Plant Cell Physiol. 28(7): 1345–1349 (1987)

Short Communication

Altered Diurnal Leaf Movements in Soybean Seedlings Treated with Triazole Growth Regulators

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Treatment of two-week old soybean (*Glycine max* (L.) Merr. cv. A2) seedlings with 250 μg of either soil-applied paclobutrazol or XE-1019 (also known as S-3307) altered diurnal leaf movements within 48 h. Leaf blade and petiole angles of primary leaves, relative to the horizontal, were consistently lower in the triazole-treated seedlings compared to controls during the daylight hours. At night, leaves were completely folded down in both treated and control seedlings, but leaves on treated seedlings reached the folded down position approximately two hours earlier than controls. A single foliar spray of 6.0 mg$\cdot$liter^{-1} GA_3 counteracted the triazole effects on leaf blade and petiole angles.

Key words: Antigibberellin — *Glycine max* — Growth regulator — Leaf movement — Leaf angle — Leaf orientation — Paclobutrazol — Triazole — XE-1019.

The triazole plant growth regulators, paclobutrazol [1-(4-chlorophenyl)-4,4-dimethyl-2(1,2,4-triazol-1-yl)pentan-3-ol] and XE-1019 [1-(4-chlorophenyl)-4,4-dimethyl-2(1,2,4-triazol-1-yl)penten-3-ol (also known as S-3307)], exhibit strong growth controlling properties in a range of plant species. These compounds induce a number of physiological responses including reduced gibberellin and sterol biosynthesis, increased chlorophyll content, altered carbohydrate status, increased tolerance to some stresses, and delayed senescence (Izumi et al. 1984, Davis et al. 1986, Fletcher et al. 1986). Many of the triazole-induced phenomena can be reversed by the application of GA_3 (Wample and Culver 1983, Mita and Shibaoka 1984, Lee et al. 1985, Steffens et al. 1985).

During some of our studies on growth and metabolic changes in paclobutrazol-treated soybean plants, we observed that leaves on treated plants exhibited different leaf orientations as compared to untreated controls. Soybean plants are known to exhibit diurnal leaf movements wherein leaf blades fold down during dark periods and then move upward during light periods. The leaf movements appear to be due to osmotic changes in the pulvinar regions and potassium may have a role in regulating the movements (Wofford and Allen 1982). In the present communication, we describe alterations in leaf movements and orientation in triazole-treated soybean plants.

Soybean (*Glycine max* (L.) Merr. cv. A2) seeds were planted in 15 cm pots filled with a peat-perlite-soil (1 : 1 : 2 by volume) growing medium. The seedlings were grown in a greenhouse under 50% shade (maximum photosynthetic active radiation of 1,100 μmol$\cdot$m$^{-2}\cdot$s^{-1}) and an approximate photoperiod of 15 h with a day/night temperature regime of about 30/20°C. Soil moisture was mantained near field capacity to eliminate the effects of moisture stress on leaf orientation. Two weeks after planting, pots were thinned to two uniform seedlings and the growth regulators were applied. At this time the primary leaves were nearly completely expanded, the first trifoliate was rapidly expanding and the second trifoliate was visible at the apex (Fig. 1). Plant height at the time of treatment was about 8 cm. The seedlings were treated with 250 μg of paclobutrazol or XE-1019 applied as a soil drench (50 ml of a 5 mg·liter^{-1} solution). Control seedlings received water only.

After treatment, petiole and leaf blade angles of the primary leaves were measured 1, 2, 3, and 7 days after treatment at approximately two hour intervals between 0700 and 2200 h. Blade and petiole angles were measured relative to the horizontal lines shown in Fig. 1 (positive and negative blade angles indicate that leaf blades were oriented above and below the horizontal, respectively). Plant height was also measured one week after treatment. In an additional experiment, plants were treated with the triazoles as described above and then one week later were sprayed to run-off with a 6.0 mg·liter^{-1} solution of GA_3.

