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A method for rapid testing of the photosynthesis-inhibiting activity of herbicides by leaf disk infiltration

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Abstract

A method for rapid detection of photosynthesis inhibitors in low concentration (0.25--1.25 ppm) was developed. The experiments were performed on disks cut from young bean leaves. The disks were infiltrated with solutions of the tested compounds and placed at the bottom of a crystalliser containing an acidic sodium carbonate solution and then illuminated. The toxicity of the tested substance was measured as the number of disks coming to the surface. It was found that linuron, monolinuron, metoksuron, atrazine and prometrine inhibited floating of the disks, whereas 2,4,5-T, MCPA and chlorpropham gave no effect. This confirms the specificity of the test which is appropriate for determining the phytotoxicity of typical photosynthesis inhibitors.

INTRODUCTION

The increase in plant production is connected with the ever wider application of plant protection agents in plant cultivation, particularly of herbicides. The toxicity of these compounds depends on their ability of inhibiting one or several metabolic processes, thus leading to the death of the plants (A u d u s, 1976; C o r b e t t, 1974). Some physiological processes occur in plants similarly as they do in animals, therefore such herbicides should be chosen which act solely on processes in plant organisms. One of these processes is photosynthesis. The toxicity of herbicides is usually determined in growth tests which do not, however, indicate whether the given compound inhibits photosynthesis (B a ń k i, 1978; K r a t k y and W a r r e n, 1971; P a r k e r, 965; I O R, 1961). Although there exist methods supplying this information, they are time-consuming or require expensive apparatus (A u d u s, 1976; B i e l e c k i and S k r a b k a, 1976; Š e s t a k et al. 1974).

As convenient material for testing photosynthesis inhibitors may serve leaf segments which go down to the bottom in solutions of photosynthesis-inhibiting compounds (T r u e l o v e et al., 1974; D a S i l v a et al., 1976). As the consequence of inhibition of the assimilation process slow penetration of the solution into the intercellular spaces occurs, causing dropping of the leaf disks to the bottom. In this way sensitivity of the particular plant varieties to herbicides can be established (G a w r o ń s k i et al., 1977; G a w r o ń s k i, 1978).

The aim of the present studies was the development of a rapid method making possible testing of the inhibitory effect of various compounds of herbicide type on photosynthesis and comparison of their phytotoxicity. In the experiments advantage was taken of the finding that leaf disks infiltrated with acidic sodium carbonate after illumination float to the surface of the solutions (W i c k l i f f and C h a s s o n, 1964; W i t h a m et al., 1971). During infiltration the intercellular spaces fill with the solution causing dropping of the disks to the bottom. Their exposure to light favours photosynthesis, owing to which the oxygen evolved expulses the solution from the intercellular spaces, causing floating of the disks to the surface. Photosynthesis inhibitors which prevent oxygen evolution should make floating upwards impossible.

MATERIAL AND METHODS

The experiments were performed with beans of the cv. 'Wiejska' (*Phaseolus vulgaris* cv. 'Wiejska'). The seeds were set in containers with sand. After germination they were transferred to pots filled with sand containing modified Richter's medium (fivefold lower amount of iron in the form of chelate), pH 6.8. The substrate moisture was maintained at 70 per cent of full water capacity. The plants in pots were placed for three weeks in a growth chamber under light of 20 000 lux intensity with relative air humidity around 60 per cent and temperature 25°C and an 8 hour period of darkness.

The procedure in all experiments was as follows: disks 7 mm in diametre were cut out between the ribs from young developed bean leaves. After keeping them in darkness for 60 min on the surface of distilled water in order to restore the physiological equilibrium of the tissue, they were infiltrated with water or the herbicide solution.

Infiltration was done in a vacuum flask of 250 ml volume containing 100 ml of the solution. Air was removed from the flask by means of an oil vacuum pump during 30 s and then pressure in it was suddenly increased by shutting off the pump. The gas from the intercellular spaces passed out of the tissue and the solution surrounding the tissues penetrated into them and the disks fell to the bottom. The procedure was repeated twice or three times, until all the disks fell to the bottom. The disks immersed in the solution were kept in darkness for time

referred to as "induction time", and then transferred to crystallisers of 150 cm^3 capacity containing 100 cm^3 of 0.05 M $NaHCO_3$ solution. The crystallisers with the disks were placed in the growth chamber under 6000 lux light at a temperature of $\pm$ 26°C. The photosynthesis-inhibiting action of the herbicide was evaluated on the basis of the number of leaf disks floating to the surface after a definite time and expressed as per cent of the control. The herbicide concentrations applied were mamy times lower than those used in field practice. As control were served leaf disks infiltrated with distilled water.

