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# EFFECT OF TOXAPHENE ON CARBON DIOXIDE ASSIMILATION AND TRANSLOCATION IN *AVENA SECALE**

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**Key Word Index**—*Avena secale*; Gramineae; oat; camphechlor; sugar metabolism; toxaphene; translocation.

**Abstract**—In susceptible oat, toxaphene inhibits photosynthetic electron flow and concomitant ATP synthesis. Although the rate of $^{14}CO_2$ assimilation is apparently not affected markedly there is an increase in dry weight of leaves contacting the pesticide. The labelling patterns in leaf sections exposed to $^{14}CO_2$ are similar for both toxaphene-treated and untreated seedlings. However, if given a period in darkness before extraction it is evident that assimilation products in leaf sections from toxaphene-treated leaves remain as small $M_r$ materials, including substantial amounts of sugars, whereas in untreated controls these were converted to polymeric materials. In toxaphene-treated seedlings the translocation of assimilation products to the roots is decreased and sucrose accumulates in the leaves.

## INTRODUCTION

Susceptibility of oat (*Avena* spp.) to toxaphene (camphechlor) in field trials suggested control by a single gene, with susceptibility dominant [1]. Studies of the biochemical mode of action of toxaphene on susceptible oat have shown that photosynthetic electron flow and photophosphorylation are both inhibited in chloroplasts isolated from seedlings a few days after spraying with the pesticide [2]. The data obtained were similar to those reported for the action of 1,1,1-trichloro-bis(*p*-chlorophenyl)ethane (DDT) on susceptible barley [3] and susceptible rye [4], which is another example of a genetically controlled reaction to an organochlorine insecticide in cereals. Though there are subtle differences, in all these situations two sites of interaction of the pesticide with the photosynthetic electron transport chain could be identified. One side was located on the oxidizing side of photosystem 2, and the second site was in the electron transport chain linking photosystem 2 to photosystem 1. Despite this striking similarity, different components in the photosynthetic lamellae, or a different site on the same component, are probably involved in the responses to DDT and toxaphene. This conclusion was based on the different responses of a range of cereal varieties to the two pesticides. In a survey to be presented elsewhere, all 13 oat varieties tested against both were susceptible to toxaphene but were resistant to DDT. This was in contrast to barley (*Hordeum* spp.) where all the varieties susceptible to toxaphene were also susceptible to DDT.

In the cases of susceptibility of barley [5] and rye [6] to DDT the inhibition of photosynthetic electron transport resulted in a decrease in assimilation of $CO_2$. Nevertheless, there was an increase in dry weight of leaves which had contacted the pesticide [5, 6] due to the apparent impairment of translocation of sugars from leaves to roots [6]. In view of the unusual nature of these relationships, similar investigations with toxaphene and susceptible oat were of evident interest.

## RESULTS AND DISCUSSION

The action of toxaphene as an inhibitor of photosynthetic electron flow and possibly as an inhibitory-uncoupler [2] must be reflected in decreased production of NADPH and ATP, necessary for $CO_2$ assimilation. Nevertheless, the dry matter content of leaves increased after toxaphene treatment (Fig. 1a). Whereas over the course of this experiment the dry weight/wet weight ratio of untreated leaves remained constant at 0.09–0.10, that for toxaphene-treated leaves increased steadily, and after 8 days this ratio was 0.16. Ethanol-extractable material in the leaves was estimated (Fig. 1b). In toxaphene-treated oat 6 days after spraying some 35% of the dry weight of the treated leaves was accounted for in this fraction. At longer times the ethanol-extractable material did not increase further as a percentage of dry weight, though the dry weight/wet weight ratio continued to increase. In untreated leaves the ethanol-extractable material also increased initially, but by the fourth day had become constant at 11% of the dry weight. The increase in extractable material in treated leaves accounts for about 75% of the increase in dry weight over the first 6 days. Parallel determinations of carbohydrate showed that in treated leaves this fraction accounted for most of the increase in ethanol-extractable material over the first few days; at longer times carbohydrates still accounted for 80% of the extractable material. The increase in carbohydrate was largely due to increases in mono- and disaccharides, and the amounts of fructosans, soluble

*Part 4 in the series "Susceptibility of Cereals to Organochlorine Pesticides and Biochemical Mode of Action". For Part 3 see ref. [2].

