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EFFECTS OF DDT ON CARBOHYDRATE METABOLISM AND TRANSLOCATION IN *SECALE* SPECIES*

SHER AKBAR† and LYNDON J. ROGERS

Department of Biochemistry and Agricultural Biochemistry, The University College of Wales, Aberystwyth, Dyfed, SY23 3DD, Wales, U.K.

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Key Word Index—*Secale cereale*; Gramineae; rye; DDT; sugar metabolism; translocation.

Abstract—An inhibition of photosynthetic electron transport in susceptible rye following treatment with DDT is accompanied by an increase in dry weight of leaves contacting the pesticide due to an accumulation of fructose, glucose, and to lesser extent, sucrose. Several days after treatment over 40% of the dry weight is due to these sugars. The assimilation of $^{14}CO_2$ by leaf segments was decreased as a consequence of DDT treatment, but labelling patterns were similar to those for leaf segments from untreated plants. However, if given a prolonged period in darkness before extraction of assimilates the leaf segments from treated seedlings retained ^{14}C in sugars and did not show the substantial decrease in extractable soluble material which was characteristic of untreated controls. In DDT-treated seedlings the translocation of metabolites from leaves to roots was severely impaired.

INTRODUCTION

The effect of DDT on susceptible rye [1], as in susceptible barley [2, 3], is to inhibit photosynthetic electron transport. Two sites of inhibition have been identified, one located on the oxidizing side of photosystem 2 and the second in the intermediate electron transport chain on the oxidizing side of photosystem 1 [1, 3]. As a consequence, cyclic and non-cyclic photophosphorylations are also inhibited [1, 2].

In barley the inhibition of photosynthetic electron transport was paralleled by an increase in dry matter content and ethanol-soluble carbohydrate in DDT-treated susceptible seedlings [4]. Similar investigations with susceptible rye have been extended to comparative studies of $^{14}CO_2$ assimilation by leaf-segments from DDT-treated and untreated seedlings, and to studies of $^{14}CO_2$ assimilation and assimilate translocation in whole seedlings.

RESULTS AND DISCUSSION

Following treatment of susceptible rye (var. Lovaspatonai) with DDT there was a steady increase in dry weight of the treated leaves and after six days the dry weight/wet weight ratio was 0.22 compared to 0.14 for untreated; the latter ratio did not change significantly over the course of the experiment (Fig. 1a). For both DDT-treated and untreated resistant rye (var. Rhayader) the dry weight/wet weight ratio remained constant (data not shown). Parallel assessment of ethanol-extractable material (Fig. 1b) showed that in the susceptible rye over 40% of the dry weight of the treated leaves was accounted for in this fraction, compared to about 10% in untreated. Such an increase in extractable material would account for over 90% of the increase in dry weight of treated leaves. When total sugars in extracts were determined it was evident that these would account for most of the material accumulated in treated leaves (Fig. 1b). Comparable results for the resistant rye showed no distinction between treated and untreated seedlings in either total extractable material or sugars, and the data obtained were similar to that for untreated susceptible rye. This latter was true for all subsequent aspects of this study and to simplify presentation these data with resistant rye are not presented.

Analyses of individual sugars in treated and untreated leaves confirmed these data (Fig. 2). In susceptible rye treated with DDT there were substantial increases in amounts of the monosaccharides glucose and fructose, about nine-fold and six-fold, respectively. While the increase in sucrose was less it was none the less significant. The total increase in dry weight from this accumulation of sugars would account for the increase in extractable material (Fig. 1) and hence for the increase in dry weight in treated leaves (Fig. 1a). The amounts of fructosans, a soluble reserve oligosaccharide, were similarly low in both treated and untreated leaves. These data are very similar to those which characterise the effect of the pesticide in susceptible barley [4]. When barley was exposed in further experiments to an appreciably higher light intensity (30 klx) after treatment there was an accumulation of starch which was retained through a succeeding period in darkness. Under the growth regime used in the present work starch did not contribute significantly to the increase in dry weight.

Plants can accumulate sugars in leaves if photosynthesis rates are high or if there is low translocation or low sink demand [5]. Treatment of wheat to decrease

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†Present address: Department of Agricultural Chemistry, NWFP Agricultural University, Peshawar, Pakistan.

