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The Study of the Effect of Amitrol on the Respiration and Activity of Some Enzymes in Poppy Plants (*Papaver somniferum* L.)

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Abstract. *Papaver somniferum* plants grown in pots in the phase of forming the first pair of leaves were treated by 6·4 ml of 0·02% solution of 3-amino-1,2,4-triazol (amitrol) per single pot, which is equal to the dose of 2 mg of amitrol per 100 cm² soil area One, four, seven and ten days after the application the effect of the herbicide on some physiological processes in the overground organs of plants was investigated.

Amitrol decreased the activity of catalase by 60% compared with control plants 1 day after the application, even though no external symptoms of damage were evident. On further action, the activity of catalase was suppressed somewhat less. The respiration was not inhibited by amitrol to such an extent as the activity of catalase. One day after the application the respiration was not suppressed significantly, at further dates the respiration activity decreased. The difference between the respiration of treated and control poppy plants was particularly evident when the oxygen consumption was referred to a single plant. Under these experimental conditions we failed to determine the activity of ascorbinoxidase and peroxidase. The activity of polyphenoloxidase was stimulated by amitrol from the fourth day after the treatment.

On the basis of these experiments we can say that poppy has not a physiologically based resistance to amitrol.

Poppy is one of the few crops for which no selective herbicides are yet available. Even though a series of different herbicides was investigated mainly in France, none of them was found sufficiently selective for use in wide practice. Certain selectivity under field experimental conditions was demonstrated by amitrol applied pre-emergently (SCHMITLIN, COT, MALLET, DUMAY 1965) which was confirmed also in our logarithmic field experiments (ZEMÁNEK 1966, unpublished) in which the herbicide was tested in a series of concentrations increasing geometrically.

For the evaluation of the actual selectivity of herbicides it is necessary to carry out not only the visual evaluation of symptoms of damage of the cultural plant or determination of yields, but also to investigate the effect of the herbicides on important physiological processes in plants. These determinations may reveal the unfavourable action of herbicides even when

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no external symptoms of damage are evident, as proved in the study of the effect of pyrazon on sugar-beet and white mustard (ZEMÁNEK, MYDLILOVÁ, BYLINSKÁ 1966). For this reason, we began to study the effect of amitrol on the physiological processes in poppy plants to determine whether in this case the selectivity is actual or relative.

Material and Methods

For the experiments a preparation containing 50% of 3-amino-1,2,4--triazol (amitrol) was used. The poppy seeds (*Papaver somniferum* L.) cultivar Hanácký modrý were sown in the soil in pots (upper diameter 9 cm) 0.5 cm deep. The soil used was from the garden in Ruzyně and had the following characteristics: pH 6.5, humus content 2.1% content of particles >0.25 mm — 12.3%, <0.01 mm — 37.7%. The plants were grown in a greenhouse (temperature 18—22° C) .The application of herbicide was carried out at a time when the poppy plants started to form the first pair of leaves (length 2—3 mm). The volume of 6.4 ml of 0.02% solution of amitrol equal to the dose of 2 mg active substance per 100 cm^2 (2 kg/ha) was put in homogeneously by drops by a pipette on the surface of soil in the pot. The pipetting was carried out carefully so that the poppy leaves were not contaminated. The effect of the herbicide on the physiological processes in poppy plants was investigated 1, 4, 7 and 10 days after the application.

The respiration intensity was determined on Warburg's respirometer. For the experiments the overground organs of plants were taken i.e. cotyledon leaves and the first pair of leaves with hypocotyl. Twenty plants were usually placed in the respirometer vessel, 0.2 ml of 10% KOH was pipetted to the central well and 0.5 ml of distilled water to the sidearm of the vessel. Further procedure was the same as described before (ZEMÁNEK, AMBROŽOVÁ 1966). The respiration was always determined in parallel on 4 manometers with treated plants and on 4 manometers with untreated plants and was expressed by metabolic quotient Q_{O_2}, i.e. by the amount of oxygen consumed in μl per 1 mg of dry weight per 1 hour. In addition, the average consumption of oxygen by a single plant per 1 hour was calculated. The significance of the difference between treated and control plants was proved by t-test. Moreover, the respiration of plants treated by amitrol was expressed in percentage of the control and was plotted graphically.

The activity of catalase was determined by the gasometric method (VOŘÍŠEK, ŠEBÁNEK 1963). The overground parts of plants (0.5 g of fresh weight) were crushed in a mortar with 0.25 g of $CaCO_3$. Then 10 ml of distilled water was gradually added and the mixture was placed in a flask into which a small vessel containing 2 ml of 3% H_2O_2 was inserted and the flask was connected to a catalasemeter. The amount of formed oxygen was read during continual agitation of the flask at one minute intervals for 5 minutes. The activity was determined alternately in control plants and in plants treated

with amitrol. Each experiment was repeated four times. The results are expressed by the amount of oxygen formed by 0.5 g of fresh plants in 5 minutes or referred to a single plant. Relative values were expressed graphically.

