DISCUSSION

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Application of CCC at 1000 and 1500 ppm to mung bean plants considerably decreased their extension growth. Similar growth retarding effects of CCC have been reported in wheat (Tolbert 1960, Linser and Kühn 1964, Page 1973, Brükner and Höfner 1980), barley (Linser *et al*. 1963, Koranteng and Matthews 1982, Naylor *et al*. 1986) tomato (Wittwer and Tolbert 1960, Adler and Wilcox 1987), cotton (Bhatt and Ramanujam 1970), *Begonia hiemalis* (Roivainen 1987), *Rhododendron obtusum* (Whealy *et al*. 1988), *Phaseolus vulgaris* (El-Fouly *et al*. 1988) and *Amaranthus caudatus* (Guruprasad and Guruprasad 1988). It is generally accepted that CCC is an inhibitor of gibberellin biosynthesis and retards the extension growth of plants by inhibiting their subapical meristematic activity (Sachs 1965) probably by blocking the cyclization of transgeranyl geranyl pyrophosphate to entkaurene (Sembdner *et al*. 1980), leading to low endogenous level of gibberellin content.
The effect of CCC on the extension growth of the shoot system was manifested in the reduction of the 3rd, 4th and 5th internodes. The 5th internode of CCC treated plants showed the highest percentage of reduction. Reduced internode length is one of the common morphological alternations pointed out by Cathey (1964) in response to CCC treatment. Similar effects of CCC on internode has been recorded in *Impatiens*

balsamina (Nanda et al. 1968), Cyperus alternifolius (Fisher 1970) cotton (Bhatt and Ramanujam 1970), soyabean (Williams and Sicardi de Mallorca 1984) and Phaseolus vulgaris (El-Fouly et al. 1988).

The elongation growth of root system was not affected by CCC treatment. However, application of CCC at 1000 ppm moderately increased the lateral growth of the root system. Such an enlargement of root system has been reported by Bragg (1982) in winter cereals.

Administration of CCC (1000 ppm) significantly increased the fresh and dry weights of shoot and root systems. Similar increase in the biomass of plants following CCC treatment has been reported in tomato (Wittwer and Tolbert 1960), carrot (Supniewska 1963), tobacco (Humphries 1963), Lolium temulentum L. (Stoddart 1965) bean, soyabean, horse bean and alfalfa (Chailakhyan and Arutyunyan 1968), rice (Chakraverty 1969), wheat (Khan and Wasti 1982, Ignatev et al.1985), barley (Koranteng and Matthews 1982, Naylor et al. 1986) and maize (Kotting et al. 1988). The total biomass production depends on the accumulation of photosynthate and this in turn is determined by the rate of carbon fixation, area of leaf surface available for photosynthesis, chlorophyll content of the photosynthetic tissue and the nitrogen fixation.

Application of CCC (1000 ppm) increased the circumference of the stem. An increase in the stem diameter has been observed in Phaseolus vulgaris (El-Fouly et al.1988). The development of thicker stem and leaves which succeed treatment with growth retardants is due to the cell enlargement in radial direction (Cathey 1964; Wheeler 1969; Mohsin and Smith 1972, and El-Fouly et al. 1988).

The root/shoot weight ratio registered its peak value on 28th day and gradually decreased thereafter showing that photosynthate accumulation in the root was faster in the early stages of growth and then it became slower in the later stages. Application of CCC at 1000 ppm significantly promoted the root/shoot weight ratio over other treatments and thus demonstrating that photosynthate accumulation is more in the root system of CCC treated plants. A similar increase in root/shoot weight ratio in bean and tomato (Tognoni et al. 1967) and a depression of shoot/root weight ratio in barley (Naylor et al. 1986) have been documented following CCC treatment.

