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EFFECTS OF SIMAZINE, ATRAZINE AND 2,4-D ON RESPIRATION RATE, SUGAR LEVEL AND AMYLASE ACTIVITY DURING GERMINATION OF *PINUS NIGRA* VAR. *CALABRICA* SCHNEID.

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Abstract

The effects of triazines and 2,4-D on the metabolism of germinating *Pinus nigra* var. *calabrica* seeds were investigated. In controls reducing sugar, respiration rate and amylase activity increased from 30 to 90 h but succrose level declined. Although simazine did not alter the rate of oxygen uptake by germinating seeds, 1000 ppm atrazine suppressed the rate of oxygen consumption. At 5 and 100 ppm, 2,4-D stimulated the aerobic respiration but 1000 ppm 2,4-D significantly reduced the oxygen uptake of germinating seeds. Trianzines did not change the reducing sugar content but 2,4-D at 100 ppm decreased the reducing asugar content at 30 h and increased it at 1000 ppm. Triazines (100 and 1000 ppm) initially decreased the sucrosse content, but afterwards elevated the sucrose level over the controls. Amount of sucrose at 30 h was reduced by 100 and 1000 ppm 2,4-D but at 60 and 90 h, 1000 ppm 2,4-D raised the sucrose content over the controls. Amylase activity was enhanced by 5 ppm 2,4-D and suppressed by higher dosages of the herbicides.

Introduction

The physiological and biochemical changes which take place in seeds at the commencement of germination and during germination are likely to be modified by factors which affect the rate and percentage of germination. In a previous study simazine and atrazine retarded the germination rate of *Pinus nigra* during the first few days, and at high concentrations (1000 and 2000 ppm) slightly reduced the final percentage germination (Shaukat. 1973). However, 2,4-D at 100 ppm and above substantially inhibited germination of *P. nigra*.

Hsueh & Lou (1947) demonstrated that 2.4-D at 100 and 1000 ppm inhibited aerobic respiration of rice and barley seeds during the germination phase 24 h after treatment. Whereas, Levari (1953) found that the respiration of germinating wheat seeds was reduced at 10 h after treatment with 2.4-D (100,500 or 1000 ppm) but that of lettuce seeds was not significantly altered by 10.25 or 100 ppm of 2.4-D, though these concentrations were inhibitory to germination.

Ashton & Uribe (1962) and Sasaki & Kozlowski (1967) showed that active ingredients of triazines do not affect respiration of germinating seeds. Funderburk & Davis (1963), however, found that respiration of seedlings was increased initially (1 to 2 h after triazine treatment) but the rate of respiration was reduced subsequently.

Apparently there are no reports on the effects of herbicides on sugar metabolism of germinating seeds, although the effect of coumarin, a germination inhibitor was studied by Poljakoff-Mayber (1952) who observed that 100 ppm coumarin inhibited the formation of reducing sugars in germinating lettuce seeds.

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The effect of 2,4-D on amylase activity varies between species, between organs of different plants and dosages; it may increase the amylase activity (Freiberg, 1955), may have no effect (Tomizawa & Koike, 1954) or inhibit the activity of amylase (Maciejewska-Potapczyk, 1955). Studies with triazines have indicated that they have stimulatory effect at low dosages (Singh & Salunkhe, 1970). The purpose of this investigation was to study the biochemical changes which take place during normal germination and also in the presence of triazines and 2,4-D.

Material and Methods

(a) *Germination conditions:* The seeds of *Pinus nigra* var. *calabrica* Schneid., obtained from Forestry Commission, U.K. were treated with 5, 100 and 1000 ppm of simazine (50% wettable powder), atrazine (50% wettable powder) and 2.4-D (pure) in petri dishes. Each of the 85mm plastic petri dishes contained 20 seeds plus either 5 ml of distilled water (control) or 5 ml of the appropriate herbicide concentration on Whatman No. 1 filter paper. The concentrations of herbicides were based exclusively on the proportion of active ingredients. Te dishes were placed in a growth chamber at 22 ± 1°C. and 55-65 % RH with 6 KLux light intensity.

