CHAPTER VI
General Discussion.

While large majority of flowering plants display reduction of flower formation due to auxin application, the pineapple plants stands as an exception by producing early flowering and in greater number when subjected to application of certain auxins. This observation leads to the assumption that the mechanism of flower formation through the agency of hormone may be different for different groups of plants. In the first group, high level of auxin causes harmful effect, whereas for the other group (pineapple) high level of auxin is an essential precondition for onset of flowering. The application of auxin antagonists, viz. TIBA (2,3,5-tri-iodobenzoic acid) (Zimmerman and Hitchcock 1942), DCA (2,4-dichloranisole) (Bonner, 1949) induced early flowering coupled with increase in flower number. Irradiation of plants with x-rays or removal of organs rich in endogenous auxin resulted in promotion of flowering. All these observations led to the thinking that any imposition of conditions that reduce endogenous auxin (IAA) content in plants will effectively induce early flowering in this category of plants. The effect of auxin antagonists is attributed to a kind of antagonistic action of these auxin homologues on native
auxin (IAA). In pineapple, on the contrary, treatment with NAA, 2,4-D and acetylene caused greater number of flowers (Clark and Kerns, 1942; Van Overbeek, 1946; and Das, 1964).

This is no doubt an enigma and to resolve this the present investigation was launched. The result of this investigation showed that application of NAA at its concentrations commencing from 10 ppm up to 300 ppm evoked increase in flowering percentage together with early flowering. Our results are in full agreement with those of the workers mentioned above. For pineapple, therefore, high auxin level is of immense need in producing flowers. Moreover, in the stem apex and leaf bases of this plant, the auxin content is fairly rich. Stem apex contains diffusible or free auxin (IAA) in high amount but leaf bases contain indoleacetaldehyde which is an auxin precursor usually derived from tryptophan after metabolic conversion by the mediation of IAA enzyme system found to be present in this plant. It is clear, therefore, that pineapple plants possess an active seat of auxin metabolism. It was also observed that horizontally placed pineapple plants (variety Cabezona) for a number of hours came to flower rapidly even without auxin application. It is explained that
this case is due to lateral movement of diffusible auxin to the lower side of the shoot due to force of gravity. This phenomenon appears to explain the auxin control mechanism of flowering in this particular plant.

This fact led Rosende (1949) to propose the hypothesis that flowering of plants is associated with maintenance of a suitable balance between natural auxin/anti-auxin/bound or inactive auxin. For plants like pineapple, a high ratio of auxin to anti-auxin is required. Application of auxin from outside, naturally shift the auxin-antiauxin balance towards a high ratio culminating in flower formation. For the other category of plants, where low value of this auxin-antiauxin ratio is necessary, flowering can be considerably induced by applying the antiauxin group which causes lowering of auxin content by competitive antagonism. As a measure of ascertaining this hypothesis of auxin/anti-auxin balance in the concept of physiology of flowering, extraction of endogenous auxin (IAA) was attempted. The results, however, are still not consistent for final acceptance of this hypothesis as a basis for flowering in plants.

The other aspect of flowering of pineapple is linked with forced flower formation by the active ingredients of smoke which are ethylene and acetylene.
This fact was noted as early as 1942 by Clark and Kerns and 1949 by Rosende. Our experiments with acetylene applied as aqueous sprays on the stem tips and leaves revealed similar results of accelerated flowering coupled with high number of flower formation. Calcium carbide dusting also induced similar effect. Calcium carbide is the source of acetylene which is released in contact with moisture.

It indicates, therefore, an identical mode of action of NAA and its homologues and that of acetylene and ethylene. This speaks in favour of the assumption that these compounds NAA and acetylene or ethylene function through common pathway in stimulating the flower formation in this plant. The recent reports of Zimmerman and Wilcoxon, 1935; "organ and Hall, 1962 and 1964; Ablese and Rubenstein, 1964 and Burg and Burg, 1966 suggest that application of auxin viz. NAA, 2,4-D and IAA to the plants causes release of ethylene gas into the tissues which induces accelerated flowering. On the basis of these reports it may be stated that NAA-induced flowering of pineapples noticed in our earlier experiments may be through the production of ethylene, the same nature of physiological action is expected. The validity of this assumption is, however, put to question due to ineffecti-
veness of either 2,4-D or IAA in inducing flower formation. In our experiments no stimulatory effect of these two auxins was observed.

Morgan and Hall (1964) reported that the release of ethylene in 2,4-D and IAA treated plants is slow. The absence of any positive results with these two auxins may perhaps be due to slow release of ethylene by these two auxins in consequence of which the optimum level of ethylene could not be reached. Weak auxins like IBA, TPA still show smaller effects in releasing the ethylene (Abbles and Rubenstein, 1964).

Gowing (1956) conducted the interaction experiments with NAA and IAA for studying the auxin action in the flowering of pineapples. The main object of this experiment was to examine if the flowering is caused due to the reduction of endogenous auxin (IAA) level after application with synthetic auxins. It's found that the stimulatory effects of low concentrations of NAA were counteracted to a certain extent by IAA. Moreover the depressing action of NAA at high concentrations was partially restored by IAA indicating thereby a kind of mutual antagonism of action. He concluded, therefore, that NAA-induced flowering in this plant is the result of the antagonistic action of NAA on the endogenous auxin concentra-
tion of this plant.

