DISCUSSION

As stated in the introductory part, this investigation was undertaken (i) to study seasonal changes in the rooting response of stem cuttings to auxin application and (ii) their modification with age, if any; (iii) to investigate the extent to which seasonal changes in the auxin effects could be ascribed to changes in the prevailing light and temperature conditions and (iv) to study the histological and biochemical changes that accrue from auxin application and their impact on the initiation and development of roots. The results obtained are proposed to be discussed under these broad heads:

Auxin Effects on Seasonal Rooting

The results presented in Experiment 1 clearly demonstrate that the rooting response of stem cuttings of 3 species under investigation varied considerably with season. Thus, water treated stem cuttings of R. tubiflorum rooted in February, May and August but not in November; those of R. sinon rooted in May but not in other months; and those of R. malabarica did not root in any month (Figs. 2-10).

The period of root initiation was also markedly affected by season. Thus, in R. tubiflorum rooting was hastened in August but was delayed in February and May, although rooting during these months was profuse. These results are in accord with those reported earlier from this
laboratory (Nanda et al., 1966). Seasonal changes in the rooting response of stem cuttings of other plants have been reported by a number of other workers (Mitchock and Zimmerman, 1930; Jiminez, 1937; Brandon, 1939; Deubler and Ferrar, 1940; Nishimura et al., 1944; Klein, 1953; Shapiro, 1957; Deolle and Mitchell, 1964; Farmer, 1966 and Nanda and Anand, 1969).

The following are some of the more significant points that have emerged from the results of this experiment.

1) Auxin effects on rooting also vary with the season. Thus, in R. tubiflorum auxins enhanced rooting in February, May and August and induced it in November. In R. malabarica, it was only 100 ppm IBA, that was able to cause some rooting in March but even this failed to induce rooting in other months. In R. simoco auxins failed to induce rooting in February, induced it in August and November but inhibited it in May, the inhibitory effect being more with IAA.

2) The period during which water-treated cuttings rooted is widened with auxin application. Thus, while control cuttings of R. simoco rooted in May and not in August and November, with auxin application these rooted in May, August as well as in November. Even in R. tubiflorum, water treated cuttings failed to root in November but IBA treated ones rooted throughout the year.

3) The seasonal rooting response of stem cuttings varies considerably with the concentration and nature of auxin
applied. For instance, in *D. sissoo*, both 10 and 100 ppm IAA were effective in May and August but only 100 ppm was effective in November. Similarly both 10 and 100 ppm IBA were effective in May but only 100 ppm in August and November. Again while cuttings of *S. malabarica* did not respond to any other auxin throughout the whole year, these responded to 100 ppm IBA in March. It is, thus, apparent that an auxin may stimulate rooting of a plant species in one season but may inhibit it in the other. Again maximum rooting of the same species may be caused by two different auxins or concentrations in two different seasons. Seasonal changes in auxin effects on rooting stem cuttings of *Salix atrocinerea* have been reported by Vieitez and Pena (1968) and in *Populus nigra* by Nanda and Anand (1970).

Klein (1953) and Tyce (1957) considered that seasonal changes in rooting may be due to the changes in endogenous growth promoting substances. The lack of root initiation in August and November in control cuttings of *D. sissoo* may be ascribed to low level of endogenous auxin at that time due to decreasing activity of meristem. The auxin level was raised with exogenous application and as a consequence of which root initiation took place. On the other hand, in May, shoot growth was active and conditions favourable for the production of endogenous auxin. Water treated cuttings, therefore, rooted well when planted in this month. An exogenous application of auxins at this time is assumed to raise the
auxin content to supraoptimal level causing considerable reduction in rooting.

**Auxin Effects on Seasonal Rooting with Age**

Stem cuttings of different ages taken from the same stock plant did not show any consistent and regular rooting pattern in case of *P. sisson* (Experiment 2). Thus, one month old cuttings rooted well; two month old ones scarcely; and 3-6 month old ones very profusely. Rooting was more or less completely nullified in 7-11 month old cuttings but occurred in 12 and 16 month old ones (Figs. 14-16). It may, however, be pointed out that in this experiment planting of cuttings of different ages happened to fall in different seasons and differences in rooting response, therefore, appear to be more due to season than to the age of the cuttings. Thus rooting was profuse during the period of active growth starting from March and lasting through September and more or less completely nullified during winter dormancy during October to February regardless of the age of the seedling stock.

