Influence of Light Regime on the Toxicity and Physiological Activity of Herbicides

Robert S. Mellor and Frank B. Salisbury
Botany and Plant Pathology Section, Colorado Agriculture Experiment Station, Fort Collins, Colorado

The response of plants to certain herbicides is influenced by the light conditions preceding or following application of the herbicide (14, 15, 16, 18, 21), but the physiological basis for the differential activity of certain herbicidal compounds in relation to the light conditions under which they are applied is uncertain in most cases.

This paper deals with 2 such light-influenced responses of plants to herbicides. One series of experiments was performed to study an observation first reported by Salisbury (21) that plants treated with 2, 4-dinitrophenol (DNP) are considerably more injured when the plants receive darkness after treatment than when they are exposed to light following application of the compound. Another series of investigations deals with the physiological activity of sodium 2, 4-dichlorophenoxyacetic acid (2, 4-D) in plants as influenced by ultraviolet light and the light regime to which plants are subjected after UV-irradiation and auxin treatment.

There are many reports in the literature concerning the effects of DNP on photosynthesis and photophosphorylation (2, 17, 26), respiration and oxidative phosphorylation (5, 7), and other energy-requiring processes involving high energy phosphate compounds (1, 19). In such studies the toxicity of DNP and related compounds is often markedly influenced by the pH at which the material is applied to plants (23), and the carrier in which the material is applied (8).

Salisbury (21) has suggested that the physiological basis for the differential toxicity of DNP to plants subjected to light or darkness after DNP treatment may lie in a difference in sensitivity of the oxidative and photosynthetic phosphorylation mechanisms to DNP. Since DNP is more phytotoxic when its application is followed by darkness, DNP may be inhibiting oxidative phosphorylation but not photophosphorylation. Thus, the level of ATP in plants left in the dark after DNP treatment would be lowered and result in observable plant damage, but plants treated and left in the light might avoid injury. Ohmura (17) and Wessels (26) have indeed demonstrated a pathway of photophosphorylation which is insensitive to DNP at concentrations which inhibit oxidative phosphorylation.

De Zeeuw and Leopold (10) have reported that tomato plants irradiated with UV light for short periods of time do not exhibit the normal epinastic responses following auxin (naphthalene acetic acid) treatment. Ultraviolet radiation given before auxin treatment was more effective in preventing epinasty in auxin-treated plants than UV radiation given after auxin treatment. In other experiments they determined that UV radiation did not significantly affect the uptake of auxin. They concluded that the prevention of auxin responses in UV irradiated plants was due to a prevention of auxin action inside the plants.

It has been noted frequently that the physiological and histological effects of UV on plant and animal tissues can be overcome or reversed by white light. For example, the inhibitory effects of UV light on such widely diverse processes as chlorophyll destruction (25), inactivation of bacteriophages (11), and growth of fungi (12) can be reversed by white light following UV radiation. The mechanism of this photoreversal is still obscure, and experimental results are difficult to interpret due to the multiplicity of physiological processes in plants affected by UV radiation.

The present work was conducted to elaborate upon these light-influenced responses of plants to 2, 4-D, DNP, and related compounds and shed new light on the physiological basis for the phytotoxicity of these compounds.

Methods and Materials

Most experiments were conducted in 2 growth chambers provided with lighting facilities and temperature controls adequate to sustain the growth of plants for extended periods of time. The chambers were illuminated with a mixed incandescent and fluorescent light source having a maximum intensity of about 1250 ft-c at plant level. Colored plastic or gelatin filters were inserted below the light source in some experiments to obtain desired wavelength

1 Revised manuscript received October 28, 1964.
2 Published with the approval of the Director, Colorado Agricultural Experiment Station, Fort Collins, Colorado as Scientific Series Paper No. 783. Report of work supported by allotments under Section 9b, 3. Bankhead-Jones, Title I, W-11. Studies on physiological and ecological factors related to weed control. Part of this work was included in a thesis by R. S. Mellor, submitted in partial fulfillment of the requirements for the M. S. degree.
regions in the visible spectrum. Spectral distribution of the red, blue and green filters used in this study are shown in figure 1.

