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EFFECTS OF CONCENTRATIONS OF TWO PHENOXYACETIC ACID HERBICIDES ON GERMINATION AND EARLY SEEDLING GROWTH OF *CUCUMEROPSIS EDULIS*

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ABSTRACT

The effects of concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on the germination and early seedling growth of *Cucumeropsis edulis* (Hook, F) Cogn. were investigated. The concentration range of 0.004-40 µm of the two herbicides significantly reduced percent germination of *C. edulis*. The lowest concentration (0.004 µm) of 2,4-D and 2,4,5-T induced a reduction in the hypocotyl and radicle lengths and the number of lateral roots of the seedlings. The medium concentration (0.4 µm) of these herbicides behaved similarly to the lower concentration and in addition induced a swelling at the hypocotyl base of the seedlings. The higher concentration (4.0 µm and 40 µm) of either 2,4-D or 2,4,5-T produced necrotic seedlings. These results are discussed with reference to the mode of action and probable mechanism of action of the two phenoxyacetic acid herbicides.

INTRODUCTION

The herbicide is an important tool in mechanized farming because of its use in the control of weeds on a large scale. One herbicide that was discovered quite early and has been used extensively all over the world is 2,4-dichloro-phenoxyacetic acid (2,4-D). This compound, in addition, is auxin-like and consequently it is of dual interest

At high concentrations, 2,4-D shows very strongly inhibitive effects and produces a wide range of growth abnormalities. Pretreatment of pine seeds with 2,4-D reduced their percentage germination (Sesaki *et al*, 1968). Inhibition of root growths together with malformations of lateral roots resulting in development of callus-like structures (Ingensiep, 1981) and development of epinasty in leaves and petioles are a few of the herbicidal effects of 2,4-D. In various commercial formulations, 2,4-D is used in selective weed control in cereals, pastures as well as non-crop lands and forests.

Low concentration of 2,4-D, however, like the auxins, are known to induce elongation of coleoptiles (Aberg and Eliasson, 1978), etiolated epicotyls of mung beans and pea seedling stems (Apelbaum and Burg, 1971).

Some other concentration of 2,4-D are used for growth regulation activities (Akobundu, 1987) such as potatoes, peas, soybeans and sugar beets.

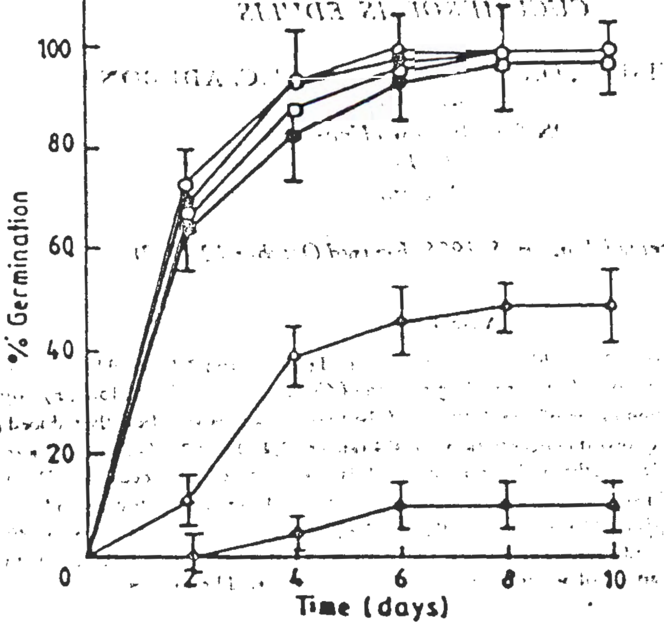

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Fig. 1: Effect of 2,4-D on the germination of *C. edulis*. O water control; ● 0,00 μM 2,4-D; □ 0.04 μM 2,4,-D; ■ 0.4 μM 2,4,-D; Δ. 4 μM 2,4-D; ▲ ·40 μM 2,4-D.

