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EFFECTS OF SIMAZINE, ATRAZINE AND 2,4-D ON $^{14}CO_2$—FIXATION AND COMPOSITION OF ^{14}C—— ASSIMILATES IN *PINUS NIGRA* VAR. *CALABRICA* SCHNEID

S. SHAHID SHAUKAT*

School of Plant Biology, University College of North Wales, Bangor, Caerns., U.K.

Abstract

The effects of root application of simazine, atrazine and 2, 4-D on $^{14}CO_2$—fixation and on the composition of ^{14}C-assimilates by young *Pinus nigra* var. *calabrica* seedling were investigated. Simazine and 2,4-D at 5 ppm substantially enhanced the rate of $^{14}CO_2$—fixation of pine needles. Higher concentrations of triazines (50,200 and 1000 ppm) and 2,4-D (200 and 1000 ppm) caused significant inhibition of $^{14}CO_2$—fixation. All the three herbicides at 100,200 and 1000 ppm invariably reduced the level of radioactivity present in a range of assimilates. However, 2,4-D (100 and 200 ppm) slightly increased the levels of ^{14}C-reducing sugars and at 1000 ppm increased the ^{14}C—malic acid content over the controls.

Introduction

Herbicides are widely used for weed control in tree seedling nurseries (Lund-Hoie, 1970) and the triazines are particularly useful for weed control in conifir nursery beds (Aldhous *et al*, 1968). Although, the triazines interfere with a number of physiological and biochemical processes (Ashton & Crafts, 1973) yet the primary mechanism whereby the triazines cause phytotoxicity is through the inhibition of photosynthetic processes such as the Hill reaction and its associated non-cyclic photophosphorylation (Bishop, 1962). Some reports indicate that chlorophenoxy-acetic acids (e.g. 2,4-D,) at herbicidal concentrations also suppress the rate of photosynthesis (Leonard *et al*, 1966; Sasasaki & Kozlowski, 1967). It has been demonstrated by Kramer & Kozlowski (1960) and Sweet & Wareing (1966) that the factors which affect photosynthesis also consequently influence overall plant growth. Further, it is well known that the growth retarding effects of triazines as well as many other herbicides depend to a great extent on photosynthetic inhibition (van der Zweep & van Oorschot, 1970). The herbicides when used for weed control in forest nurseries, inspite of their selective action, may seriously affect the growth of the tree seedlings at dosages necessary for effective weed control (Winget *et al;* 1963; Aldhous *et al*, 1968).

Bearing these considerations in mind the effects of two chloro-omino-triazines (viz. Simazine and atrazine) and a phenoxyacetic herbicide (2,4-D) on $^{14}CO_2$—fixation and the composition of ^{14}C— assimilates of *Pinus nigra* were studied.

Materials and Methods

$^{14}CO_2$—fixation study:

Seeds of *Pinus nigra* var. *calabrica* Schneid were sown in John Innes No. 1 compost at a rate of 10 seeds per 15 cm diameter pot. A 'Captain' fungicide treat-

*Present address: Department of Botany University of Karachi, Karachi-32, Pakistan.

ment was applied at the time of sowing to prevent damping off of the seedlings. Seedlings were raised in a heated glasshouse and given a 16 h daylength, natural light being supplemented by light from Philip's 400 Watt mercury vapour incandescent lamps (type MBFR) and thinned to 2 per pot 6 weeks after sowing. Ten mililitres of 5,50,200 or 1000 ppm of either simazine (2-chloro-4, 6-bis (ethylamino) -s-triazine), atrazine (2-chloro-4-ethylamino-6-isoprophylamino-s-triazine) or 2,4-D (2,4-dichlorophenoxyacetic acid) were applied to the root system of each seedling 8 weeks after sowing. Six replicate plants were used for each treatment. One week after herbicide treatment, the plants were fed with 5 μ Ci of $^{14}CO_2$ which were supplied to the entire shoot system by enclosing the pot in a polythene bag and liberating $^{14}CO_2$ by the action of N HCl on $Na_2{}^{14}CO_3$ on on a planchette (Lovell *et al*, 1972). The seedling were harvested after 30 minutes: the basipetal translocation of $^{14}C_2$ assimilates during this period was found to be negligible. The needles were oven dried, weighed and the radioactivity estimated quantitatively by the method outlined by Lovell (1969) using a 'Tracerlab' automatic Geiger-Muller proportional counter. Observations of symptoms of developing herbicidal toxicity, if any, were made routinely.

