

Desmedipham Phytotoxicity to Sugarbeets (*Beta vulgaris*) Under Constant Versus Variable Light, Temperature, and Moisture Conditions¹

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Abstract. The type of growth chamber environmental regime used altered the response of sugarbeets (*Beta vulgaris* L. 'USH-9') to desmedipham [ethyl *m*-hydroxycarbanilate carbanilate(ester)]. No difference occurred in growth retardation following treatment at different times of day when standard growth chamber constant day/night temperature and light cycles were used. When temperatures were programmed and light intensity varied during the day/night cycles, approximating out-of-doors daily cyclic conditions, the herbicide caused greater injury following morning treatments and less injury following late afternoon treatments. Data obtained in the growth chamber under the typical constant day/night pattern did not agree with field derived information; data obtained using the programmed temperature and varying light regime agreed well with field data and with known action of desmedipham. Plants stressed for moisture after treatment were less retarded than plants turgid at treatment; but both showed relationships to time of day at treatment similar to those noted above. Moisture stress prior to treatment diminished growth retardation by desmedipham, and reversed the treatment time of day effect.

INTRODUCTION

Changes in environmental conditions can profoundly alter the activity of herbicides (6, 8). High temperatures and high light intensities have been shown to increase the injury to sugarbeets following treatment with phenmedipham (methyl *m*-hydroxycarbanilate *m*-methylcarbanilate) (4) and desmedipham (2). The time of day at treating is not generally recognized as causing major differences in herbicide activity. MCPA [(4-chloro-*o*-tolyl)oxy]acetic acid has, however, been shown to cause greater injury when applied to peas (*Pisum sativum* L.) in the late afternoon than when applied in the early morning (14). Recent reports have indicated that injury to sugarbeets treated with phenmedipham and desmedipham in the field was greater when treatments were made in the early morning than in the late afternoon³ (10).

Assessment of the effects of environmental variables are normally conducted in growth chambers utilizing a temperature regime that is constant at a higher value during the day and constant at a lower value during the night (5). Light is

normally on or off on the same day/night time cycle. Downs and Hellmers (5) argued that programmable temperature probably offers no advantage over growing plants under the constant higher day temperature and constant lower night temperature cycle. We had reason to suspect that the response of sugarbeets to desmedipham was not the same when constant versus varying temperature and light cycles were used. This paper reports on the response of sugarbeets to desmedipham when applied in the morning, mid-day or late afternoon in relation to constant versus varying temperature and light cycles. Determinations of the interactions with moisture stress before and after treatment were also undertaken.

MATERIALS and METHODS

Sugarbeet seeds were soaked in water for 24 h and planted in soil in 210 ml plastic pots. Seedlings were grown in a growth chamber at a temperature varying continuously according to a sine-wave pattern between 15 C at night and 35 C at noon (Figure 1). Pots were watered daily to field capacity with 0.5 strength Hoagland solution (9). Day and night lengths were 16 and 8 h, respectively. Light intensity measured with a Lambda Instrument LI-185 Quantum Sensor at plant height was 500 microeinsteins m⁻²sec⁻¹ (approximately 20 klux). Plants were treated with 1.1 kg/ha of desmedipham at the cotyledon or 2-leaf stage of growth (first or third true leaves approximately 2 mm long) using a greenhouse beltsprayer. Times of treatment were after 3, 7, or 11 h of light exposure, corresponding to 0900, 1300 and 1700 h (Figure 1). Different groups of plants were exposed to different combinations of the following temperature and light intensity conditions during the 24 h period following the first treatment time: day and night temperatures constant at 35 and 15 C respectively; temperature varying according to the sine-wave pattern between 35 and 15 C; light intensity constant during the 16 h day at 500 microeinsteins m⁻²sec⁻¹; light at half intensity during the first 3 and last 5 h (Figure 1). The four possible combinations of the above temperature and light intensity conditions were used.