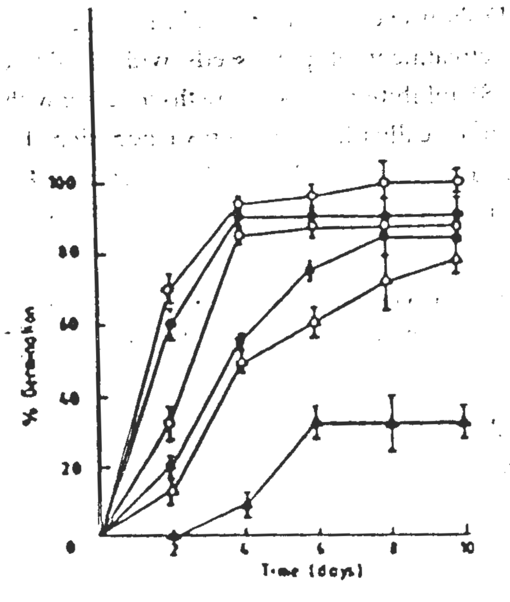

Fig. 2: Effect of 2,4,5-T on germination of *C. edulis*. O water control; ● 0.004 μM 2,4,5-T; □ 0.04 μM 2,4,5-T; ■ 0.4 μM 2,4,5-T; Δ 4 μM 2,4 5-T; ▲ 40 μM 2,4,5-T.

Another phenoxycarboxylic acid which resembles 2,4-D in many of its effects but with greater herbicidal action in some woody species, is 2,4,5-trichlorophenoxyacetic acid (Bovey, 1980). As a herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is used for pine site preparation and release from competition (Michael, 1980). The presence of highly toxic chemical, dioxin as impurity in the synthesis of 2,4,5-T has, however, led to its withdrawal from the market for some time now.

Cucumeropsis edulis (Hook, F.) Cogn., a member of the cucurbitaceae is an important crop species used in the preparation of vegetable stews in West-African countries. It is, therefore, widely cultivated in these places, very often intercropped with maize and cassava. Under such condition, *C. edulis* has an additional advantage of reducing weed pressure on the farm because of its straggling growth.

Although 2,4-D and indeed many of the phenoxyacetic acid herbicides have been used for well over 5 decades now, their mechanisms of action are still ill-understood. The object of this investigation was to study the effects of 2,4,5-T on the germination, radicle and hypocotyl growths and on the number of lateral roots of *C. edulis* during course of its early growth. This is with the view of producing useful result that can lead to an increased understanding of the associated mechanisms of action of these herbicides.

MATERIALS AND METHODS

Seed preparation:

The seeds of *C. edulis* were bought from a local market in Ile-Ife, Osun state. They were sterilized with 25% sodium hypochlorite for five minutes, washed with three changes of distilled water then finally soaked in distilled water for two hours. The seeds were then used to carry out experiments as follows:

(1) Effects of 2,4-D and 2,4,5-T on germination.

The following serial concentrations of 2,4-D were prepared: 0.004 µm, 0.04 µm 0.4 µm and 40 µm. Seeds were planted in petri dishes lined with two layers of Whatman No 1 filter paper moistened with 8 mls of each test solution. In each treatment there were three replicates of 20 seeds each. The seeds were incubated in the dark at $25^0C \pm 2^0C$, recording the germination at 2 day-intervals over a 10-day period. The emergence of the radicle from the testa was the criterion adopted for germination.

Effects of 2,4-D and 2,4,5-T on seedling growth

The seeds were planted in petri dishes and the experimental procedures and conditions were the same as in the germination experiment described above. At the end of an incubation period, the hypocotyl and radicle lengths of the seedlings were measured using a metre rule. The diameter

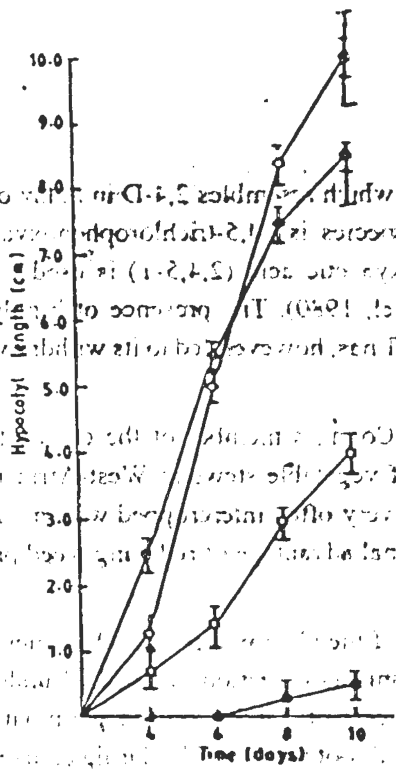

Fig. 3: Effect of 2,4-D on the hypocotyl length of *C. edulis*. O water control; ● 0.004 μ M 2,4-D; □ 0.04 μ M 2,4-D; ◘ 0.4 μ M 2,4-D