In order to establish optimal conditions for testing the photosynthesis-inhibiting activity several experiments were performed to successively establish: (1) the mode of introduction of the herbicide into the tissue, (2) the time of induction, that is the time during which the disks should be kept in the herbicide solution in darkness and (3) the time of exposure to light of the disks for making photosynthesis possible and their floating to the surface of the $NaHCO_3$ solution (CO_2 source).

The experiments were run in three replications. Observations on the floating of the leaf disks to the surface of the solution were carried out in each replication on 30 disks chosen randomly from a larger lot when they were transferred to the crystallisers with $NaHCO_3$. The numerical results expressed as per cent of the control were statistically elaborated by analysis of variance at $p = 0.05$. The significance of the differences was evaluated by Duncan's multiple range test allowing the establishment of homogeneous groups. Only the final results of statistical analysis are given in the tables, the numerical results not differing significantly belonging to homogeneous groups are denoted by the same letter.

RESULTS

Mode of introduction of the herbicide into the tissue

Two methods were compared in the experiment of monolinuron introduction. This substance is a photosynthesis inhibitor and was applied in several concentrations: (a) the leaf disks were infiltrated with distilled water before placing in monolinuron solution, (b) the disks were infiltrated not with water but with monolinuron solution before placing them in the same solutions. Induction time was 60 min and the time of exposure to light in the growth chamber 20 min. The mode of introduction of the herbicide into the tissue had a significant effect on its inhibitory action (Table 1). Leaf segments infiltrated with monolinuron solution lost their ability of floating up to the surface to a higher degree than did the disks infiltrated with distilled water and then placed in analogous herbicide solutions. The effect was noticeable at higher monolinuron concentrations.

Table 1

Photosynthesis-inhibiting activity of monolinuron in dependence on the mode of its introduction into the tissue
(Number of disks coming to surface as % of control)

Monolinuron concentration ppm	Disks infiltrated with	
	distilled water[1]	monolinuron[2]
0.25	100[a]	100[a]
0.75	95[a]	93[a]
1.00	86[a]	70[b]
1.25	76[a]	44[b]

[1]Disks infiltrated with distilled water and kept in darkness in monolinuron solution. [2]Disks infiltrated with monolinuron solution and kept in it in darkness.
The letters denote groups uniform at p = 0.05 for all concentrations.

Time of keeping infiltrated disks in darkness – induction time

Various induction times were applied from 20 to 120 min. In view of the results of the previous experiment, the leaf disks were infiltrated with two extreme monolinuron concentrations 0.25 and 1.25 ppm.

Prolongation of the induction time has a significant influence enhancing the inhibitory effect of monolinuron (Table 2). The dependence of floating up of the disks on the induction time was most pronounced in the case of the 1.25 ppm monolinuron concentration. Every 20 min prolongation of the induction time reduced significantly the number of disks coming to the surface. For the 0.25 ppm concentration the inhibitory action of the herbicide was noticeable as late as after 100 and 120 min of induction. Under the conditions of the experiment as optimal induction time may be considered 120 min. At this time a distinct difference could

Table 2

Photosynthesis-inhibiting activity of monolinuron in dependence on induction time
(Number of disks coming to surface as % of control)

Monolinuron concentration ppm	Induction time, min					
	20	40	60	80	100	120
0.25	100[a]	100[a]	100[a]	97.8[a]	93.0[b]	86.7[c]
1.25	93.3[a]	75.5[b]	44.5[c]	22.2[d]	11.1[e]	6.7[e]

Letters denote groups uniform at p = 0.05 for all concentrations.

be seen between the control and the 0.25 ppm concentration. The floating to the surface of disks treated with the highest concentration (1.25 ppm), however, was not completely inhibited.

Time of exposure of leaf disks to light

The exposure time varied from 10 to 40 min and monolinuron was applied in all concentrations from 0.25 to 1.25 ppm. For the leaf disks infiltrated with monolinuron the longest exposure time 120 min was adopted, according to the results of the above described experiment.

