	C†	2	2
" (")	75	30°-20°	6	5	S†	0	0
" (")	82	30°-20°	305	284	C*	117	22
			808	572		224	32
II. *Clone derived from grafted scion taken from mature tree*							
8057 (171)	Unknown	30°-30°	23	0	–	–	–
" (172)		30°-30°	6	5	C†	8	0
" (178)		30°-30°	24	10	C†	35	0
" (5065)		20°-20°	160	122	C†	62	19
			213	137		105	19

* = Deep-frozen pollen † = Fresh pollen

‡ = pollen stored in dessicator at room temperature

survived to maturity (7.7% of the potential 1,525 from the original 305 flower buds). Most of these were on the proximal spurs 15, 17 and 19, with a few on spurs 29, 32, 34 and 36 (Figure 2). Only a single fruit attained maturity from self-pollination, and none developed from any of the plants flowering later than May.

(b) *Pollen germination and storage*

Pollen from flowering branches of mature trees in Nigeria was dried for 3 days and tested in a range of sucrose solutions, with or without 0.01% boric acid (Table 3). Virtually no germination took place without boric acid, and the most favourable concentration of sucrose was 20%. A second test, run the next day with this sucrose concentration throughout, showed that pollen germination was inversely related to the concentrations of boric acid used, 0.005% giving 26.1% germination (Table 3).

Preliminary tests suggested that pollen could be stored in a deep-freeze, so whenever trees flowered in Scotland dried samples were stored at -25°C. Depending on the sizes of the different collections, viability tests using 20% sucrose and 0.005% boric acid were done at 0, 1, 2, 4, 8 and 16 wk. These confirmed that pollen could be stored at low temperatures with no significant loss of the ability to germinate. Corresponding pollen samples kept in a dessicator at room temperatures lost viability within one week. It was also found that the proportion of germinable pollen grains differed greatly from flower to flower. Moreover, only deep-frozen pollen samples with more than 18% germination *in vitro* gave successful cross-pollination.

(c) *Seed germination*

Seeds in 51 mature fruits germinated, 15.5% of those produced from cross-pollinations with fresh or deep-freeze stored pollen. Most viable seeds in the detailed study came from spurs 15 and 17 (Fig. 2). Germination and seedling growth appeared normal, and there were several instances of two embryos germinating in a single fruit. There were no indications of seed dormancy. The few fruits from self-pollinations did not germinate.

Discussion

It is clear from this study that small, easily-managed trees of *T. scleroxylon* can reproduce at an early age, yielding mature fruit, viable seeds and normal seedlings following cross pollination with fresh and deep frozen pollen. Since there appears to be no viable self-pollination (Howard and Bowen, 1977), controlled crossing does not require emasculation or bagging. Considerable progress in overcoming obstacles to tree breeding has therefore been made, although it is not yet possible to induce flower initiation predictably. This is possible however in certain other forest tree genera, particularly those of the Cupressaceae and Taxodiaceae, by applying plant hormones to small, genetically uniform rooted cuttings (Longman, 1978). Here again the desired crosses can easily be made, yielding normal seedlings.

In attempting to elucidate the factors responsible for stimulating precocious flowering of *Triplochiton scleroxylon*, it is possible that the 19½ h photoperiods in the glasshouses might have contributed, since there are known to be a proportion of long-day plants in the tropics (Mathon, 1975). It is probably more significant that flowering occurred after shoot extension had ceased and many leaves senesced. Furthermore, flowering tended to be more frequent in plants transferred to cooler environments and/or watered with cold water. This suggests that soil temperatures may be important, perhaps affecting carbohydrate source/sink relationships or

the hormone balance by inhibiting root growth. In West Africa, water stress in the short-dry season might have a similar effect, but deliberate withholding of watering in the glasshouses has not so far induced any flowering. Pronounced clonal variation suggests that a solution may come through concentrating research on naturally prolific clones (Longman, 1981), for example the "juvenile" 8001, 8002 and 8045 and the mature 8057.