(b) *Determination of respiration rate.*—The respiration rate was measured at 30,60 and 90 h of imbibition by a direct warburg method (Umbreit *et al*, 1957). The main compartment of Warburg vessel contained 10 germinating seeds and 1 ml of distilled water. The centre well contained 0.2 ml of 20% KOH solution and a 4 cm^2 folded piece of Whatman No. 40 filter paper, to prevent the spillage of KOH outside the well. The water bath containing the manometers was maintained at 25°C. Readings were taken at 20 min. intervals for 1 h. Each treatment and control was replicated thrice.

(c) *Determination of sugars.*—The seeds were treated and germinated as described above. Analysis was carried out on seeds at 30,60 and 90 h after the start of imbibition.

For the extraction of sugars a method based on Grant & Fuller (1968) was adopted. Forty seeds (from 2 Petri dishes) were thoroughly ground in 80% ethanol and the mixture brought to 40 ml by further addition of ethanol and then boiled for 1 min. in order to denature the enzymes (viz. invertase, amylase, etc.). A further extraction for 3 h at 70°C was then carried out before centrifugation at 4000 rpm for 15 min. The supernatant together with the washings of the residue were re-centrifuged at 4000 rpm for 10 min. and then reduced to approximately 3 ml. at 40°C in a rotary vacuum evaporator. This was made upto 20 ml with distilled water and was used for analysis. Two ml of the extract were added to 2 ml of the DNS (3:5) di-nitrosalicylic acid) reagent. The mixture was heated in a boiling water bath for 5 min., cooled in running tap water and made upto 10 ml with distilled water. The absorbance was measured between 400-700 nm. The optical densities at 525 nm were compared with standard solutions of glucose.

For the analysis of sucrose, 1 ml of the extract was added to 1 ml of water. To this mixture 0.2 ml of invertase concentrate (340 E.U./ml) were added and left at a temperature of 20°C for 20 min. before adding 2 ml DNS reagent, after which the analysis was performed as above.

(d) *Measurement of amylase activity.*—The material used was similar to that described for the measurement of oxygen uptake. Amylase activity of seeds was ascertained at 30,60 and 90 h after the commencement of imbition and measured by an agar-gel diffusion method described by Clum (1967) with minor modifications. The starch agar substrate was prepared by adding 1 g of soluble starch and 10 g Oxoid No. 3 to one litre of boiling distilled water. The mixture was stirred for 3 min. and poured into 90mm Petri dishes, @ 25 ml/dish. An amylase extract was prepared by macerating 20 germinating seeds (from one Petri plate) in 25 ml ice cold 0.2 N acetate buffer (pH 5.3). The macerate was centrifuged at 4000 rpm for 10 min. The clear surpernatant was made upto 49 ml with the buffer and 1 ml chloramphenicol solution (5000 ppm) was added to give the extract a final concentration of 10 ppm chloramphenicol to control the growth of micro-organisms without seriously affecting the metabolism of tissue or tissue extract (Sabota *et al*, 1968). Four Whatman No. 1 filter paper discs (each 9 mm in diameter) were evenly placed on agar-gel plate. Each disc received 10 µl of amylase extract. The plates were then placed at 21°±1°C for 7 days. After this the discs were removed and plates flooded with I_2KI solution. The diameter of the clear zones were measured twice at right angles. Each treatment and control was replicated three times.

Results

(i) *Metabolism of untreated germinating seeds:*

The respiration of control seeds during the germination period (upto 90 h) increased rapidly with time (Fig. 1) . The reducing sugar content and amylase activity gradually increased with time but the sucrose content decreased during this period (Fig. 1).

(ii) Effects of herbicides on the aerobic respiration rate:

Respiration of germinating seeds, in general, increased with time (Fig. 2). Simazine did not significantly alter the rate of oxygen consumption of the germinating

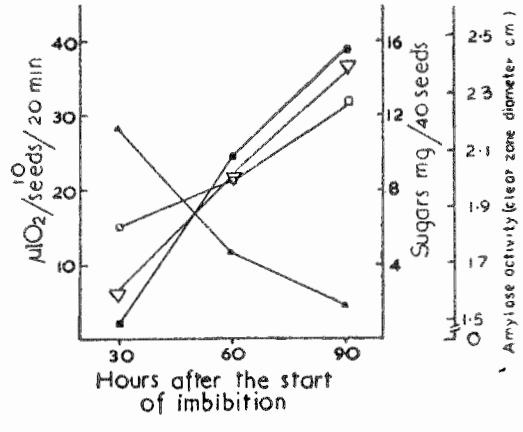

Fig. 1. Changes in sugars, respiration rate and amylase activity in untreated control seeds of *inus nigra* during germination. Respiration (□ —— □); reducing sugars (■ —— ■); sucrose (▲ —— ▲); amylase activity (△ —— △).