It appeared that the time of exposure to light of the disks was a very essential element after induction (Table 3). After a short 10 min exposure part of the disks came to the surface of the solution only when the lowest concentration of monolinuron (0.25 ppm) was applied. Longer illumination periods caused the appearance of an increasing number of disks on the surface, most marked at a concentration of 0.75 ppm (all differences were significant). The relation between the number of floating disks and the herbicide concentration was inversely proportional. The disks coming to the surface were most numerous after 40 min, the 0.25 ppm concentration having no detectable effect on oxygen evolution, and even at the highest concentration 1.25 ppm a small number of leaf disks appeared on the surface. It was concluded from this experiment that the most suitable time of exposure to light in the tests is 20 min, at this time the differences in the effect of increasing herbicide concentrations were most pronounced. The above described experiments gave grounds to establish the following procedure in testing the phytotoxicity of herbicides on leaf disks:

(a) cutting of disks and placing them in darkness for 60 min in distilled water,

Table 3

Photosynthesis-inhibiting activity of monolinuron in dependence on time of exposure to light

(Number of disks coming to surface as % of control)

Time of exposure to light min	Monolinuron concentration, ppm				
	0.25	0.50	0.75	1.0	1.25
10	60.9^{c}	0^{c}	0^{d}	0^{c}	0^{b}
20	83.3^{b}	54.4^{b}	22.2^{c}	12.2^{b}	3.3^{ba}
30	90.7^{b}	68.9^{b}	36.7^{b}	37.8^{a}	4.4^{ba}
40	100^{a}	78.9^{a}	44.5^{a}	41.1^{a}	7.8^{a}

Letters denote groups uniform at $p = 0.05$ for all concentrations

(b) infiltration of the disks with a solution of the tested substance,

(c) keeping of the infiltrated disks in this solution for 120 min in darkness (induction time),

(d) transfer of the disks to a crystalliser with 100 cm^3 of 0.05 M $NaHCO_3$ so that they should not shade one another and exposure to light in a growth chamber for 20 min at 26°C.

Checking of the developed method

The activity of eight herbicides was determined by the above described method: linuron, metoksuron, monolinuron, atrazine and prometrine proved to be typical photosynthesis inhibitors (C o r b e t t, 1974), whereas 2,4,5-T, chlorpropham, MCPA have no direct influence on this process. All CO_2 assimilation-inhibiting herbicides exhibited a high activity (Table 4). With increase of the concentration of these substances the number of leaf disks floating to the surface of the solution diminished considerably. Similar results were obtained when using molar herbicide concentrations (Fig. 1).

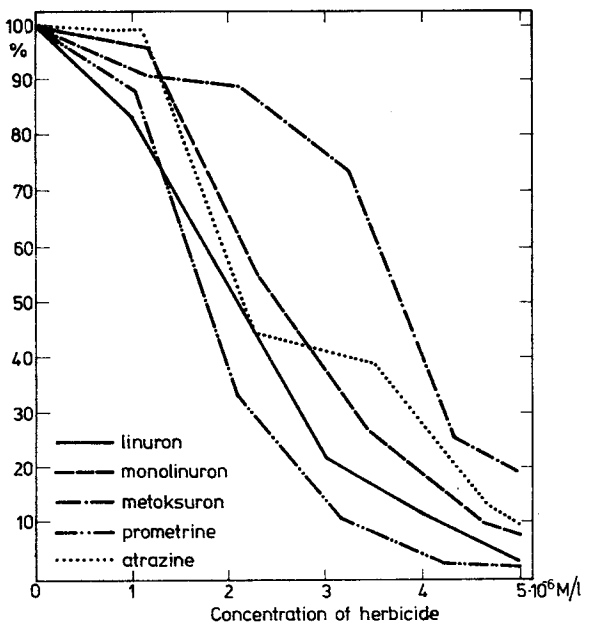

Fig. 1. Photosynthesis-inhibiting activity of some herbicides (Number of disks coming to surface as per cent of control)

The highest photosynthesis inhibiting activity was noted in the case of prometrine and the lowest for metoksuron. Linuron in a 0.25 ppm concentration, similarly as prometrine, showed a higher toxicity than other herbicides, but at

higher concentrations monolinuron was equal to it. Atrazine applied in the lowest concentration did not inhibit oxygen evolution, in higher concentration its inhibitory activity was higher or lower than that of linuron and monolinuron in dependence on the concentration.