That differences in rooting response in Experiment 2 were due to seasonal changes and not due to age of the seedlings is apparent from the results of Experiment 3, in which seeds were sown periodically and stem cuttings from seedlings of varying age groups were planted simultaneously. Rooting occurred in all cases regardless of age of the seedling stock.
stock although the number of roots produced and their length increased with age both in *B. tubiflorum* and *D. sissoo* (Figs. 19-25). This was inspite of the fact that the number of fibrous cells in pericyclic ring increased with age in *D. sissoo* and secondary wood in both. It would, thus, appear that differences in age of the seedlings falling within about 18 months in these species did not affect rooting to any appreciable extent. However, rooting of stem cuttings was markedly influenced when differences in age of the stock plant were more marked. This is clearly brought out when the results of rooting of these 3 species obtained in Experiment 2 are compared with those obtained in Experiment 1. In Experiment 2, stem cuttings were taken from seedlings, while in Experiment 1, these were taken from seedlings in the case of *B. tubiflorum* and from branches of fully grown trees in the case of *D. sissoo* and *S. malabarica*. The rooting behaviour of stem cuttings of *B. tubiflorum* was similar in the two experiments but differed in the case of *D. sissoo* and *S. malabarica*. Thus, water treated cuttings of *D. sissoo* did not root in August in Experiment 1, but these rooted in Experiment 2. Similarly control cuttings of *S. malabarica* did not root in any planting in Experiment 1 but rooted up to an age of 4 months in Experiment 2. These results are in conformity with the
earlier findings in the case of *P. sinuosa* (Singh, 1967). It was found that stem cuttings from seedlings rooted readily while those taken from branches of about the same age from a fully grown tree did not. This difference in the rooting response of two type of cuttings has been ascribed to the high degree of sclerification and the presence of pericycle and bast fibres in the latter (Singh, 1967). In the present investigation also, in *S. malabarica* the number of phloem fibres increased considerably and the cortex formed a thick ring of tanniferous cells with the increase in age.

Evidence is available in literature to show that the rooting of cuttings decreases with age of the mother plants (*Nizov, 1944; Allen and McComb, 1965; Sax, 1962 and Guanme and Nelson, 1965*) and this decrease is attributed to a decrease in the reactivity of auxins with age of the mother plant (*Gorter, 1961*) and also to some anatomical changes in stem structure (*Beakbane, 1961; Goedin, 1965*).

**Aein and GA. Effects on Rooting under Varying Licht and Temperature Conditions**

(1) Determinative role of photoperiod

The results of Experiments 4-9 clearly demonstrate that light has a determinative role to play in rooting stem cuttings. In *B. jubiflorum* root initiation did not take place at all in cuttings from LD plants subsequently exposed to LD conditions and occurred only when either
the mother plants or the cuttings were subsequently exposed to SD conditions. The number and length of roots increased with an increase in the number of SD cycles but decreased with an increase in the number of LD cycles given to the mother plants or to the cuttings (Experiments 4 and 5). In *D. alisca* and *S. malabarica* also, the rooting was more profuse on cuttings taken from SD grown plants than from LD grown ones (Experiment 7). Even when the plants were grown under natural conditions of photoperiod and temperature but the cuttings taken from them were subsequently exposed to varying photoperiods, the results were similar. Thus, rooting was earlier and the number of roots higher on cuttings under SD than under ND conditions, there being no rooting at all under LD condition even till the termination of experiment after 56 days (Experiment 8).

The decrease or lack of root initiation under LD conditions may be ascribed to the production of some inhibitor(s) as postulated by Nitsch and Nitsch (1950) and Nitsch (1963). It may, however, be noted that the number of roots produced on cuttings taken from SD and ND grown plants increased with time when exposed to LD conditions subsequently. This is contrary to what would be expected if some inhibitor(s) was(were) produced under LD conditions. Alternately, it would appear that SD favours the synthesis and accumulation of some factor(s)
which are favourable for rooting and which are not produced under LD conditions and as a consequence of which there was a decrease in root initiation under LD condition.