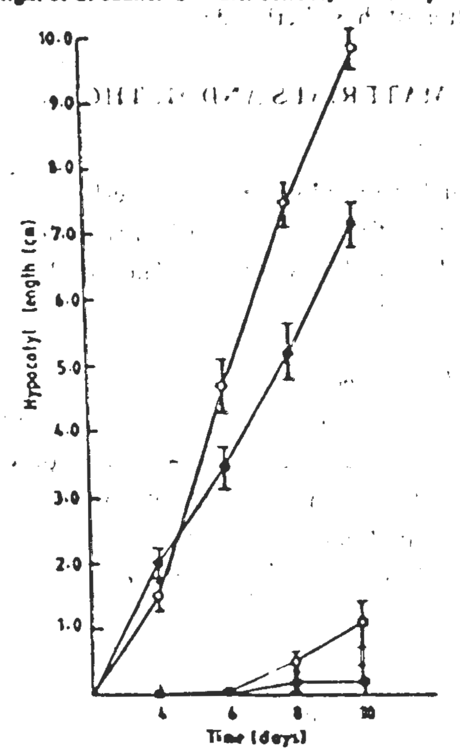

Fig. 4: Effect of 2,4,5-T on the hypocotyl length of *C. edulis*. O water control; ● 0.004 μ M 2,4,5-T; □ 0.04 μ M 2,4,5-T; ■ 0.4 μ M 2,4,5-T.

of the hypocotyl base of the seedlings was also measured with the aid of micrometer screw gauge (Yousif *et al*, 1979). The number of lateral roots of the seedlings were also counted.

The data obtained from three replicates of each experiment repeated thrice were pooled together and comparison of the treatment means were carried out using analyses of variance. As for the percent germination data, they were transformed into arcine values prior to analyses.

RESULTS AND DISCUSSION

The seeds of *C. edulis* which were non-dominant showed almost 100% germination by day 4, and more than 50% germination was observed just two days after planting. The results of the germination studies conducted on *C. edulis* seeds treated with 2,4-D and 2,4,5-T are shown in figures 1 and 2, respectively. After 10 days of incubation, the percent germination of the seeds planted in all the concentrations of 2,4-D investigated were significantly lower than that of the control ($P \leq 0.05$). With reference to 2,4,5-T, the germination studies on the other hand, showed that by day 10, there was no significant difference in percent germination between the control seeds and those incubated in 0.004 µm, 0.04 µm or 0.4 µm; only the seeds treated with high concentrations (4 µm & 40 µm) significantly inhibited germination of the seeds. Thus, the two herbicides differed significantly with respect to their effect on germination.

Previous workers (Yousif, 1977; Sanchez-Torres, 1984) have reported that lower concentrations of 2,4-D have in some instances inhibited germination of *Cucurbita pepo* seeds, particularly during the early days of incubation. Indole auxins and auxin-type herbicides inhibit seed germination and this inhibition is concentration dependent and varies with the plant species studied (Aberg and Eliassan, 1978). The minimum concentration of 2,4,5-T that significantly reduced germination of *C. edulis* seeds was higher than that of 2,4-D, although this does not appear to be the case with woody species (Bovey, 1980).

The water germinated seedlings, 10 days after planting, were healthy looking and their hypocotyls were long and rather straight except at the sub-hook zone. The hypocotyls which were appropriately cylindrical were uniform in the diameter from below the subhook region down to the basal region. Treated seedlings however, varied from being robust to rather thin and hypocotyls showed pronounced swelling or reduction in diameter at the same base, depending on the concentration of the herbicides. Figures 3, and 4 show the effects of 2,4-D and 2,4,5-T respectively on the extension growth of the hypocotyl of *C. edulis* seedlings. In both herbicides the lowest concentration (.004 µm) did not have any significant effect on the hypocotyl length of seedlings ($P \leq .05$).