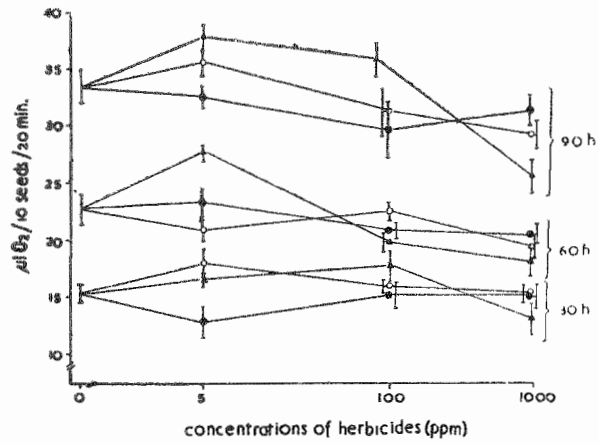

Fig. 2. The effect of simazine, atrazine and 2,4-D on the oxygen uptake of germinating seeds of *Pinus nigra*. (● —— ●) Simazine; (○ ——○) atrazine; (▲——▲) 2,4-D Measurements were taken 30, 60 and 90 h of imbibition.

seeds. Atrazine at 5 ppm slightly stimulated respiration at 30 h but subsequently did not affect oxygen uptake. At 1000 ppm, atrazine slightly inhibited oxygen consumption at 90 h. The effect of 2,4-D on respiration was distinctly different from that of the triazines: 2, 4-D at 5 ppm significantly stimulated the O_2 absorption at 60 and 90 h. At 100 ppm 2, 4-D slightly increased the oxygen consumption at 30 and 90 h. However, 1000 ppm 2, 4-D markedly suppressed oxygen uptake of the germinating seeds at 60 and 90 h. ($p<0.01$).

(iii) *Effect of herbicides on the sugar content:*

The effects of herbicides on reducing sugar and sucrose levels are graphically presented in Figs. 3 and 4 respectively. Reducing sugar content of the seeds increased from 30 to 80 h while the level of sucrose in general, declined during this period. Neither atrazine nor did simazine change the reducing sugar content of the germinating seeds at any of the concentrations used. Whereas. 100 ppm 2, 4-D at 30 h decreased the reducing sugars to almost half that of control level and at 1000 ppm nearly doubled the amount as compared to the control. Except for these changes, the herbicides. in general, had little effect on the amount of reducing sugars in the germinating pine seeds. The sucrose content, however, was invariably altered by the herbicides in general. Simazine at 100 ppm reduced the sucrose content at 30 h, whereas at 60 and 90 h 1000 ppm simazine increased the sucrose level in germinating pine seeds. A similar trend was depicted by atrazine. At 100 and 1000 ppm atrazine substantially decreased the sucrose content at 30h but had no effect at 60 h. In 90 h germinated seeds greater amounts of sucrose were found in 100 and 1000 ppm atrazine treatments than in the controls. Like the triazines 2,4-D (100 and 1000 ppm) gave rise to lower levels of sucorse than did control seeds at 30 h. This response being more pronounced than that induced by the triazines. At 60 h there was a slight increase at 1000 ppm 2,4-D. However, at 90 h, unlike the triazines, 100 ppm 2,4-D reduced the

