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Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update

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Herbicides targeting grass plastidic acetyl coenzyme A carboxylase (ACC) are effective selective graminicides. Their intensive use worldwide has selected for resistance genes in a number of grass weed species. Biochemistry and molecular biology have been the means of determining the herbicidal activity and selectivity toward crop plants of ACC-inhibiting herbicides. In recent years, elucidation of the tridimensional structure of ACC and identification of five amino acid residues within the ACC carboxyl transferase domain that are critical determinants for herbicide sensitivity shed light on the basis of ACC-based resistance to herbicides. However, metabolism-based resistance to ACC-inhibiting herbicides is much less well known, although this type of resistance seems to be widespread. A number of genes thus endow resistance to ACC-inhibiting herbicides, with the possibility for various resistance genes that confer dominant resistance at the herbicide field rate to accumulate within a single weed population or plant. This, together with a poor knowledge of the genetic parameters driving resistance, renders the evolution of resistance to ACC-inhibiting herbicides unpredictable. Future research should consider developing tactics to slow the spread of resistance. For this purpose, it is crucial that our understanding of metabolism-based resistance improves rapidly because this mechanism is complex and can confer resistance to herbicides with different target sites.

Key words: ACCase, acetyl-CoA carboxylase, aryloxyphenoxypropionate, cyclohexanedione, target site, molecular modeling, mutation, metabolism, cross-resistance, fitness, genetic polymorphism, interspecific crosses, selection.

Weeds are a continuing problem in agriculture. The introduction of selective herbicides since the late 1940s has greatly facilitated farmers' work by suppressing the need for manual weeding. However, reliance on herbicides for weed control has resulted in shifts in the weed flora and, more importantly, in the selection of herbicide-resistant weed populations. This is particularly true for herbicides with a single target, such as herbicides inhibiting acetyl coenzyme A carboxylase (ACC). ACC inhibitors are a group of commercially important, very effective, selective graminicides that are applied postemergence. They are also known as group A herbicides (Anonymous 2004) or group 1 herbicides (Mallory-Smith and Retzinger 2003). Since their introduction in the late 1970s, the ACC-inhibiting herbicides have been widely used worldwide to control a number of grass weed species (Devine and Shimabukuro 1994). As a consequence, they rapidly selected, and are still selecting, resistant plants within grass weed species. Since 1994, when resistance to ACC-inhibiting herbicides was last reviewed in detail (Devine and Shimabukuro 1994), the number of grass weed species in which resistant plants have been reported has increased from 9 to > 34 (Heap 2004). Resistance to ACC-inhibiting herbicides has now been reported in 26 countries. The estimates of cultivated land surfaces concerned by this resistance vary between 3 to > 4.6 million ha (Heap 2004). Along with the development of resistance to ACC-inhibiting herbicides, our understanding of the mechanisms endowing this resistance has progressed substantially, largely owing to the use of molecular biology techniques. The purpose of this review therefore is to summarize what we have learned about ACC and the evolution of grass weed populations toward resistance to ACC-inhibiting her-

bicides during the last decade. Available data about ACCs and ACC inhibitors and their mode of action will be reviewed; the focus is set first on the mechanisms endowing resistance and second on the evolution of resistance in grass weeds.

Plant ACCs

ACC Isoforms

ACC (EC 6.4.1.2) is a biotinylated enzyme that catalyzes the ATP-dependent carboxylation of acetyl coenzyme A into malonyl coenzyme A in a two-step, reversible reaction: $\text{HCO}_3^- + \text{ACC-biotin} + \text{ATP} \leftrightarrow \text{ACC-biotin-CO}_2 + \text{ADP} + \text{Pi}$, and $\text{ACC-biotin-CO}_2 + \text{acetyl coenzyme A} \leftrightarrow \text{ACC-biotin} + \text{malonyl coenzyme A}$.

This reaction is the first step in the synthesis of fatty acids. In plants, large amounts of malonyl coenzyme A are needed in the plastids to sustain *de novo* fatty acid biosynthesis. Malonyl coenzyme A is also needed in the cytosol for a variety of reactions, including the elongation of very long chain fatty acids, the synthesis of secondary metabolites (e.g., flavonoids, anthocyanins), and the malonylation of secondary metabolites (Harwood 1988). The plastid envelope not being permeable to malonyl coenzyme A, this led to the hypothesis that at least two ACC isoforms are present in the cytosol and in the plastids of plants, respectively. It was not before the mid-1990s that the structure of ACC isoforms was clearly understood (Konishi and Sasaki 1994; Konishi et al. 1996; Sasaki et al. 1995; also see Inclendon and Hall [1997] for a history of ACC structure elucidation). All ACC isoforms contain three catalytic domains, namely the biotin carboxyl-carrier (BCC), the biotin carboxylase

FIGURE 1. Acetyl coenzyme A carboxylase (ACC) isoforms present in plants. TP, transit peptide; ACC functional domains: BCC, biotin carboxyl-carrier; BC, biotin carboxylase; CT, carboxyl transferase. Note that the TP could be cleaved after the plastidic ACC isoform has been imported into the chloroplast.

(BC), and the carboxyl transferase (CT) domains (Nikolau et al. 2003). The BC domain catalyzes the ATP-dependent carboxylation of a biotin group covalently linked to a lysine (Lys) residue in the BCC domain. The CT domain thereafter catalyzes the transfer of the carboxyl group from biotin to acetyl coenzyme A.

The plastidic ACC isoform accounts for > 80% of the total ACCase activity in leaves (Ashton et al. 1994; De Prado et al. 2000; Egli et al. 1993). In most plant species, it is a heteromeric enzyme composed of four distinct subunits: two for the CT domain and one for each of the BC and BCC domains (Figure 1). Readers are referred to recent reviews (Nikolau et al. 2003; Sasaki and Nagano 2004) for detailed descriptions of heteromeric ACC. In contrast, the ACC isoforms occurring in the cytosol in all plants and in the plastids and mitochondria in *Poaceae* (grasses) are large, homomeric enzymes carrying all three functional domains on a single polypeptide (Focke et al. 2003; Nikolau et al. 2003; Figures 1 and 2). All homomeric ACCs share a high degree of sequence conservation. The major difference between homomeric ACC isoforms is the presence of about 100 extra amino acids at the *N*-terminal end of the plastidic and mitochondrial isoform (Figure 2) that likely form a

dual-targeting transit peptide sequence (Peeters and Small 2001). The transit peptide enables this ACC isoform to be directed to chloroplasts and mitochondria.

The distinction between grasses and other plants might not be clear-cut however. The plastids of some species in the dicotyledonous family *Geraniaceae* do not contain a heteromeric ACC and probably contain a homomeric isoform of this enzyme instead (Christopher and Holtum 2000). Furthermore, the plastids of dicotyledonous species such as *Brassica napus* L. and *Arabidopsis thaliana* L. contain both a heteromeric and a homomeric ACC isoform (Nikolau et al. 2003).

Tridimensional Structure of Homomeric ACC

Two homomeric ACC polypeptides have long been proposed to associate in a 500-kDa homodimer to yield the active ACC enzyme (Egli et al. 1993), although there has been some controversy regarding the possible role of low molecular mass proteins as being part of the active ACC (Inclendon and Hall 1997). The dimeric structure of active homomeric ACC has recently been established by crystallization of a functional CT domain from yeast ACC in com-

FIGURE 2. Schematic representation of a plastidic homomeric acetyl coenzyme A carboxylase (ACC) showing the three functional domains (BCC, biotin carboxyl-carrier; BC, biotin carboxylase; CT, carboxyl transferase) and the transit peptide (TP) that is absent in cytosolic ACCs. The five amino acid residues critical for sensitivity to ACC-inhibiting herbicides have been referenced after the sequence from black-grass plastidic ACC (EMBL accession AJ310767).

TABLE 1. Complete higher plant acetyl coenzyme A carboxylase sequences.

Species	Isoform	Length (amino acids)	EMBL/Genbank/DDBJ accession number	Source
Blackgrass	Plastidic	2320	AJ310767	cDNA
<i>Arabidopsis thaliana</i> L.	Plastidic ^a	2375	AF062308	gDNA
<i>A. thaliana</i>	Cytosolic	2254	AF062308	gDNA
Canola	Plastidic ^a	2304, 2321	X77576, AJ131865	gDNA
Soybean	Cytosolic	2261	L42814	gDNA
Alfalfa	Cytosolic	2257	L25042	cDNA
Rice	Cytosolic	2267	AC092548	gDNA
Foxtail millet	Plastidic	2321	AF294805	cDNA
Wheat	Plastidic	2253	AF029895	cDNA
Wheat	Cytosolic	2260	U39321	gDNA
Corn	Plastidic	2325	U19183	cDNA

^a On the basis of the presence of a putative transit peptide.

plex with its substrate, acetyl coenzyme A (Zhang et al. 2003). The ACC CT dimer consists of the head-to-tail arrangement of two CT monomers, creating two active site cavities per dimer. Acetyl coenzyme A enters these cavities to undergo carboxylation into malonyl coenzyme A (Zhang et al. 2003).