Also, in the two herbicides, there was significant reduction in the hypocotyl length of the seedlings that grew in the medium concentration i.e. .04µm and 0.4 µm ($P \leq 0.05$). At the higher

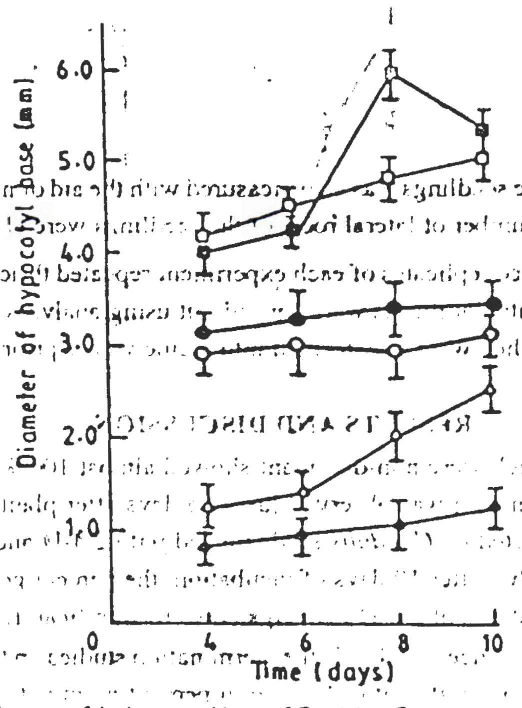

Fig. 5: Effect of 2,4-D on the diameter of the hypocotyl base of *C. edulis*. O water control; ● 0.004 μ M 2,4-D; □ 0.04 μ M 2,4-D; ■ 0.4 μ M; 2,4-D; Δ 4 μ M 2,4-D; ▲ 40 μ M 2,4-D.

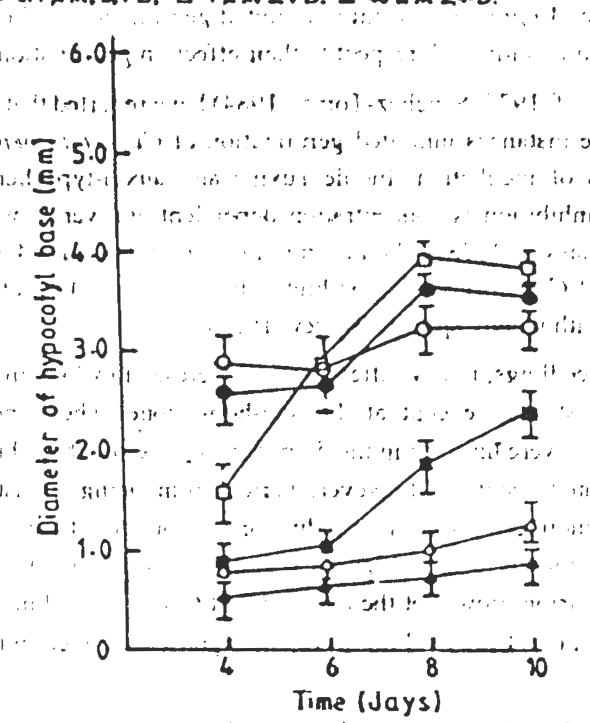

Fig. 6: Effect of 2,4,5-T on the diameter of the hypocotyl base of *C. edulis*. O water control; ● 0.004 μ M 2,4,5-T; □ 0.04 μ M 2,4,5-T; ■ 0.4 μ M 2,4,5-T; Δ 4 μ M 2,4,5-T; ▲ 40 μ M 2,4,5-T.

concentrations (4.0 µm and 40 µm) of 2,4-D or 2,4,5-T, there was no measurable hypocotyl as the seedling development hardly progressed beyond radicle protrusion stage.

The concentration - dependent effect of 2,4-D on the extension growth of the hypocotyl has been reported previously in soybean(Key *et al.*, 1966), pea (Apelbaum and Burg, 1972) and in *C.pepo* (Sanchez-Torres, 1984).

The graphs of figures 5 and 6 represent the effects of 2,4-D and 2,4,5-T respectively on the growth of the diameter of the hypocotyl base of *C. edulis* seedlings. The lowest concentration of 2,4-D (0.004 µ m) had no significant effect on the hypocotyl base of the seedlings while the higher concentration did have significant effect ($P \leq 0.05$). The medium concentration (.04 and 0.4 µm) caused the hypocotyl base of the seedling to swell whereas the high concentration (4 µm and 40 µm) produced the opposite effect i.e a reduction in the diameter of the hypocotyl base. In the case of 2,4,5-T, concentrations of 0.4 µm, 4 µm and 40 µm significantly reduced the diameter of the hypocotyl base and only 0.04 µm induced a swelling at the hypocotyl base of the seedling ($P \leq .05$) The lowest concentration of 2,4,5-T(.004 µm) was not significantly different from water control with respect to the diameter of the hypocotyl base.