Some plants exposed to constant temperature and constant light or varying temperature and varying light were stressed for moisture before or after treatment; others were maintained at field capacity. Moisture stress was measured with a pressure chamber (11). Measurements were made at the times of treatment for 2-leaf plants stressed before treatment, which occurred after 48 (0900), 52 (1300), or 56 (1700) h without irrigation; these values were compared with concurrent

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measurements of non-stressed controls. Stress measurements were made after 48 h without irrigation in all plants stressed after treatment. Cotyledon stage plants were not watered for an additional 24 h period.

Some control plants were harvested on treatment day (pretreatment controls), other control plants and treated plants were grown for 7 days following spraying, cut at soil level, dried at 80 C, and weighed. Posttreatment gain in shoot weight was determined by subtracting the dry weight of the pretreatment control from the 7-day harvest weight. Percent of posttreatment growth in treated plants was calculated by dividing their gain in dry weight by that of the posttreatment controls. All different exposure groups consisted of 10 replications (10 pots with 4 plants per pot). Two exposure groups (temperature/light intensity/moisture stress combinations) were grown simultaneously; the experiment consisted of a sequence of four such simultaneous growth chamber runs. The validity of comparisons between each sequence of this series of growth chamber runs was evaluated by an analysis of variance of the controls. There was no significant difference (at 5% level) due to sequence number. Statistical significance of the treatment results was determined for non-stressed plants by a factorial analysis with time of treatment, temperature and light intensity as factors. The factors analysed statistically for stressed plants were moisture stress, temperature and light conditions.

In a separate experiment plants used in determining photosynthetic inhibition were grown in a greenhouse and treated with desmedipham as described above, but at the 4-leaf stage of growth. These plants were treated with desmedipham at the same time and were not subjected to variable conditions. Growth conditions were: average day and night temperatures 30 and 20 C, respectively, maximal light intensity 2000 microeinsteins $m^{-2}sec^{-1}$, average day and night lengths 16 and 8 h. Following treatment, plants were transferred to growth chambers and placed in the dark or in the light (490 microeinsteins $m^{-2}sec^{-1}$) at a temperature of 30 C. The plants were exposed to $^{14}CO_2$ at regular intervals after treatment. Inhibition of CO_2 fixation was determined as described previously (2). The average of 5 replications (pots with 4 plants per pot) of treated and control plants was used to compute percent inhibition at each, light or dark, exposure time. Dark and light exposed plants were placed under the same light conditions prior to measurement for a sufficient length of time to allow stomatal openings to equalize. Results were subjected to an analysis of variance with time of harvesting and light or dark exposure as main effects.

RESULTS and DISCUSSION

Sugarbeets grown under the constant temperature and light intensity regime showed no significant differences in posttreatment growth when sprayed with desmedipham at different times of the day (Figure 2). When light intensity or temperature were varied (Figure 1) to simulate outdoor conditions the plants treated earlier in the light period were more severely injured (Figure 2). The difference in desmedipham-induced injury between morning and the late afternoon treatments was even larger when light and temperature were varied simultaneously. Although the growth of the plants per se was little

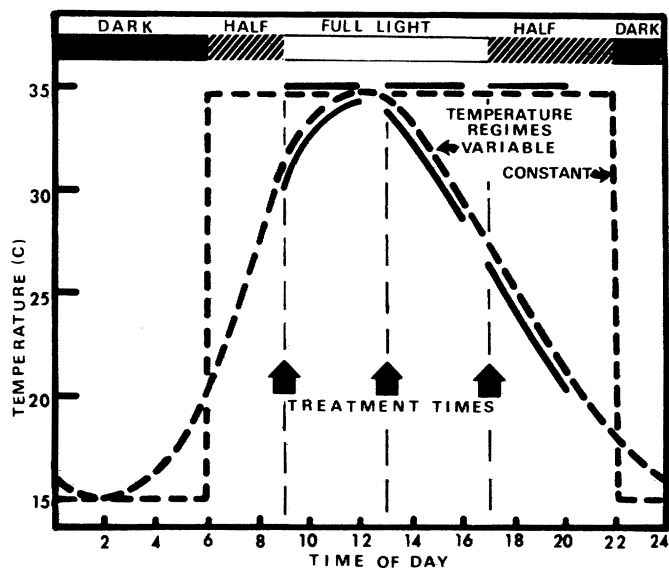


Figure 1. Temperature and light regimes during the 24 h treatment period. Solid black bars along abscissa indicate darkness. Under constant light conditions plants were exposed to a light intensity of 500 microeinsteins $m^{-2}sec^{-1}$ from 0600 to 2200 h. Under variable light conditions light intensity was dimmed to half intensity during the first 3 and last 5 h of the 16 h day, as indicated by the hatched bars. Constant or variable temperature regimes are indicated by square or sine wave curves. Treatment times are shown by annotated arrows. The 3 h after treatments, which lead to maximal photosynthetic inhibition, are shown as solid black lines along the temperature curves.