The source of near UV light was a lamp which transmitted over a range of 3407 to 3888 A and peaked at 3600 A. This lamp was suspended 60 cm above the plant material. Another UV light source, used especially in the 2,4-D photoreversal experiments, consisted of two 15 w. T-8 Westinghouse Sterilamps, each with an initial rating of 38 μw/cm² at a distance of 1 m from the lamps. Approximately 95% of the radiation from this source lies in the 2537 A unit region of the spectrum (27). This UV light source was also suspended 60 cm above the tops of the plants.

Pinto beans, Phaseolus vulgaris L. var Scout, were grown on 16-hour photoperiods in the growth chambers at 23 to 25°. The plants (10–12 days old) were used when the primary leaves were about half expanded and before appreciable development of the first trifoliate leaf.

Chemicals were applied to the plants by dipping the upper 5 to 8 cm of the shoot and the primary leaves into 200 to 250 ml of solution (containing Tween 20 detergent). In some experiments 2,4-D was applied in a single 50 lambda drop to the base of 1 of the primary leaves at the point where the lamina is attached to the petiole.

Subsequent growth inhibition and toxicity of the chemicals were evaluated in several ways. In the case of the DNP-treated plants, injury was evaluated on the basis of the inhibition of fresh weight increase compared to untreated controls 5 to 12 days after chemical treatment. Inhibition of fresh weight increase in the treated plants resulted from a general inhibition of growth and also from drying out of the tissue injured by the applied chemicals.

The response of plants to 2,4-D as influenced by various treatments was evaluated by measuring the epicotyl curvature of treated plants 24 hours after the application of 2,4-D, according to the method of Day (9).

The sodium salt of 2,4-D used in this study was obtained from the J. T. Baker Chemical Company, Phillipsburg, N. J., and the the DNP was secured from the Eastman Kodak Company, Rochester, N. Y.

The catechol used in this study was a special grade of the material that had been sublimed to remove the free phenol. This material was obtained from Gestur Johnson, Chemistry Department, Colorado State University.

Experiments and Results

Toxicity of DNP in Relation to Light Conditions after Treatment. Figure 2 shows the results of a typical experiment in which DNP was applied to plants, half of which were then placed in the dark for 24 hours while the other half remained in the light. The fresh weights of leaves from treated plants receiving darkness following application of DNP were considerably reduced compared to leaves left in the light following treatment. Although there was slight injury to plants left in the light, a 10-fold higher concentration was required in the light to produce damage equivalent to that of dark-grown plants.

Toxicity of DNP in Relation to Light Conditions

Table I. Effect of pH on Differential Toxicity of DNP in Relation to Light Regime before and after Application of Chemical

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Light intensity prior to treatment ft-c</th>
<th>Conditions after treatment</th>
<th>24 Hr dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNP** pH 7.0</td>
<td>1250</td>
<td>2.37 ± .14</td>
<td>2.22 ± .04</td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>2.48 ± .13</td>
<td>2.04 ± .05</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.60 ± .17</td>
<td>1.88 ± .22</td>
</tr>
<tr>
<td>DNP** pH 3.3</td>
<td>1250</td>
<td>1.60 ± .07</td>
<td>0.81 ± .11</td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>1.60 ± .19</td>
<td>0.91 ± .11</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1.23 ± .06</td>
<td>0.61 ± .15</td>
</tr>
<tr>
<td>Control</td>
<td>1250</td>
<td>2.71 ± .09</td>
<td>2.61 ± .14</td>
</tr>
<tr>
<td></td>
<td>850</td>
<td>2.73 ± .14</td>
<td>2.54 ± .17</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.51 ± .24</td>
<td>2.60 ± .30</td>
</tr>
</tbody>
</table>

* Mean and standard error of 6 replications of paired leaves.