The experiments were conducted using a completely randomized design with at least four plants per treatment. Each experiment was conducted at least twice.

Both paclobutrazol and XE-1019 inhibited shoot elongation. Mean plant heights ($\pm$standard error) one week after treatment were 10.1 ± 0.7, 8.1 ± 0.6, and 8.1 ± 0.1 cm for the control, paclobutrazol, and XE-1019 treatments, respectively. Similar growth inhibition of soybean by paclobutrazol has been reported earlier (Sankhla et al. 1985). It has generally been found that XE-1019 is somewhat more active than paclobutrazol in inhibiting shoot growth of most species (Bassi et al. 1986), but this was not the case with the application rates used with soybean in the present study. The foliar GA_3 spray completely reversed the triazole-induced growth inhibition so that plants treated with the triazoles plus GA_3 had similar heights as untreated controls within 10 to 14 days after the GA_3 application (data not presented). Similar

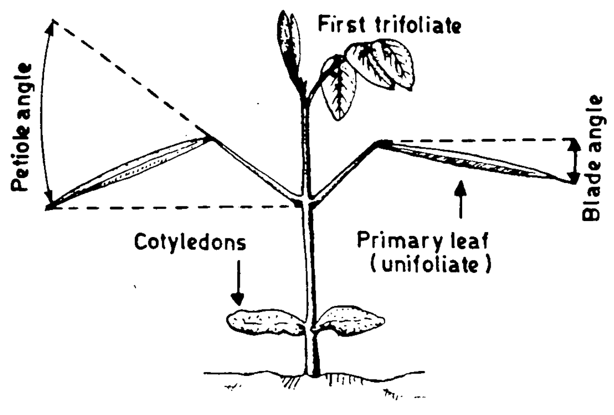

Fig. 1

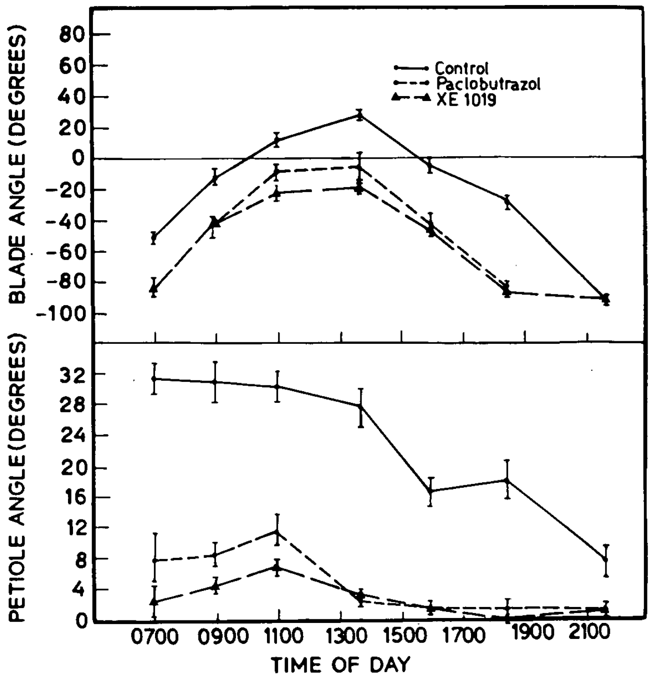

Fig. 2

Fig. 1 Drawing of a two-week old soybean seedling showing how petiole and blade angles were measured relative to the horizontal.

Fig. 2 Blade and petiole angles of the primary leaves of control and triazole-treated soybean seedlings throughout the daytime hours. Seedlings were treated one week prior to the measurements. Bars indicate standard error of mean.

reversal of paclobutrazol-induced growth inhibition by GA_3 has been observed with other species (Wample and Culver 1983, Steffens et al. 1985).