affected by the growth chamber light or temperature regime it was clear that the physiological processes in the plants in relation to desmedipham uptake, activity and/or deactivation must have been altered. The blanket statement that there are no advantages to growing plants under variable regimes rather than constant regimes (5) thus does not seem to be universally valid. If the type of phenomenon reported here occurs with other herbicides, or other externally applied growth altering substances, especially those that might alter photosynthetic processes, then interpretation of results obtained using conventional growth chambers should not be assumed to be also applicable to field conditions. The previously noted (10) field responses of sugarbeets to desmedipham sprayed at different times of day were duplicated in the growth chamber only when the variable regimes were used; constant regimes provided data not consistent with the field information. Our data indicate that researchers studying effects of herbicides in relation to temperature and light should consider utilizing variable temperature and light regimes.

The experiment, in which the magnitude of posttreatment photosynthetic inhibition was determined at regular intervals (Figure 3), showed maximal inhibition to occur 3 h after treatment. Higher temperatures and light intensities have previously been shown to increase injury caused by desmedipham (2).

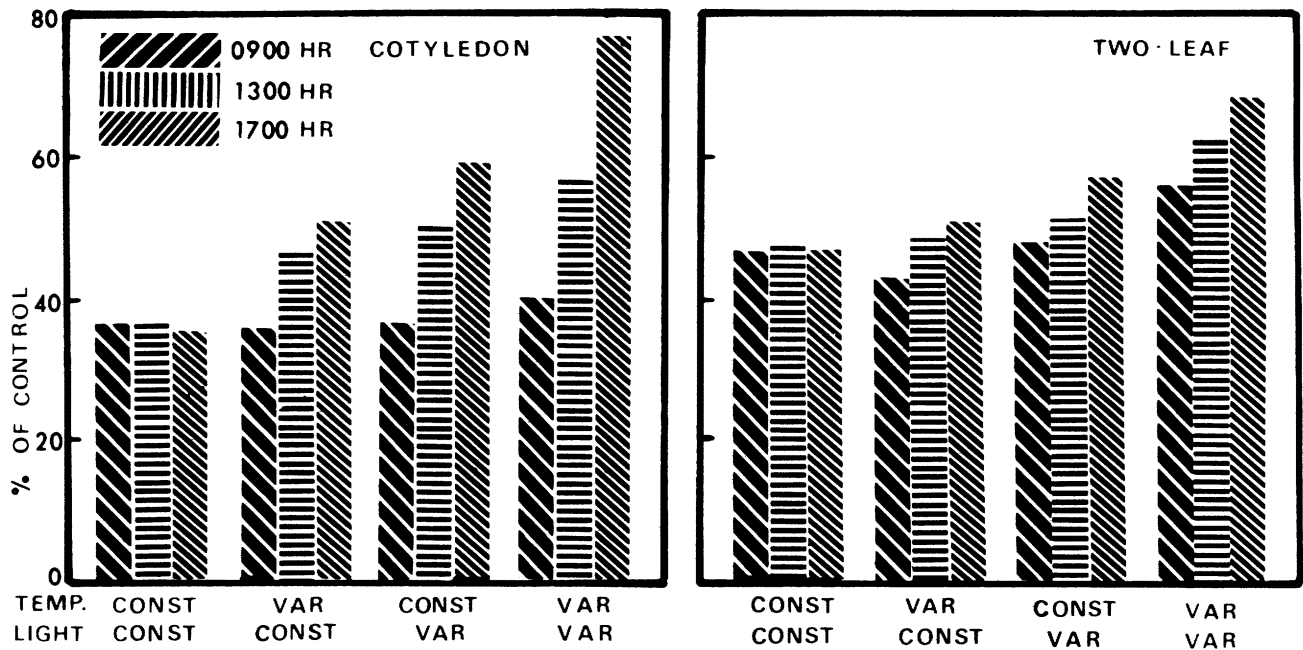


Figure 2. The effect of differing temperature and/or light intensity regimes on sugarbeet growth following treatment with 1.1 kg/ha of desmedipham at different times of the day. Percent of control represents the ratio of posttreatment gain in dry weight of treated plants to that of the controls. The stage of seedling growth at treatment is noted on the appropriate histogram. See text for explanation of temperature and light cycles employed.

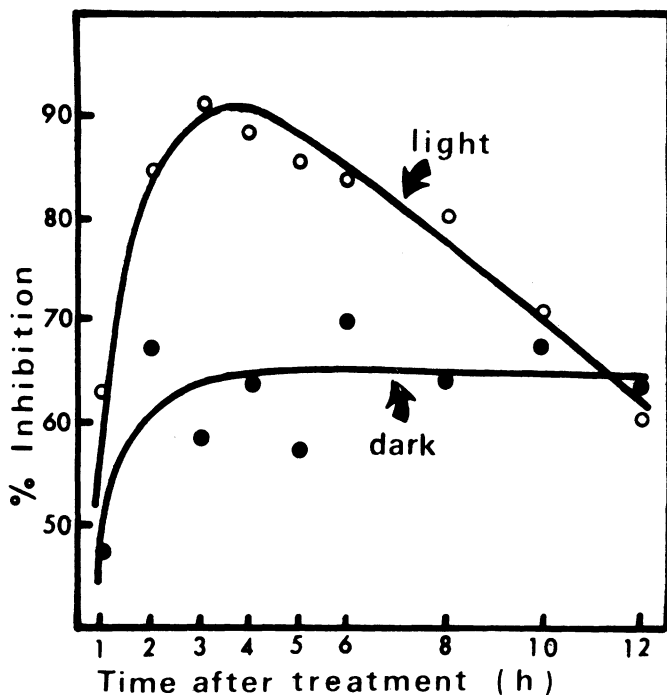