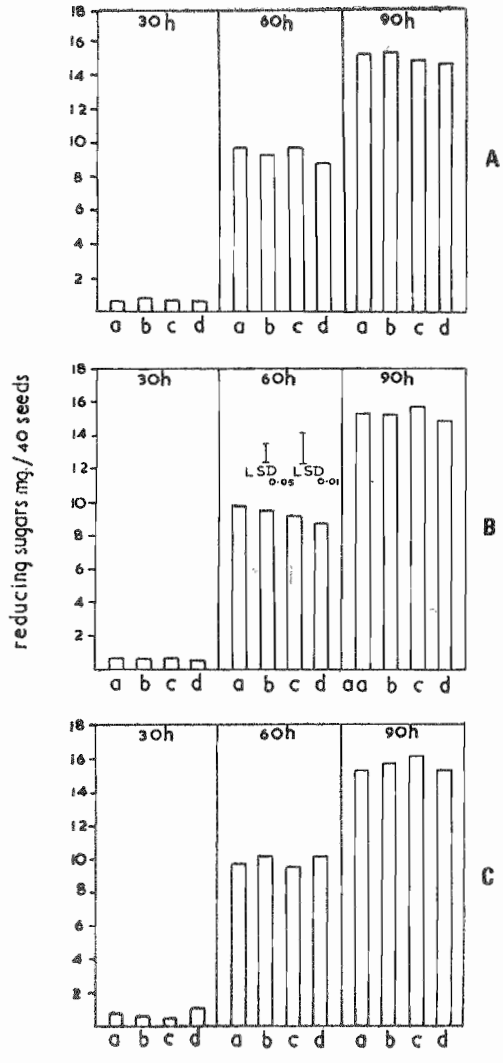

Fig. 3. The effect of simazine, atrazine and 2,4-D on reducing sugar content of germinating seeds of *Pinus nigra*. A, simazine; B, atrazine; C, 2,4-D. a, control; b, 5 ppm; c, 100 ppm; d, 1000 ppm. Measurements were taken 30, 60 and 90 h after the start of imbibition.

sucrose content to almost half that of control, whereas seeds treated with 1000 ppm 2,4-D (which did not germinate) had slightly higher level of sucrose as compared to controls.

(iv) *Effect of herbicides on the amylase activity:*

The results of amylase activity, as expressed by the diameter of the clear-zone on starch-agar plates are given in Table 1. Amylase activity, in general, increased

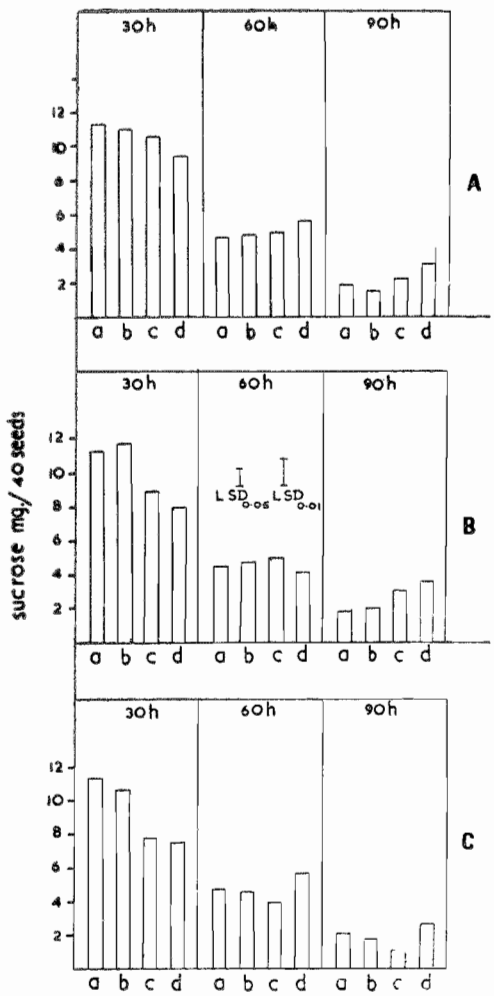

Fig. 4. The effect of simazine, atrazine and 2,4-D on sucrose content of germinating seeds of *Pinus nigra*. A, simazine; B, atrazine; C, 2,4-D. a, control; b, 5 ppm; d, 1000 ppm.

gradually during the period of observation. Triazines at 5 ppm did not affect the amylase activity. At 30 h. only 1000 ppm simazine significantly inhibited the amylase activity ($p<0.05$). However, at 60 and 90 h both simazine and atrazine substantially suppressed the activity of this enzyme. Though a sublethal dose of 2,4-D (5ppm) markedly stimulated the amylase activity, higher concentrations (100 and 1000 ppm 2,4-D) significantly reduced the amylase activity over the controls ($p < 0.01$).

Discussion

The control as well as treated seeds showed a definite pattern of metabolic changes during germination. The control seeds showed an increase in oxygen uptake, a decline in the level of sucrose but an increase in reducing sugars coupled with a gradual increase in amylase activity (which is presumably breaking down starch and dextrin reserves) during the process of germination.