Natural Variation at Homomeric ACCs

Homomeric ACCs are large proteins consisting of around 2,300 amino acids (Figure 2), meaning that the corresponding cDNAs are about 7,000 bp in length. To further complicate the situation, the genes encoding plant homomeric ACCs have a complex intron–exon structure. The coding sequence of the gene encoding wheat (*Triticum aestivum* L.) cytosolic ACC is interrupted by no less than 28 introns (Podkowinski et al. 1996), whereas those of the gene encoding the plastidic ACC in blackgrass (*Alopecurus myosuroides* Huds.) and green foxtail [*Setaria viridis* (L.) Beauv.] are interrupted by 32 introns (Délye, unpublished data). This means entire ACC genes are around 15,000 bp in length. These are good reasons why so few plant ACC genes are fully known to date (Table 1). In diploid grasses, a single nuclear gene (Gornicki et al. 1997; Huang et al. 2002) generally encodes the plastidic and mitochondrial homomeric ACC isoform, although two genes might exist in *Lolium* spp. (Huang et al. 2002). The situation seems more complex for the cytosolic ACC isoform, which might be encoded by several genes distinct from that encoding the plastidic and mitochondrial isoform. At least five distinct genes encoding cytosolic ACC isoforms have been identified in hexaploid wheat (Faris et al. 2001). As a consequence of all that precedes, few data are available on variability of ACC genes.

From the ACC sequences available, it appears that hom-

omeric ACC proteins are highly conserved among and within species. Amino acid sequences of grass plastidic ACCs share > 80% identity. They display > 65% identity with the amino acid sequences of plant cytosolic ACCs, and as much as 52% identity with the amino acid sequence of a cytosolic isoform from an organism as phylogenetically distant as yeast. This data, together with a recent study that revealed an excess of synonymous vs. nonsynonymous nucleotide variation within the CT domain of blackgrass plastidic ACC (Délye et al. 2004b), strongly suggest that ACC is a gene under purifying selection (i.e., that nonsynonymous mutations cannot occur anywhere within the gene without deleterious consequences).

Herbicides Inhibiting ACCs

Structure and Mode of Action of ACC-Inhibiting Herbicides

ACC inhibitors are a group of commercially important herbicides enabling efficient management of grass weed species. They belong to two chemical families: aryloxyphenoxypropionates (APPs), also known as “fops,” and cyclohexanediones (CHDs), also known as “dims” (Secor et al. 1989). Although APP and CHD molecules all consist of a hydrophobic carbon skeleton with polar substituents, the molecules in the two families display rather dissimilar structures (Figure 3). APPs and CHDs show nearly competitive inhibition with respect to the substrate acetyl coenzyme A, which suggests a binding site at ACC CT site (Burton et al. 1991; Rendina et al. 1990). Because APPs and CHDs are mutually exclusive inhibitors, it was proposed that their binding sites on ACC CT overlap but are not the same (Burton et al. 1991; Rendina et al. 1990). ACC inhibition will block fatty acid biosynthesis; consequently, cell membrane integrity will be altered, thus causing metabolite leakage and rapid plant death (Devine and Shimabukuro 1994). The generation of cytotoxic hydroperoxides as a secondary consequence of the disruption of fatty acid biosynthesis has also been proposed to be a primary cause for the toxicity of ACC-inhibiting herbicides (Cummins et al. 1999).

The activity of APPs and CHDs against ACC is limited by strict structural requirements (Turner and Pernich 2002; Webb et al. 2000). For instance, only *R*(+) enantiomers of APPs are active against ACC, whereas *S*(–) enantiomers are not (Gerwick et al. 1988; Rendina et al. 1988; Walker et

FIGURE 3. Generic structures of the aryloxyphenoxypropionate (APP, right) and cyclohexanedione (CHD, left) herbicides. Developed formulas of commercial compounds are available in Heap (2004).

al. 1988). Large substituents at positions R1 and R4 (Figure 3) in CHDs confer high inhibitory activity against ACC, whereas large substituents at position R2 are associated with poor inhibitory activity (Webb et al., 2000).

Recent studies have confirmed the mode of action of APPs and CHDs. First, heterologous expression of chimeric ACCs in yeast have shown that some determinants of sensitivity to APPs and CHDs were located on a 400-amino acid fragment of wheat plastidic ACC encompassing a section of the CT domain (Nikolskaya et al. 1999). Further works that will be discussed more in detail in the "Molecular basis of resistance to ACC inhibitors" section showed that two regions of the CT domain of plastidic ACC are critical for sensitivity to APPs and CHDs (Délye et al. 2002c, 2003, 2005; Zagnitko et al. 2001). The definite evidence that APPs and CHDs bind to the homomeric ACC CT domain came from the determination of the three-dimensional structure of yeast cytosolic ACC CT domain dimers co-crystallized with bound APP molecules (Zhang et al. 2004b). APPs were shown to bind inside the active site cavity of ACC CT dimers. Binding of one APP molecule inside one of the two active sites of a CT dimer caused conformational changes in the structure of the whole dimer (Zhang et al. 2004b). These changes were incompatible with the binding of acetyl coenzyme A and malonyl coenzyme A molecules for catalysis, in agreement with biochemical data showing that APPs are nearly competitive inhibitors of acetyl coenzyme A (Burton et al. 1991; Rendina et al. 1990; Zhang et al. 2004b). Conformational changes exposed the core of the CT domain dimer bound with an APP molecule, which could facilitate the binding of a second APP molecule inside the second active site of the CT dimer (Zhang et al. 2004b). This agrees with a previous demonstration of positive cooperativity for APP binding to homomeric cytosolic ACCs obtained by analysis of the kinetics of herbicide binding (Herbert et al. 1996b).

The use of tridimensional protein modeling that is based on the structure of the yeast cytosolic ACC CT domain and the similar ACC-inhibiting features of CHDs and APPs allowed the proposition that, in plant plastidic homomeric ACCs, CHDs bind inside the CT dimer active site cavity like APPs to exert their ACC-inhibiting effect (Délye et al. 2005). However, a major limit to the use of models is that crystallographic data for the tridimensional structure of the ACC CT domain have been obtained from the yeast homomeric cytosolic ACC, which is considerably less sensitive to APPs and CHDs than the grass plastidic isoform (Joachimik et al. 1997; Nikolskaya et al. 1999; Table 2). Furthermore, homomeric plastidic ACC isoforms from grasses do not exhibit cooperativity for APP binding (Herbert et al. 1996b). Thus, if the general binding mode of ACC inhibitors inside the CT active site cavity is very likely similar for both plastidic and cytosolic isoforms given the conservation among homomeric ACC proteins, it clearly displays differences in the detail of the interactions involved (Délye et al. 2005).

Molecular and biochemical data have clearly established that the CT domain of the homomeric ACC is the primary target site for APPs and CHDs. Alternative modes of action proposed, such as the possibility that these herbicides are auxin antagonists that bind to putative cell membrane receptors to induce rapid depolarization of the membrane and

cell death via oxidative damage (Ratterman and Balke 1988; Shimabukuro et al. 2001), can thus be discounted. Although APPs and CHDs do depolarize cell membranes in vitro (e.g., Holtum et al. 1994; Ratterman and Balke 1988; Vila-Aiub et al. 2003), this might not occur in vivo at herbicide field rates. Depolarization of the cell membrane is very likely a secondary effect after ACC inhibition and the ensuing disruption of the fatty acid biosynthesis pathway, as has been proposed before (Di Tomaso 1994; Incledon and Hall 1997).

Selectivity of ACC-Inhibiting Herbicides Toward Crop Plants

Selectivity on the Basis of Differences in ACC Sensitivity

At field rates, APPs and CHDs strictly target grass species, apart from a few exceptions discussed before. This can be related to the high sensitivity of chloroplastic homomeric ACC to these herbicides (Table 2). Field rates of APPs and CHDs do not affect most other plant species or animals. The basis for selectivity toward the vast majority of non-grass plant species resides in the insensitivity of the heteromeric ACC that is present in the plastids of these species to APP and CHD action (Alban et al. 1994; Burton et al. 1987). The structure of heteromeric ACCs is highly different from that of homomeric ACCs (Figure 1), which is very likely the basis for heteromeric ACC insensitivity. Other organisms, such as animals or fungi, only contain homomeric ACC isoforms. Considerable differences have been observed in the activity of distinct APP or CHD molecules against a given homomeric ACC isoform, as well as among homomeric ACC isoforms for a given ACC inhibitor (Table 2). Although some of the differences observed among studies for a given ACC isoform and a given herbicide might be attributed to differences in ACC assays, as shown by Shukla et al. (1997a), this is likely not the case for comparisons performed within a given study, and this is certainly not the reason why cytosolic homomeric ACC isoforms are consistently found to be less sensitive by several orders of magnitude to APPs and CHDs than the rather similar homomeric plastidic isoforms from grasses (Table 2). It has been proposed elsewhere that the efficacy of a given ACC-inhibiting herbicide toward a given homomeric ACC isoform will depend on how well the inhibitor molecule fits inside the CT active site cavity (Délye et al. 2005). For instance, the *S*(-) enantiomer of ACC molecules cannot adopt a conformation fitting inside the CT active site because of the methyl group on the chiral carbon, whereas the *R*(+) enantiomer can, which is the basis for the *R*(+)/*S*(-) selectivity of APP enantiomers (Zhang et al. 2004b). A likely explanation for the differences in sensitivity observed among homomeric ACC isoforms is thus the occurrence of differences in the access to, and the fit inside, the CT active site cavity of ACC inhibitor molecules. In this respect, it should be mentioned that the plastidic ACC from the parasitic protozoan *Toxoplasma gondii* is rather sensitive to APPs but not to CHDs (Table 2), which enabled the proposition that APP-derived molecules could be used in treatments for some human and animal protozoa-related diseases (Jelenska et al. 2002). Apart from a few exceptions, the selectivity of APPs and CHDs against grasses is therefore based on the differ-

TABLE 2. Sensitivity of wild-type acetyl coenzyme A carboxylase (ACC) from eukaryotic organisms to ACC-inhibiting herbicides on the basis of ACC enzyme assays.^a