** 3 × 10⁻³ M DNP.
before Treatment. Plants were placed in the dark for various periods of time just prior to DNP application and then given a standard 16-hour dark period after the DNP treatment. The results indicate that increasing the length of the dark period preceding DNP treatment increases the phytotoxicity of the chemical (fig 3). Because of the enhanced effect of DNP following a period of darkness, all subsequent experiments reported in this paper were performed after the plants had received a standard 8-hour dark period in the growth chamber.

Toxicity of DNP in Relation to the Length of Darkness following Treatment. Bean plants were treated with 1.5 × 10^{-3} M DNP, a concentration which gave a pronounced differential effect of light or darkness following treatment (fig 2). Following application of the chemical, plants were placed in the dark for various periods ranging from 2 to 24 hours. The results shown in figure 4 indicate that fresh weight, evaluated 8 days after treatment, decreased with increasing darkness up to a maximum of about 16 hours.

Toxicity of DNP in Relation to pH. The results of an experiment in which 3 × 10^{-3} M DNP was applied to plants in solutions of pH 3.3 or 7.0 are recorded in table I. Following treatment, the plants were placed in the dark or were given light intensities of 500, 850 or 1250 ft-c. The data of table I clearly indicate that DNP is considerably more toxic when applied in acid solution. When DNP is applied in neutral solutions the differential response to light and darkness is diminished.

Influence of Light Quality on Phytotoxicity of DNP. Plants were treated with 2 × 10^{-3} M DNP and then placed for 24 hours in the dark or in white, red, green, blue, or UV light. Only 1 wavelength region could be tested at a time with the 2 growth chambers, but results of experiments with all wavelengths of radiation are summarized in figure 5. It is evident that red light was nearly as effective as white light in preventing injury from DNP application. Green and UV light were only slightly more effective than darkness in preventing injury, while blue light had an intermediate effect.

Effect of Light Regime on the Phytotoxicity of Polyphenols. A cursory survey of various polyphenols including catechol, resorcinol, pyrogallol and orcinol pointed up an interesting and significant re-

---

**Fig. 2.** Fresh weights of leaves from plants treated with various concentrations of DNP followed by light or darkness (24 hours) compared with the control. Nine plants were included in each treatment and the points represent the total fresh weight of 18 leaves 6 days after treatment with DNP.

**Fig. 3.** Influence of darkness before DNP treatment on the toxicity of DNP (2 × 10^{-3} M). Except for 1 group of plants (DNP + light) which received no darkness, plants received a standard 16-hour dark period after DNP treatment. Nine plants were included in each time treatment and the points represent the fresh weight of 18 leaves 1 week after treatment with DNP. Control plants received no chemical.

**Fig. 4.** Fresh weights of leaves from plants treated with DNP and given dark periods of various duration compared to the control (untreated plants placed in the light for 24 hours). Plants were treated with 1.5 × 10^{-3} M DNP. Twenty plants were included in each time treatment and the points represent the total fresh weight of 40 leaves 1 week after treatment with DNP.
remain in the light. The results shown in figure 6 are essentially opposite to those shown in figure 2. That is, damage due to catechol was more severe if plants remained in the light following treatment than if they were placed in the dark.

![Figure 5](image)

**Fig. 5.** Comparison of various wavelengths of light in the prevention of DNP injury. Symbols indicate the mean fresh weight ± 2 standard errors of 16 replicates of paired leaves treated with $2 \times 10^{-3}$ M DNP and receiving different colored light (as indicated), white light (W) or darkness (D) for 24 hours after treatment with DNP. The control symbol (C) refers to untreated plants receiving 24 hours of white light during the experimental period.

relationship between light and the toxicity of one of these compounds. In 1 experiment bean plants were treated with $10^{-2}$ M solutions of each of the polyphenols and half of the plants receiving each chemical were given either a 24-hour dark period or left in the light for the same period of time. The results of a typical experiment are shown in table II. Of the chemicals surveyed, only catechol was significantly toxic at the concentration used. In contrast to DNP, however, catechol was considerably more toxic to plants when they received light after treatment than when they received darkness.

