CAVELIER
ABOGADOS

A

Señor Director

NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 16-028064- -00001-0000
Fecha: 2016-05-16 16:27:22 Dep: 2020 DIR.NUEVASCF
Tra: 11 PATENTEIYII Eve: 378 FASENACIONALI
Act: 329 CTOINFORMACION Folios: 49

QL

Trámite: 011 **Evento:** 378 **Actuación:** 329

Referencia: Expediente 16028064

Asunto: Solicitud de patente para: "*PLANTAS TOLERANTES A HERBICIDAS*".

Solicitante: BASF AGROCHEMICAL PRODUCTS B.V.

Fecha de solicitud: 5 de febrero 2016

MEMORIAL COMPLEMENTARIO Y MODIFICACIÓN A LAS REIVINDICACIONES

ANDRÉS RINCÓN USCÁTEGUI, identificado como aparece al pie de mi firma, en mi condición de apoderado de la sociedad solicitante de la patente de la referencia, dando alcance al memorial radicado el 5 de febrero de 2016 ante este Despacho, deseo presentar un nuevo pliego reivindicatorio y complementar la información y los argumentos presentados.

I. MODIFICACIONES AL PLIEGO REIVINDICATORIO

Con el fin de atender las observaciones realizadas por el examinador de manera preliminar al examen de patentabilidad, el solicitante decide aportar un nuevo capítulo reivindicatorio conformado por 12 reivindicaciones en las que se han incluido las siguientes modificaciones:

1. La nueva reivindicación establece una tolerancia a 100 g ai/ha de cicloxidim, de haloxifop y de tepraloxidim. Dicha tolerancia está soportada en la descripción (ver WO2011/028832 A2) en por lo menos los párrafos [0115], [0218] (incluyendo Tabla 1) y [0271] (el cual es parte del Ejemplo 2 y en la Figura 17 de la memoria descriptiva. De modo que, es claro que la modificación realizada no amplia el alcance de la solicitud inicial.

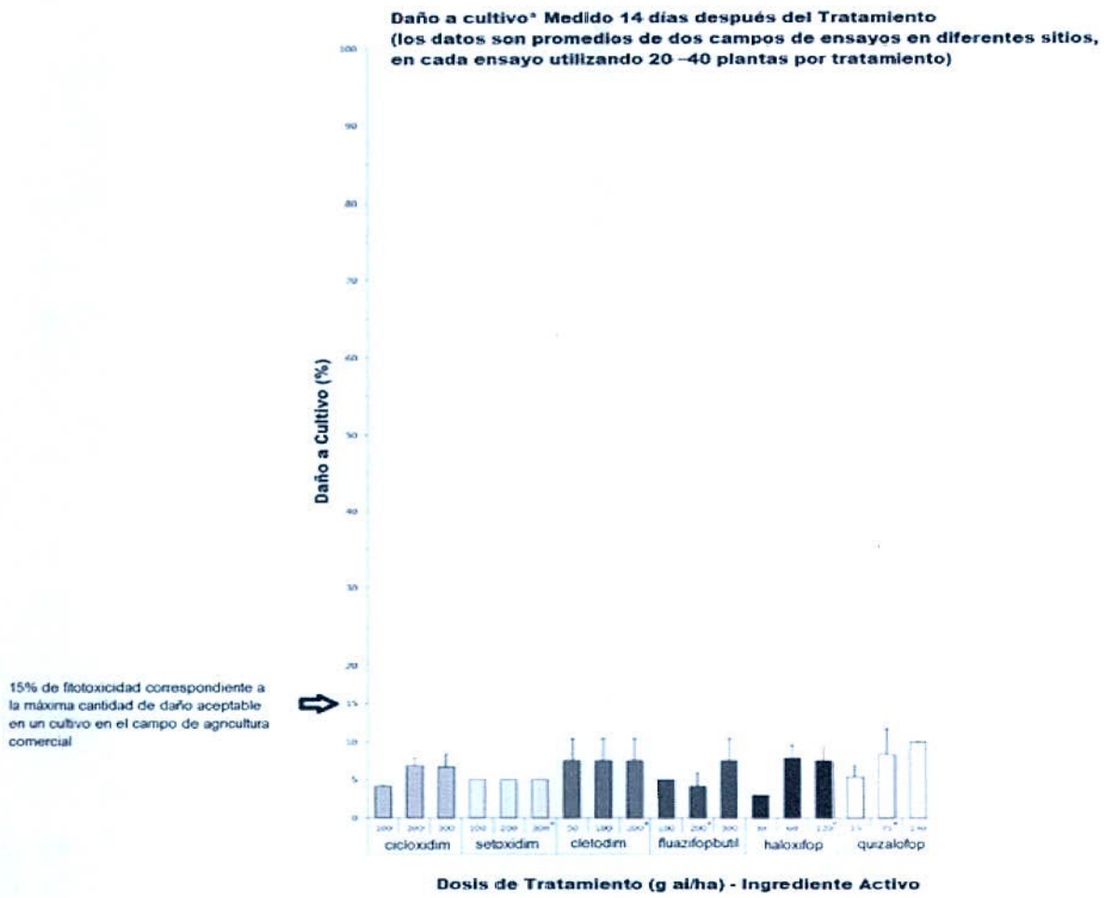
2. Se añadieron las nuevas reivindicaciones 5 a 10, las cuales especifican en mayor detalle algunas características de la invención. Lo anterior, de acuerdo con las enseñanzas de la descripción tal como fue originalmente presentada.