Species	ACC isoform	Range of ACC I_{50} values observed (μ M)											References
		APPs					CHDs						
		Cd	Dc	Fx	Fz	Hx	Qz	Ct	Cx	Sx	Tk		
Blackgrass	Plastidic	4.0–10.9	0.4–5.2	< 0.2–1.0	1.2–8.3	1.2–2.7	0.06	0.8–3.9	1.3–6.7	2.1–9.0	0.9	Delye et al. 2003, 2005; Menéndez and Prado 1996, 1999; Moss et al. 2003; De Prado et al. 2004	
<i>Avena</i> spp.	Cytosolic	NA	35.0	NA	NA	NA	NA	NA	NA	NA	NA	Menéndez and De Prado 1999	
	Plastidic	NA	0.2–35.9	2.1–5.9	12.1	NA	NA	NA	3.9	2.3–4.7	1.1–4.1	Cocker et al. 2000; Ma- nechote et al. 1997; Shukla et al. 1997a	
<i>Digitaria ischea-</i> <i>mum</i> Schreb. Muhl.	Plastidic	NA	NA	4.9	NA	NA	1.3	NA	NA	3.4	NA	Kuk et al. 1999	
Large crabgrass	Plastidic	NA	NA	NA	4.1–5.8	0.9–1.2	NA	1.1	NA	3.5–8.2	2.8–3.7	Hidayat and Preston 1997; Vollenberg and Stoltenberg 2002a	
Goosegrass	Plastidic	NA	NA	1.0	5.6	NA	NA	1.5	NA	3.8	NA	Leach et al. 1995	
<i>Hordeum lepor-</i> <i>inum</i> Link.	Plastidic	NA	0.6	NA	10.2	1.7	NA	NA	NA	16.0	2.7	Matthews et al. 2000	
<i>Lolium</i> spp.	Plastidic	NA	0.2–21.2	NA	3.3–22.8	0.4–2.0	0.07	0.9–1.7	2.7	1.4–9.0	< 0.2–1.7	Christopher and Holtum 1998; Cocker et al. 2001; De Prado et al. 2000; Evenson et al. 1997; Gronwald et al. 1992; Kuk et al. 2000; Preston et al. 1996; Tardif et al. 1993, 1996; Tardif and Powles 1994; Yu et al. 2004	
Giant foxtail	Cytosolic	NA	> 125.0–210.0	NA	NA	1050.0	NA	250.0	NA	200.0	NA	De Prado et al. 2000; Evenson et al. 1997	
	Plastidic	NA	NA	NA	5.8	NA	NA	1.3	NA	6.2	NA	Vollenberg and Stolten- berg 2002a	
	Plastidic	NA	0.1–0.6	0.6–4.4	3.4	1.8	0.08–2.1	0.6–3.2	1.2	0.8–7.7	0.3	De Prado et al. 2004; Marles et al. 1993; Shukla et al. 1997b	
Corn	Cytosolic	NA	95.0	NA	NA	NA	NA	NA	NA	NA	NA	De Prado et al. 2004	
	Plastidic	NA	NA	NA	5.0–10.0	0.07–< 1.0	0.02–0.04	NA	NA	5.0–10.0	NA	Egli et al. 1993; Herbert et al. 1996a, 1996b; Parker et al. 1990; Price et al. 2003	
	Cytosolic	NA	NA	NA	1500.0	1.0	48.0–60.0	NA	NA	> 10.0	NA	Egli et al. 1993; Herbert et al. 1996b; Price et al. 2003	

TABLE 2. Continued.

Species	ACC isoform	Range of ACC I_{50} values observed (μ M)										References
		APPs					CHDs					
		Cd	Dc	Fx	Fz	Hx	Qz	Ct	Cx	Sx	Tk	
Wheat	Plastidic	NA ^b	NA	1.0	NA	< 1.0–2.0	0.2	NA	NA	< 1.0–< 10.0	NA	Gornicki and Haselkorn 1993; Joachimiak et al. 1997; Konishi and Sasaki 1994
Tall fescue <i>Festuca rubra</i> L.	Cytosolic	NA	NA	NA	NA	250.0	NA	NA	NA	> 500.0	NA	Joachimiak et al. 1997
	Plastidic	NA	NA	NA	NA	5.8	NA	NA	NA	6.9	NA	Stoltenberg et al. 1989
	Plastidic ^c	NA	NA	NA	> 50.0	118.0	2.0	NA	NA	> 1,000.0	NA	Stoltenberg et al. 1989; Herbert et al. 1996a
Blue fescue (var. <i>glauca</i>)	Plastidic ^c	NA	NA	NA	283.0	NA	NA	NA	NA	688.0	NA	Catanzaro et al. 1993
<i>Poa annua</i> L.	Plastidic ^c	NA	NA	NA	50.0	NA	0.1–1.0	NA	NA	20.0–25.0	NA	Herbert et al. 1996a; Price et al. 2003
<i>Vulpia bromoides</i> L. Gray	Plastidic ^c	NA	22.0	NA	73.0	19.0	NA	NA	NA	> 100.0	8.0	Yu et al. 2004
<i>Pisum sativum</i> L.	Cytosolic	NA	7.0	40.0	NA	150.0	9.0–57.0	NA	NA	> 1,000.0	NA	Alban et al. 1994; Christopher and Holtum 1998; Herbert et al. 1996b; Konishi and Sasaki 1994
<i>Cyclotella cryptica</i>	Cytosolic	NA	NA	NA	NA	> 100.0	NA	23.0	NA	NA	NA	Roessler 1990
<i>Toxoplasma gondii</i>	Plastidic	5.0	NA	NA	NA	5.0	NA	NA	NA	> 400.0	NA	Jelenska et al. 2002
Yeast	Cytosolic	> 400.0	NA	NA	NA	> 400.0	NA	NA	NA	> 400.0	NA	Jelenska et al. 2002
<i>Rattus</i> sp.	Cytosolic	NA	NA	NA	NA	> 500.0	NA	NA	NA	> 500.0	NA	Joachimiak et al. 1997
						NA	NA	All CHDs, 18.0–40.0				Seng et al. 2003

^a APP, aryloxyphenoxypropionate; Cd, clodinafop; CHD, cyclohexanedione; Ct, clethodim; Cx, cycloxydim; Dc, diclofop; Fx, fenoxaprop; Fz, fluazifop; Hx, haloxyfop; I_{50} , herbicide concentration that inhibits ACC activity by 50%; Qz, quizalofop; Sx, sethoxydim; Tk, tralkoxydim.

^b Abbreviation: NA, Not assayed.

^c Plastidic ACC inherently tolerant of ACC-inhibiting herbicides.

ences in sensitivity existing among plastidic homomeric ACC and other ACC isoforms.

Selectivity on the Basis of Herbicide Metabolism

Grass crops such as wheat, corn (*Zea mays* L.), or rice (*Oryza sativa* L.) contain plastidic homomeric ACC isoforms with sensitivity to APPs and CHDs similar to that of plastidic ACCs from grass weeds (Table 2). However, ACC-inhibiting herbicides are used to control weeds in these crops (e.g., the APPs fenoxaprop and diclofop or the CHD tralkoxydim). The selectivity of CHDs is solely based on the capacity of the grass crop to rapidly metabolize the herbicide in question into inactive products. APPs are applied as relatively inactive carboxylic acid esters (e.g., fenoxaprop-ethyl, diclofop-methyl) to enhance their penetration in plants (Devine and Shimabukuro 1994). The ester bond is hydrolyzed by esterase activity to release the acid, herbicidally active form of APPs. An esterase involved in the activation of APPs has recently been fully characterized in blackgrass (Cummins and Edwards 2004). The selectivity of APPs against grass weeds is based on a more rapid hydrolysis of the ester to the active acid form of APPs in grass weeds (Cummins and Edwards 2004; Hill et al. 1978; Jeffcoat and Harries 1973) and a faster metabolism of the active herbicide in grass crops (Shimabukuro et al. 1979).

The capacity to metabolize ACC-inhibiting herbicides can be either inherent to the grass crop or triggered by safeners. Safeners are chemicals exhibiting a high botanical and chemical specificity. They suppress the phytotoxic effects of herbicides toward crop plants without altering their activity against weeds. Safeners have recently been reviewed in detail elsewhere (Davies and Caseley 1999; Hatzios and Burgos 2004). They trigger genes or metabolic pathways with a role in general plant response to stress in a manner similar to other abiotic or biotic factors like pathogens, drought, or heat shock (Hatzios and Burgos 2004). In particular, safeners stimulate glutathione-*S*-transferase (GST), cytochrome P450 (pigment absorbing at 450 nm)-dependent monooxygenase (CYP), and glucosyl transferase activities degrading herbicides. The mechanisms of herbicide degradation will be discussed in the "Molecular basis of resistance to ACC inhibitors" section because they also endow grass weed resistance to ACC-inhibiting herbicides. It should however be noted that different enzymes can be involved in degradation of herbicides in the same family. For example, in wheat, constitutively expressed CYP activity metabolizes the APP herbicide diclofop and the CHD tralkoxydim via aryl hydroxylation and hydroxylation of the *p*-methyl group, respectively (Hadfield et al. 1994; McFadden et al. 1989), whereas a safener-induced GST isoform detoxifies the APP fenoxaprop via glutathione conjugation (Tal et al. 1993). It should be noted that not all ACC-inhibiting herbicides are selective toward grass crops. Poor metabolism by the crop or lack of a suitable safener prevents some APPs (e.g., haloxyfop) or CHDs (e.g., clethodim or sethoxydim) to be applied in grass crops.