The results of the joint action of NAA and IAA studied in our investigation revealed that similar pattern of antagonism between NAA and IAA, the stimulatory effect of the former being depressed by IAA which in its turn showed no visible stimulatory effect. This observation appears to support the views expressed by Gowing. Further proof of this hypothesis was sought by applying MH and TIBA, which are well known auxin antagonists and studying their nature of activity. Contrary to their highly stimulatory effects on the flowering in tomato and Xanthium, there was neither any change in the flower number nor was there acceleration of flowering. This fact tends to rule out the well founded hypothesis of reduction of endogenous auxin (IAA) level as a pre-condition for flowering. This conflicting results called for further interaction studies between auxins, and between one auxin and another auxin- antagonists. In the experiment with NAA and 2,4-D, the effect of NAA acting alone gave significant results in its stimulation. 24-D always caused inhibition even at concentration as low as 10 ppm. This action of 2,4-D is at variance with that of Van Overbeek (1946) who showed marked stimulation in flowering of pineapple with this auxin. In our experiments
consistent inhibition was depicted. The joint action of NAA and 2,4-D showed a kind of antagonism of action in that 2,4-D alleviated the stimulation of NAA and for this the net result of the interaction is always in a magnitude lower than that of NAA acting alone. Such an interaction between auxins may be due to their action on two different focal points independently. Perhaps the inhibiting influence of 2,4-D is greater than the stimulatory effect of NAA with the net result of reduction in flowering percentage. This interaction experiment was extended to between one auxin (NAA) and other auxin-antagonist (MH) for greater precision about the pattern of interaction, since these two compounds are with opposing action, and antagonism between them is almost a certainty. The effect of NAA had been consistently stimulatory raising the flowering percentage to the upper limit of 100 percent. MH also indicated a slight but not statistically significant stimulation of flower formation. The combined effect, on the other hand, was a slight depression on the flowering percentage. The action of NAA appeared to suffer a bit in presence of MH. The statistical analysis of the interaction shows that it was significant. This fact suggests that in the flowering of this plant antagonism of action is displayed, indicating
thereby a competition in the metabolic pathways culminating in flowering.

Lastly the effect of the two compounds, each compound influencing promotion of flower formation together with high flowering percentage was investigated. These compounds were NAA and acetylene.

Acetylene is an allied hydrocarbon to ethylene. Ethylene proved to be physiologically a highly active gas in respect of inducing early flowering in pineapple plant, in accelerating ripening of a number of fruits. It also stimulates new root formation, breaks dormancy of potato tubers, promotes activity of enzyme systems and stimulates respiration, besides causing epinastic curvature in the leaf petioles of potato and tomato plants. Apples, avocados and few other fruits are shown to produce ethylene in their tissues, specially during climacteric rise of the respiration of the fruits. Moulds like *Penicillium digitatum* produces ethylene in amount sufficient to accelerate colour development in as many as 500 sound green lemons. Acetylene and propylene also cause epinasty on tomato petioles. In sprouting of seed potatoes ethylene chlorohydrin proved to be highly potent.

The above mentioned observations bring out an interesting relation between ethylene and auxin. In
certain physiological action, there often appears to be a great similarity between auxins and unsaturated hydrocarbons. Their identical behaviour is eloquently proved by their inducing spinastic curvature of leaf petioles, acceleration of the activity of enzyme systems, namely that of amylase, proteolytic protein splitting enzymes and respiration. The other phenomenon in which both auxins and unsaturated have identical mode of physiological activities are flower formation in pineapples, fruit ripening and root formation as noted above.

Despite of these close similarity of physiological action between the two kinds of compounds, there exists a sharp dissimilarities of actions between them in regard to the following aspects. Unlike auxins unsaturated hydrocarbons will not cause elongation. In case of seed dormancy of potato, their effect is widely different in that auxin prolonges dormancy, whereas ethylene hydrochloride accelerates it. Presumably ethylene compounds reduce endogenous auxin level in tubers and thereby promote development of eyes (buds). In abscession also auxins and unsaturated hydrocarbons have opposite effects. While auxin prevents abscission, ethylene and related compounds stimulates it. Here perhaps also a case of lowering of auxin level induced by unsaturated hydrocarbon.
Controversy exists as to whether ethylene should be classified as a hormone or not. Van Overbeek is of the opinion that ethylene may be placed under the group hormone but he disfavours the idea of calling it an auxin.

Burg and Burg undertook investigation on this controversial problem and on the basis of their results they have reported that initiation of flowering in pineapple is controlled by ethylene rather than auxin, the latter functioning indirectly to stimulate flowering by inducing ethylene formation (Burg and Burg, 1966).

In our interaction experiments between acetylene and NAA, where acetylene was treated prior to NAA so as to induce lowering of auxin level, if there be any, followed by NAA. A clear out antagonism of action in stimulating flower formation in pineapple was observed. This antagonism may be interpreted in the light of opposing action between unsaturated hydrocarbons and auxin. But fact remains that the phenomenon of competitive antagonism generally takes place between two physiologically identical compounds.

All these facts lean heavily to the conclusion that NAA and acetylene have common metabolic pathways through which flowering of pineapple is promoted by them.