One week after treatment, both triazole compounds strongly influenced primary leaf orientation throughout the day (Fig. 2). At 0700 h, both blade and petiole angles were 20 to 30 degrees lower in treated plants compared to controls. These differences persisted throughout most of the daylight hours. Primary leaf blades on triazole-treated plants never attained a positive blade angle during the day whereas control leaves had blade angles of about +30 degrees around 1300–1400 h. By about 1900 h, primary leaves on triazole-treated plants were completely folded down (blade angle of −90 degrees) whereas control leaf blade angles were still relatively high (about −30 degrees). By 2130 h, however, leaves on both control and treated plants were completely folded down. Leaves on all plants remained folded down throughout the night which is typical for soybean. Control petiole angles, however, remained at least a few degrees higher than those on treated plants even during the night. Although not quantified, it was observed that movement and orientation of the first trifoliate leaf was influenced by the triazoles in the same manner as the primary leaves.

To determine the rapidity of the triazole effects on leaf orientation, blade and petiole angles of the primary leaf were measured 1, 3, 5 and 7 days after treatment at three times during the day. The triazoles had little influence on blade and petiole angle at 0900 h on the first or second day after treatment (Fig. 3). By 1700 h of the second day (48 h after treatment), however, blade angles were about 10 degrees more negative on treated plants compared to controls. Hence, the

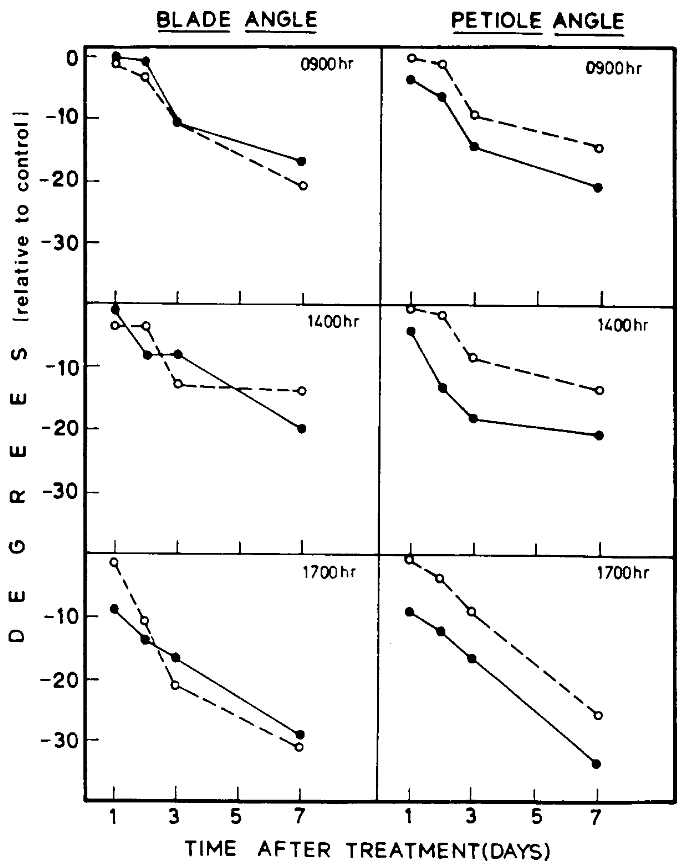

Fig. 3 Blade and petiole angles of the primary leaves of triazole-treated soybean seedlings at various times after treatment. Angles are expressed relative to the control (–●– paclobutrazol; –○– XE-1019).

Table 1 Blade and petiole angles of the primary leaves of control and triazole-treated soybean seedlings at various times after a foliar spray of 6.0 mg·liter^{-1} GA_3