Figure 3. Inhibition of CO₂ fixation in sugarbeets. Greenhouse grown plants were treated with 1.1 kg/ha of desmedipham at the 4-leaf stage and kept in the light or dark following treatment for the times indicated.

These facts suggested an interaction between the conditions prevailing during this critical (initial 3 h) inhibition period and the magnitude of the injury. In the time-of-day-at-treatment experiment, inhibition and temperature maxima under the variable temperature regime coincided, at 1200 h, only for the 0900 h treatment. Temperatures were decreasing (Figure 1) during the critical inhibition period for the later treatments. Plants treated at 0900 h under the variable light regime received 8 h, those treated at 1300 h only 4 h, and those treated at 1700 h received 0 h of full light after treatment, followed in all cases by 5 h of reduced light (Figure 1). Peak photosynthetic inhibition thus occurred with less or no high light intensity following treatments made later in the day. Full light and high temperature, however, occurred during the maximal inhibition period regardless of the time of day at treatment when the temperature and light regimes were constant. The period of maximal light and temperature the next day would seem not to be critical, as the plants were showing substantial recovery by that time, Figure 3 and as reported previously (2).

The adverse effects of exposure to high temperatures or to longer periods of bright light were more pronounced in plants at the cotyledon stage (Figure 2) than in the larger plants, indicating greater uptake and/or lesser ability to inactivate the herbicide in the younger plants. The main effects due to time of day at treatment and to light intensity were statistically significant, in cotyledon stage plants, at the 0.1% level, and

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Table 1. Change in water status of 2-leaf stage sugarbeet seedlings stressed before and after herbicide treatment.