Safeners also enhance the elimination of herbicide metabolites via vacuole sequestration or incorporation into cell walls (Hatzios and Burgos 2004). Although it is proposed that most of the safener action has to do with gene transcription enhancement, very little is known to date about

the perception and transduction mechanisms involved in the protective action of safeners.

Molecular Basis of Resistance to ACC Inhibitors

The high number of herbicide resistance cases reported thus far (Heap 2004) and the information passed around by researchers and herbicide manufacturers should not obliterate the fact that, to be effective, herbicides must be applied properly on weed populations. The efficacy of ACC-inhibiting herbicides against grass weeds could vary substantially depending on the growth stage of the targeted weeds (Milner et al. 2001). The "in-field" susceptibility to ACC inhibitors of grass weeds at a growth stage theoretically susceptible to ACC-inhibiting herbicides depends on the physiology of the weeds during and after herbicide application, and that physiology depends in turn on environmental factors such as the weather (Collings et al. 2003).

The selection exerted by highly effective herbicides results in weed populations that are no longer controlled by the herbicide doses originally applied. This phenomenon is known as "resistance to herbicides." When properly applied, herbicides inhibiting ACC kill > 95% of sensitive plants in grass weed populations (Foster et al. 1993). They have consequently been used repetitively for years on vast cropping surfaces as the sole way of controlling grass weeds, which rapidly lead to the selection of grass weed populations able to withstand field-rate doses of ACC-inhibiting herbicides (Devine and Shimabukuro 1994). Resistance to herbicides has been defined in several ways, the most satisfactory possibly being "the inheritable ability of a weed not to be controlled by an herbicide" (Gressel 1990, p. 174). This definition encompasses both mechanisms that reduce the sensitivity of weeds to herbicides and that modify ecological traits so that weeds are enabled to dodge herbicides. The following terms will be used hereafter: cross-resistance for resistance to several herbicides endowed by a single gene, multiple resistance for resistance to several herbicides endowed by more than one gene, and tolerance (Anonymous 1998) for species that intrinsically cannot be controlled by ACC-inhibiting herbicides. Resistance factors (RFs) are ratios between concentrations of an herbicide that exert a similar effect on resistant and sensitive plants, respectively.

Mechanisms of resistance to ACC-inhibiting herbicides can be divided into two categories: ACC-related and metabolism-based. These two categories of mechanisms are also involved in selectivity of ACC-inhibiting herbicides toward crops, as discussed previously. Numerous studies, mostly based on biological and enzymological data, have been conducted on a number of grass weed species in which plants resistant to ACC-inhibiting herbicides have been identified. Both target-related and metabolism-based mechanisms underlying resistance to APPs and CHDs are best and most thoroughly understood in a single grass weed species, blackgrass.

ACC-Related Mechanisms of Resistance

These mechanisms confer resistance to ACC-inhibiting herbicides by either rendering plastidic ACC activity resistant to the herbicide action or by increasing the herbicide-

sensitive plastidic ACC activity, thus making up for the inhibiting action of the herbicide.

Mutations within Homomeric Plastidic ACC

Since the early 1990s, a number of biochemical studies have contributed to show that homomeric plastidic ACC isoforms with altered sensitivity to APPs, CHDs, or both are a mechanism of resistance to these herbicides that is widespread among and within grass species (Table 3). However, the identification of mutations involved in altered sensitivity was not achieved until recently. The five variable amino acids with a role in sensitivity to ACC-inhibiting herbicides identified so far are listed in Table 4. An updatable version of this table will be maintained at the International Survey of Herbicide Resistant Weeds Web site (www.weedscience.org). Researchers are encouraged to add new data to this list.

Although highly conserved among species, homomeric ACC sequences differ in length (Table 1) because of non-conserved additions or deletions and because of the presence of the dual-targeting peptide in plastidic isoforms (Figure 2; Table 1). This leads to a variation in the position of amino acid residues of interest among species. Furthermore, only partial ACC sequences are available for several grass species (e.g., wild oat [*Avena fatua* L.], *Lolium* sp.). Blackgrass is the grass for which ACC has been most thoroughly studied on a biochemical and molecular biology basis (Délye et al. 2002a, 2002b, 2003, 2005; Menéndez and De Prado 1999; Moss et al. 2003; Price et al. 2004) and in which the highest number of ACC mutations has been identified (Table 4). To avoid confusion in future ACC studies, it is proposed that ACC amino acid substitutions be referenced with the corresponding position in blackgrass plastidic ACC (EMBL/Genbank/DBJ accession AJ310767), as in this review (Table 4).

Caution should be taken when comparing RFs derived from herbicide concentrations that inhibit ACC activity by 50% (I_{50} values), as discussed in the “Selectivity of ACC-inhibiting herbicides toward crop plants” section. However, results of published studies enable a rough separation of three groups of cross-resistance patterns conferred by mutant plastidic ACC isoforms (Table 3). One group of mutant ACC isoforms confers a very high RF to the CHD sethoxydim, but much lower RFs to other CHDs and to APPs. This cross-resistance pattern has been associated in four grass species with an isoleucine (Ile) to leucine (Leu) substitution at position 1781 that has been selected by sethoxydim alone or in association with APPs (Tables 3 and 4). A second group of mutant ACC isoforms that has been selected by APPs only confers high RFs to APPs but low RFs or no resistance at all to CHDs (Table 3). This cross-resistance pattern is conferred by a tryptophan (Trp) to cysteine (Cys), an Ile to asparagine (Asn), or a glycine (Gly) to alanine (Ala) substitution at positions 2027, 2041, or 2096, respectively, in blackgrass (Tables 3 and 4). It should be noted that an Ile to valine (Val) substitution at position 2041 did not confer a high RF to the APP clodinafop, in contrast with the Ile to Asn substitution at position 2041 (Table 4; Délye et al. 2003). In a third group, mutant ACC isoforms selected by APPs or CHDs confer high RFs to all APPs and CHDs assayed (Table 3). In blackgrass, this cross-resistance pattern is associated with an aspartic acid (Asp) to Gly sub-

stitution at position 2078 (Tables 3 and 4). The last subdivision in Table 3 contains mutant ACCs with various cross-resistance patterns that did not fit elsewhere. These patterns are highly diverse and are likely associated with mutations distinct from those already known.

All five variable amino acid residues associated with resistance to ACC-inhibiting herbicides are located within the active site cavity of the ACC CT domain (Délye et al. 2005). From tridimensional models derived from yeast homomeric cytosolic ACC, only the substitution at position 2041 directly interfered with herbicide binding. Given the likely tight fit of ACC inhibitor molecules within the cavity of the CT active site (see the section “Selectivity of ACC-inhibiting herbicides toward crop plants”), it has been proposed that the other four mutations cause resistance by hampering herbicide access to its binding site, by altering the spatial shape of the herbicide binding site via small allosteric changes, or by both mechanisms (Délye et al. 2005). That such a subtle change as the Ile to Leu substitution at position 1781 has such a drastic effect on the sensitivity to sethoxydim (Table 3) clearly illustrates the tight fit of the herbicide within the CT active site cavity. Although the cavity of the CT active site is constituted by almost equal contributors from two distinct regions of the CT domain (Zhang et al. 2003), one determinant for herbicide resistance (residue 1781) occurred in a region of highly conserved three-dimensional structure, whereas the four other known to date were clustered in a more variable region encompassing amino acid positions 2027 to 2096. It is probable that additional, yet unknown, mutations exist there that confer altered sensitivity to ACC inhibitors.

Homomeric Plastidic ACC Inherently Tolerant to ACC-Inhibiting Herbicides

Several grass species are tolerant of ACC-inhibiting herbicides because a plastidic homomeric ACC intrinsically displaying a sensitivity to these molecules that is in the range of what is usually observed for cytosolic homomeric ACCs (Table 2). Tolerant plastidic ACCs are found at least in the *Festuca*, *Poa*, and *Vulpia* genera (Table 2). The molecular basis for tolerance at the enzyme level is unknown. Furthermore, not all species within a genus may contain a tolerant homomeric plastidic ACC: red fescue (*Festuca rubra* L.) and blue fescue (*Festuca ovina* var. *glauca* Lam. Koch.) contain a tolerant plastidic ACC, whereas the closely related species tall fescue (*Festuca arundinacea* Shreb.) does not (Cattanzaro et al. 1993; Stoltenberg et al. 1989). Grasses tolerant to ACC-inhibiting herbicides are not major weeds. However, *Lolium* spp. are major weeds that are normally sensitive to ACC-inhibiting herbicides but readily hybridize with *Festuca* species (Morgan et al. 2001). Such hybridizations might result in the introgression of tolerant plastidic ACCs from *Festuca* sp. into *Lolium* sp., as has been suspected in Italian *Lolium* spp. populations (Dinelli et al. 2004).

Increase in Homomeric Plastidic ACC Activity

This atypical resistance mechanism has only been reported in johnsongrass (*Sorghum halepense* L.; Bradley et al. 2001). The authors observed similar I_{50} values for the plastidic ACC isoforms from resistant and sensitive plants but higher plastidic ACC-specific activity in resistant plants.