Study of ^{14}C—assimilates:

Seedling of *P. nigra* var. *calabrica* were raised in glasshouse as described above and thinned to two per pot at 8th week after sowing. The seedlings were treated with 10 ml of 100,200 or 1000 ppm of simazine, atrazine or 2,4-D when 9 weeks old. One week after herbicide application each pot (containing 2 seedlings) was supplied with 50 μCi of $^{14}CO_2$, a radiolabel of 50 μCi was found to be the optimum amount for the purpose of autoradiography of radiochromatograms (see below). The seedlings were harvested after 4h, chopped into small fragments and boiled in 80% ethanol for 10 minutes. To accomplish thorough extraction of ^{14}C– metabolites, the extract was kept overnight. Subsequently the extract was centrifuged twice at 4000 rpm and the supernatant was reduced down to 1 ml at 40°C under reduced pressure. The extract was made upto 2 ml with the addition of 50% ethanol. Whatman No. 1 filter papers were loaded with 20 μl of the extract and the descending unidimensional chromatography was performed using the solvent system n-butanol– acetic acid-water 120:30:50 v/v/v (Smith, 1961). The composition of ^{14}C– assimilates was ascertained by the autoradiography of the chromatograms; the later being exposed to Kodirex X-ray films for 3 days. Sugars were identified with the aid of p-anisidine-HCl, ammonical silver nitrate, neptho-rescorcinol and aniline– diphenylamine reagents; amino acids by using BDH ninhydrin spray, and organic acid by aniline-xylose reagent (Block *et al*, 1958). In addition, co-chromatography was performed and Rf– values calculated and matched with the reported ones (Smith, 1961).

Results

(a) *Effects of herbicides on $^{14}CO_2$– fixation:* Simazine at higher concentrations (50 ppm and above) caused a marked reduction in the rate of $^{14}CO_2$ - fixation as indicated by lower levels of specific activities at these dosages (Fig. 1). Whereas, at sublethal dose (5 ppm) simazine significantly augmented the photofixation of pine needles ($p<0.05$)'

Like simazine, atrazine at higher dosages also significantly suppresed the rate of photo-fixation of $^{14}CO_2$ ($p<.01$). However, the adverse effects of simazine

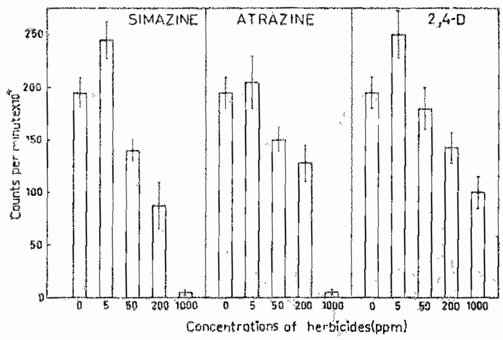


Fig. 1. The effect of herbicide treatment on $^{14}CO_2$—fixation by pine needles. The results are expressed as specific activity values.

were slightly more pronounced in comparison to atrazine as depicted by greater reduction in $^{14}CO_2$- assimilation. Atrazine at 5 ppm, unlike simazine did not alter the rate of fixation. Visible symptoms of damage to the needles following 5 and 50 ppm simazine or atrazine treatment were practically unnoticeable during the entire period of the experiment. The appearance of plants treated with 200 or 1000 ppm simazine or atrazine varied little from the control upto 45 days after treatment. Subsequently, however, colour changes were discernible; the tips of the needles turned yellow and slight wilting was apparent on the 7th days. The toxicity symptoms developed in triazine treatments were substantially more pronounced at 1000ppm.

