Rhythmic Changes in the Sensitivity of Cotton Seedlings to Herbicides

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ABSTRACT

Cotton (Gossypium hirsutum L.) seedlings that were grown under a photoperiod of 12 hours darkness and 12 hours light showed oscillations in their sensitivity to the herbicides sodium 5-(2-chloro-4-trifluoromethyl)-phenoxy)-2-nitrobenzoate (acifluorfen), butyl 2-(4-(5-(trifluoromethyl)-2-pyridinyl)oxy)phenoxy)propanoate (fluazifop) and 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide (bentazon). Sensitivity was expressed in appearance of necrotic areas on the cotyledons and in decreased growth of the shoot. The seedlings were least sensitive in the beginning and middle of the light period, then the sensitivity increased and reached its maximum during the beginning and middle of the dark period and then declined. Seedlings grown from germination under continuous light exhibited very small or no oscillations in sensitivity. The oscillations in sensitivity were entrained by one cycle of darkness and light. A cycle of 12 hours darkness and 12 hours light triggered the greatest oscillations while either increasing or decreasing the duration of the dark period resulted in smaller oscillations. Apparently, these oscillations in sensitivity to herbicides were endogenously controlled since after entrainment they continued irrespective of the light conditions.

MATERIALS AND METHODS

Cotton (Gossypium hirsutum L.) seeds were germinated and grown for 7 d in plastic pots (12.5 cm in diameter, 12 cm in height) filled with peat and irrigated with distilled H₂O. The seedlings were grown in growth chambers at 29°C under certain photoperiods which are specified in each experiment. The light source (700 ft-c) was a combination of regular incandescent light and fluorescent light (F48T12-CW-VHO, Sylvania, Danvers, MA).

The herbicides sodium 5-(2-chloro-4-trifluoromethyl)-phenoxy)-2-nitrobenzoate (acifluorfen), butyl 2-(4-(5-(trifluoromethyl)-2-pyridinyl)oxy)phenoxy)propanoate (fluazifop) and 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide (bentazon) were applied by spraying the seedlings until run-off. The injury caused by the herbicides was evaluated by degree of damage to the cotyledons as expressed by appearance of necrotic areas after several days. It was scored from 1 which represents no visible necrotic areas on the cotyledons to 5 which represents completely necrotic cotyledons. At the time of herbicide application, the seedlings had only two cotyledons and had not yet developed shoots. Resumption of growth was determined by measuring the shoot weight several days after the herbicide application.

RESULTS

Cotton seedlings grown under a photoperiod of 12 h light and 12 h darkness showed daily oscillations in their sensitivity to the herbicides bentazon, acifluorfen, and fluazifop. Maximal sensitivity to the three herbicides, as observed by development of necrotic areas in the cotyledons (scored as degree of damage) occurred about 4 to 8 h after the beginning of the dark period. Later in the dark period, the sensitivity of the cotyledons began to decrease and it was much lower during the light period (Fig. 1). The seedlings were treated with herbicides before the development of the shoots. The subsequent development of the shoots was affected by bentazon and acifluorfen, depending on the time of their application. The maximal inhibition of shoot growth was achieved when these herbicides were applied during the first and middle parts of the dark period. During the last part of the dark period the growth inhibition began to be smaller, and during the following light period it was the smallest (Fig. 1). Fluazifop exhibited a very small effect on subsequent shoot growth. However, it almost completely stopped the growth of the cotyledons when applied in the sensitive stage during the first and middle parts of the night. Application of fluazifop during the last part of the dark period and during the light period was less effective in inhibition of cotyledon growth (Fig. 1).

Application of various concentrations of bentazon to seedlings...
Fig. 1. Oscillations in the response of cotton seedlings to bentazon (1 mM), acifluorfen (1 mM), and fluazifop (10 mM). Seedlings were grown under a photoperiod of 12 h darkness and 12 h light for 4 d from germination, then treated with the herbicides at various times and immediately transferred to continuous light. The degree of damage to the cotyledons was scored 2 d after treatment with acifluorfen or fluazifop and 4 d after treatment with bentazon. The fresh weight of the shoots or cotyledons was measured 5 d after the treatment with bentazon or fluazifop and 8 d after the treatment with acifluorfen. (--) Dark period, (○) light period; (●) degree of damage, (□) fresh weight.