In an experiment analogous to the first one described above with DNP, catechol was applied to plants at 3 different concentrations. Plants were then placed in the dark for 24 hours or allowed to

![Figure 6](image)

**Fig. 6.** Response of plants to catechol concentration in relation to the light conditions plants received after treatment. Twelve plants were included in each treatment and the points represent the total fresh weight of 24 leaves weighed 12 days after application of the chemical. The control line refers to untreated plants which received 24 hours of darkness following the time the other plants were treated.

**Modification in the Response of UV Irradiated Plants to 2,4-D.** Eighty uniform bean plants were divided into 4 groups and given the following treatments: 2,4-D (applied by dipping 1 of the primary leaves), 2,4-D followed by 23 minutes of UV irradiation, 23 minutes of UV irradiation followed by 2,4-D treatment, or 23 minutes of UV alone. Half of the plants in each group were then placed in the dark for 24 hours and the other half of the plants received about 8 hours of solar radiation in the greenhouse. The mean epicotyl curvature of each group of plants 24 hours after treatment is shown in table III.

Plants treated with 2,4-D and irradiated with UV light exhibited epinastic responses only if the plants were returned to the light following treatment. Plants placed in darkness following treatment showed none of the epinastic responses associated with 2,4-D treatment. The response was essentially the same whether the 2,4-D was applied before or after UV irradiation.

The leaves which were irradiated and placed in the dark acquired a slight bronzed appearance 24 to 36 hours after irradiation, particularly the leaf to which the 2,4-D was applied. Leaves of plants which received the same amount of irradiation but were left in the light in the greenhouse showed no visible irradiation injury and looked in all re-
Table III. Effect of Ultraviolet Radiation and Light Conditions after Treatment on Response of Bean Plants to 2,4-D

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conditions after treatment</th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 4-D**o</td>
<td>488 ± 6.2</td>
<td>41.5 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>2, 4-D + 23 min UV</td>
<td>29.9 ± 4.1</td>
<td>3.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>23 min UV + 2, 4-D</td>
<td>30.1 ± 3.42</td>
<td>1.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>23 min UV</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Mean and standard error of epicotyl curvature of 10 plants.
** 5 × 10⁻³ m 2,4-D applied by dipping 1 primary leaf in the 2,4-D solution.

spects like the leaves of nonirradiated plants. The leaf that received the 2,4-D treatment was often noticeably curled, even on those plants which did not exhibit any appreciable epicotyl curvature. This experiment was repeated with similar results 4 times.

UV Time Series. Bean plants were irradiated with UV light for different lengths of time (0–25 min). 2,4-D was applied (single 50 lambda drop on a primary leaf), and the plants were placed in the dark. Control groups received zero or 25 minutes of UV radiation 2,4-D treatment and light (1000 ft-c) in the growth chamber.

The epinastic response of bean plants to 2,4-D decreased with increasing exposure to UV light (fig 7). Five minutes of irradiation decreased the epinastic response by more than 40%, and plants irradiated with UV 20 minutes and left in the dark virtually failed to respond to 2,4-D treatment. Plants which had received 25 minutes of UV radiation followed by light did respond to 2,4-D treatment.

2,4-D Concentration Series. Plants were irradiated with UV for 24 minutes prior to applications of 2,4-D in 3 concentrations, 10⁻², 10⁻³, 10⁻⁴ M (single drop on 1 primary leaf). Half of the plants receiving each concentration of 2,4-D were left in the dark 24 hours, along with nonirradiated plants treated with the same concentrations of 2,4-D. The other half of the irradiated plants was placed in the light in growth chambers, along with nonirradiated plants receiving only 2,4-D treatment. Figure 8 shows the mean epicotyl curvature of the plants in each treatment.