The herbicide 2,4-D at 5 ppm, like simazine, significantly stimulated the photosynthetic rate ($p<.01$). At 50 ppm, 2,4-D did not show any significant effect but at 200 and 1000 ppm significantly retarded the $^{14}CO_2$- fixation rate of pine needles ($p<.05$). However, the reduction caused by 2,4-D at 200 and 1000 ppm was relatively of much smaller order in comparison to that induced by triazines at these dosages.

(b) *Effects of herbicides on the composition of ^{14}C– assimilates:*—The results of this study are given in Plates 1-3. The ^{14}C- label, in general was present in hexose phosphate, unknown I, sucrose, glucose—fructose (the spots of these two metabolites were not distinguishable individually, except in Plate I, hence they will be collectively referred to as reducing sugars), and malic acid. In addition a little ^{14}C– incorporation in chlorophyll was also observed. Although, no quantitative estimation of ^{14}C– metabolites was performed and qualitative changes due to various treatments were imperceptible in the radiochromatograms, yet from the intensity of various metabolite spots some marked differences in the levels of ^{14}C– assimilates among treatments and controls were easily discernible.

It is evident from the radiochromatograms that simazine atrazine and 2,4-D at all the concentrations used (viz. 100,00 and 1000 ppm) invariably decreased the level of radioactivity present in a range of assimilates. In general, triazines caused a greater reduction in ^{14}C– assimitales than did 2,4-D. The degree of reduction of radiolabel was greater at higher dosages. The heaviest labelling, in general, occurred

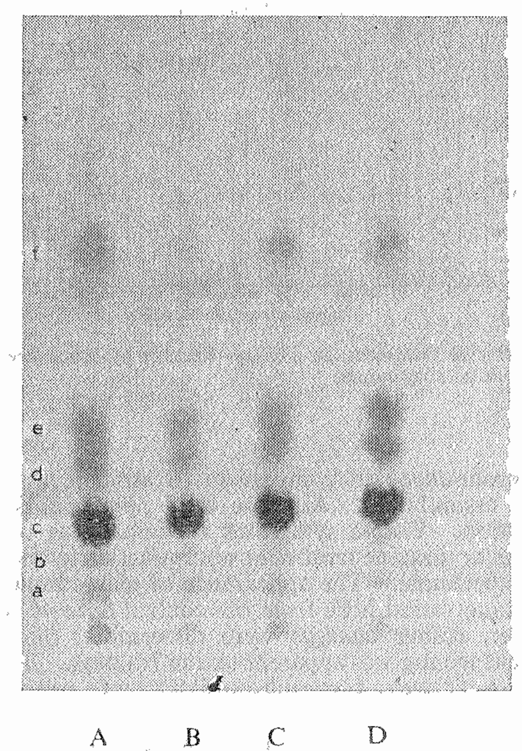
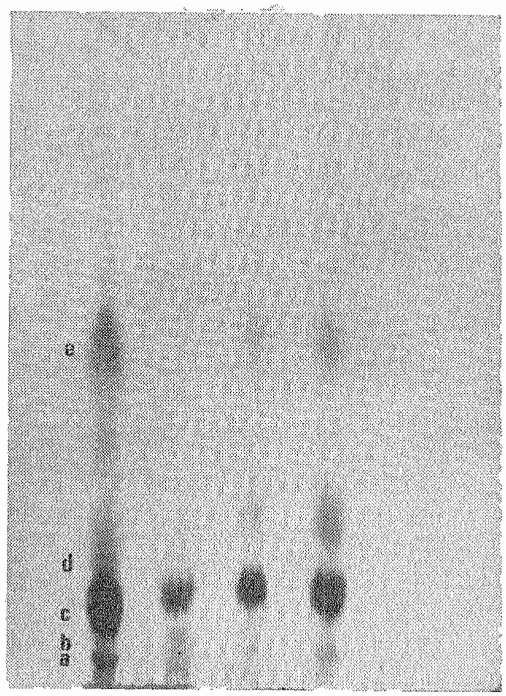


Plate 1. Autoradiograph showing the effect of 100 ppm herbicides on the composition and levels of ^{14}C—assimilates of pine needles.