(ii) Determinative role of temperature

The absence of rooting in *P. sissoo* and *S. malabarica* under LD conditions can be attributed to the prevailing low temperature as no significant differences in rooting could be observed on stem cuttings exposed to different photoperiodic conditions, when the experiment was repeated in July (Experiment 9). That the differential rooting response in these two experiments is primarily due to differences in the prevailing temperatures is borne out from the results of Experiments 15 and 16. It was observed that stem cuttings exposed to 15°C did not root even with auxin application while those exposed to 25 and 35°C rooted profusely.

(iii) Auxins substitute for SD requirement

Another significant point that emerges from this investigation is that the SD requirement for rooting of stem cuttings of *P. tubiflorum* could be substituted by treatment with IAA and IBA as roots were initiated on cuttings that were taken from LD plants but were treated with these auxins even when these were planted under LD condition subsequently (Experiment 6). The lack of rooting
in untreated cuttings under LD condition may, therefore, be ascribed to a low concentration of endogenous auxin under this condition. These results are, however, not in accord with the postulation of other workers that higher rooting response of cuttings of some plants under LD may be due to higher concentration of endogenous auxins under this photoperiod (Gorter, 1957; Harada and Mitsch, 1959; Harada, 1962; Raadte et al., 1963).

(iv) Stimulation of rooting by GA$_3$

The effect of GA$_3$ in stimulating rooting (Experiment 6) is very interesting as GA$_3$, in general, is reported to be inhibitory to rooting (Brian, 1957; Brian et al., 1962; Kato, 1958; and Chailakhyan and Nekrasova, 1958). Chardenon and Taris (1963) reported that GA$_3$ in very low concentrations promoted rooting. The results presented in Experiment 6, on the other hand, demonstrate that rooting in B. subiflorum increased with the increase in the concentration of GA$_3$.

(v) Role of intensity and quality of light

The light influences rooting of stem cuttings of these species by virtue of its intensity and quality as well and the results are very interesting. Thus, in B. subiflorum, rooting decreased with a decrease in the intensity of light. The roots produced on cuttings taken from plants raised under 1,000 and 100 ft.c. light
intensity were very thin and slender. The effectiveness of auxin was also determined by the intensity of light. Thus, rooting increased with the concentration on cuttings exposed to high light intensity, but was inhibited on those exposed to light of low intensity. Runkova (1962) also reported better rooting of IAA treated cuttings of bean under light of high intensity although the control ones rooted more under light of low intensity. Cumming (1959) also reported that there was an increase in the number of roots produced on red clover when the intensity of light was increased. Ouellet (1967) reported that although the number of roots produced increased, their growth was weak in cuttings exposed to light of low intensity.

On the other hand, in B. sieva and S. malabarica, the rooting increased with the decrease in the intensity of light, auxins causing root inhibition and the inhibitory effect increasing with concentration. Ouellet (1960) found low light intensity favourable for rooting and ascribed this effect to a decrease in the rate of transpiration which controlled the premature wilting of leaves. Heide (1968) reported that root initiation was promoted but their elongation was inhibited by high light intensity.

The quality of light is also an important factor for rooting of stem cuttings of these species. Thus, in cuttings of B. tubiflorum root initiation was better under
red light and IAA increased it, the effect increasing with concentration but IBA inhibited it under all light qualities. On the other hand, the rooting of stem cuttings of *D. sissoo* was not affected by the quality of light. Auxins increased the number of roots produced though inhibited their elongation under different qualities of light. Blue light increased the rooting response of *S. malabarica* when cuttings were treated with auxins.