The inhibitory effect of UV light on the response of plants to 2,4-D extends over a wide concentration range. At high concentrations (10⁻² M) there was some epinasty exhibited in those plants receiving darkness after treatment, but the total response is significantly less than when the plants are placed in the light after treatment. As usual in this study, lower 2,4-D concentrations produced little epinasty in plants given darkness after UV irradiation.

Reversal of UV Effects by White Light. It seemed apparent that white light following UV irradiation would effectively reverse the effects of UV on the response of plants to 2,4-D. In 1 experiment, summarized in table IV, the effect of 1 hour of white light following UV radiation and 2,4-D treatment was investigated.

Plants which received 1 hour of white light in the growth chambers before being placed in the dark 24 hours (treatment 5) showed considerable epinasty compared to plants which were placed in uninterrupted darkness (treatment 4) for 24 hours. Thus, 1 hour of white light was adequate to partially reverse the effects of UV radiation.

Discussion

The results of this study confirm the observation of Salisbury (21) that the phytotoxic properties of DNP are influenced by the light regime following application of the chemical. In addition, the present investigation indicates that at least 3 other factors, the light regime prior to DNP treatment, pH at which the material is applied, and the quality of light after DNP treatment, have a significant influence on the differential toxicity of DNP.

The experimental results shown in figure 5 are of particular interest since they, in part, may account for the differential toxicity of DNP in light and darkness and provide a test for our original hypothesis regarding the physiological basis of DNP injury. Black et al. (6) showed that red and blue light are most effective and green least effective in bringing about photosynthetic phosphorylation. If the prevention of injury due to DNP is based on maintaining the ATP level in the leaves by photo-

Table IV. Reversal by White Light of Effects of Ultraviolet Radiation on Epinastic Response of Bean Plants to 2,4-D

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Light conditions after treatment</th>
<th>Degree of curvature* (After 24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2, 4-D**</td>
<td>Light</td>
<td>34.0 ± 6.1</td>
</tr>
<tr>
<td>2. 2, 4-D</td>
<td>Dark</td>
<td>48.5 ± 7.8</td>
</tr>
<tr>
<td>3. 30 min UV + 2, 4-D</td>
<td>Light</td>
<td>38.6 ± 7.0</td>
</tr>
<tr>
<td>4. 30 min UV + 2, 4-D</td>
<td>Dark</td>
<td>9.5 ± 0.3</td>
</tr>
<tr>
<td>5. 30 min UV + 2, 4-D + 1 hr light (1000 ft-c)</td>
<td>Dark</td>
<td>23.8 ± 3.42</td>
</tr>
<tr>
<td>6. 30 min UV + 2, 4-D + 1 hr dark</td>
<td>Light</td>
<td>27.0 ± 5.7</td>
</tr>
</tbody>
</table>

* Mean and standard error of epicotyl curvature of 10 plants.
** 3 × 10⁻⁴ M 2,4-D applied in a single drop (50 lambda) to 1 primary leaf.
Although catechol and DNP are both reported to function as uncouplers of oxidative phosphorylation (13), the influence of light upon their phytotoxicity is completely different. Lieberman and Biale (13) concluded from their studies concerning the inhibition of oxidative phosphorylation by phenols that only the oxidized form of catechol (o-benzoquinone) inhibited the process, and that the reduced form was innocuous. Schaal and Johnson (22) found that the most effective polyphenols inhibiting the growth of Streptomyces scabies were those that auto-oxidized readily to the quinone, such as catechol. Enzyme systems capable of catalyzing the oxidation of polyphenolic compounds have recently been reported by Sisler and Evans (24), and the enzyme polyphenol oxidase, also widely distributed in fungi and higher plants, functions in catalytic breakdown of catechol in plants. Thus, it seems likely that the toxicity of catechol in these studies was actually due to a light-catalyzed production of a quinone or a labile hydroperoxide.