Time without irrigation		Water stress at end of stress period following spraying.				Time without irrigation		Water stress at spraying; plants stressed before spraying.			
Time of day	(h)	TcLc ^a		TvLv ^b		(h)	TcLc ^a		TvLv ^b		
		turgid (Bars)	stressed (Bars)	turgid (Bars)	stressed (Bars)		turgid (Bars)	stressed (Bars)	turgid (Bars)	stressed (Bars)	
0900	48	-8.1	-13.3	-8.0	-13.0	48	-7.6	-13.4	-7.0	-13.2	
1300	52	-8.8	-14.8	-8.2	-13.8	48	-8.0	-13.6	-7.1	-13.4	
1700	56	-9.6	-17.7	-9.4	-16.6	48	-8.6	-14.2	-7.1	-13.2	

^aTcLc – Temperature and light constant.

^bTvLv – Temperature and light varying.

those due to temperature at the 0.5% level. The interaction between light regime and time of day at application was highly significant (at 0.1% level) indicating that the response due to spraying at different times of day differed significantly depending on the light regime used. Light effects were also significant at the 0.1% level with plants treated at the 2-leaf stage, but temperature and time of day effects, although discernible, were less pronounced (significant only between the 20 and 10% level and at the 10% level respectively) and showed the same trends as those obtained with the smaller plants (Figure 2). Light regime therefore seemed to be the more important variable than temperature, which should probably be expected in relation to a photosynthetic inhibition phenomenon.

The effects of pre- and posttreatment moisture stress differed in magnitude and pattern. Plants treated at the 2-leaf stage were visibly wilted at the end of the stress periods. The differences in water potential between plants stressed before treatment and unstressed plants, measured at the time of treatment, increased rapidly toward the end of the second day of the stress period. These differences were slightly more pronounced in plants under the constant than under the variable temperature-light intensity regime (Table 1). Cotyledon stage plants were not visibly wilted after 72 h without irrigation and pressure chamber measurements showed no water deficit differences between stressed and non-stressed plants. Stressed cotyledon stage plants, however, were distinctly yellowish in color and growth of their first true leaves was retarded by 50%, as determined by measurement of their blade lengths (approximately 4 mm for stressed and 8 mm for unstressed plants). Differences in the water potentials between plants stressed after treatment and unstressed plants in the 2-leaf stage were also alike regardless of the time of treatment, as they were not irrigated for the same length of time (Table 1).

Plants stressed for moisture following desmedipham treatment showed responses in relation to time of treating similar to those which were not stressed (Figures 4 and 5). This confirmed that variable growth chamber regimes did not provide the same herbicide-plant response as constant regimes. The magnitude of the herbicidal retardation was somewhat less for the plants stressed for moisture after treatment than for those which were fully turgid. Under constant temperature and light conditions plants stressed for moisture after treat-

ment showed no time of day effect in relation to desmedipham treatment (Figure 4). There was, however, a pronounced decrease in activity with later treatment times when temperature and light were varied (Figure 5).

Pretreatment moisture stress decreased desmedipham-caused growth retardation in comparison with fully turgid plants in all cases (Figures 4 and 5) except for the 1700 h treatment under variable temperature and light intensity conditions in the smaller plants. Increasing growth retardation with later treatment times may be explained by progressive herbicide-independent drought injury. The influence of time of day at desmedipham application was reversed by moisture stress prior to treatment. The later applications caused less injury than those made earlier (Figures 4 and 5). This was observed even under constant temperature and light conditions. The main effects of the analysis of variance due to moisture stress were significant at the 0.5% level and due to temperature and light at the 5% level. The interaction between time of day and moisture stress was significant at the 5% level. In the experiment which established the time of maximal photosynthetic inhibition, the effects due to the time of harvesting after treatment and to light and dark exposure were significant at the 5% level.

It appears unlikely that decreased herbicide penetration due to stomatal closure in plants under stress at the time of treatment caused the reversal in injury due to desmedipham, as plants kept in the dark were also shown to suffer high photosynthetic inhibition within 3 h after treatment (Figure 3). One possible explanation of the counter effect of moisture stress to desmedipham injury may be due to the decrease in Hill reaction activity in water stressed plants (7). Water deficits are known to reduce pigment formation (13), and particularly that of the chlorophyll-protein complex containing chlorophyll b, which is mainly associated with photosystems II (1). Hill reaction inhibitors such as desmedipham have been shown to disrupt the photosynthetic electron transport chain in the photosystem II region (3). With photosystem II inhibited, secondary reactions, such as the photooxidation of chlorophyll (12) could also be reduced. Water stress thus might constitute a condition which triggers a protective mechanism (the slowdown of photosystem II activity) during which degradation of the herbicide could still proceed.