The induction of swelling by 2,4-D on susceptible seedlings is well established. Apelbaum and Burg (1972) reported swellings at the subhook of pea seedlings while swellings at the hypocotyl base of the honey mesquite (Meyer,1970) and pine seedlings have been observed. The subapical swellings in soybean (Key *et al,* 1966) and pea (Apelbaum and Burg, 1972) were attributable mainly to radial expansion of cells. In contrast, Meyer (1970) and Wu *et al*., (1971) demonstrated that the 2,4-D induced swelling at the hypocotyl base of the honey mesquite and pine seedlings must be due to cell proliferation. A similar study conducted on *C. pepo* by Yousif *et al* (1979) showed that both cell proliferation and cell enlargement were involved in the swelling produced at the hypocotyl base of the seedlings.

Seedlings incubated in 2,4-D and 2,4,5-T were significantly reduced in their radicle length compared to those of seedlings incubated in water (Figs. 7 and 8).

The lowest concentration of 2,4-D and 2,4,5-T inhibited the radicle length of the seedlings more than 50%. After 10 days of incubation of the seedlings in the high concentration of the herbicides (0.4 µm and 4 µm) the radicles of the seedlings which just penetrated the seed coat became necrotic.

Water control seedlings were found to possess very healthy, long lateral roots, averaging 25 in number by day 10. Compared with these, the lateral roots of the seedlings with the two herbicides were rather short in length and fewer in number (Figs 9 and 10). There were no lateral roots produced by the radicle of the seedlings incubated in 0.4 µm solutions of either herbicides. The

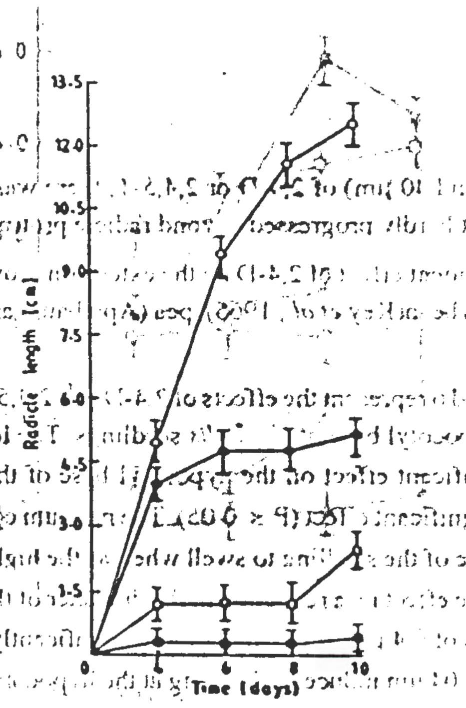

Fig. 7: Effect of 2,4-D on the length of C. edulis O water control; ● 0.004 µ M 2,4-D; □ 0.04 µ M 2,4-D;

■ 0.4 µ M 2,4-D.

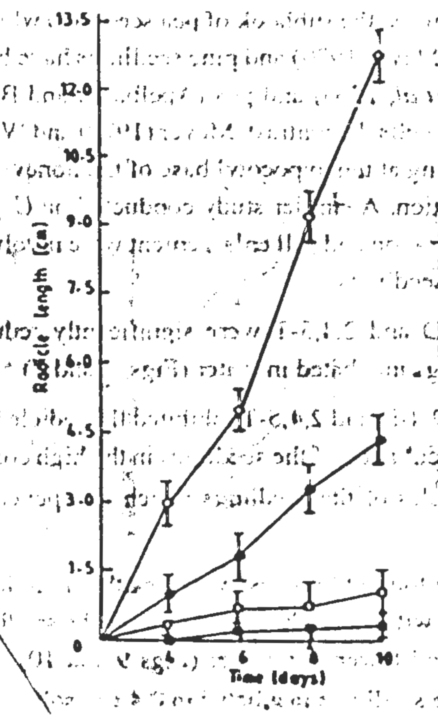

Fig. 6: Effect ... □ 0.0

... length of C. edulis. O water control; ● 0.004 µ M 2,4,5-T; □ 0.04 µ M 2,4,5-T;