The experiments shown in figure 7 and 8 indicate that the response of plant tissues to 2,4-D is considerably modified when the plants have been subjected to brief periods of UV radiation, and that the response of irradiated plants to 2,4-D depends on the light regime to which they are subjected after irradiation. These results are similar to those of de Zeeuw and Leopold (10) with tomatoes. In their investigations, however, the auxin (naphthalene acetic acid) responses of tomato plants were prevented by UV radiation even when the plants were given white light after irradiation and auxin treatment. The histological effects of UV radiation on bean leaf tissue in relation to the light conditions following irradiation as reported by others (3, 25) were also observed in this study.

The results of experiments such as those summarized in tables III and IV suggest that a photo-reversal mechanism similar to that reported to be of widespread occurrence in both plants and animals (4, 11, 12, 25) may be operative in bringing about the observed reversal by white light of the inhibitory effects of UV on the response of bean plants to 2,4-D.

The UV light used in this study could not have destroyed the 2,4-D directly and probably did not prevent its uptake, since the results were essentially the same whether the 2,4-D was applied before or after the plants were irradiated. Furthermore, plants which received 2,4-D before irradiation exhibited epinasty if they were returned to the light.

Summary

The influence of the light regime under which plants are grown on the toxicity and physiological activity of 2,4-dinitrophenol and 2,4-dichlorophenol has recently been presented by Ross (20) and Wessels (26).

### Fig. 7. The effect of the amount of UV radiation on the response of bean plants to 2,4-D treatment. The points represent the mean epicotyl curvature of 15 plants measured 24 hours after 2,4-D treatment. The lower line shows the epicotyl curvature of plants receiving various amounts of UV followed by darkness (24 hours). The upper line represents the epicotyl curvature of plants receiving either zero or 25 minutes of UV followed by light.

### Fig. 8. The effect of UV radiation on the response of bean plants to various concentrations of 2,4-D applied before irradiation (24 min). Each point represents the mean epicotyl curvature of 10 plants measured 24 hours after application of 2,4-D. The light and dark lines refer to plants which received either 24 hours of light or darkness after 2,4-D treatment but no UV radiation.
oxoaeic acid was studied under conditions of controlled light and temperature.

The phytotoxicity of 2,4-dinitrophenol increased with the amount of darkness plants received after application of the chemical up to 12 to 16 hours, at which time almost maximum injury from a given concentration of the compound had occurred. Plants receiving light after 2,4-dinitrophenol treatment were relatively uninjured. The light regime under which plants were grown prior to 2,4-dinitrophenol treatment and the pH at which the 2,4-dinitrophenol was applied also had a significant influence on the toxicity of the chemical.

White or red light was more effective than green or blue in preventing damage from 2,4-dinitrophenol. This is in agreement with the hypothesis that prevention of 2,4-dinitrophenol damage by light is due to the maintenance of high adenosine triphosphate levels through photophosphorylation.

Damage due to various herbicides chemically similar to 2,4-dinitrophenol is also prevented to varying degrees by light. Catechol and perhaps other polyphenols seem to act in a manner just opposite to 2,4-dinitrophenol in relation to the light environment. Catechol was considerably more toxic when plants received light after application of the compound.

Bean plants subjected to short periods of ultraviolet radiation (2537 Å) fail to exhibit the usual epinastic responses to 2,4-dichlorophenoxyacetic acid if placed in the dark subsequent to treatment. Treated plants placed in the light, however, exhibited the normal epinastic responses associated with auxin treatment. Ultraviolet radiation was effective in preventing epinasty whether applied before or after 2,4-dichlorophenoxyacetic acid treatment.

The results suggest that a photoreversal mechanism similar or identical to that reported to bring about the reversal of other ultraviolet effects in various organisms is also operative in reversing the effect of ultraviolet light on the response of bean tissue to 2,4-dichlorophenoxyacetic acid.

Literature Cited