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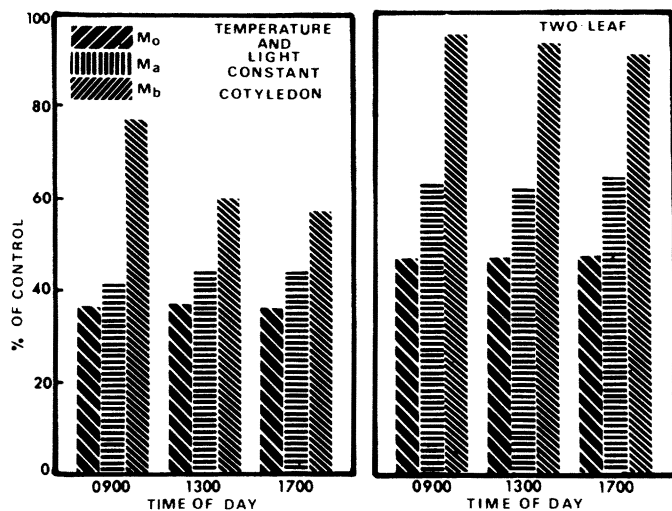


Figure 4. The effect of moisture stress on sugarbeet growth following treatment with 1.1 kg/ha of desmedipham at different times of the day at constant temperature and light intensity. Percent of control represents the ratio of posttreatment gain in dry weight of treated plants to that of the controls. Moisture levels indicated as: Mo = no moisture stress; Mb = moisture stress before treating; Ma = moisture stress after treating. Growth stage at treating is indicated on the appropriate histogram.

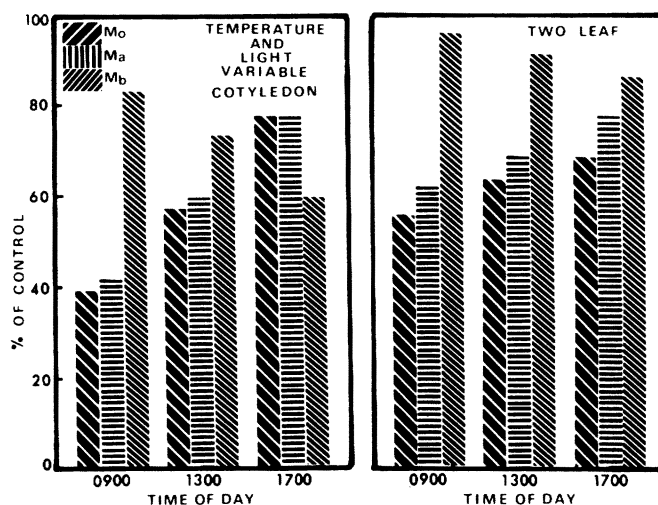


Figure 5. The effect of moisture stress on sugarbeet growth following treatment with 1.1 kg/ha desmedipham at different times of the day at variable temperatures and light intensities. Percent of control represents the ratio of posttreatment gain in dry weight of treated plants to that of the controls. Moisture levels indicated as: Mo = no moisture stress; Mb = moisture stress before treating; Ma = moisture stress after treating. Growth stage at treating is indicated on the appropriate histogram.

The herbicide response in relation to time of day at treating reported here is opposite to that shown previously for response of peas to MCPA (14). There is, however, no conflict in these two apparently opposite findings. The MCPA response to time of day was explained on the basis of differing translocation patterns at different times of the day. The desmedipham-time-of-day-interaction can be explained in relation to the rapid inhibition of photosynthesis, and associated effects, in high light intensity, followed by detoxification in the dark. These results emphasize that the physiology of the plant and the mechanism of action of the herbicide must be understood if effects of the environment, here time of day, are to be explained. The data presented indicate that spraying desmedipham in the afternoon and evening should provide better crop tolerance than spraying in the morning.

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