As in West Africa (Howland and Bowen, 1977), but contrary to the report of Jones (1975*a*), flowers in the glasshouses usually opened in late afternoon. The proportion of flower buds developing to maturity in Nigeria was estimated at 0.01 – 0.1%, contrasting with 7.7% in the glasshouses. The first flowers successfully pollinated on an inflorescence appears to have the best chance of producing viable seed, especially if the fruits occur on spurs with a competitive advantage, such as the largest or most proximal. Self-thinning of fruits is common among tropical trees, but the relatively low value of 15.5% seed germination achieved in the glasshouses emphasise that there is still much to learn about the reproductive biology of *T. scleroxylon*. It is also appropriate to recall that while natural precocious flowering is a great asset in research, induced reproduction is to be preferred in building improved clone mixtures for commercial forestry.

TABLE 3

Effects of sucrose and boric acid on % germination of *T. scleroxylon* pollen previously stored over silica gel for 3-4 days and incubated at about 20°C.

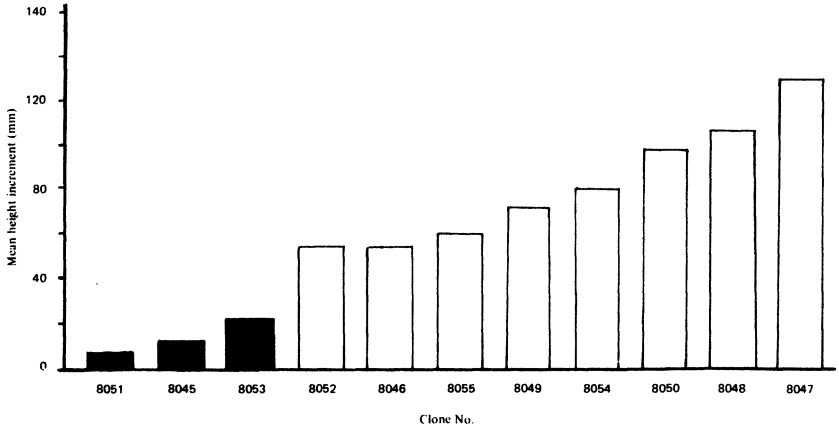
a) Test 1, after 2 h.

	Sucrose concentration (%)				
	10	*15*	*20*	*25*	*30*
Without boric acid	0	0	0	0.1	0
With 0.01% boric acid	0	0.2	12	4.0	1.0

b) Test 2, after 7 h.

	Boric acid concentration (%)					
	0.005	*0.01*	*0.02*	*0.04*	*0.08*	*0.16*
With 20% sucrose	26	13	8.3	6.0	4.9	4.4

Fig. 1 – Height increments (mm) from September to April of flowering (■) and nonflowering (□) *T. scleroxylon* clones grown in a cool glasshouse (20°C).



