Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 67 (2006) 2561–2567
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Carboxylesterase activities toward pesticide esters in crops and weeds

Markus Gershater [a], Kate Sharples [b], Robert Edwards [a,*]

[a] Crop Protection Group, School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK
[b] Syngenta, Jealott's Hill, International Research Station, Bracknell RG42 6EY, United Kingdom

Received 19 June 2006; received in revised form 18 September 2006

Dedicated to the memory of Professor John R. Bowyer (1955–2006) and his many contributions to herbicide research and plant biochemistry.

Abstract

Proteins were extracted from maize, rice, sorghum, soybean, flax and lucerne; the weeds *Abutilon theophrasti*, *Echinochloa crus-galli*, *Phalaris canariensis*, *Setaria faberii*, *Setaria viridis*, *Sorghum halepense* and the model plant *Arabidopsis thaliana* and assayed for carboxylesterase activity toward a range of xenobiotics. These included the pro-herbicidal esters clodinafop-propargyl, fenoxaprop-ethyl, fenthioprop-ethyl, methyl-2,4-dichlorophenoxyacetic acid (2,4-D-methyl), bromoxynil-octanoate, the herbicide-safener cloquintocet-mexyl and the pyrethroid insecticide permethrin. Highest activities were recorded with α-naphthyl acetate and methylumbelliferyl acetate. Esters of *p*-nitrophenol were also readily hydrolysed, with turnover declining as the chain length of the acyl component increased. Activities determined with model substrates were much higher than those observed with pesticide esters and were of limited value in predicting the relative rates of hydrolysis of the crop protection agents. Substrate preferences with the herbicides were typically 2,4-D-methyl > clodinafop-propargyl > fenthioprop-ethyl, fenoxaprop-ethyl and bromoxynil-octanoate. Isoelectric focussing in conjunction with staining for esterase activity using α-naphthyl acetate as substrate confirmed the presence of multiple carboxylesterase isoenzymes in each plant, with major qualitative differences observed between species. The presence of serine hydrolases among the resolved isoenzymes was confirmed through their selective inhibition by the organophosphate insecticide paraoxon. Our studies identify potentially exploitable differences between crops and weeds in their ability to bioactivate herbicides by enzymic hydrolysis and also highlight the usefulness of Arabidopsis as a plant model to study xenobiotic biotransformation.

Keywords: Arabidopsis; Detoxification; Herbicide bioactivation; Maize (*Zea mays* L.); Pesticides; Rice (*Oryza sativa*); Sorghum (*Sorghum bicolor*); Soybean (*Glycine max*); Serine hydrolases; Weeds; Xenobiotics

1. Introduction

Several major classes of herbicides are applied in the field as inactive esters which are then hydrolysed within plant tissues to release the phytotoxic acid or alcohol (Hassall, 1990). Good examples of this metabolic activation are seen with the methyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D-methyl, **1**), bromoxynil-octanoate (**5**) and esters of the aryloxyphenoxypropionates (AOPPs) such as clodinafop-propargyl (**2**), fenthioprop-ethyl (**3**) and fenoxaprop-ethyl (**4**) (Fig. 1). Additionally, other agents used in crop protection, such as the herbicide safener cloquintocet-mexyl (**7**) and the pyrethroid insecticide permethrin (**8**) (Fig. 1), can also undergo ester hydrolysis as a route of primary metabolism. In the case of pyrethroids, hydrolysis following application on the crop abolishes their activity as insecticides (Preiss et al., 1988). It can therefore be seen that the hydrolysis of crop protection agents in plants is important in determining their bioactivity by mediating either their metabolic activation or detoxification (Hassall, 1990).

* Corresponding author. Tel.: +44 191 334 1318; fax: +44 191 334 1201.
E-mail address: Robert.Edwards@durham.ac.uk (R. Edwards).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.09.019