TABLE 3. Summary of published resistance factors computed as herbicide concentrations that inhibit acetyl coenzyme A carboxylase (ACC) activity by 50% (I_{50} ratios) between wild-type and mutant resistant plastidic ACC isoforms on the basis of enzyme assays.^a

R/S I ₅₀ ratios (RFs)												
Species ^b	Selecting herbicide ^c	APPs					CHDs					References
		Cd	Dc	Fx	Fz	Hx	Qz	Ct	Cx	Sx	Tk	
Very high RFs to sethoxydim, lower RFs to other herbicides												
Blackgrass (Leu ₁₇₈₁)	U	NA ^d	11.0	> 27.5	41.7	NA	NA	NA	NA	> 12.2	> 11.1	Moss et al. 2003
Wild oat UM1 (Leu ₁₇₈₁)	U	NA	10.1	9.8	NA	NA	NA	NA	NA	105.0	10.1	Shukla et al. 1997a
Large crabgrass	Fz, Sx	NA	NA	NA	10.3	NA	NA	5.8	NA	66.0	NA	Volenberg and Stoltenberg 2002a
Giant foxtail	Sx	NA	NA	3.0	NA	NA	11.3	5.7	NA	192.0	NA	Shukla et al. 1997b
	Fz, Sx	NA	NA	NA	10.6	NA	NA	4.8	NA	319.0	NA	Volenberg and Stoltenberg 2002a
Green foxtail UM131 (Leu ₁₇₈₁)	Dc, Sx	NA	NA	6.9	NA	NA	4.2	2.4	NA	423.0	NA	Délye et al. 2002e; Shukla et al. 1997a
Green foxtail	Sx, Hx	NA	20.0	NA	102.0	13.9	NA	5.8	416.7	625.0	NA	De Prado et al. 2004
Corn	Sx	NA	NA	NA	NA	20.5	NA	NA	NA	> 38.0	NA	Parker et al. 1990
High RFs to APPs, low RFs to CHDs												
Blackgrass (Asn ₂₀₄₁)	Fx, Cd	202.5	50.5	37.0	NA	77.5	NA	2.5	4.5	NA	NA	Délye et al. 2003
(Cys ₂₀₂₇)	Fx, Cd	90.5	21.5	53.0	NA	19.0	NA	3.0	5.0	NA	NA	Délye et al. 2005
(Ala ₂₀₉₆)	Fx, Cd	57.5	10.0	20.5	NA	16.0	NA	6.5	9.0	NA	NA	Délye et al. 2005
<i>Avena sterilis</i> ssp. <i>ludoviciana</i> Durieu	U	NA	3.7	8.2	NA	NA	NA	NA	0.8	NA	1.6	Cocker et al. 2000
<i>Digitaria ischaemum</i>	Fx	NA	NA	5.9	NA	NA	41.3	NA	NA	2.2	NA	Kuk et al. 1999
Italian ryegrass	Dc	NA	27.7	NA	NA	9.1	10.0	1.5	NA	0.9	NA	Gronwald et al. 1992
	Dc	NA	19.0	NA	NA	5.4	NA	2.3	NA	1.8	NA	De Prado et al. 2000
Rigid ryegrass VLR69	U	NA	29.0	NA	4.3	20.0	NA	NA	NA	1.2	1.0	Preston et al. 1996
WLR96	Dc	NA	> 217.0	NA	> 6.9	> 56.0	NA	NA	NA	R	6.0	Tardif et al. 1996
<i>Lolium temulentum</i> L.	Dc	NA	> 168.0	NA	NA	NA	NA	NA	NA	10.0	NA	Kuk et al. 2000
High RFs to APPs and CHDs												
Blackgrass (Gly ₂₀₇₈)	Fx, Cd	125.5	23.0	53.0	NA	35.0	NA	36.0	83.5	NA	NA	Délye et al. 2005
	APPs	NA	NA	NA	NA	NA	83.0	NA	NA	> 42.0	NA	Price et al. 2004
Goosegrass	Fz	NA	NA	24.5	> 90.0	NA	NA	4.4	NA	20.5	NA	Leach et al. 1995
Rigid ryegrass SLR3	Sx	NA	> 36.5	NA	≥ 2.9	20.0	NA	NA	NA	7.8	> 9.5	Tardif et al. 1993
SLR 31	U	NA	6.0	NA	55.0	15.0	NA	NA	NA	26.0	14.0	Tardif and Powles 1994
Green foxtail	Sx	NA	46.7	48.3	NA	NA	60.3	31.0	NA	50.0	31.3	Marles et al. 1993
Other cross-resistance patterns												
Wild oat UM33	U	NA	1.2	10.5	NA	NA	NA	NA	NA	5.0	1.7	Shukla et al. 1997a
Blackgrass	U	NA	2.6	NA	3.8	5.1	NA	1.5	1.6	2.1	NA	Menéndez and De Prado 1996
<i>Hordeum leporinum</i>	U	NA	0.7	NA	0.9	0.8	NA	NA	NA	4.1	1.4	Matthews et al. 2000
Italian ryegrass	U	NA	2.9	NA	8.8	NA	NA	NA	19.8	NA	3.5	Cocker et al. 2001

^a Ala, alanine; APP, aryloxyphenoxypropionate; Asn, asparagine; Cd, clodinafop; CHD, cyclohexanedione; Ct, clethodim; Cx, cycloxydim; Cys, cysteine; Dc, diclofop; Fx, fenoxaprop; Fz, fluazifop; Gly, glycine; Hx, haloxyfop; Leu, leucine; Qz, quizalofop; RF, resistance factor; Sx, sethoxydim; Tk, tralkoxydim; U, unknown.

^b Weed lines are indicated, and mutant ACC alleles conferring resistance are given when known.

^c Not assayed.

TABLE 4. Amino acid substitutions within plastidic homomeric acetyl coenzyme A carboxylase (ACC) and associated cross-resistance patterns observed at the whole-plant level.^a

Amino acid residue ^b		Resistance ^c											References
		APPs					CHDs						
Wild-type	Resistant	Weed	Cd	Dc	Fx	Fz	Hx	Ct	Cx	Sx	Tk		
Ile ₁₇₈₁	Leu	Blackgrass	S	R	R	R	S	S	R	R	R	Délye et al. 2002b, unpublished data; Moss et al. 2003	
	Leu	Wild oat	ND	R	ND	ND	ND	ND	ND	R	ND	Christoffers et al. 2002	
	Leu	Green foxtail	ND	R	R	ND	ND	ND	ND	R	R	Délye et al. 2002e; Heap and Morrison 1996	
	Leu	<i>Lolium</i> sp.	S	R	R	ND	ND	ND	R	ND	ND	Délye et al. 2002b; Tal and Rubin 2004	
Trp ₂₀₂₇	Cys	Blackgrass	R	ND	R	ND	R	S	S	ND	ND	Délye et al. 2005	
Ile ₂₀₄₁	Asn	Blackgrass	R	ND	R	ND	R	S	S	ND	ND	Délye et al. 2003	
	Asn	<i>Lolium</i> sp.	R	R	ND	ND	R	ND	S	ND	ND	Délye et al. 2003	
	Val	<i>Lolium</i> sp.	S	ND	ND	ND	R	ND	S	ND	ND	Délye et al. 2003	
Asp ₂₀₇₈	Gly	Blackgrass	R	ND	R	ND	R	R	R	ND	ND	Délye et al. 2005	
	Ala	Blackgrass	R	ND	R	ND	R	S	S	ND	ND	Délye et al. 2005	

^a Ala, alanine; APR, aryloxyphenoxypyrone; Asn, asparagine; Asp, aspartic acid; Cd, clodinafop; CHD, cyclohexanedione; Ct, clethodim; Cx, cycloxydim; Cys, cysteine; Dc, diclofop; Fx, fenoxaprop; Fz, flazifop; Gly, glycine; Hx, haloxyfop; Ile, isoleucine; Leu, leucine; Sx, sethoxydim; Tk, tralkoxydim; Trp, tryptophan; Val, valine.

^b Amino acid number is standardized to blackgrass plastidic homomeric ACC (EMBL AJ310767).

^c S and R, respectively, indicate that plants containing at least one copy of the ACC mutant allele are sensitive or resistant to the corresponding herbicide either in the field or in bioassays (see text for comment). ND, not determined at the whole-plant level.

Their interpretation was an overproduction of the plastidic ACC isoform in the resistant plants. However, given that the specific activity of the plastidic ACC per milligram of ACC protein in resistant plants was threefold higher than that in sensitive plants, it seems more likely that resistance to ACC-inhibiting herbicides in johnsongrass was due to the presence of a plastidic ACC isoform with increased intrinsic catalytic activity. The molecular basis for this increase in ACC activity remains unknown.

Metabolism-Based Mechanisms of Resistance

Herbicides are external factors that apply powerful stresses on plants. As a consequence, they trigger plant pathways of general response to stress. Metabolism-based detoxification of herbicides in grasses can thus be viewed as a deviation of enzymes normally involved in the proceeding of endogenous products so as to (1) prevent a concentration in active herbicide molecules sufficient to cause metabolic damage to be reached at the target site, or (2) make up for the herbicide deleterious effects, or both. Such enzymes enable grass crops to withstand field applications of ACC-inhibiting herbicides. They also exist in grass weeds. Variation in their regulation, specificity, or both confers metabolism-based resistance to ACC-inhibiting herbicides in grass weeds.

Metabolism modifies the chemistry of herbicide molecules, and therefore their properties. The quicker a plant is capable of altering herbicide molecules, the more resistant it is to these herbicides. Metabolism of herbicides can be described as a three-phase process (for review, see Kreuz et al. 1996; Van Eerd et al. 2003). In a first phase, the herbicide is generally transformed into a more water-soluble and less toxic molecule via oxidation, hydrolysis, or reduction. Notable exceptions for this are APP esters that are activated in their acidic phytotoxic form in the first phase of their metabolism pathway, as discussed in the "Selectivity of ACC-inhibiting herbicides toward crop plants" section. A second phase involves conjugation of an herbicide or its metabolite to the tripeptide glutathione (γ -Glu-Cys-Gly), a sugar, or an amino acid, which further decreases the toxicity of the parent molecule. In a third phase, metabolites from the second phase are further conjugated and eliminated by exportation to the vacuoles or incorporation into the cell walls.