Table 1. Dose Response of Cotton Seedlings to Bentazon at Their Sensitive and Resistant Stages

<table>
<thead>
<tr>
<th>Bentazon (mM)</th>
<th>Shoot Fresh Wt at Following Time (h)</th>
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<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>0.25</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>0.50</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>1.00</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>2.00</td>
<td>0</td>
</tr>
</tbody>
</table>

During the sensitive or resistant stages showed that the inhibition of shoot growth that was caused during the sensitive period was achieved in the resistant period only with a 2-fold higher concentration (Table 1). Any concentration above 0.25 mM inflicted more damage to the cotyledons of seedlings in the sensitive stage. Maximal difference between the response of seedlings during the sensitive and resistant stages was achieved after treatment with 1 mM bentazon while the higher and lower concentrations caused smaller differences. Treatment with 0.25 mM bentazon had almost no visible effect on the cotyledons (Fig. 2).

The response of the seedlings to bentazon was similar if, after the application of the herbicide, they were kept under the normal photoperiod or were transferred to continuous light. Seedlings that were transferred to continuous darkness, even for 4 d after the treatment with bentazon, did not show any visible symptoms. However, after transfer to continuous light the response to bentazon was similar to that observed in seedlings that were transferred immediately to continuous light or kept in continuous darkness (III). The degree of damage to the cotyledons was scored 4 d after the treatment with the herbicide. Seedlings that were kept in continuous darkness were transferred after 4 d to continuous light, and the degree of damage to the cotyledons was scored 4 d later (□); (□) dark period.
Fig. 4. Entrainment of the oscillations in the response of cotton seedlings to bentazon (1 mM). Seedlings were grown under continuous light for 4 d from germination, then exposed to one cycle of darkness/light. Bentazon was applied at various times during the second cycle. After the bentazon treatment, all the seedlings were transferred to continuous light. The degree of damage to the cotyledons was scored 4 d after the treatment with the herbicide. (□) Dark period, (■) light period; (●) degree of damage, (■) fresh weight.

Entrainment with one cycle of various dark/light combinations. Exposure to one cycle of 12 h darkness and 12 h light resulted in the largest changes in the sensitivity to bentazon as reflected by the degree of damage to the cotyledons and development of the shoots. Under such entrainment the maximal damage occurred when bentazon was applied 4 h after the beginning of the dark period. Later in the dark period the sensitivity decreased and it remained low during the light period. Prolonging, as well as shortening the dark period from 12 h resulted in smaller changes in the sensitivity to bentazon. Moreover, after exposure to dark periods longer than 12 h, the maximal sensitivity occurred at a later stage during the dark period.

Detailed kinetics of the development of the sensitive phase to bentazon, after one cycle of 12 h darkness and 12 h light, showed a quick rise in the sensitivity which reached its maximum 2 h from the beginning of the dark period. After about 3 h of maximal sensitivity it began to decrease gradually and reached a low level after 4 h (Fig. 5).

Seedlings that were grown under cycles of 12 h darkness and 12 h light for 4 d from germination were transferred to inverted light periods, i.e. the dark period was changed to light period and the light period was changed to dark period. Under these new conditions the seedlings responded to bentazon according to the chronological time and not according to the immediate light conditions (Fig. 6) As usual, the degree of damage to the cotyledons and inhibition of shoot growth were highest 4 h after the beginning of the cycle and then became lower, although the dark and light periods were exchanged (Fig. 6).

DISCUSSION

The sensitivity of cotton seedlings to three herbicides (bentazon, acifluorfen, and fluazifop) as expressed by appearance of necrotic areas on the cotyledons and decreased shoot growth was related to the time of application. Similar relations between plant sensitivity to herbicides and the time of application have previously been reported for these and several other herbicides and plant species (1, 4, 7, 8, 14). In the present work we showed that such oscillations in sensitivity to the herbicides were rhythmical because first, they did not occur when the seedlings were grown under continuous light from germination; second, they were entrained by a certain photoperiod; and third, after they had
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been entrained they continued irrespective of the immediate light conditions.

Light is required for the expression of the toxic effect of bentazon and acifluorfen (9, 10, 13). However, this light requirement was not involved in the timing of the sensitivity. The sensitivity was determined only by the time of herbicide application, irrespective of the immediate light conditions after the herbicide application.

The long term response of cotton seedlings to the herbicides was determined only by the time of application, irrespective of light conditions after the herbicide application. The same pattern of response was observed with three herbicides (bentazon, acifluorfen, and fluazifop) which are known to have different primary modes of action (6, 9, 10, 13). Therefore, it seems that the mechanism which contributes to the oscillations in the sensitivity is nonspecific. Current studies are aimed to clarify this mechanism.

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