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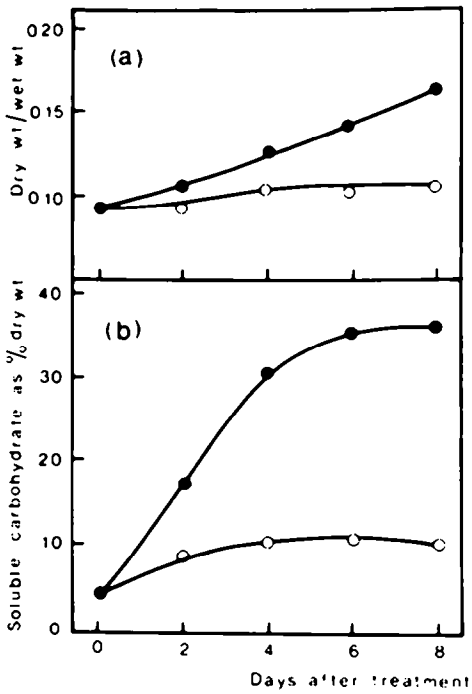


Fig. 1. Effect of toxaphene on dry weight and ethanol-extractable carbohydrate. Susceptible seedlings were treated with toxaphene and sampled at 2 day intervals. Figure 1a shows the data for dry weight/wet weight ratio and Fig. 1b that for ethanol-extractable carbohydrate. Closed symbols are data for treated seedlings and open symbols represent untreated controls.

reserve oligosaccharides, were insignificant in both treated and untreated leaves.

Analyses of individual sugars in treated and untreated leaves (Fig. 2) substantiated these conclusions. At day 0 amounts of fructose were negligible and glucose and

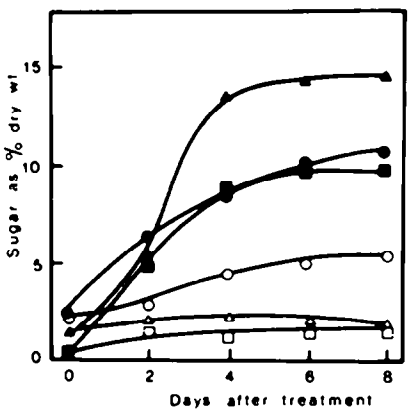


Fig. 2. Effect of toxaphene on the principal components of the ethanol-extractable carbohydrate fraction from susceptible oat. Seedlings were treated with toxaphene and individual sugars estimated at intervals up to 8 days. Closed symbols are data for treated seedlings and open symbols are for the corresponding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

sucrose accounted for about 2% of the dry weight. Following toxaphene treatment, substantial amounts of glucose, fructose and sucrose were accumulated. In untreated leaves there was a significant increase in sucrose but amounts of the monosaccharides remained low. In treated compared to untreated leaves, glucose and fructose had increased seven-fold and sucrose had doubled within 6 days of spraying seedlings with toxaphene. These data were entirely consistent with that obtained in the earlier and quite separate experiments summarized in Fig. 1 and indicate that sugars would account for essentially all the ethanol-extractable material from treated leaves.

These results are similar to those characterizing the effect of DDT on susceptible rye [2], where a consequence of the inhibition of photosynthetic electron transport was decreased $CO_2$ assimilation, changes in patterns of assimilation products, and an impairment of translocation. These aspects were also investigated in susceptible oat seedlings treated with toxaphene.

Experiments were carried out with leaf sections or intact seedlings, under conditions of steady state photosynthesis, in $^{14}CO_2$ at atmospheric concentration in air. After 30 min exposure to $^{14}CO_2$, and in some cases a subsequent period in darkness, leaf and root material was separately extracted and the $^{14}C$ assimilated into soluble products measured. Individual compounds were separated by 2D-TLC, located by autoradiography, and their radioactivity determined [7]. Representative data for the most informative experiments are shown in Table 1.