Treatment	Days after GA_3 treatment					
	1 Time of day (h)			3 Time of day (h)		
	0900	1400	1700	0900	1400	1700
Blade angle (degrees)						
Control	−6.0±8.3	−12.8±2.3	−31.3±6.2	−17.8±7.1	−22.5±6.7	−17.6±6.1
Control+GA_3	−12.8±4.9	−6.8±3.0	−24.5±5.3	−8.0±5.3	−7.8±2.0	−7.5±2.5
Paclo	−54.0±8.6	−47.8±5.9	−81.3±4.7	−69.7±3.7	−60.7±3.9	−83.3±6.7
Paclo+GA_3	−21.5±6.7	−24.5±1.8	−38.8±3.2	−34.3±2.5	−38.3±1.9	−37.3±4.2
XE-1019	−47.8±4.4	−44.8±1.7	−67.0±4.1	−69.8±8.9	−56.0±6.8	−72.3±8.9
XE-1019+GA_3	−32.3±3.7	−22.0±1.7	−35.8±2.0	−27.8±5.5	−23.3±8.7	−40.0±4.6
Petiole angle (degrees)						
Control	12.0±2.8	3.3±1.2	5.0±2.9	11.5±2.3	11.8±3.2	6.8±2.5
Control+GA_3	21.5±4.8	12.5±5.8	18.0±4.8	22.0±3.9	24.5±3.4	25.3±6.0
Paclo	−11.5±3.6	−15.5±3.0	−23.0±2.4	−17.7±5.6	−9.8±2.5	−14.0±5.4
Paclo+GA_3	3.5±1.3	1.5±0.6	1.8±0.9	4.5±1.5	5.5±2.5	1.5±1.5
XE-1019	−0.3±2.3	−5.5±4.0	−5.3±2.9	−2.3±1.4	−2.5±1.7	−2.5±1.3
XE-1019+GA_3	6.0±1.7	5.3±1.5	2.8±0.9	5.8±1.9	9.0±2.5	5.3±1.4

Triazole treatments were made one week prior to GA_3 application. Triazoles were applied at a rate of 250 μg per 15 cm pot. Plus/minus values indicate standard error of mean.

triazole-induced changes in leaf orientation were quite rapid and occurred before growth inhibition was visible.

A single foliar spray of 6.0 mg·liter^{-1} GA_3 partially reversed (within 24 h) the triazole effects on leaf blade and petiole angle (Table 1). By 0900 h on the day following the GA_3 spray, blade and petiole angles were condiderably more positive on triazole-treated plants sprayed with GA_3 compared to those that were not sprayed. By three days after the GA_3 foliar spray, leaf blade angles on plants treated with triazoles plus GA_3 were 25 to 45 degrees more positive than those on plants that were not sprayed with GA_3. Likewise, petiole angles were about 8 to 20 degrees more positive on triazole-treated plants sprayed with GA_3 compared to those that were not sprayed. Hence, like other triazole-induced phenomena, altered leaf movements on soybean can at least be partially counteracted by GA_3.

The altered leaf movements in triazole-treated soybeans may, at least in part, explain why treated plants had much less biomass than untreated controls in previous studies using larger and older plants (Sankhla et al. 1985, 1986). Because the triazoles reduced the erect habit of soybean leaves during the daylight hours, it is possible that canopy light absorption and hence photosynthesis were reduced compared to untreated control plants. Although paclobutrazol did not affect soybean net photosynthesis in a laboratory study where leaves were placed perpendicular to incoming light (Sankhla et al. 1985), the triazoles may indirectly reduce whole-plant photosynthesis by altering leaf orientation and light absorption.

The response of soybean leaves to the antigibberellin-like triazoles is consistent with the previously reported effects of GA_3 on leaf movements of other legumes such as *Albizzia julibrissin* (McEvoy and Koukkari 1972) and *Mimosa pudica* (Williams and Raghavan 1966). In these species, GA_3 inhibited the dark-induced closure of pinnules. The mode by which the triazoles enhanced leaf closure of soybean in the present study is unknown, but may be related to changes in potassium content of the pulvinar region which is thought to be an important factor regulating leaf movements (Wofford and Allen 1982). Further work is needed to test this possibility.

The authors thank ICI Americas and Chevron Chemical for supplying the triazole compounds. We also thank Drs. B. N. Smith and R. D. Horrocks for financial support.

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(Received February 25, 1987; Accepted July 21, 1987)