A variety of enzymes are involved in herbicide metabolism (Kreuz et al. 1996; Van Eerd et al. 2003). Among the enzymes involved in the first phase of herbicide metabolism, CYPs have been shown to be involved in metabolism-based resistance to ACC-inhibiting herbicides. CYPs are heme proteins belonging to multigenic families. They mostly catalyze the NADPH₂-dependent mono-oxygenation of a variety of molecules. The reader is referred to reviews for an overview of the gene organization, the structure and the functions of CYPs (Werck-Reichhart and Feyereisen 2000), and the role of CYPs in herbicide metabolism (Kreuz et al. 1996; Werck-Reichhart et al. 2000). Although an increase in CYP activity has been demonstrated to play a role in resistance to ACC-inhibiting herbicides in a number of studies that used various CYP inhibitors (Table 5), no CYP gene endowing resistance to ACC-inhibiting herbicides has been characterized to date. The use of CYP inhibitors will block the first phase of herbicide metabolism, thus inhibiting the provision of herbicide metabolites for further me-

TABLE 5. Metabolism-based resistance to acetyl coenzyme A carboxylase (ACC)-inhibiting herbicides in grass weeds.^a

Species	Population ^b	Enzyme activity involved in metabolism-based resistance	Cross-resistance to herbicides with other modes of action	ACC-based resistance ^c	References
Blackgrass	Burgundy	CYP	Acetolactate synthase	Yes	Letouzé and Gasquez 2001
	OxfordSI, LincsE1,	NI	Photosystem II ^d	Yes (Leu ₁₇₈₁)	Cocker et al. 1999; Hall et al. 1997; Moss et al. 2003
	NottsA1				Cummins et al. 1999; Hall et al. 1997
	Peldon	GST, NI	Photosystem II, ^d peroxidizing herbicides	No	
Wild oat <i>Avena sterilis</i> Large crabgrass <i>Hordeum leporinum</i> Italian ryegrass Rigid ryegrass	RB	GST	No	No	Letouzé and Gasquez 2003
	RC	CYP, GST	No	No	Letouzé and Gasquez 2003
	NE Spain	CYP, GT	Photosystem II ^d	Yes	Menéndez and De Prado 1996
	T11, Dorset	NI	NA	No	Cocker et al. 2000
	NAS4	CYP	NA	Yes	Maneeshore et al. 1997
	Murray Bridge	NI	NA	No	Hidayat and Preston 1997
	Artherton	NI	NA	Yes	Matthews et al. 2000
	EssexA1, LincsA1, WilsB1	CYP, GST	Photosystem II ^d	No	Cocker et al. 2001
	SLR31	CYP	Acetolactate synthase ^d	Yes (Leu ₁₇₈₁)	Preston and Powles 1998; Villalobos et al. 2005
				Yes	Preston et al. 1996
<i>Lolium</i> spp.	VLR69	CYP, NI	No	Yes	
	Roma94, Toscana97	CYP, NI	NA	No	Bravin et al. 2001

^a CYP, cytochrome P450; GST, glutathione-S-transferase; GT, glucosyltransferase; NA, not assayed; NI, enzyme activity involved in metabolism-based resistance not identified.^b Population in which one or several mechanisms of resistance have been identified.^c Present together with metabolism-based resistance in the weed population investigated.^d May be multiple resistance.

tabolism reactions. Such studies thus demonstrate that CYPs are involved in metabolism-based resistance to ACC-inhibiting herbicides, but not that they are solely responsible for this resistance.

Among the enzymes involved in the second phase of herbicide metabolism, glucosyltransferases (GTs) and GSTs have been shown to be involved in metabolism-based resistance to ACC-inhibiting herbicides. GTs catalyze the *O*-glucosylation of CYP-hydroxylated molecules. GT activity is well known in grass crops such as wheat (Van Eerd et al. 2003). The role of GT activity in resistance to ACC-inhibiting herbicides has been shown solely in the blackgrass population *A. myosuroides* 'Peldon', in which resistance was shown to be partly the result of a constitutive increase in both CYP and GT activities (Brazier et al. 2002). GSTs catalyze the ATP-dependent conjugation of first-phase herbicide metabolites to glutathione. Like CYPs, GSTs belong to multigenic families. Active GST enzymes are mostly homo- or heterodimers, with various dimer combinations coexisting in plants. The gene organization and the structure and functions of GSTs have recently been reviewed elsewhere (Frova 2003), as has their role in metabolism-based resistance to herbicides in weeds (Reade et al. 2004). It has been shown in blackgrass that an increase in GST activity could be associated with a decrease in sensitivity to APPs (Milner et al. 2001; Reade and Cobb 2002). Interestingly, at least a part of this increase in GST activity observed in resistant blackgrass plants was due to the constitutive overexpression of a gene encoding a GST isoform that did not metabolize APPs. Instead, this isoform protected blackgrass cells from the cytotoxicity of APPs resulting from the production of hydroperoxides issued from the disruption of fatty acid biosynthesis by reducing these hydroperoxides to the corresponding, less toxic alcohols via a secondary glutathione-peroxidase activity (Cummins et al. 1999).

A number of genes are clearly involved in metabolism-based resistance to ACC-inhibiting herbicides, which renders their study fairly complicated. Besides, cross-resistance patterns associated with metabolism-based resistance are quite unpredictable, and the range of herbicide molecules concerned is broader than in the case of ACC-related resistance. Indeed, in several weed species, CYP or GST activities have been associated with resistance to ACC- and acetolactate synthase-inhibiting herbicides, ACC- and photosystem II-inhibiting herbicides (Preston 2004), or ACC-inhibiting and peroxidizing herbicides (Cummins et al. 1999; Table 5). However, metabolism-based cross-resistance to herbicides with different modes of action has so far been clearly demonstrated only in the case of ACC-inhibiting and peroxidizing herbicides (Cummins et al. 1999) and in one case for ACC- and acetolactate synthase-inhibiting herbicides (Letouzé and Gasquez 2001). Other studies merely proved that inhibition of a given CYP or GST activity restored or increased sensitivity to herbicides with different target enzymes. The inhibitors used were not specific for a single CYP or GST isoform, and a given CYP or GST activity can be a result of several distinct isoenzymes. Thus, these studies did not prove that resistance to herbicides with different target sites was due to a single CYP or GST gene, thereby not excluding the possibility for multiple resistance. The range of herbicides concerned with metabolism-based multiple or cross-resistance being rather unpredictable, manag-

ing weed populations resistant to ACC-inhibiting herbicides because of enhanced herbicide metabolism by switching to herbicides with a different mode of action could prove tricky.

Membrane Repolarization: Not a Resistance Mechanism

Recovery of transmembrane potential has been reported in several cases as a mechanism of resistance to ACC-inhibiting herbicides (Vila-Aiub et al. 2003; for a review, see Holtum et al. 1994) in the context of the hypothetical interaction of these herbicides with a putative binding site on cell membranes that was discussed in the section "Structure and mode of action of ACC-inhibiting herbicides". However, as discussed there, it seems more than likely that the membrane repolarization observed in some resistant plants is a secondary consequence of a resistance mechanism rather than the mechanism itself (Shukla et al. 1997a). In particular, repolarization might result from transmembrane pumps excreting herbicide metabolites toward vacuoles or cell walls.

Evolution of Resistance to ACC-Inhibiting Herbicides

The development of herbicide resistance in weeds is an evolutionary process. Herbicide treatments of weed populations result in the selection of any gene(s) conferring individual plants the capacity to withstand herbicides at the rate used in the field and to reproduce successfully thereafter. Under herbicide selection, the genetic composition of the weed population changes so that the frequency of resistance genes increases. The population genetic factors influencing the evolution of herbicide resistance in weed populations have been reviewed elsewhere (Jasieniuk et al. 1996). Prominent among them are genetic control of resistance, the initial frequency of resistance genes in weed populations, inheritance of resistance, the fitness penalty associated with resistance genes, and gene flows among weed populations. As discussed hereafter, not very much is known about these factors in the case of resistance to ACC-inhibiting herbicides.

Genetic Control of Resistance

Identification of Resistance

Before studying the evolution of resistance in a weed species, it is necessary to ensure that this resistance does exist. This requires assessing herbicide effects under standardized conditions that enables good herbicide efficacy. A number of bioassays based on a differential response of resistant and sensitive plants to discriminating doses of ACC-inhibiting herbicides have been developed for this purpose (Beckie et al. 2000; Boutsalis 2001; Letouzé and Gasquez 1999; Retrum and Forcella 2002; Tal et al. 2000). These assays, based either on seed (e.g., Letouzé and Gasquez 1999) or the whole plant (e.g., Boutsalis 2001), enable the detection of a phenotype (i.e., herbicide-resistant) but do not provide data about the gene(s) endowing it.

It should be mentioned here that, depending on the bioassay used, cross-resistance patterns observed at the enzyme and at the plant levels might not be identical. For instance,

plants containing mutant ACC isoforms with substitutions at positions 2027, 2041, and 2096 that displayed low RFs to CHDs at the enzyme level (Table 3) were found to be sensitive to CHDs in a seedling-based bioassay (Délye et al. 2003, 2005). In such bioassays, weed seeds are germinating in an herbicide solution, which makes the plants face herbicide doses much higher than is expected to occur in field conditions. It is therefore possible that these plants could survive field rates of CHDs.