Estas modificaciones se ajustan al artículo 34 de la Decisión 486 de 2000 y no constituyen una ampliación de la protección que correspondería a la divulgación contenida en la solicitud inicial.

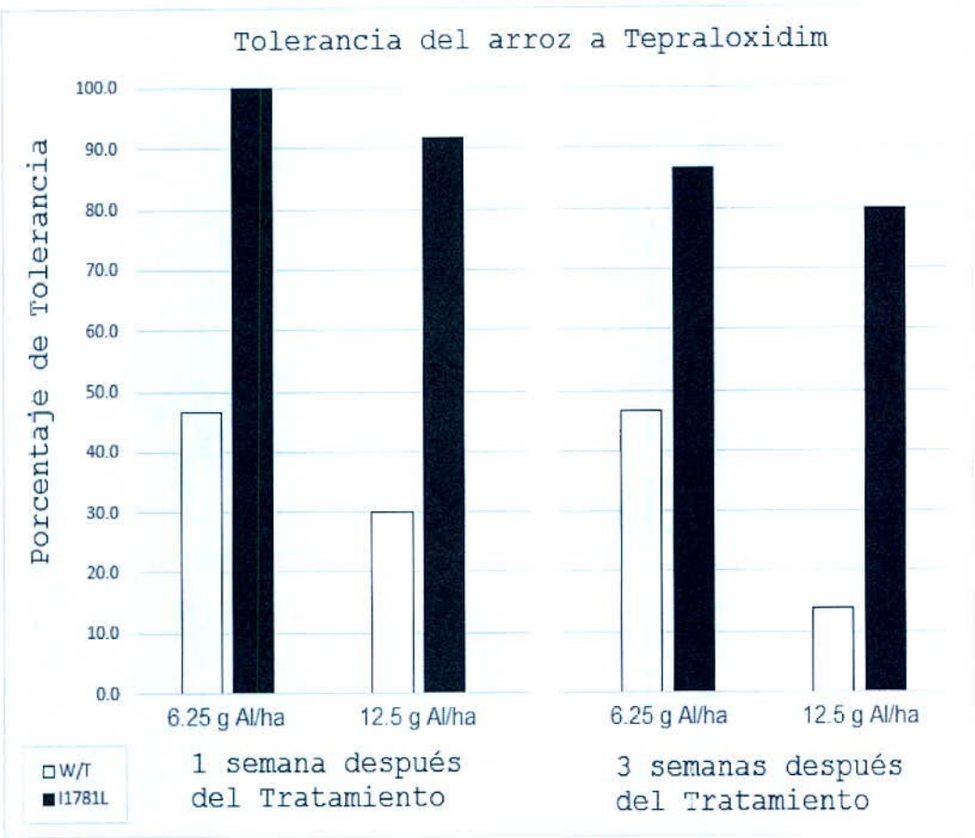
II. EN CUANTO A LA NOVEDAD Y EL NIVEL INVENTIVO

Se presentan a continuación dos gráficos que demuestran resultados alcanzados por la presente solicitud, los cuales además comprueban ventajas técnicas de la invención con respecto al arte previo.