The data in the first two columns summarize the incorporation of $^{14}C$ into ethanol extractable material in leaf sections of untreated (BuO) and toxaphene (BtO) treated oat. There was only a slight decrease in total $^{14}C$ assimilation into treated leaves, and the patterns of incorporation into the 13 major metabolites were similar, though glycolate and PEP were elevated in untreated leaf sections and alanine was less. Sucrose formed the major $^{14}C$-labelled component. Thus though photosynthetic electron flow is inhibited and levels of NADPH and ATP depleted, there has been no marked effect on $^{14}CO_2$ assimilation under the conditions of this experiment. If leaf sections were left in darkness for 12 hr before the assimilation products were analysed the total amounts of $^{14}C$ in untreated and toxaphene-treated samples were $400 \times 10^3$ and $610 \times 10^3$ dpm, respectively. The fall in $^{14}C$ in untreated leaf sections is consistent with that seen for similar experiments with rye [6] and can be explained by the conversion of photosynthesis products into insoluble polymeric material during the dark period. These changes are evidently decreased substantially in the treated leaves, and reflect the decreased energy status which results from the inhibition of photosynthetic electron flow. There were also marked changes in the incorporation patterns after this period in darkness. In untreated leaf sections the amount of sucrose decreased substantially and some glucose and fructose were formed. Other changes were decreases in malate and increases in HMP, alanine, glutamate and probably aspartate, though amounts of this were somewhat variable in different experiments. In leaf sections from toxaphene-treated plants overall quantities of sugars were the same or somewhat elevated, malate decreased, and HMP, glutamate and PEP increased as a result of the dark period. Comparing the assimilation patterns for untreated and treated leaf sections indicated higher amounts of sugars and lowered

Table 1. Effect of toxaphene on $^{14}C$ assimilation into ethanol-extractable products in oat

| Compounds isolated | $^{14}C$ incorporated ($10^{-3}$ × dpm)* | | | | | |
|---|---|---|---|---|---|---|
| | BuO | BtO | Bu20 | Bt20 | Bu20r | Bt20r |
| Total | 940 | 822 | 524 | 742 | 484 | 310 |
| PGA | 8.8 (0.9) | 3.8 (0.5) | 6.3 (1.2) | 4.0 (0.5) | 3.1 (0.6) | 3.0 (1.0) |
| HMP | 0.9 (0.1) | 2.9 (0.3) | 27.3 (5.2) | 11.1 (1.5) | 7.9 (1.7) | 5.5 (1.9) |
| Sucrose | 429.7 (45.7) | 333.7 (40.6) | 55.2 (10.5) | 246.9 (33.0) | 67.1 (13.9) | 44.0 (14.5) |
| Glucose | Neg | Neg | 13.7 (2.6) | 85.4 (11.5) | 51.8 (10.8) | 37.5 (12.4) |
| Fructose | Neg | Neg | Neg | 15.0 (2.0) | 47.9 (9.9) | 25.3 (8.4) |
| Glycolate | 17.3 (1.8) | 1.3 (0.2) | 5.1 (1.0) | 2.8 (0.4) | 5.2 (1.0) | 3.1 (1.0) |
| Glycine | 57.1 (6.0) | 55.0 (6.6) | 57.8 (11.0)† | 31.2 (4.2)† | 55.4 (11.5)† | 31.2 (10.3)† |
| Serine | 83.6 (8.9) | 55.7 (6.7) | 96.4 (18.3) | 39.0 (5.2) | 49.7 (10.3) | 20.1 (6.4) |
| Glycerate | 7.7 (0.8) | 5.5 (0.7) | 5.9 (1.1) | 4.2 (0.6) | 4.2 (0.9) | 2.2 (0.7) |
| Alanine | 11.7 (1.2) | 27.8 (3.4) | 43.0 (8.2) | 29.7 (3.4) | 26.8 (5.1) | 14.2 (4.5) |
| Malate | 95.6 (10.1) | 104.4 (12.7) | 3.9 (0.7) | 6.0 (0.8) | 9.8 (2.0) | 9.2 (3.0) |
| Aspartate | 18.2 (1.9) | 16.6 (2.0) | 18.3 (3.5) | 18.6 (2.5) | 13.2 (2.7) | 12.0 (3.8) |
| Glutamate | 9.7 (1.0) | 11.1 (1.3) | 44.9 (8.2) | 26.8 (3.6) | 8.2 (1.7) | 6.2 (2.0) |
| PEP | 14.3 (1.5) | 3.4 (0.4) | 7.3 (1.4) | 15.7 (2.1) | 4.8 (1.0) | 8.7 (2.8) |

Four primary leaf sections of toxaphene-treated or untreated susceptible oat two days after spraying were arranged in frames with their cut bases in water, illuminated (100 $\mu E/m^2/sec$; 25°) and flushed with normal air for 30 min then with $^{14}CO_2$-air, containing 1 $\mu Ci$ $^{14}C/L$ for 30 min (both at 0.8 l/min). BuO, BtO indicate Blyth untreated (u) or toxaphene-treated (t) leaf sections extracted immediately after $^{14}CO_2$ assimilation. The data for Bu20 and Bt20 were using 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 (Bu20 and Bt20) or root (Bu20r and Bt20r). For whole seedlings, ethanol-soluble material as mg and as % of dry wt (in parentheses) was Bu20, 9.7 (17.1); Bu20r, 9.7 (20.5); Bt20, 12.6 (30.2); Bt20r, 6.0 (13.7).