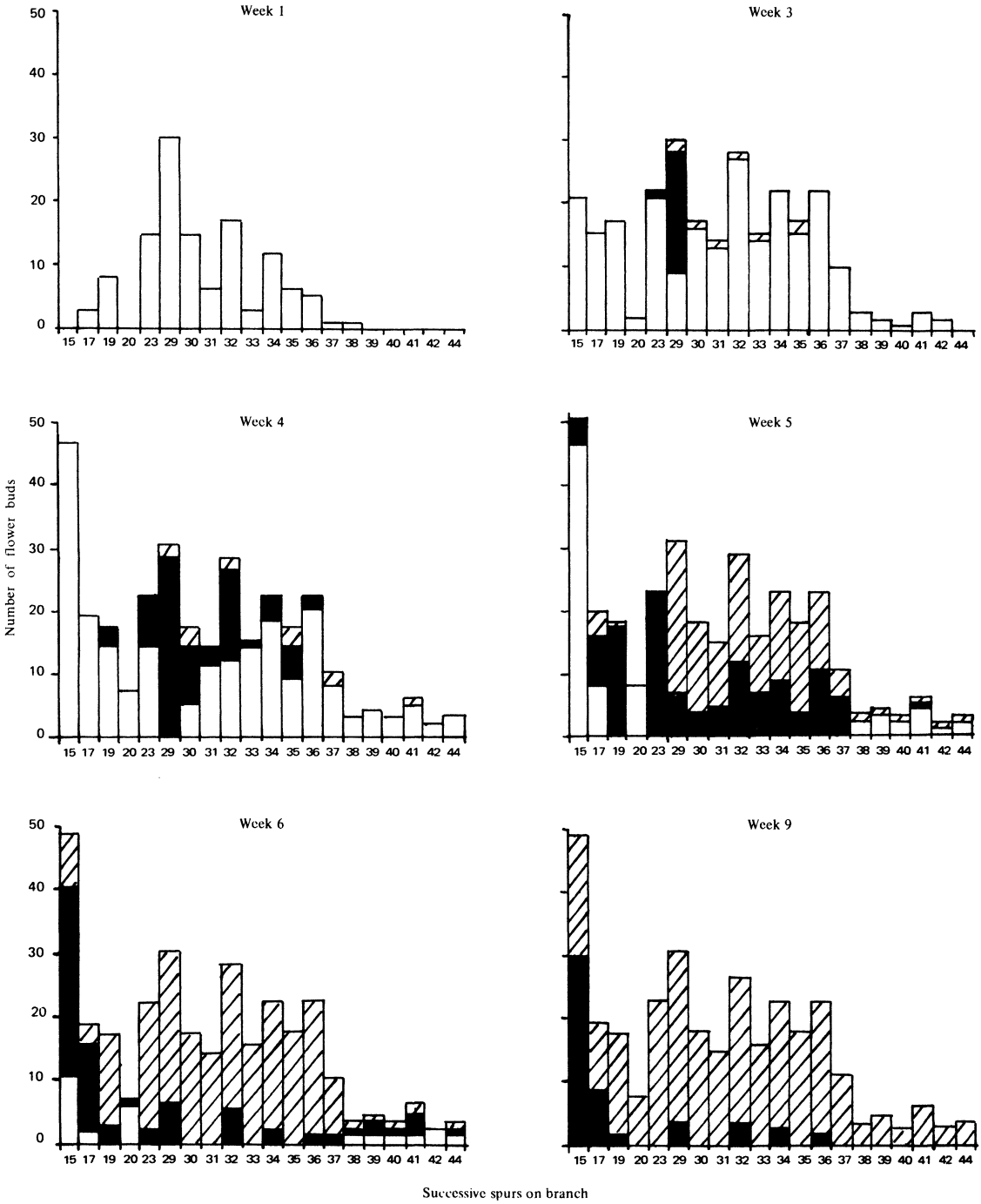


Fig. 2 – Numbers of flower buds, and their development, on spurs of a *T. scleroxylon* inflorescence (clone 8002) growing in a tropicalised glasshouse near Edinburgh (April to August 1980).

□ = Nos. of flower buds ▨ = Nos. of flower buds or fruits aborted ■ = Nos. of developing fruits