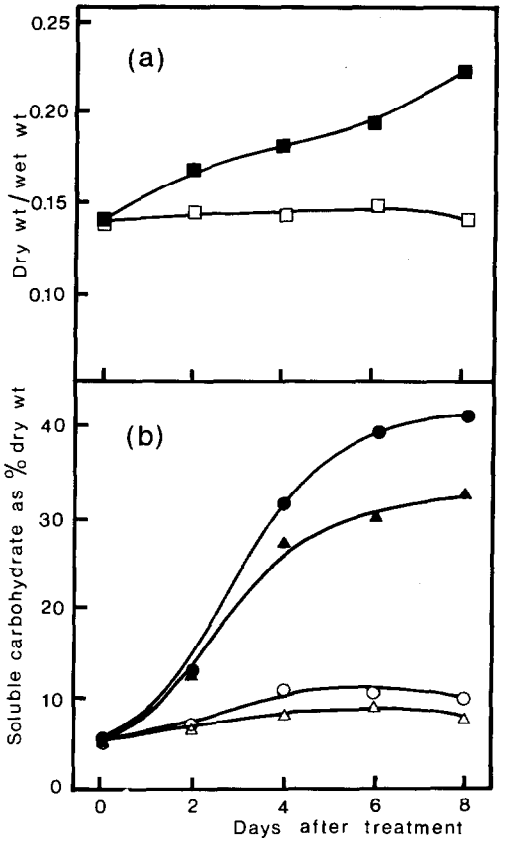

Fig. 1. Effect of DDT on dry weight and ethanol-soluble carbohydrate. Susceptible seedlings were treated with DDT and sampled at intervals up to 8 days. Figure 1a shows the data for dry weight/wet weight ratio. Closed symbols are data for treated seedlings, and open symbols are untreated controls. In Fig. 1b ○, ● represent ethanol-soluble carbohydrate and △, ▲ represent total sugars. Results for treated resistant rye were similar to those for untreated susceptible rye.

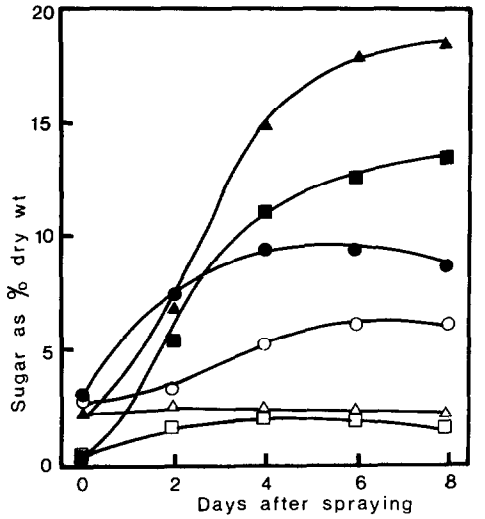

Fig. 2. Effect of DDT on the principal components of the ethanol-soluble carbohydrate fraction from susceptible rye. Susceptible seedlings were treated with DDT and individual sugars estimated at intervals up to 8 days. Closed symbols are data for treated seedlings, and open symbols are untreated controls. ○, ● Sucrose; △, ▲ glucose; □, ■ fructose.

translocation [6] resulted in a decreased CO_2 assimilation which was accompanied by accumulation of carbohydrate. Sucrose, glucose and fructose were all at elevated levels and in total were about the same as seen in the present study. Amounts of starch were also raised. The decreased CO_2 fixation was attributed to an end product inhibition of photosynthesis by the accumulated sugars. Increase in soluble carbohydrates accounted for an increase in dry matter of the flag leaf following ear removal to reduce sink demand [7]. Accumulation of sugars to comprise about 14% of the dry weight of the wheat shoot occurred in response to growth at low temperatures [8].

The recognition of these marked disturbances in amounts of central metabolites in rye in response to DDT prompted further investigations of $^{14}CO_2$ assimilation and of the subsequent distribution of ^{14}C amongst metabolites. In these studies leaf sections, or in some experiments whole seedlings, were exposed to $^{14}CO_2$ at atmospheric concentration. After a period of steady-state photosynthesis the tissue was extracted and ^{14}C assimilated into soluble products assessed before individual compounds were separated by 2D-TLC. Radioactive areas corresponding to individual compounds were precisely located by autoradiography and radioactivity determined. The methodology is described fully elsewhere [9].