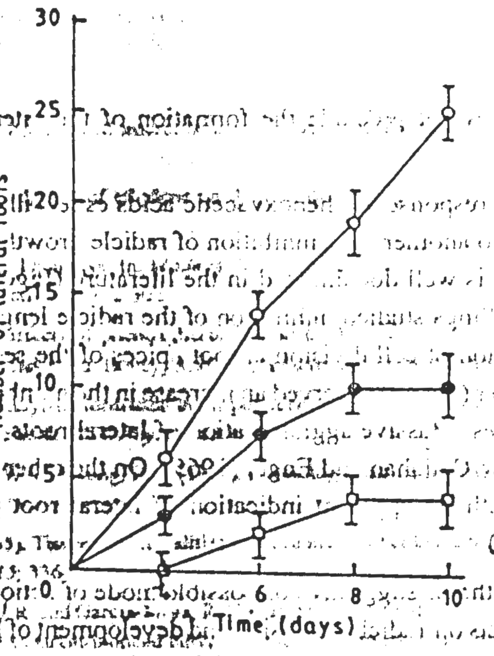

Fig. 9: Effect of 2,4-D on the number of lateral roots of *C. edulis*. ○ water control; ● 0.004 μ M 2,4-D; □ 0.04 μ M 2,4-D.

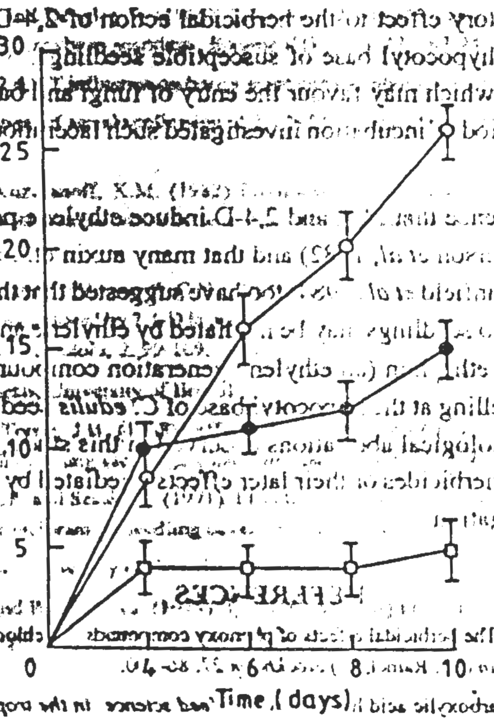

Fig. 10: Effect of 2,4,5-T on the number of lateral roots of *C. edulis*. ○ water control; ● 0.04 μ M 2,4,5-T. □ 0.004 μ M 2,4,5-T;

observation, however, does not preclude the formation of the lateral roots primordia in the affected seedlings.

The behaviour of roots in response to phenoxyacetic acids especially 2,4-D and 2,4,5-T seems to vary from one species to another. The inhibition of radicle growth by 2,4-D and 2,4,5-T,like the principal native auxin is well documented in the literature (Ingensiep,1981; Pinfield *et al.* 1984). In most of the seedlings studied, inhibition of the radicle length by these herbicides has been ascribed to suppression of cell division at root apices of the seedlings (Burg *et al*, 1971; Pinfield *at al.*, 1984). Meyer (loc.cit) observed an increase in the number of lateral roots produced by 2,4-D- treated mesquites. Massive agglomeration of lateral roots developing near the tip of bent grass was reported by Callahan and Engel (1965). On the other hand, with pine seedlings a cellular proliferation with no apparent indication of lateral root primordia formation was observed (Wu *et al.*, 1971).

An important observation that is suggestive of possible mode of action of 2,4-D and 2,4,5-T is their strong inhibitory effects on radicle extension and development of lateral roots. These effects are sufficient for pre-emergence use of these herbicides since both radicle and lateral roots are very crucial for establishment of seedlings in the soil.

Another possible contributory effect to the herbicidal action of 2,4-D and 2,4,5-T may be the excessive swelling of the hypocotyl base of susceptible seedlings. Such growth could lead to rupture of the outer tissue which may favour the entry of fungi and bacteria (Ashton and Craft, 1981), although for the period of incubation investigated such lacerations were uncommon in the seedlings *C. edulis*.

There is considerable evidence that IAA and 2,4-D induce ethylene production in plant tissues (Burg and Burg, 1968; Grierson *et al*, 1982) and that many auxin effects are now attributable to ethylene (Mulkey, 1982). Pinfield *et al* (1984) too have suggested that the 2,4-D induced swelling of hypocotyl base of *C. pepo* seedlings may be mediated by ethylene and were able to mimic this response by application of ethephon (an ethylene generation compound) to *C. pepo* seedlings. Thus the induction of a swelling at the hypocotyl base of *C. edulis* seedlings by 2,4-D or 2,4,5-T or indeed any of the morphological aberrations observed in this study, may either be due to the immediate effects of these herbicides or their later effects, mediated by ethylene, a matter which will require further investigation.

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