* % total activity in ethanol-extracted products given in parentheses. Neg., negligible amounts.

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

serine, and to lesser extent aspartate and glutamate, in the latter as being the most significant changes. These data are not presented in detail since they reflect broadly the patterns seen in the subsequent experiments with intact seedlings, which proved more informative in other respects.

In the final series of experiments toxaphene-treated and untreated seedlings were allowed to fix $^{14}CO_2$ under identical conditions and were then held in darkness for 20 hr before analyses separately of leaves and root tissue. The data for total $^{14}C$ assimilation (last four columns of Table 1) showed that untreated (sum of Bu20 and Bu20r) and treated seedlings (sum of Bt20 and Bt20r) were virtually identical in this respect and in total ethanol-extractable material. This supported the conclusion from the earlier leaf section experiments (BuO and BtO) that assimilation of $CO_2$ was not affected significantly as a result of toxaphene treatment and the resulting partial inhibition of NADPH and ATP formation [2].

For untreated seedlings, the exposure to 20 hr darkness was accompanied by severe depletion in sugars and malate. A number of compounds have increased, notably HMP, alanine and glutamate. Changes in all these were greater than for leaf sections given 12 hr darkness. There were also substantial increases in glycine and serine, where incorporations had been similar in leaf sections extracted immediately or after 12 hr darkness, and, subject to the earlier reservation, also into aspartate.

The labelling pattern for treated leaves was indistinguishable to that for leaf sections given 12 hr darkness before extraction of assimilates. For these seedlings the difference in initial labelling pattern was again a decrease in malate and increased HMP, glutamate and PEP. The extractable material from these leaves (4 days after spraying) accounted for 30% of their dry weight, in very good agreement with the earlier experiments (Fig. 1). The conclusion is that even when high levels of sugars are present there is no evidence of a regulatory control on $CO_2$ assimilation. The possibility of control of sucrose synthesis through lowered ATP/ADP ratio [8] would not be attractive since in these leaves ATP will have been depleted by the action of toxaphene as an inhibitory-uncoupler of photosynthetic electron flow [2].

There were some very evident differences in labelling pattern in the leaves of untreated and treated seedlings. In the latter, sugar levels remained high and there were appreciable amounts of glucose and fructose. In contrast, amounts of HMP, glycine, serine, alanine and glutamate were lower. Toxaphene, though not inhibiting $^{14}C$ assimilation, has thus had a marked effect in depressing metabolic interconversions; in particular, sugars remain unmetabolized except for invertase-catalysed formation of glucose and fructose.

In roots the patterns of incorporation were similar in untreated (Bu20r) and toxaphene-treated (Bt20r) seedlings. The only significant differences were lower serine and higher PEP in the latter. Amounts of all three sugars were broadly comparable. This differs from similar experiments with rye [6] susceptible to DDT where sucrose in the roots was very low and monosaccharides comprised

most of the sugars present. Of more direct interest were the observations that translocation of $^{14}C$-labelled metabolites in treated seedlings had been significantly decreased. A comparison of the total $^{14}C$ in the extractable material from roots compared with that for the whole seedling gave values of 29% for treated seedlings compared with 48% for untreated seedlings. This is in agreement with the analyses of extractable material as a % of dry weight; *ca* 14% and 21% for roots from treated and untreated plants, respectively.

The earlier data for the effect of toxaphene on photosynthetic electron flow in susceptible oat [2], and that now presented for accompanying effects on $CO_2$ assimilation and translocation, are in close accord with those for the effects of DDT on susceptible rye [4, 6], though there the effect on translocation was more severe [6].

Several factors can promote sugar accumulation in leaves [9]. Decreasing translocation in wheat by a low temperature regime or chilling the base of the leaf resulted in accumulation of sugars and starch, and as a result an end product inhibition of photosynthesis and decreased $CO_2$ assimilation [10]. A low sink demand in wheat can also cause sugar accumulation as shown by the increase in dry matter of the flag leaf following ear removal [11].