The data in the first two columns of Table 1 summarize the incorporation of ^{14}C in total and into major metabolites. In leaf sections from treated susceptible rye assimilation was less than in those from untreated seedlings and this difference lay well outside that which might be attributed to variability in sampling. This was consistent with the earlier observation [1] that photosynthetic electron flow is inhibited by treatment with DDT. However, the patterns of incorporation (LuO and LtO in Table 1) were very similar with sucrose, probably predominantly in the cell vacuole [10], forming the major ^{14}C component. That patterns of metabolites are so similar would suggest that there has been no significant affect on enzymes involved in CO_2 fixation [4]. If leaf sections were left in darkness for 12 hr before the products of assimilation were extracted and estimated the total ^{14}C in extractable material from DDT-treated and untreated seedlings was similar (560×10^3 and 680×10^3 dpm, respectively), though the latter was still somewhat higher. This suggests that in the untreated tissue some products of photosynthesis have been converted during the dark period into insoluble polymeric material. These changes are absent, or considerably diminished, in the treated leaves. For the leaf sections from untreated seedlings incorporation patterns changed markedly as a result of the period in darkness. Levels of PGA, glycine, malate and sucrose were less, decreases in the latter compensated for by appearance of fructose and glucose through invertase action [11]. Aspartate and glutamate also increased substantially. These data are not shown in the table since they are also evident in the studies with intact seedlings (Table 1), which proved more fruitful in other respects. However, on comparing leaf sections from treated and untreated plants after the dark period major differences in

Table 1. Effect of DDT on ^{14}C assimilation into ethanol-extractable products in rye

Compounds isolated	^{14}C incorporated (10^{-3} × dpm)*					
	LuO	LtO	Lu20	Lt20	Lu20r	Lt20r
Total	1280 (100)	500 (100)	1125 (100)	820 (100)	355 (100)	44 (100)
PGA	26.3 (2.0)	19.5 (3.8)	6.3 (0.6)	3.6 (0.4)	1.8 (0.5)	0.7 (1.5)
HMP	13.2 (1.0)	7.5 (1.5)	19.3 (1.7)	17.4 (2.1)	7.6 (2.1)	1.3 (3.0)
Sucrose	630.0 (49.0)	224.0 (44.7)	182.0 (16.0)	194.0 (23.7)	11.0 (3.1)	1.9 (4.2)
Glucose	Neg (<0.1)	Neg (<0.1)	248.0 (22.0)	156.4 (19.0)	85.7 (24.0)	8.5 (19.4)
Fructose	Neg (<0.1)	Neg (<0.1)	106.8 (9.4)	59.5 (7.2)	64.6 (18.0)	5.9 (13.5)
Glycolate	7.0 (0.5)	3.4 (0.7)	10.6 (0.9)	10.3 (1.3)	4.3 (1.2)	1.0 (2.3)
Glycine	54.2 (4.0)	25.0 (5.0)	19.2 (1.7)†	6.3 (0.8)†	17.5 (4.9)†	1.9 (4.2)†
Serine	73.8 (5.7)	35.8 (7.0)	72.2 (6.4)	65.5 (7.9)	27.0 (7.6)	3.1 (7.2)
Glycerate	12.2 (0.9)	5.4 (1.0)	0.7 (0.2)	2.9 (0.4)	2.6 (0.7)	0.9 (2.1)
Alanine	16.2 (1.3)	8.7 (1.7)	36.7 (3.2)	12.1 (1.4)	13.6 (3.8)	1.6 (3.5)
Malate	92.1 (7.0)	30.9 (6.0)	19.8 (1.7)	11.6 (1.4)	6.0 (1.7)	1.0 (2.2)
Aspartate	20.9 (1.6)	8.2 (1.6)	44.5 (3.9)	23.2 (2.8)	7.9 (2.2)	1.7 (3.8)
Glutamate	15.2 (1.2)	6.3 (1.3)	51.6 (4.6)	31.0 (3.7)	5.7 (1.6)	1.5 (3.4)
PEP	9.7 (0.7)	4.1 (0.8)	15.8 (1.0)	13.6 (1.6)	3.0 (0.8)	0.8 (1.9)

Four primary leaf sections of DDT-treated or untreated susceptible rye two days after spraying were arranged in frames with their cut bases in water, illuminated (*ca* 100 μE m^{-2} sec^{-1}; 25°) and flushed with normal air for 30 min then with $^{14}CO_2$-air, containing 1 μCi ^{14}C l^{-1} for 30 min (both at 0.8 l min^{-1}). LuO, LtO indicate Lovaspatonai untreated (u) or DDT-treated (t) leaf sections extracted immediately after $^{14}CO_2$ assimilation. The data for Lu20 and Lt20 were using two intact seedlings taken three days after spraying and kept in the dark for 20 hr after $^{14}CO_2$ assimilation before extraction of the leaves (Lu20 and Lt20) or root (Lu20r and Lt20r).