In the present investigation CCC at a concentration of 1000 ppm significantly increased the area and thickness of the leaves. Anatomical studies revealed that this increase in thickness of the leaves of CCC treated plants is due to an expansion of the mesophyll tissue. This could be a reason that plants sprayed with CCC produced larger leaves. A similar increase in the thickness of leaves due to the expansion of mesophyll tissue following CCC application has been reported in cotton (Bhatt and Nathan 1970) and kidney bean (El-Fouly *et al*. 1988). Increased leaf area following CCC treatment has also been observed in mustard (Humphries 1963), tomato (Wittwer and Tolbert 1960), *ipomoea batatas* (Kabi and Sarma 1973) and kidney bean (El-Fouly *et al*. 1988). A concomittant increase in the leaf area index was also observed in the CCC treated plants (1000 ppm) over the control plants. This increase in leaf area index can be attributed to the increased leaf area of the plants which received CCC spray.

Treatment of plants with CCC (1000 ppm) increased their total chlorophyll content. Many investigators agree that plants treated with CCC produce greener leaves with high chlorophyll content. Increase in chlorophyll content following treatment with CCC has been reported in tomato (Wittwer and Tolbert 1960), tobacco (Humphries 1963), cotton (Bhatt and Ramanujam 1970, El-Fouly and Ashour 1970), barley (Badanova and Levina 1970) and wheat (Höfner *et al*. 1984, and Farrahi-Aschtiani *et al*. 1987). Stoddart (1965) observed that chlorophyll production in *Lolium temulentum* L. was stimulated in presence of CCC. He also found a correlation between chlorophyll production and protein accumulation. Knypl (1967) observed that CCC arrested chlorophyll degradation in kale leaf tissue by retarding the degradation of already existing proteins. Humphries (1968) suggested that promotion of chlorophyll content in response to treatment with CCC is a consequence of growth retardation and accumulation of protein in leaves due to restricted mobilization to the shoot. Bhatt and Nathan (1970) in their studies with cotton suggested that the increase in chlorophyll content in leaves of plants treated with cycocel (CCC) can be attributed to the expansion of mesophyll tissues.

The highest value of net assimilation rate (NAR) was attained on 42nd day **for** all the treatments. Like the leaf area and leaf area index, net assimilation rate of mung bean was also increased by CCC administration (1000 ppm) as compared to control. This increase in net assimilation rate (1000 ppm) obtained in plants which received 1000 ppm CCC spray could be due to the promotive effect of CCC on leaf area, leaf area index.

The net primary productivity rose linearly with net assimilation rate. The net primary productivity was at its peak on 42 day in all the treatments. The higher net primary productivity recorded in the CCC treated (1000 ppm) plants might have resulted from the higher net assimilation rate and leaf area index obtained following CCC treatment.

The analysis of shoot and root systems revealed that the maximum accumulation of total soluble sugars and starch occurred in 1000 ppm CCC treated plants, irrespective of the number of CCC ~~administration~~ treatments. The build up of total soluble sugars and starch in response to CCC application has been documented earlier by Stoddart (1964,1965) in *Phleum pratense* L.

and *Lolium temulentum* L. and Knapp *et al*. (1987) in wheat. In *Lolium temulentum*, after 7 days of CCC treatment, total soluble sugar content was found twice as that of the control and this ratio was maintained until the end of 14 days of experiment. It is argued that this higher concentration of total soluble sugars and starch could be resulting from the increased leaf area and chlorophyll content (Stoddart, 1965). In the present study also a significant increase in the leaf area, chlorophyll content and net assimilation rate was recorded in plants receiving CCC treatment. No direct evidence is, however, available at the moment to advance a precise explanation for the CCC induced accumulation of total soluble sugar and starch. Nonetheless, as suggested by Stoddart (1965), it is quite probable that the high content of total soluble sugars and starch could be an outcome of the increased area of leaf as well as chlorophyll content of the photosynthetic tissue.

Maximum number of nodules were present on 42nd day in all the treatments. After the 42nd day the number of nodules started decreasing, though the fresh and dry weights of the nodules continued to increase till the 56th day. The decrease in the number of nodules after 42nd day might be due to a reduction in the rate of nodule formation and senescence of early formed nodules. CCC at 1000 ppm significantly increased the number as well as the nodule mass per root system as compared to other treatments. This increase in the number and mass of nodules observed in CCC (1000 ppm) treated plants might be due to the availability of more carbohydrates to the root system from the shoot. A similar increase in the number of nodules following CCC treatment has been observed in alfalfa (Chailakhyan and Arutyunyan 1968) and soyabean (Williams and Sicardi de Mallorca 1984).