A, Control; B, Simazine; C, Atrazine; D, 2,4-D. a, hexose phosphate; b, unknown I; c, sucrose; d, glucose; e, fructose; f, malic acid.

in sucrose in comparison to other photosynthates. The amount of ^{14}C - sucrose was reduced by triazines as well as 2,4-D. At 100 ppm this decrease was very small for triazines and marginal in case of 2,4-D. Nevertheless at dosages of 200 and 1000 ppm a marked depletion was readily apparent. The amounts of ^{14}C– sucrose were slightly lesser in simazine than in atrazine treatments at 100 and 200 ppm. The level of ^{14}C– reducing sugar (i.e. glucose and fructose) was considerably diminished by the triazines at 100 and 200 ppm (Plate 1 and 2) and at 1000 ppm ^{14}C-reducing sugars were completely absent in the extracts of simazine and atrazine treated seedlings (Plate 3). However, 2,4-D at 100 and 200 ppm slightly increased the level of ^{14}C– reducing sugars (Plate 1 and 2) but at 1000 ppm this level was not markedly different from that of controls (Plate 3). At 200 ppm, simazine decreased the labelled reducing sugars considerably more than did atrazine (Plate 2). The level of ^{14}C– malic acid declined in the triazine treatments; greater decrease occurred at higher dosages and at 100 ppm no label could be found in malic acid. 2,4-D at 100 and 200 ppm slightly abated the incorpoation of radiolabel into malic acid but at 1000 ppm 2,4-D relatively greater amount of ^{14}C– malic acid was found over the controls.



A B C D

Plate 2. Autoradiograph showing the effect of herbicides on the composition and levels of ^{14}C— assimilates of pine needles. A,B,C, and D, same as in plate I.
a, hexose phosphate; b, unknown I; c, sucrose; d, reducing sugars; e, malic acid.

Discussion

A significant reduction in $^{14}CO_2$— fixation occurred as a consequence of root application of simazine or atrazine at 50,200 and 1000 ppm concentrations. It is well known that triazine toxicity is primarily due to the effect on photosynethetic process which is adversely affected by triazines owing to the uncoupling of the Hill reaction and its associated non-cyclic photophosphorylation (Bishop, 1962), by modification of leaf anatomy (Kozlowski & Clausen, 1966), chloroplast damage (Hill *et al*, 1968), closure of stomata (Walker & Zelitch, 1963) or by reduction in chlorophyll content (Singh & West, 1966). In contrast, at low dosage (5 ppm) simazine enhanced the rate of photofixation though atrazine at the same dose had no such effect. Similarly, using sublethal concentration of simazine (5 ppm in nutrient solution), Lund-Hoie (1969) obtained an increase in the photosynthetic rate of *Picea abies* seedlings. The reason for this stimulation of CO_2— fixation is not known but certain hypothetical possibilities can be raised: a) It has been demonstrated by Agha & Anderson (1967) that simazine at low concentration increases the chlorophyll content in certain species; this would consequently lead to greater rate of photofixation. b) Furthermore, low dosages of triazines are known to enhance the respiration rate (Goren and Monselise, 1965), moreover, at such

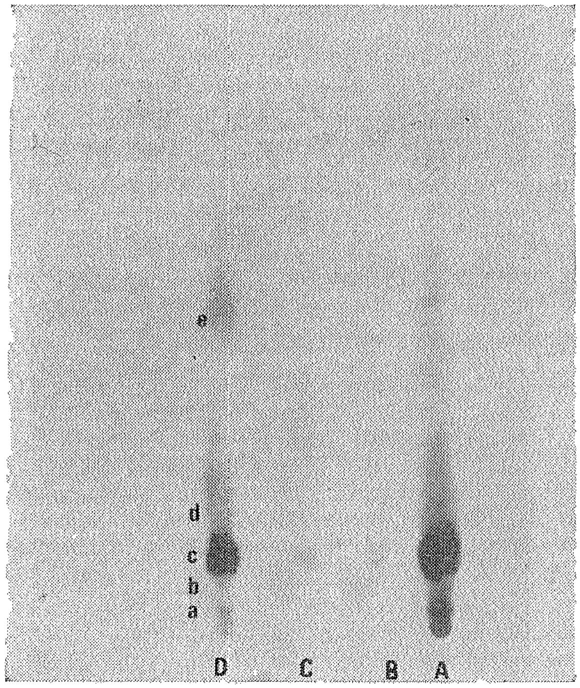


Plate 3. Autoradiograph showing the effect of 1000 ppm of herbicides on the composition and levels of ^{14}C—assimilates of pine needles. A,B,C and D, same as in Plate 1. a,b,c,d, and e, same as in Plate 2.