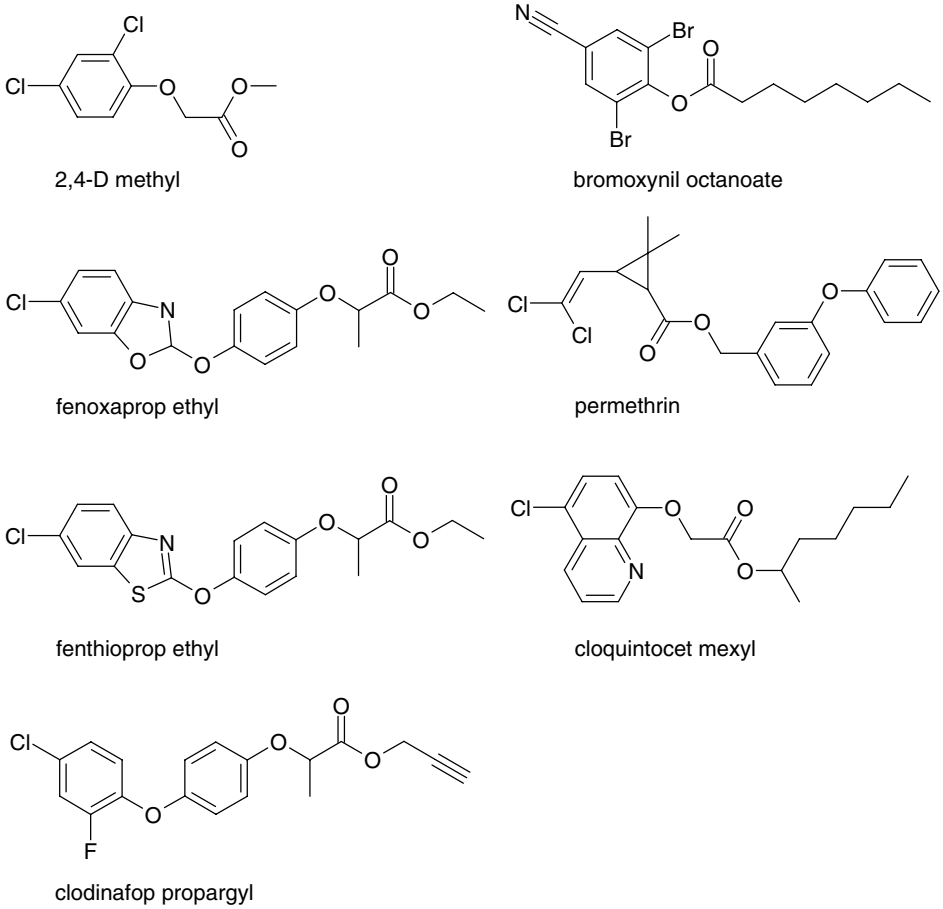

Fig. 1. Pesticide esters used in this study. Compounds are numbered in the text as **1**, 2,4-D-methyl; **2**, fenoxaprop-ethyl; **3**, fenthioprop-ethyl; **4**, clodinafop-propargyl; **5**, bromoxynil-octanoate, **6**, permethrin; **7**, cloquintocet-mexyl.

The AOPPs represent one of the best described examples of hydrolysis-mediated activation of a pro-herbicidal ester. Thus, whereas wild oats (*Avena fatua* L.) rapidly hydrolyse flamprop-isopropyl to the phytotoxic flamprop acid, this reaction is much slower in barley (*Hordeum vulgare* L.), allowing the crop to safely detoxify the herbicide (Jeffcoat and Harries, 1975). In contrast, the rapid build up of active flamprop in wild oats leads to toxicity, with this bioactivation contributing to the selective graminicidal action of this herbicide. More recent studies with the sulphonyl urea herbicide imazamethabenz-methyl have also demonstrated the importance of relative rates of hydrolysis in determining herbicide-resistance in wild oat (Nandula and Messersmith, 2000). Thus, susceptible populations hydrolysed imazamethabenz-methyl to the phytotoxic imazamethabenz acid much more rapidly than herbicide-resistant wild oat.

There is a long history of xenobiotic-hydrolysing carboxyesterases being described in plants using model colorimetric substrates such as α-naphthyl acetate. Although these old studies did not ascribe specific functions to these enzymes, major differences in esterase isoenzyme content between plant species and even within cultivars were demonstrated (Cherry and Katterman, 1971). Esterase expression has also been recorded to change during plant development (Frossard and Voss, 1978; Chandra and Toole, 1977), and in response to stress and infection (Muarlidharan et al., 1996; Baudouin et al., 1997; Wäspi et al., 1998). While these qualitative changes in expression are suggestive of important functions for these enzymes in plant growth, development and stress tolerance, very few studies have been directed at their functional characterisation.

We are interested in identifying esterases in crops and weeds which are active in pesticide metabolism. In earlier studies, we demonstrated esterase activities toward the herbicides diclofop-methyl, binapacryl and bromoxynil-octanoate in wheat (*Triticum aestivum* L.), and the competing weeds black-grass (*Alopecurus myosuroides* L.) and wild oat (Cummins et al., 2001). Esterase activities toward the herbicides were significantly higher in the weeds than in wheat, most markedly with the AOPP herbicide diclofop-methyl. The respective enzymes have been shown to be expressed in the apoplast in both wild oat (Holl et al., 1986) and wheat (Haslam et al., 2001) and have recently been identified and cloned from black-grass (Cummins and Edwards, 2004). Based on sequence analysis, the 40 kDa esterase from black-grass showed