The maximum activity of nitrogenase (acetylene reduction) was recorded on 42nd day of the growth period in all the treatments. CCC application at a concentration of 1000 ppm considerably enhanced the acetylene reduction, though the other two concentrations of CCC (500 and 1500 ppm) tested did not bring about any increase as compared to the control. It is well known that CCC is an inhibitor of gibberellin biosynthesis in plants. At this juncture it is interesting to note the observation made by Williams and Sicardi de Mallorca (1984) that application of GA_3 to soyabean reduced the number of nodules and their acetylene reducing activity where as CCC administration significantly increased the number of nodules with out evoking any effect on nitrogenase activity. They also observed that CCC significantly reduced the endogenous gibberellin content. The mechanism of stimulation of nitrogenase activity by CCC is not yet clearly understood. It has been reported that activity of nitrogenase requires energy supply primarily in the form of carbohydrate from the photosynthesis of the host plant (Hardy et al. 1968; Gordon et al. 1985). Thus the availability of photosynthate is considered as a major factor limiting symbiotic nitrogen fixation (Bergersen 1971, Lawn and Brun 1974). It is, therefore, presumed that the higher rate of acetylene reduction recorded in mung bean plants in the present investigation might be due to the availability of more photosynthate to the root nodules following CCC treatment.

The total nitrogen content of both shoot and root systems was found to increase till the end of the experiment in all the treatments. The maximum accumulation of nitrogen was recorded in the plants treated with 1000 ppm CCC irrespective of the number of sprays given. The increase in total nitrogen content obtained following CCC treatment (1000 ppm) in mung bean in the present investigation might be due to the higher nitrogenase activity (acetylene reduction) of the nodules of CCC treated plants. It has been reported that CCC treatment resulted in a 36 % increase in the nitrogen content of leaves while the stem nitrogen content recorded a two fold increase in tobacco plants (Humphries 1963).

The content of total ureides in the shoot and root system increased progressively till the end of the experiment. A conspicuous increase in the amount of ureides was observed in the shoot and root systems of plants which received

CCC spray (1000 ppm). Ureides are the major nitrogenous compounds transported in the xylem of nitrogen fixing soyabean plants and several other legumes (McClure and Israel 1979; Thomas and Schrader 1981; Atkins et al. 1982.) including mungbean. It is a known fact that nitrogen contained in these ureides arises predominantly from symbiotic nitrogen fixation in root nodules (Fujihara and Yamaguchi 1978). McClure et al. (1980) observed a positive correlation between relative ureide concentration and rate of nitrogen fixation in soyabean. Van Berkum et al. (1985) correlated ureide concentration in the young stem and plants with the rate of acetylene reduction, nodule mass and above ground nitrogen accumulation. In the present investigation, the accumulation of ureides in the mungbean plants coincides with that of total nitrogen. It is therefore presumed that the higher rate of ureide accumulation in the mungbean plants treated with 1000 ppm CCC might have resulted from the higher rate of nitrogen fixation as evidenced by the higher nitrogenase activity (acetylene reduction).

The total protein content of the shoot and root systems, as observed in the case of total soluble sugars, starch, total nitrogen was found to be significantly increased by the pre flowering and pre and post flowering application of 1000 ppm CCC. An increase in protein content of the plants in response to CCC treatment has been reported in *Lolium temulentum* L. (Stoddart 1965) and bean (Kharanyan 1969). The increase in the protein content of plant observed under the influence of CCC could be due to the production of more soluble carbohydrates (Stoddart 1965) or due to the increased accumulation of protein owing to a slower rate of protein decomposition brought about by CCC (Kharanyan 1969). The increase in total protein content following CCC treatment in mungbean in the present study could be attributed to the higher rate of carbohydrate and nitrogen accumulation in the CCC treated plants.