Discriminating among Resistance Mechanisms

Unraveling the genetic control of resistance to ACC-inhibiting herbicides is a prerequisite to understanding its evolution. Of particular importance is the necessity to discriminate between ACC-based and metabolism-based resistance at the plant level because ACC-based resistance will be selected solely by ACC-inhibiting herbicides, whereas metabolism-based resistance might be selected by herbicides with alternative target sites (see the section "Metabolism-based mechanisms of resistance"). In the absence of a quick and simple ACC enzyme assay, it has been proposed that the CHD sethoxydim can be used routinely as an indicator of the presence of ACC-based resistance because it is not exposed to metabolism-based resistance (Moss et al. 2003). However, three out of the five mutant ACC alleles identified so far appear to confer resistance to APPs only (Table 4), which renders sethoxydim a rather unreliable indicator of ACC-based resistance. ACC-based resistance is expressed in pollen, whereas metabolism-based resistance is not (Letouzé and Gasquez 2000; Richter and Powles 1993). A pollen-based bioassay thus seems a more reliable routine test to identify ACC-based resistance. Alternatively, polymerase chain reaction (PCR)-based assays enabling specific detection of resistant ACC alleles have been developed in blackgrass (Délye et al. 2002a, 2002b, 2003, 2005), *Lolium* sp. (Délye et al. 2002b; Tal and Rubin 2004), or green foxtail (Délye et al. 2002c) and could be adapted to other grass weed species provided that resistant ACC alleles are characterized. Conversely, one enzyme-linked immunosorbent assay (ELISA) that allows the detection of metabolism-based resistance from enhanced GST activity has been developed in blackgrass (Reade and Cobb 2002).

Coexistence of Various Resistance Genes within a Single Weed Population/Plant

It appears that in most cases in which the cause of resistance has been identified, resistance to ACC-inhibiting herbicides is the result of a mutant plastidic ACC (Tables 3 and 5). The prevalence of ACC-based resistance cases might partly be a consequence of the relatively straightforward identification of resistant ACC isoforms by biochemical or molecular methods, whereas that of the diverse enzymes underlying metabolism-based resistance is more problematic. This means that, when ACC-based resistance is identified, metabolism-based resistance could be present as well, although not identified. In the case of ACC-related resistance, different resistant ACC alleles have been found in a single blackgrass population, and even in a single plant (Délye et al. 2004a, 2005). Besides, where the mechanisms of resistance to ACC-inhibiting herbicides have been investigated in detail, it was found that ACC-related and metabolism-

based resistance mechanisms can coexist within a single weed population (Table 5) or even a single weed plant (Letouzé and Gasquez 2001; Manechote et al. 1997; Vila-Aiub et al. 2005). At least in weeds such as blackgrass or *Lolium* spp., in which it has been studied extensively, resistance to ACC-inhibiting herbicides thus appears to be a complex trait, governed by probably more than five mutant ACC alleles and a certainly higher number of metabolism genes. In this respect the broadly used terms “resistant population” and “resistant biotype” are misleading. Indeed, resistant populations/biotypes are often treated as homogeneous entities, although they contain individuals that could be resistant to a given herbicide by different mechanisms, thus displaying heterogeneous cross-resistance patterns and resistance factors. Furthermore, resistant populations/biotypes most often contain a certain proportion of sensitive plants. Given the complexity of the genetic control of resistance to ACC-inhibiting herbicides, resistance should rather be considered at the level of the plant. Population studies should consider the frequency of resistant plants and the variety of RFs or resistance mechanisms present within a weed population.

Mutation to Resistance and Initial Frequency of Resistant Plants in a Weed Population

The gene encoding plastidic ACC has been proposed to be under purifying selection because the vast majority of nucleotide substitutions observed there were silent or synonymous (Délye et al. 2004b). This suggested that not any amino acid replacement, and therefore not any mutation conferring resistance to ACC-inhibiting herbicides, can occur and be maintained in the field. This is consistent with the vital metabolic role of plastidic ACC. However, although it has been shown that Leu₁₇₈₁ and Asn₂₀₄₁ mutant ACC alleles each evolved from several distinct, independent origins in blackgrass (Délye et al. 2004a, 2004b), there is no published data about the frequency of occurrence of mutations in the gene encoding plastidic ACC. The same is true for any mutation at loci conferring resistance to ACC-inhibiting herbicides. The frequency of weed plants resistant to ACC-inhibiting herbicides before their initial application is poorly known as well. This is mostly because of the huge number of plants that must be screened in an unsprayed population to obtain reliable estimates of this frequency. The initial frequency of weed plants resistant to ACC-inhibiting herbicides has only been estimated in Australian rigid ryegrass (*Lolium rigidum* Gaud.) populations and U.S. wild oat populations, where it fluctuated between 3% and < 0.2%, depending on the population (Matthews and Powles 1992; Mengistu et al. 2003). The number and the nature of the gene(s) endowing resistance in these populations were not determined.

Inheritance of Resistance

The gene encoding plastidic ACC and the genes involved in metabolism-based resistance are nuclear, meaning they can be propagated both by seed and pollen. ACC-based resistance was found to be dominant over sensitivity in wild oat (Kibite et al. 1995), blackgrass (Letouzé and Gasquez 2001), and foxtail millet [*Setaria italica* (L.) Beauv.; Wang and Darmency 1998]. In both studies, a single discriminat-

ing dose of herbicide was used. When a range of herbicide doses was used, ACC-based resistance was found to be dominant over sensitivity in foxtail millet (Wang and Darmency 1997) and partially dominant over sensitivity in corn (Marshall et al. 1992; Parker et al. 1990), wild oat (Murray et al. 1995, 1996), and rigid ryegrass (Tal et al. 2004; Tardif et al. 1996). At herbicide doses much higher than field rate, ACC-based resistance was also found to be partially dominant over sensitivity in Italian ryegrass (*Lolium multiflorum* Lam.; Betts et al. 1992) and giant foxtail (*Setaria faberi* Herrm.; Volenberg and Stoltenberg 2002b). In wild oat, ACC-based resistance was dominant over sensitivity at low herbicide doses but partially dominant at higher doses (Seefeld et al. 1998). Partial dominance of ACC-based resistance over sensitivity along a range of herbicide doses seems consistent with the ACC-inhibiting mode of action (see the section “Structure and mode of action of ACC-inhibiting herbicides”). The point is, however, that ACC-based resistance is dominant at field rates (herbicide doses selecting for resistance genes in weed populations). As a consequence, plants homozygous and heterozygous for ACC-based resistance all survive field-rate treatments, although the effects of these treatments on their respective seed production has not been compared to date. Dominance of metabolism-based resistance to ACC-inhibiting herbicides has only been studied in blackgrass populations in which a CYP-based resistance was shown to be dominant over sensitivity at a single, discriminating herbicide dose (Letouzé and Gasquez 2001). It can thus be expected that most genes endowing resistance to ACC-based herbicides confer a resistance that is dominant over sensitivity at the herbicide field rate.

Herbicide Selective Pressure and Fitness Cost

At field rate, ACC-inhibiting herbicides display a killing rate for sensitive plants ranging from 95 to 97% (Foster et al. 1993). Any gene enabling an individual plant to survive herbicide application will thus strongly be selected for. However, the particular herbicide(s) exerting selective pressure is not a reliable predictor for the resistance pattern(s) that will be selected. As an example, the five resistant mutant ACC alleles identified in blackgrass confer distinct cross-resistance patterns (Table 4), although they have essentially been selected for by the APPs fenoxaprop, clodinafop, or both (Délye et al. 2004a, 2005). Similarly, resistance to CHDs in wild oat has been selected by both APPs and CHDs, and not solely by CHDs (Légère et al. 2000). When other than ACC-based resistance mechanisms are involved, the relationship between selecting herbicides and selected resistance patterns is still more unpredictable. For instance, the use of APPs can result in the selection of multiple or cross-resistance to herbicides with other sites of action (Cummins et al. 1999; Friesen et al. 2000; Letouzé and Gasquez 2001; Preston 2004).

After selection, the outcome of resistance genes in weed populations depends on their pleiotropic effects on the fitness of individual plants in the absence of herbicide. Fitness can be defined as survival and reproductive success in a given environment. Determining the relative fitness of resistant and sensitive plants is therefore crucial to understand the evolution of the genetic structure of weed populations. Fitness studies require the comparison of major traits in the life cycle of weeds (e.g., seed germination, vegetative growth

rate, seed production) between resistant and sensitive plants and the assessment of the ecological consequences in the field of the differences observed. Ideally, comparisons should be performed in a range of environmental conditions between plants containing or not containing a clearly identified resistance gene and otherwise displaying close genetic backgrounds. Few studies provided data about the fitness of plants resistant to ACC-inhibiting herbicides. No differences were found in growth or seed production between resistant and sensitive plants in giant foxtail and large crabgrass [*Digitaria sanguinalis* (L.) Scop.; Wiederholt and Stoltenberg 1996a, 1996b]. However, neither study is really informative because the gene(s) endowing resistance was not identified, and resistant and sensitive plants were issued from distinct populations. This is also true for a study comparing resistant and sensitive plants respectively originating from two distinct populations of goosegrass (*Eleusine indica* L. Gaertn.; Marshall et al. 1994). The “weakly inheritable” resistant phenotype studied there might have been under polygenic control, and the higher resource allocation toward seed production observed in the resistant plants could not be clearly associated with resistance. In a study comparing the germination of rigid ryegrass seeds from individual plants of population SLR31 that were sensitive or resistant because of either increased CYP activity or both a resistant mutant Leu₁₇₈₁ ACC allele and increased CYP activity, seeds from the latter type of plants were found to require light and higher temperatures to germinate (Vila-Aiub et al. 2005). This enabled the authors to propose that the plants from this particular population could be controlled in the field by seed burial via shallow cultivation so as to maximize their fitness penalty.