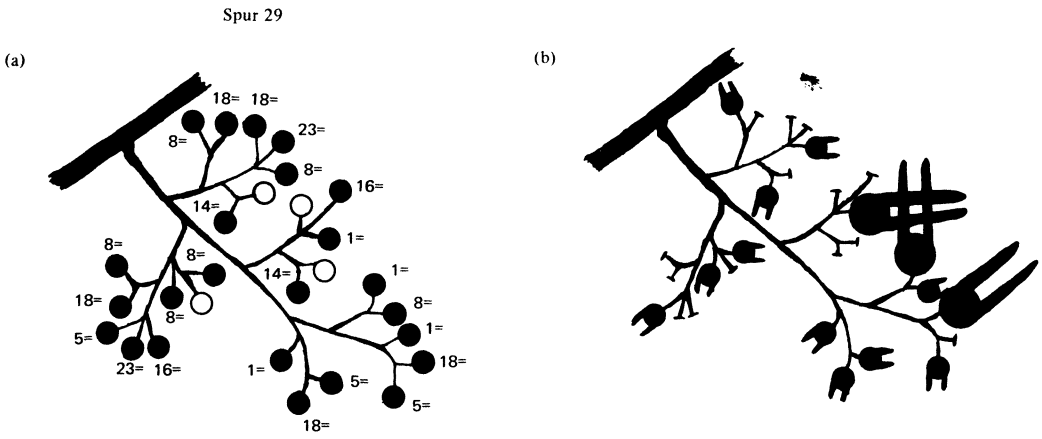


Fig. 3 – The numbers, positions, order of opening and fates of flower buds on Spur 29 of a *T. scleroxylon* plant (clone 8002) in April – August 1980.

(a) ○ = developing flower buds (unpollinated)
● = pollinated flowers (number = order of pollination)

(b) = developing fruits (2 weeks after pollination but subsequently aborted).
= developing fruits (6 weeks after pollination and retained to maturity).
T = abscinded fruits.

REFERENCES

ASHIRU, M.O. (1975). Some aspects of work on insect pests of leaves and fruits of *Triplochiton scleroxylon* K. Schum. *Proceedings of the symposium on variation and breeding systems of* Triplochiton scleroxylon (*K. Schum*). Federal Department of Forest Research, Ibadan, Nigeria, 42-52.

BORCHERT, R. (1976). The concept of juvenility in woody plants. *Acta Horticulturae,* **56**, 21-36.

BOWEN, M.R., HOWLAND, P., LAST, F.T., LEAKEY, R.R.B. and LONGMAN, K.A. (1977). *Triplochiton scleroxylon:* its conservation and future improvement. *FAO Forest Genetic Resources Information No. 6,* 38-47.

GBILE, Z.O. (1975). Taxonomic studies of *Triplochiton scleroxylon* K. Schum. *Proceedings of the symposium on variation and breeding systems of* Triplochiton scleroxylon (*K. Schum*), Federal Department of Forest Research, Ibadan, Nigeria, 19-27.

HALL, J.B. and BADA, S.O. (1979). The distribution and ecology of Obeche (*Triplochiton scleroxylon*). *Journal of Ecology,* **67**, 543-564.

HOWLAND, P. (1975). Vegetative propagation methods for *Triplochiton scleroxylon* K. Schum. *Proceedings of the symposium on variation and breeding systems of* Triplochiton scleroxylon (*K. Schum*). Federal Department of Forest Research, Ibadan, Nigeria, 99-109.

HOWLAND, P. and BOWEN, M.R. (1977). *Triplochiton scleroxylon* K. Schum and other West African tropical hardwoods. *West African Hardwoods Improvement Project, Research Report 1971-1977,* Forestry Research Institute of Nigeria, 154pp.

JONES, N. (1974). Records and comments regarding the flowering of *Triplochiton scleroxylon* K. Schum. *Commonwealth Forestry Review,* **53,** 52-56.

JONES, N. (1975 *a*). Observations on *Triplochiton scleroxylon* K. Schum. Flower and fruit development. *Proceedings of the symposium on variation and breeding of* Triplochiton scleroxylon (*K. Schum*), Federal Department of Forest Research, Ibadan, Nigeria, 28-37.

JONES, N. (1975 *b*). Techniques for assessing quality of *Triplochiton scleroxylon K. Schum.* fruits. *Proceedings of the symposium on variation and breeding of* Triplochiton scleroxylon (*K. Schum*), Federal Department of Forest Research, Ibadan, Nigeria, 60-73.

JONES, N. (1976). Some biological factors influencing seed setting in *Triplochiton scleroxylon K. Schum.* In: *Tropical Trees: Variation, Breeding and Conservation.* (Eds. Burley, J. & Styles, B.T.), Academic Press, London, 125-134.