* % total activity in ethanol-extractable products given in parentheses. Neg, Negligible amounts.

†Included as glycine but R_f in the first dimension in chromatography somewhat lower than expected.

labelling pattern were evident. In particular, there were decreased incorporations into malate, aspartate, and glutamate in leaf sections from treated plants, balanced by higher amounts of sucrose. Raffinose, tentatively identified, and an unidentified disaccharide or oligosaccharide, were present in recognisable amounts in leaf sections from untreated plants but only trace amounts were seen in those from treated seedlings.

Useful data for defining differences in metabolism between DDT-treated and untreated seedlings during a dark period following photosynthetic $^{14}CO_2$ assimilation came from experiments with intact seedlings. Here treated and untreated seedlings were allowed simultaneously to assimilate $^{14}CO_2$ but were held in darkness for 20 hr before analyses separately of leaves and roots cut at soil level. These data are summarized in the last four columns of Table 1. At the end of the dark period treated seedlings contained less ^{14}C in extractable material (sum of Lt 20 and Lt 20r) than untreated plants (sum of Lu 20 and Lu 20r). In contrast to studies with leaf sections, differences in labelling patterns in leaves from treated and untreated seedlings in this experiment were less clear cut. Though in the former there was on a percentage basis more sucrose, the decreases in malate, aspartate and glutamate were arguably within experimental variability and a decrease in alanine was more significant. The extractable material from leaves of the treated seedlings accounted for 32% of their dry weight, in good agreement with data for plants of the same age given in Fig. 1b. Thus even in the presence of high levels of sugars $^{14}CO_2$ assimilation into sucrose and hence its monosaccharide constituents in treated rye continues unabated and there is no indication of any regulatory control in sucrose synthesis. It has been suggested that a lowered ATP/ADP ratio, such as results from inhibition of photosynthetic electron flow by the pesticide [1], might be effective in this respect [12].

When extractable material from the roots of treated and untreated plants was quantitated (Lt 20r and Lu 20r in Table 1) it was clear that translocation to the former had been severely inhibited. Total dpm in the soluble fraction of roots of treated seedlings was only about 10% that in roots from untreated. By comparing total ^{14}C in the extractable material from roots with the total assimilated into soluble material by the seedling the effect on translocation can be estimated, albeit crudely. For untreated seedlings about 24% of the ^{14}C had been translocated to the roots at the conclusion of the experiment, compared to only 5% for pesticide-treated plants. This is consistent with the observation that soluble material in roots contributed 20% to the dry weight of roots from untreated plants compared to 12% for treated, in contrast to the situation in leaves. The labelling pattern on a percentage basis differed with respect to a number of metabolites, but interpretation might be unfruitful because of the low amounts of ^{14}C in roots of treated seedlings and possible losses in recovery of some zones. There is, as expected, only a small quantity of sucrose but amounts of glucose and fructose are substantial.

The means by which sucrose synthesis in the plant is regulated is still unclear [12, 13]. A suggestion with considerable support is that because of its dependence on the availability of orthophosphate sucrose synthesis is governed by the rate of photosynthesis. Since sucrose is formed outside the chloroplast and the membrane of this is impermeable to it [14] control must be exercised at the cytoplasmic level. Both sucrose phosphatase [15] and

sucrose phosphate synthetase [16] are inhibited by sucrose, and elevated levels of this could lead by mass action effect to an increase in triose-phosphates. Sequestering of phosphate by sucrose-phosphate, hexose-phosphate and triose phosphates would decrease orthophosphate in the cytoplasm available for exchange with triose phosphates across the chloroplast envelope. Orthophosphate in the chloroplast would be lowered. Increased amounts of triose phosphates in the cytoplasm would themselves inhibit export of these from the chloroplast and favour starch formation. These and other factors [17] would contribute to a decline in photosynthesis.

However, in the $^{14}CO_2$ assimilation experiments reported in Table 1 (LtO, cf. LuO) no accumulation of triose phosphate or of sugar phosphates was evident, even against a background of considerable accumulation of sucrose and its constituent sugars. Our interpretation of the succession of events in DDT-treated rye is as follows. DDT treatment of susceptible rye results in an inhibition of photosynthetic electron transport [1] and a consequent decrease in ATP and NADPH available for carbohydrate formation. The outcome is a decrease in net CO_2 assimilation, but sugars are nevertheless formed, though in smaller amount. However, there is only limited translocation to the roots and the sucrose remains in the leaf. This and derived glucose and fructose are not depleted during subsequent dark (night) periods, and additional sugars are accumulated during intervening illuminations. As a result the dry weight of treated leaves steadily increase but growth and development of the seedling itself is adversely affected and the plant ultimately becomes chlorotic and dies.