The increase in oxygen uptake as germination proceeds in the germinating pine seeds agrees with the findings of Nyman (1963). Of the two triazines used here only atrazine induced changes in respiration rate and this corresponds to the results of Sasaki & Kozlowski (1967) who demonstrated that the respiration rate of 4-6 day-old *Pinus resinosa* seedlings was only inhibited by atrazine and not by simazine. The greater toxicity observed for atrazine, viz. greater inhibition of germination percentage and rate and emergence percentage, in comparison to simazine partly substantiates this observation (Shaukat, 1973).

The increase in oxygen uptake induced by 2,4-D at 5 and 100 ppm supports the finding of Williams & Dunn (1961). However, the oxygen consumption was remarkably suppressed by 1000 ppm 2,4-D which corresponds with the results of Hsueh & Lou (1947).

A gradual decrease in the sucrose content and an increase in the reducing sugar content with time during germination in treated as well as in control pine seeds was observed. Somewhat similar changes, have been noticed by Hattori & Shiroya (1951) and Simancik & Simak (1968) in *Pinus thumbergi* and *P. sylvestris*

TABLE 1. Effect of herbicides on amylase activity of germinating *Pinus nigra* seeds, in terms of clear zone diameters (cm.) Standard errors are given against means.

Treatments	Dose ppm	Hours of imbibition		
		30	60	90
Control	0	1.58±0.16	2.01±0.17	2.38±0.13
Simazine	5	1.63±0.18	1.89±0.12	2.56±0.09
,,	100	1.71±0.23	1.69±0.07	1.72±0.17
,,	1000	1.30±0.08	1.77±0.20	1.68±0.15
Atrazine	5	1.62±0.18	2.10±0.14	2.27±0.13
,,	100	1.55±0.22	1.79±0.11	1.88±0.24
,,	1000	1.53±0.13	1.55±0.19	1.61±0.09
2,4-D	5	1.73±0.10	2.36±0.15	2.67±0.11
,,	100	1.88±0.16	1.72±0.07	1.50±0.17
,,	1000	1.40±0.13	1.46±0.18	1.38±0.26

respectively. Neither simazine nor did atrazine produce any change in the reducing sugar content of germinating pine seeds, although both of them did inhibit amylase activity as a consequence of which lesser amounts of reducing sugars should be expected in the treatments. However, Bradbeer (1958) and Beevers (1961) have shown that storage fat in the seeds is converted into carbohydrates during germination. This could be the possible reason for the increase in reducing sugar content in the triazine treatments, inspite of the inhibition of amylase activity.

Sucrose was found to be present in lesser quantity at 30 h but greater at 90h in seeds treated with 100 and 1000 ppm 2.4-D or atrazine than the controls. In case of 2,4-D this could be due to initial stimulation and a later inhibition of invertase activity as observed by Tomizawa & Koike (1954) in rice and sweet potato. Howver, Hofmann & Schmeling (1953) using *Vicia* and *Taraxacum* and Flood *et al* (1970) using chicory root found increased invertase activity after 2,4-D treatment. Others (Neely *et al*, 1950) were unable to find any effect of 2,4-D on invertase activity.

Whether the similar change in sucrose content exhibited by the triazine treated seeds are also due to such enzymatic changes, is uncertain as nothing is known about the effect of triazines on invertase activity.

Amylase activity was inhibited by the triazines as well as by 2,4-D at 100 and 1000 ppm at 60 and 90 h. As the radicle grows rapidly during this period it needs hydrolysed food material for its development. Consequently inhibition of amylase activity could result in the suppression of root growth. The decrease in amylase activity as a result of 2,4-D treatment (100 and 1000 ppm) corroborates the findings of Tomizawa & Koike (1954) and Maciejewska-Potapaczyk (1955). The increase in amylase activity in 5 ppm 2.4-D treatment correlated very well with the observation that shoot growth was stimulated and no reduction in dry weight occurred at this concentration (Shaukat, 1973). Increase in amylase activity at low dosages of 2,4-D has also been demonstrated by Tomizawa & Koike (1954). On the other hand, low concentrations (5 ppm) of simazine and atrazine did not increase the amylase activity, which is contrary to the observations of Singh & Salunkhe (1970). Furthermore, the significant inhibition of amylase activity at 100 and 1000 ppm of both simazine and atrazine found in the present study seems to be previously unreported.

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