**Auxin Effects on Seasonal Rooting and Biochemical Changes**

The biochemical changes in stem cuttings planted in different seasons are rather interesting. In all the 3 species, the starch content of stem cuttings was high prior to planting but decreased with time, the relative decrease varying with the species, the season, and the auxin treatment. Thus, the decrease was the fastest in August, slow in October and the slowest in December so that the starch content of cuttings in the respective treatments remained highest in December, was less in October and the lowest in August. It is significant that in December, the starch content did not change much with time in *D. sissoo* and *S. malabarica* while on the other hand it decreased appreciably in *B. jubiflorum* although the decrease was less than in October. It may also be noted that the decrease in starch content, in general, was faster in auxin treated than in control
cuttings. The seasonal changes in auxin effects on rooting of stem cuttings of these species also exhibited similar differences. Thus, in *D. sissoo* and *S. melanocarpa* roots were produced earlier in auxin-treated than in control cuttings both in August and October, being earlier of the two months in August. In both these species, roots were not produced at all in December while in *B. tubiflorum*, these were produced in December also particularly with auxin application although rooting was delayed. It would, thus, appear that seasonal rooting response of stem cuttings is closely related with the disappearance of starch, the low rooting corresponding with high and profuse rooting with low starch content of cuttings. Bausor (1942) also reported that there was a decrease in starch content by 'rhizocaline' during rooting. An increased break-down of starch to sugars in leaves, stem and roots of plants has also been reported by other workers (Borthwick et al., 1937; Alexander, 1938; Stuart, 1938 and Beal and Whiting, 1945). Jusufov et al. (1965), also found a decrease in starch content during rooting. Nanda and Anand (1969) have also shown that seasonal changes in starch content of cuttings of *Populus nigra* are related with their starch content.

The disappearance of starch is closely related with the activity of hydrolytic enzymes which cause the mobilisation of reserve food materials (Experiment 18).
Thus, the hydrolytic activity of enzymes was very high in August and October when rooting occurred. There was no activity in stem cuttings of *D. sissoo* and *S. malabarica* in December. It may be noted that cuttings of these species failed to root in this planting. However, hydrolytic enzymes were activated within two days even in December in stem cuttings of *B. jubiflorum* and earlier with auxin application than in control. Rooting in this species occurred in December also.

That the rooting response of stem cuttings was closely related with the mobilisation of reserve food materials is also apparent from seasonal changes in the content of soluble carbohydrates, the trends of changes being markedly different from those of starch. Thus, the content was low prior to planting, increased within 2 days indicating mobilisation of starch into soluble carbohydrates but decreased subsequently indicating their utilisation in rooting. The subsequent decrease in general occurred first of all in IBA, then in IAA and last of all in control cuttings.

That mobilisation of reserve food materials is involved in rooting is also clear from changes that occurred in the contents of total, soluble and protein nitrogen in different plantings. Thus, the protein nitrogen was high earlier but decreased subsequently, the content not differing much from control with auxin
application in R. jubiforme but being lower than the control with auxin application in R. sissone and S. malabarica, clearly indicating that degradation of proteins occurred when roots were initiated. This is also apparent from the fact that the contents of total and soluble nitrogen increased during the earlier stages. The fall in the level of total and soluble nitrogen contents after 2-4 days clearly shows that soluble nitrogen was utilised in the initiation and development of roots. The association of rooting of cuttings with such factors as carbohydrates, nitrogenous compounds, auxins, rooting co-factors and vitamins have been reported by other workers (Doak, 1941; Pease, 1943 and Hess, 1961). These factors tend to accumulate near the base of the cuttings where certain cells become meristematic and initiate roots (Vemman, 1962). It has also been shown that mobilisation of starch by auxins is brought about by an increase in the activity of hydrolytic enzymes which mobilise sugars and pass them to the site of root initiation (Doak, 1941; Braske and Nickell, 1952; Hort and Cowie, 1953; Venis, 1964; Ali and Westwood, 1966; Nanda et al., 1967, 1969). Nanda et al. (1967) proposed that auxin-induced effects on rooting may be mediated through mobilisation of reserve food materials by the enhanced activity of hydrolytic enzymes. Thus, appropriate levels of carbohydrates and auxins are essential for supply of energy necessary for the initiation and development of roots and therefore any factors that
influence the mobilisation of reserve food materials influence the initiation and development of roots as well. The results on auxin effects on seasonal changes in hydrolytic activity of enzymes presented in Experiment 18 lend support to this postulate. The additional evidence of the relationship between rooting and mobilisation of reserve food materials has been provided more recently by Nanda et al. (1969) in their studies on seasonal changes in the rooting response of Populus nigra, Hibiscus rosa-sinensis and other plants.

It has also been shown in this laboratory that the activity of hydrolytic enzymes is enhanced by auxins and is profoundly influenced by the prevailing light and temperature conditions suggesting, thereby, that the seasonal changes in the rooting response of stem cuttings are determined by the physio-morphological status of the branches that is controlled by the prevailing light and temperature conditions and which governs the production of endogenous auxin and this effect is mediated primarily through its effect on the mobilisation of reserve food materials caused by the enhanced activity of hydrolytic enzymes.