Sucrose is the form in which carbohydrate is translocated and it is likely that phloem loading is facilitated by cotransport with protons in a symport system [17]. This transport may be hampered because of the decreased energy status of the cell [18], and in rye treated with DDT we have shown that ATP levels are decreased [1]. Similarly, the effects of triazine [19] and bipyridyl [20] herbicides on CO_2 assimilation and translocation have also been explained by their effect on photosynthetic electron flow and thereby the energy status of the leaf.

The contrary view that the primary site of action is an inhibition of sugar utilization or translocation and this causes an inhibition of photosynthetic electron flow should also be considered. Experiments with leaf discs of sugar beet showed that supplying sucrose exogenously led to a decrease in incorporation from $^{14}CO_2$ into sucrose, though starch was formed. Only at high sucrose concentrations was photosynthesis inhibited [21]. However, in intact plants the evidence for a direct link between accumulation of sucrose in leaves and decreased rate of photosynthesis is not convincing [5, 12], and for tobacco (*Nicotiana tabacum*) photosynthesis was unaffected by a three-fold increase of sugar [22]. In wheat an accumulation of carbohydrate following ear removal did not result in an inhibition of photosynthesis [7], in contrast to an earlier report [23]. Other results suggest that translocation in sugar beet is more dependent on the amount of carbon as sucrose in the leaf than on photophosphorylation [24].

The view that increased sugars might cause the observed inhibition of electron flow and thus of photophosphorylation in rye treated with the pesticide would, however, be in conflict with the observation that DDT will inhibit these photosynthetic activities *in vitro* without significant quantities of sugars being present. The interpretation we favour is therefore that the primary site of action of DDT in rye is photosynthetic electron flow and the decreased CO_2 assimilation, accumulation of sugars and lack of translocation all derive from this lesion.

Finally, it might be noted that the effects of DDT on susceptible rye are sufficiently similar to those shown by barley to suggest the same gene could be responsible. Since in principle the extent of inhibition of photosynthetic electron flow and thereby sugar accumulation could be controlled by choice of treatment (dosage, length of exposure to pesticide) these systems may be useful as tools in mechanistic studies clarifying source-to-sink processes.

EXPERIMENTAL

Treatment of plants with DDT. The methods are described in the preceding paper [1].

Extraction of soluble products. The leaves of three plants (*ca* 3 g wet weight) were homogenized in hot 80% EtOH; the leaves of a fourth plant were used to determine the wet wt:dry wt ratio. After homogenization, extracts were centrifuged at 1000 *g* for 10 min to remove cell debris, and the ethanolic solns were then carefully reduced to dryness by vacuum distillation at 36° in a rotary evaporator. The residue was extracted by gentle agitation at 25° with deionized H_2O. After removal of undissolved solids by filtration through a sintered glass funnel total carbohydrate in an appropriate aliquot was determined by PhOH–H_2SO_4 reagent [25] and the remaining extract was again reduced to dryness before soln in 5 ml 80% EtOH and transfer to a previously weighed vessel. The extract was concd to *ca* 0.5 ml under a stream of N_2 and the remaining liquid removed by freeze-drying to determine the weight of EtOH-extractable material.

Estimation of sugars. The sugars present (almost entirely glucose, fructose and sucrose) in extracts desalted on an 8 g deAcidite E (free base form)-Zeokarb 225 (H^+ form) column, after the centrifugation stage during extraction, were separated by chromatography on Whatman No. 1 paper using EtOAc–HOAc–H_2O (3:3:1, by vol.) as solvent. After location of zones on indicator strips by *p*-anisidine hydrochloride reagent [26] the individual sugars were eluted by H_2O and estimated by PhOH–H_2SO_4 reagent [25].

$^{14}CO_2$ assimilation studies. The procedures for leaf sections are given in ref. [9]. When whole seedlings were used these were gently uprooted, and held upright in suitably large boiling tubes adapted for gas flow as in the sample chamber used for leaf sections. Preparation of ^{14}C assimilate extracts, separation of metabolites by 2D-TLC and assay of radioactivity are as in ref. [9].

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