Chlorpropham, 2,4,5-T (trichlorophenoxyacetic acid), MCPA (4-chloro-2--methylphenoxyacetic acid) had no effect in any of the concentrations used.

Table 4

Photosynthesis-inhibiting activity of some herbicides
(Number of disks coming to surface as % of control)

Herbicide	Herbicide concentration, ppm				
	0.25	0.50	0.75	1.0	1.25
Linuron	83.3^{c}	54.4^{c}	22.2^{d}	12.2^{d}	3.3^{c}
Metoksuron	91.1^{ba}	87.8^{b}	73.3^{b}	24.5^{b}	14.5^{b}
Monolinuron	96.7^{a}	55.5^{c}	26.7^{d}	10.0^{d}	3.3^{c}
Atrazine	98.9^{a}	44.4^{d}	38.9^{c}	16.7^{c}	1.1^{c}
Prometrine	87.8^{cb}	34.5^{e}	11.1^{e}	4.2^{e}	2.2^{c}
Chlorpropham	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
2,4,5-T	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
MCPA	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Letters denote groups uniform at $p = 0.05$ for each concentration.

DISCUSSION

The application of acidic sodium carbonate as CO_2 source in photosynthesis for the immersed leaf disks infiltrated with herbicides allowed the evaluation of their photosynthesis-inhibiting activity which was manifested in the differing capability of the disks to evolve oxygen. The oxygen displaced the herbicide solution introduced previously by infiltration into the intercellular spaces, and owing to this the disks came to the surface of the solution.

The results obtained in the present experiments gave grounds for establishing optimal conditions for testing the activity of photosynthesis inhibitors. It was found that infiltration of the disks with a solution of the tested substance and a long induction time, that is period of keeping in darkness after infiltration cause a stronger inhibition of oxygen evolution. This is probably connected with the opportunity of penetration of a greater amount of the herbicide into the cells.

Investigation of the activity of the chosen herbicides confirmed that the developed test is appropriate for determining the phototoxicity of potential photosynthesis inhibitors. The negative results in the case of MCPA, 2,4,5-T and

chlorpropham indicate that this method is not suitable for testing herbicides of the auxin group, the toxicity of which consists in inhibition of other metabolic processes then photosynthesis (A u d u s, 1976; C o r b e t t, 1974).

The basic advantage of the here described method is the rapid testing of herbicide activity and the simple procedure. By taking advantage of the phenomenon of floating of leaf disks infiltrated with herbicide solutions the time of testing was shortened to about 3.5 h with concentrations of 0.25-1.25 ppm. Evaluation of herbicide activity on the basis of falling of the leaf disks to the bottom in the herbicide solution lasts 9-12 h for concentrations of 0.1-0.5 ppm (T r u e l o v e et al., 1974; G a w r o ń s k i et al. 1977) and for lower concentrations it has to be prolonged to 24 h (T r u e l o v e et al., 1974; D a S i l v a et al., 1976). The here presented method not only allows detection of photosynthesis inhibitors, but what is equally important, makes possible precise comparison of their activity which changes with the increase of concentration of the herbicide solution.

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Metoda testowania aktywności herbicydów hamujących fotosyntezę za pomocą infiltracji krążków z liści

S t r e s z c z e n i e

Opracowano szybką metodę wykrywania inhibitorów fotosyntezy w niskich stężeniach 0,25-1,25 ppm. Doświadczenia przeprowadzono na krążkach wyciętych z młodocianych liści fasoli. Krążki infiltrowano roztworami badanych związków i umieszczano na dnie krystalizatora zawierającego roztwór kwaśnego węglanu sodu, a następnie oświetlano. Miarą toksyczności badanej substancji był procent krążków wypływających. Stwierdzono, że linuron, monuron, metoksuron, atrazyna i prometryna hamowały wypływanie krążków, a 2,4,5-T, chloroprofam i MCPA nie wykazywały aktywności. Potwierdza to specyficzność testu nadającego się do określania fitotoksyczności typowych inhibitorów fotosyntezy.