LADIPO, D.O., LEAKEY, R.R.B., LONGMAN, K.A. and LAST, F.T. In press. A study of variation in *Triplochiton scleroxylon* K. Schum: some criteria for clonal selection. *Proceedings of IUFRO Symposium on genetic improvement and productivity of fast growing tree species,* Sao Paulo, Brazil.

LEAKEY, R.R.B. *In litt.* Physiological studies for tropical tree improvement and conservation. Stockplant factors affecting root initiation in cuttings of *Triplochiton scleroxylon* K. Schum.

LEAKEY, R.R.B., CHAPMAN, V.R. and LONGMAN, K.A. In pres *a*. Physiological studies for tropical tree improvement and conservation. Factors affecting root initiation in cuttings of *Triplochiton scleroxylon K. Schum.*

LEAKEY, R.R.B., LAST, F.T., LONGMAN, K.A., OJO, G.O.A., OJI, N.O. and LADIPO, D.O. In press *b*. *Triplochiton scleroxylon:* a tropical hardwood for plantation forestry. *Proceedings of IUFRO symposium on genetic improvement and productivity of fast growing tree species,* Sao Paulo, Brazil.

LINHART, Y.B., MITTON, J.B., BOWMAN, D.M., STURGEON, K.B. and HAMRICK, J.L. (1979). Some genetic consequences of differential reproduction in populations of forest trees. In: *Flowering and Seed Development in Forest Trees* (Ed. Bonner F.), USDA Forest Service, Starkville, U.S.A., 41-50.

LONGMAN, K.A. (1976 *a*). Conservation and utilization of gene resources by vegetative multiplication of tropical trees. In: *Tropical Trees: Variation, Breeding and Conservation* (Eds: Burley J. & Styles B.T.) Academic Press, London, 19-24.

LONGMAN, K.A. (1976 *b*). Some experimental approaches to the problem of phase change in forest trees. *Acta Horticulturae,* **56,** 81-90.

LONGMAN, K.A. (1978). Control of flowering for forest tree improvement and seed production. *Scientific Horticulture,* **30,** 1-10.

LONGMAN, K.A. (1981). Possibilities of controlled reproduction in trees. In *Forest & Woodland Ecology: An account of research being done in I.T.E.,* I.T.E. Symposium No. 8, Eds: Last, F.T. and Gardner, A.S., Institute of Terrestrial Ecology, Cambridge, 102-104.

LONGMAN, K.A., LEAKEY, R.R.B., HOWLAND, P. and BOWEN, M.R. (1979). Physiological approaches for utilizing and conserving the genetic resources of tropical trees. *Proceedings of 3rd World Consultation on Forest Tree Breeding,* Canberra, 1977, Vol. 2, 1043-1054.

MACGREGOR, W.D. (1934). Silviculture of the mixed deciduous forest of Nigeria. *Oxford Forestry Memoires, No. 18*, 64-67.

MACKENZIE, J.A. (1959). Phenology of *Triplochiton, Technical Note Federal Department of Forest Research, Ibadan, Nigeria*, No. 1, 5pp.

MATHON, C.C. (1975). *Ecologie du developpement et phytogeographie.* Faculte des Sciences de l'Universite, Poitiers, France.

ODEYINDE, M.A. (1975). Observations on the smut infection of flowers and fruits development of *Triplochiton scleroxylon K. Schum. Proceedings of the symposium on variation and breeding of* Triplochiton scleroxylon (*K. Schum*), Federal Department of Forest Research, Ibadan, Nigeria, 53-56.

PHARIS, R.P., and KUO, C.G. (1977). Physiology of gibberellins in conifers. *Canadian Journal of Forest Research*, 7, 299-325.

TAYLOR, C.J. (1960). *Synecology and Silviculture in Ghana.* Thomas Nelson & Sons Ltd.