The activity of ascorbinoxidase, polyphenoloxidase and peroxidase was determined by the method of POVOLOCKAYA and SEDENKO (1955). These enzymes oxygenate ascorbic acid in the presence of pyrocatechine and at optimal pH (5; 7.5). The amount of the unoxygenated ascorbic acid was determined with 2,6-dichlorophenolindophenol. The activity of enzymes is thus expressed in mg of 2,6-dichlorophenolindophenol necessary for the titration of enzyme extract prepared from 1 g of fresh weight of plants.

Results

The results of the experiments are presented in Tables 1 and 2 and relative values expressed in per cent of catalase activity and respiration of control plants are shown in Fig. 1. The symptoms of damage to poppy plants by amitrol were apparent as a slight yellowing of the leaves 4 days after the application. Clear symptoms (chlorose of leaves and retarded growth of the plants) were visible in some cases after 7 days but mainly after 10 days when several plants died.

Amitrol affected the catalase activity 1 day after the application. The activity was lowered by 60% compared with the control plants, even though no external symptoms of damage were evident. The catalase activity was

Table 1

Effect of amitrol on catalase activity in poppy plants

Number of days after application	ml O_2 per 0.5 g per 5 min.			ml O_2 per 1 plant per 5 min.	
	control	amitrol	t-value	control	amitrol
1	24·8	9·8	57·9	0·82	0.30
4	25·3	12·9	19·9	0·92	0·45
7	24·7	11·6	4·86	1·41	0·60
10	22·1	16·1	4·17	1·47	0·69

Table t-value is 2.45 at P = 0.05 and 3.71 at P = 0.01

affected also at further dates of determination, with the tendency (when evaluating the enzyme activity according to fresh weight) that the suppression of activity was not so pronounced compared with control plants mainly 10 days after the application, though in this interval the difference between the activity of treated and untreated plants was also highly significant. This is perhaps due to the fact that after 10 days some plants died, so that the plants selected for the measurement were more resistant to the action of the herbicide.

Table 2
Effect of amitrol on the respiration of poppy plants

Number of days after application	µl O_2 per 1 mg dry weight per 1 hour			µl O_2 per 1 plant per 1 hour		
	control	amitrol	t-value	control	amitrol	t-value
1	3·36	3·13	1·54	2·97	3·03	0·45
4	2·74	2·41	15·73	3·50	2·90	2·42
7	2·80	2·45	7·03	5·10	3·80	7·84
10	3·38	2·80	3·18	8·20	4·15	6·80

The course of respiration was somewhat different. On the whole, we can say that the respiration was not suppressed by amitrol to such an extent as the catalase activity. One day after the application, the respiration was not evidently suppressed but decreased gradually on the further action of the herbicide. The difference between the respiration of treated and untreated plants was especially perceptible when the oxygen consumption was referred to a single plant. In this case the suppression of the growth of plants by amitrol exerted influence upon the respiration rate.

Under these experimental conditions we failed to determine the activity of ascorbinoxidase and peroxidase with the applied method. The activity determined was either very low or none. We determined only the activity

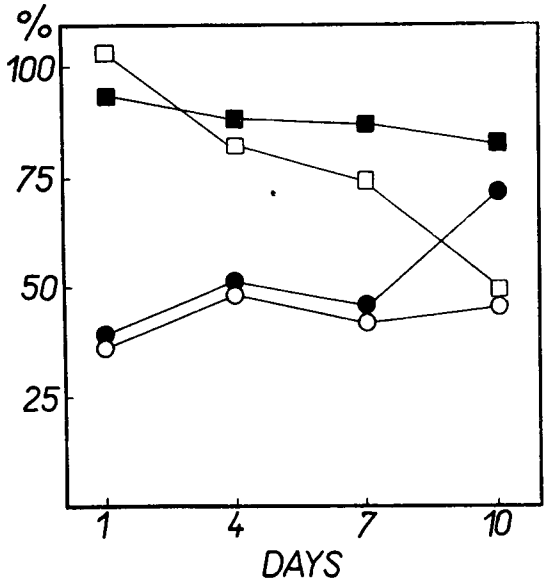

Fig. 1. Respiration and catalase activity of poppy plants (in per cent of activity of control plants) 1 day, 4, 7, 10 days after the application of amitrol
●——● catalase activity (per fresh weight of plants)
○——○ catalase activity (per single plant)
■——■ respiration (per dry weight of plants)
□——□ respiration (per single plant)

of polyphenoloxidase; its relative values for the plants treated by amitrol compared with control plants were: after 1 day 94%, after 4 days 142%, after 7 days 184%, after 10 days 156%. The activity of polyphenoloxidase was thus stimulated by amitrol.