A current view is that if sucrose, formed extrachloroplastically, accumulates there is inhibition of sucrose phosphate synthetase [12] and sucrose phosphate phosphatase [13]. There will be, through mass action effects, increases in hexose- and triose-phosphates. This sequestering of phosphate leads to a fall in orthophosphate level in the cytoplasm. This decreases transport of triose phosphates from the chloroplast since orthophosphate is exchanged for triose-phosphate across the chloroplast envelope. A depression in photosynthetic electron flow will reflect the lack of orthophosphate for the coupled photosynthetic phosphorylation, and a decrease in $CO_2$ assimilation, since this requires ATP and NADPH. Phloem loading of sucrose is thought to involve cotransport with protons [14] and if this is inhibited by decreased energy status of the cell, translocation will be impaired.

The relationships between photosynthetic energy conservation, $CO_2$ assimilation, sucrose synthesis and translocation have been reviewed elsewhere [8]. The implications from the present data on the effect of toxaphene regarding these mechanisms for control of photosynthesis have been already made in the context of similar data for the response of rye to DDT [6]. They can now be extended in one respect and that is to suggest that these inter-relationships may not be tightly linked. Thus in oat treated with toxaphene, levels of HMP and triose phosphate remain low even in the presence of large quantities of sucrose (e.g. Bt20 in Table 1). The experiments also suggest that even when photosynthetic electron flow is inhibited significantly there is no corresponding decline in $CO_2$ assimilation and sugars accumulate in the leaves. The first responses to the decreased energy status of the plant are in decreased leaf metabolism or translocation of the sugars derived from photosynthesis.

The sequence of events which follows the treatment of oat with toxaphene may be as follows. Photosynthetic electron transport is inhibited, so depleting ATP and NADPH. Though rates of formation of these are decreased they are nevertheless sufficient to support $CO_2$ assimilation to about the same extent as in untreated plants. Sugars are formed, but the lowered energy status of the cell means that translocation to the roots is decreased and further metabolism of sugars in the leaf does not occur. Each successive light period promotes the formation of additional sugars and after several days these form a substantial proportion of the dry weight of the leaves. The observation that translocation is not so severely affected as with comparable inhibitions of electron transport in rye treated with DDT [6] may be part of the explanation why toxaphene-treated oat can often show a slow recovery from pesticide injury.

The magnitude of the inhibition of photosynthetic electron flow by DDT or toxaphene can be regulated by dosage level. An extension of the experiments described earlier in that respect and in regard to environmental parameters (e.g. light, temperature) could therefore provide a framework for clarifying the inter-relationships between energy status, assimilation, sugar metabolism and translocation.

The origin of the genetic response in cereals to organochlorine pesticides is of interest. Though both DDT and toxaphene have been used widely, cereal crops are unlikely to have been sufficiently exposed to these pesticides to be subject to selection pressures. Possibly susceptibility, the dominant character, is one expression of the gene coding for a component of the photosynthetic membrane. A parallel would be the sensitivity to triazines in weeds. Here the herbicides act at the 32 k D protein in the $Q_B$ region of the photosynthetic electron transport chain and resistance of some weeds has arisen through selection of plants where the herbicide binding site is altered [15]. Recently, it has been shown that three residues in the 32 k D protein can be independently altered in weed biotypes to produce three distinct patterns of resistance to s-triazines and other herbicides acting at this site [16]. As a result, weed biolypes with varying levels of resistance to s-triazines display quite different cross-resistances to these other herbicides. The genetic change to give resistance to an organochlorine pesticide in cereals could therefore be of a single base in the gene coding for a photosynthetic membrane component. Different reactions to toxaphene and DDT in the various cereal types might be due to separate changes in the structure of the binding site of a membrane polypeptide. Cereals also show selective resistance to some herbicides. *Avena fatua* is sensitive to chlorfenprop-methyl while *A. sativa* and *A. sterilis* are resistant, and varietal differences in response of *T. aestivum* varieties to chlortoluron have been reported [17]. It is likely that genes conferring resistance to other pesticides may be widespread in agricultural crop species and their exploitation in breeding programmes for improved varieties could give the potential for crop protection against agrochemicals used in pest control.

## EXPERIMENTAL

*Treatment of plants with DDT.* The methods are described in the preceding paper [2].

*Extraction of soluble products.* The leaves of three plants (*ca* 3 g wet wt) 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 [18] 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) as solvent. After location of zones on indicator strips by *p*-anisidine hydrochloride reagent [19] the individual sugars were eluted by $H_2O$ and estimated by $PhOH-H_2SO_4$ reagent [18].

*$^{14}CO_2$ assimilation studies.* The procedures for leaf sections are given in ref. [7]. 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 were as in ref. [7].

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