Different fitness penalties can be associated with different resistance genes and even with different mutant alleles of a gene encoding an herbicide target enzyme (Roux et al. 2004). Similarly, different fitness penalties can be associated with the various mutant ACC alleles conferring herbicide resistance (Table 4). Indeed, investigators in a study conducted on nine populations of blackgrass suspected that fenoxaprop selected Leu₁₇₈₁ ACC alleles rather than Asn₂₀₄₁ alleles (Délye et al. 2004a). Because both types of alleles confer resistance to fenoxaprop (Table 4), it was proposed that a higher fitness penalty might be associated with Asn₂₀₄₁ ACC alleles (Délye et al. 2004a). This hypothesis must be checked by more extensive studies. It is consistent with the Ile₁₇₈₁ to Leu substitution most often observed among species (Table 4), suggesting that limited or no fitness penalty might be associated with this particular resistant ACC allele. Conversely, a strong reduction in ACC activity that could have consequences on fitness has been observed in blackgrass for Cys₂₀₂₇ and Gly₂₀₇₈ ACC alleles (Délye et al. 2005). Constitutive, metabolism-based resistance like that observed in blackgrass (Brazier et al. 2002; Cummins et al. 1999) is also likely to result in a fitness penalty, but there is no published study to date to check this point.

Weed Mating Systems and Gene Flows among Populations

Resistance to ACC-inhibiting herbicides has developed in highly selfing species such as green foxtail, as well as in highly outcrossing species such as blackgrass. However, the

mating system of species is of importance because a variety of resistance genes will accumulate more rapidly in individual plants in outcrossing species. Similarly, gene flow by pollen or seeds among populations that evolved different resistance genes might be crucial for the evolution of resistance at the species level. The relative contribution of pollen and seeds to gene flow within and among populations clearly depends on the species considered. In the mostly selfing species wild oat, resistance gene flows occur among weed patches and distinct fields. The large-sized seeds of *Avena* spp. seem a more efficient vector for resistance genes than pollen (Andrews et al. 1998; Cavan et al. 1998a; Murray et al. 2002); however, cross-pollination appeared to occur both between and within the two species, thus likely playing a role in the evolution and spread of resistance to ACC-inhibiting herbicides (Cavan et al. 1998a). In the mostly outcrossing species blackgrass, gene flows both among weed patches in a field and among distant fields were estimated to be quite low (Cavan et al. 1998b; Délye et al. 2004a, 2004b). Nevertheless, molecular data suggested that long-distance gene flows existed until recently and might still exist among blackgrass populations (Délye et al. 2004a, 2004b). It can be proposed that the small seeds of blackgrass contaminating cereal crop or grass forage crop seed batches would mediate these gene flows. Data obtained from *Avena* spp. and blackgrass suggest a major role of seeds in the propagation of resistance to ACC-inhibiting herbicides in these species. Large-scale studies need to be conducted before this point is definitely proved.

Is the Evolution of Resistance to ACC-Inhibiting Herbicides Predictable?

From the data obtained in blackgrass, *Avena* spp. and *Lolium* spp., resistance to ACC-inhibiting herbicides appears to be a complex trait under an oligogenic or polygenic control. Resistance genes are mostly dominant at herbicide field rates and can accumulate in a single plant. On the other hand, spraying programs are most often designed at the field level. At the level of the range of a weed species, spraying programs thus create a mosaic of selective pressures that select for a likely large variety of resistance genes, with associated cross-resistance patterns that are little predictable. Local weed populations adapted to local spraying programs will thus emerge. The emerging picture is that of resistance to ACC-inhibiting herbicides that likely evolves following a “patchy” frame, as observed in wild oat (Beckie et al. 1999, 2002) and suspected in blackgrass (Délye et al. 2004a). Besides, as suggested by a recent survey conducted in wild oat (Mengistu et al. 2003), the selective pressure of ACC-inhibiting herbicides, if constantly applied, would likely result in the rapid (Délye et al. 2004b) selection of many resistance genes, leading to an increasingly complex genetic control of resistance and, in fine, to the emergence of local, highly fit, resistant phenotypes that might also be resistant to herbicides with alternative modes of action. All this renders a prediction for the global evolution of resistance unachievable.

What Now?

Herbicides strongly contribute to secure yield and are indispensable in traditional, mechanized crop farming. How-

ever, the increase in costs to bring new products on the market (Cobb and Kirkwood 2000), the growing public concern of the use of pesticides, and the lack of herbicides under development with new modes of action, combined with the development of resistant weed populations, represent a growing threat to the most effective way for farmers to control weeds. Three research topics appear relevant with regard to ACC-inhibiting herbicides.

Slowing the Development of Resistance

An obvious way of delaying the increase in frequency of resistant plants in a weed population is not to use herbicides selecting for resistance to control this population. To do so, quick resistance diagnosis tests such as the PCR- or ELISA-based assays mentioned in the "Genetic control of resistance" section are potentially useful resistance management tools because their rapidity enables the avoidance of spraying herbicides that will further select resistance during the growing season when resistance is detected. Field studies showed that breaking the life cycle of weeds, and consequently reducing the abundance of weed populations, is an efficient way of slowing up the development of resistance to ACC-inhibiting herbicides. This can be achieved by various cultural methods, such as ploughing or incorporation of perennial forage crops, that reduce weed abundance and facilitate less frequent herbicide use, as demonstrated in blackgrass (Chauvel et al. 2001) and wild oat (Beckie et al. 2004). When ecological differences between resistant and sensitive plants are known, cultural methods can also be devised so as to maximize the fitness penalty in resistant plants, as proposed in rigid ryegrass (Vila-Aiub et al. 2005).

Alternate use of herbicides with different target sites is predicted by models to significantly reduce the selection for resistance (Jasieniuk et al. 1996), as theorized for blackgrass and wild oat (Cavan et al. 2000, 2001). Another possibility is spraying tank-mixed herbicides with different modes of action (Diggle et al. 2003). Such tactics must however consider broad-spectrum, multiple, or cross-resistance from metabolism-based resistance mechanisms occurring at the level of the population or the plant. With application of tank-mixed herbicides, the possible antagonism between herbicides with different modes of action must also be considered (Barnes and Oliver 2004).

Developing New ACC-Inhibiting Herbicides

APPs and CHDs are very useful selective graminicides. In this respect, new molecules inhibiting the enzyme activity of all homomeric plastidic ACC isoforms, either sensitive or resistant to APPs, CHDs, or both, would have obvious commercial appeal. Derivatives of APP and CHD molecules have been found to be good leads for developing such molecules (Rendina et al. 1990; Shukla et al. 2004). Substrate analogs, such as the one proposed as a basis for the development of antibacterial agents targeting heteromeric ACC (Levert and Waldrop 2002), might also be of interest. Another approach involves high-throughput, random screening of molecules for anti-ACC activity. In the absence of a microassay for ACC activity, a pilot screen that uses antibody mimics of homomeric ACCs to identify such molecules has been developed (Webb and Hall 2002). In contrast to this approach, the availability of the tridimensional structure of

the CT domain of homomeric ACC (Zhang et al. 2003) should enable the rational design of ACC inhibitors. The CT domain in plastidic homomeric ACC is under purifying selection (Délye et al. 2004b). Thus, the probability of simultaneous occurrence of mutations conferring resistance to APPs and CHDs and to molecules with a distinct binding site inside the CT active site cavity is expected to be extremely low. Bipiperidylcarboxamide derivatives are potent inhibitors of mammal homomeric ACC isoforms (Harwood et al. 2003). A tridimensional model study showed that one of these compounds bound at a site not overlapping the binding site for APPs in the yeast homomeric ACC CT domain (Zhang et al. 2004a), thus making bipiperidylcarboxamide derivatives potential candidates for the development of new ACC-inhibiting herbicides. However, tridimensional models for the homomeric ACC CT domain are based on the structure of yeast homomeric ACC (Zhang et al. 2003). As discussed in the "Structure and mode of action of ACC-inhibiting herbicides" section, the binding mode of APPs and CHDs to grass plastidic homomeric ACC likely differs in detail from what is observed in yeast. A tridimensional structure of the CT domain of a grass plastidic homomeric CT domain is thus needed for an effective rational design of new ACC-inhibiting herbicides.

Unraveling Metabolism-Based Resistance

Molecular biology recently provided for a rather thorough comprehension of the mechanisms of ACC-based resistance to herbicides. More data is now needed about the ecological consequences (e.g., fitness penalty) of ACC-based resistance. However, ACC-based resistance is only one facet of resistance to ACC-inhibiting herbicides. A complete understanding of this phenomenon implies studying metabolism-based resistance. In contrast to ACC-based resistance, our knowledge of metabolism-based resistance is rather poor. Metabolism-based resistance is likely widespread. It could have heavy deleterious consequences for weed control, primarily because of the broad range of present and future herbicide molecules that can be affected. It seems therefore clear that the new challenge for weed scientists dealing with resistance to herbicides in general is to unravel the basis and assess the ecological consequences of metabolism-based resistance in weeds. Given the diversity of mechanisms involved, this task will certainly prove harder to complete than understanding target-based resistance mechanisms. In the near future, weed genomics (Basu et al. 2004) and molecular ecotoxicology (Sandermann 2004) might well provide the keys to metabolism-based resistance to herbicides, as did "standard" molecular biology for target-based resistance.

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