doses increase in the activity of adenosine triphosphatase has also been reported (Singh & Salunkhe, 1970). A relatively greater amount of energy available would not only augment the loading of assimilates into the conducting tissue but also the subsequent translocation of these organic products (Ziegler, 1956). A very close correlation between the rate of translocation and photosynthesis has often been noted (Hofstra & Nelson, 1969). It is probable, therefore, that the stimulation of photofixation y the low concentration of simazine could have occurred indirectly owing to the enhancement of the rate of translocation of assimilates.

Photofixation was also suppressed by 2,4-D at higher dosages (200 and 1000 ppm) confirming earlier reports of the inhibition of photosynthetic rate by 2,4-D (Sasaki & Kozlowski, 1967). The lesser degree of reduction caused by 2,4-D in comparison to triazines supports the view that photosynthetic inhibition does not constitute the primary action of chlorophenoxyacetic acid herbicides (Robertson & Kirkwood, 1970). The inhibition of $^{14}CO_2$– fixation caused by 2,4-D presumably is in part due to the uncoupling of the Hill reaction (Moreland & Hill, 1962; Chkanikov *et al*, 1966). Evidences also suggest that this inhibition could also be induced indirectly by the closure of the stomata (Bradbury & Ennis, 1952), damage to the assimilation tissues and chloroplasts (Lee *et al*, 1967; Hallam, 1970), disruption of vascular system resulting in a reduction in water or assimilate conduction (Rubin & Gitsaenko, 1968) or by depletion in the chlorophyll content (Kiselev, 1966).

The stimulation in the rate of $^{14}CO_2$– fixation obtained in 5 ppm 2,4-D treatment strengthenes the results of earlier workers (cf. Wort, 1964). This enhancement of photosynthetic rate was probably correlated with the increase in chlorophyll content at this concentration (Shaukat, 1973).

All the three herbicides brought about a reduction in the level of ^{14}C– assimilates presumably owing to the supperession of $^{14}CO_2$– fixation. The levels of assimilates obtained in various treatments corresponded closely with the respective $^{14}CO_2$– fixation rates.

The level of ^{14}C– sucrose declined in all the treatments and this decrease was more pronounced at higher concentrations. However, ^{14}C– sucrose synthesis was not completely checked even a. 1000 ppm of simazine or atrazine. Zweig & Ashton (1962) studied the effect of atrazine on ^{14}C– assimilates in bean leaves and reported that 10 ppm atrazine completely prevented the sucrose synthesis 8h after treatment. The present results are in contrast with this finding since some ^{14}C– sucrose was detected in 1000 ppm atrazine or simazine treatments even 7 days after herbicide application.

Apparently triazines had no effect on the quality or the relative amounts of labelled assimilates, yet the total amounts were substantially reduced. This suggests that $^{14}CO_2$– fixation was decreased in proportion to the number of photosynthetic units (quantatropes) inactivated.

The amount of ^{14}C– sucrose declined in 100,200 and 1000 ppm 2,4-D treatments but a substantial rise occurred in the level of ^{14}C– reducing sugars. This result corroborates the earlier findings (c.f. Robertson & Kirkwood, 1970). The labelled hexose phosphate was also decreased by 2,4-D which indicates that the products of glycolytic cycle were also affected. At 100 and 200 ppm 2,4-D the radio activity in malic acid was considerably abated but this was substantially raised by 1000 ppm 2,4-D. The accumulation of malic acid at 1000 ppm 2,4-D agrees with the results of Mostafa & Fang (1971) and Matlib *et al* (1972) who observed inhibition of the oxidation of Kreb's cycle substrates including malate by phenoxyacetic herbicides which resulted in the accumulation of such substances.

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