Discussion

In these experiments we proved that amitrol applied to the roots of poppy plants affects the physiological processes in the overground organs of these plants. The activity of catalase was especially very strongly decreased in the first day after the application of the herbicide (almost by 60%), on further action (4, 7, 10 days) the inhibition decreased. This result is in keeping with the results of MAKOVCOVÁ (1965) with white mustard showing that the unfavourable effect of amitrol on the plant gradually decreases. The respiration of poppy plants was reduced by amitrol much less than the activity of catalase, viz. in the range of 7—17%, which agrees also with MAKOVCOVÁ's data (1965); however, we found continued suppression of the respiration of poppy plants while she had found a stimulation of the respiration 5—6 days after the application. These differences may be due to different kinds of plant tested (white mustard), to the application of amitrol in the earlier stage of development (initial stage of germination) and to the different procedure in growing the plants. The activity of polyphenoloxidase was stimulated by amitrol, which is very similar to the stimulation of the activity of peroxidase found in white mustard as a result of the action of amitrol (MAKOVCOVÁ 1965) or pyrazone (ZEMÁNEK, AMBROŽOVÁ 1967).

From the results presented we may conclude that poppy is not physiologically resistant to amitrol. The resistance is only relative, so that under unfavourable external conditions (light soil, washing of the herbicide with rain water to the seed of poppy etc.) the culture may in field conditions be damaged by amitrol.

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Rostliny máku setého pěstované v kořenáčích byly ošetřeny ve fázi prvého páru pravých listů pomocí pipety množstvím 6,4 ml 0,02% 3-amino-1,2,4-aminotriazolu (amitrolu) na 1 kořenáč, což odpovídá dávce 2 mg amitrolu na 100 cm². Za 1 den, za 4, 7 a 10 dní po aplikaci byl stanovován vliv herbicidu na některé fyziologické pochody v nadzemních částech rostlin.

Amitrol snížil aktivitu katalázy o 60 % ve srovnání s neošetřenými rostlinami již za jeden den po aplikaci, přestože v této době nebyly patrny ještě vůbec žádné vnější symptomy poškození. V dalších termínech hodnocení byla aktivita katalázy potlačována o něco méně. Respirace nebyla inhibována amitrolem v takovém měřítku jako aktivita katalázy. Za jeden den po aplikaci respirace průkazně potlačena nebyla, teprve v dalších termínech měření se respirační činnost snižovala. Rozdíl mezi respirací ošetřených a neošetřených rostlin máku byl zvláště patrný při přepočtu spotřeby kyslíku na jednu rostlinu. Aktivitu askorbinoxidázy a peroxidázy se nepodařilo za daných pokusných podmínek zjistit. Aktivita polyfenoloxidázy byla amitrolem stimulována počínaje čtvrtým dnem po ošetření rostlin.

Na základě těchto pokusů lze usuzovat, že mák setý nemá fyziologicky podmíněnou odolnost vůči amitrolu.

Й. ЗЕМАНЕК, Я. АМБРОЖОВА, Центральный институт растениеводства, Прага: **Изучение влияния амитрола на дыхание и активность некоторых ферментов у растений мака (*Papaver somniferum* L.).** — Biol. Plant. **9** : 270—275, 1967.

Растения мака, выращиваемые в горшках, были в фазе первой пары листьев обработаны при помощи пипетки 6,4 мл 0,02%-ного раствора 3-амино-1,2,4-аминотриазола (амитрола) в расчете на 1 горшок, что соответствует дозе 2 мг амитрола на 100 см² поверхности. Определялось влияние гербицида на некоторые физиологические процессы в надземных частях растения через 1, 4, 7 и 10 дней после обработки.

Амитрол понизил активность каталазы на 60% уже через 1 день после обработки, хотя в это время никакие симптомы повреждения еще не проявились. В последующие дни активность каталазы была подавлена несколько слабее. Дыхание подавлялось амитролом в меньшей мере, и лишь при определении в более поздние сроки. Различия в потреблении кислорода растениями, обработанными и не обработанными гербицидом, оказались особенно выразительными при пересчете потребления кислорода на одно растение. Разницу в активности аскорбиноксидазы и пероксидазы при данных условиях опыта определить не удалось. Активность полифенолоксидазы стимулировалась амитролом, начиная с 4-го дня после обработки.

На основании опытов можно сделать заключение, что мак не является физиологически устойчивым по отношению к амитролу.