The area of the source leaf increased stedily till 52nd day and it remained almost constant thereafter. CCC spray (1000 ppm) to mungbean significantly increased the area of the source leaf (subtending leaf). In Phaseolus vulgaris, Zea mays and Hordeum vulgare Hawker and Walker (1978) studied the relationship between leaf expansion rates, invertase activity and reducing sugar concentrations and suggested a role for invertase in the growth of the leaves. In the present study CCC (1000 ppm) has been found to increase considerably the activity of invertase in the source leaf. The invertase activity of leaf tissue of CCC treated plants on 42nd day recorded 48 % increase over the control. El-Fouly et al. (1988) also observed a similar enhancement of invertase activity and increased leaf area in kidney bean following CCC treatment. One of the possible reasons for the increased leaf area might be the expansion of mesophyll tissues as evidenced by the anatomical studies. Increased leaf area of the subtending leaf following CCC treatment might therefore be due to a positive role of CCC on the expansion of mesophyll tissue by stimulating invertase activity.

The observed increase in the total soluble sugars and reducing sugars following CCC treatment may be attributed to the increased activity of amylase, and invertase in the source leaf. A similar increase in amylase and invertase activity following CCC treatment has been reported in wheat (Hassan <u>et</u> <u>al</u>. 1975; Firgany <u>et</u> <u>al</u>. 1980).

The dry weight of the source leaf progressively increased till the 52nd day and started declining thereafter. The accumulation of starch, total protein and nitrogen in the source leaf also showed a similar trend. Conversely, the dry weight and the subsequent build up of starch, total protein and nitrogen in pods increased till their maturity. The highest rate of dry matter accumulation in pods was observed between the 52nd and 62nd day coinciding the decline of dry matter in the source leaf. Administration of CCC at 1000 ppm significantly increased the dry weight and the contents of starch, total protein and nitrogen of the source leaf as well as of pods. The increased dry matter production and the concomittant increase in the starch, total protein and nitrogen content in the source leaf of the CCC treated plants may be attributed to high photosynthetic rate of the source leaf as evidenced by its area and chlorophyll content. The increase in the dry weight of the pods of the plants treated with CCC (1000 ppm) is manifested by the increase in the number of seeds per pod though seed weight is not altered by CCC significantly. The increased invertase and amylase activity due to CCC application indicates that CCC alters carbohydrate metabolism in the leaf and might be stimulating the mobilization and translocation of photosynthate from the source to sink resulting in the increased seed number and pod weight.

The increase in the number of seeds per plant observed under the influence of CCC ~~has been~~ was found to be due to an increase in the number of pods as well as the number of seeds per pod. The number of pods per plant and the number of seeds per plant were increased by 32.5 and 48.5 % respectively over the control following 1000 ppm CCC spray. Similar increase in seed yield in responses to CCC administration has been reported in kidney bean (Gunasena and Clemants 1970, El-Fouly et al.1988). However, no significant increase in 1000 seed weight could be observed. The number of pods per plant and seeds per pod are the main factors contributing to the yield increase. Generally higher seed yield is related to higher number of seeds per plant. There is considerable evidence to show that seed yield is principally a function of seed number produced and not seed size (Evans 1976). El-Fouly et al. (1988) suggested that in kidney bean, increased leaf growth and diameter of vascular elements in addition to cell shortening may lead to more mobilization of photosynthates towards actively growing reproductive parts (normal sink) leading to more pod formation, more seed filling and consequent more seed yield per plant in response to CCC treatment.

It may be concluded from the present studies that CCC enhances growth (biomass) and yield of mung bean by

1. reducing shoot extension growth and enhancing root growth (dry weight)
2. improving root/shoot weight ratio
3. increasing leaf area and leaf area index (LAI) and total chlorophyll content resulting in an increase in net assimilation rate (NAR) and net primary productivity (NPP) as evidenced by a significant increase in dry matter production.
4. improving nitrogen fixing ability as evidenced by the increased number of nodules per plant and nitrogenase activity resulting in more nitrogen and protein content.
5. promoting the production of flowers as evidenced by the increased number of pods per plant.
6. improving the translocation of photosynthates from the source to the sink.