Primer Gráfico de Barras



Segundo Gráfico de Barras



Con respecto al primer gráfico de barras, se muestra % fitotoxicidad/datos de daño a cultivo donde 15% corresponde al corte para una tolerancia comercialmente relevante, es decir la máxima cantidad de daño aceptable en un cultivo en el campo de la agricultura comercial. Por otra parte, el segundo grafico muestra el porcentaje de toleración del arroz a Tepraloxidim con respecto al tiempo de tratamiento.

Adicionalmente, se anexa al presente memorial copia de la Declaración realizada por el Dr. Dale Carlson, co-inventor de la presente invención, que fue presentada en la USPTO en el trámite de la solicitud equivalente a la solicitud Colombiana, ya que ambas hacen pare de la misma familia de patentes. En dicha declaración se discute la dificultad en la obtención de las líneas de arroz reivindicadas. En particular, se resalta que no representa una rutina la utilización de métodos aceptados en el arte para generar la materia reivindicada tal como muestran los documentos de la Declaración donde se indica cómo técnicas de mutagénesis de semillas tradicionales, luego de múltiples intentos, fallaron en generar las plantas de arroz deseadas con tolerancia a tepraloxidim. Finalmente, la

materia reivindicada fue generada por selección sobre cicloxidim. Esto es, resulta co (Délye, 2005)mpletamente inesperado y sorprendente que las líneas de arroz seleccionadas sobre cicloxidim exhibieran tolerancia a tepraloxidim.

Adicionalmente, se presentan dos referencias adjuntas escritas por Delye et al. para consideración del Examinador. Ambas referencias revelan que la mutación I1781L en múltiples especies de pastos falla al conferir tolerancia a haloxifop. Para su conveniencia, se han resaltado las porciones relevantes en ambas referencias donde se revela la falla de la mutación I1781L para conferir tolerancia al haloxifop (por ej. ver Tabla 4 en Delye et al., Weed Science, 53: 728-746 (2005) y páginas 796 y 801 en Delye et al., Plant Physiology, 137:794-806 (2005)).

Basado en la revelación de estas dos referencias de Delye et al., se solicita que incluyan en los argumentos a presentar al Examinador, que una persona con conocimientos comunes en la materia hubiera dejado aparte la mutación I1781L en una proteína de ACCasa de una planta de arroz para obtener tolerancia a haloxifop. En consecuencia, la ACCasa plastídica de arroz reivindicada por el Solicitante resulta no obvia para una persona con conocimientos en el arte. De la misma forma, la solución técnica ofrecida por la ACCasa plastídica de arroz reivindicada por el Solicitante, es decir la tolerancia a haloxifop, resulta también no obvia para una persona con conocimientos en el arte. De hecho, la solución técnica ofrecida por la ACCasa plastídica de arroz reivindicada por el Solicitante presenta un notable progreso en el campo debido las fallas determinadas por otros expertos del arte como se encuentra evidenciado en los documentos de Delye et al. quien fracasó en producir pastos tolerantes a haloxifop con la mutación I1781L.

III. PETICION

Solicito a ese Despacho tener en cuenta el nuevo pliego reivindicatorio y la información aquí suministrada al momento de expedir una acción oficial evaluando la patentabilidad de la invención.

IV. ANEXOS

1. Nuevo pliego reivindicatorio conformado por 12 reivindicaciones.

2. Recibo por valor de $80.000 por concepto de dos reivindicaciones adicionales a la décima.

3. Formulario de modificaciones en solicitud pendiente

4. Recibo por concepto de presentación de modificaciones a las reivindicaciones por valor de $ 164.000

5. Copia del documento: Délye, C. (2005). Molecular Bases for Sensitivity to Acetyl-Conezyme A Carboxylase Inhibitors in Black-Grass. Plant Physiol. Vol. 137.

6. Copia del documento: Délye, C. (2005). Weed resistance to acertyl coenzyme A carboxylase inhibitors: an update. Weed Science.

7. Copia y traducción de la declaración realizada por el Dr. Dale Carlson, co-inventor de la presente invención, que fue presentada en la USPETO en el trámite de una solicitud equivalente.

Señor Director,

ANDRÉS RINCÓN USCÁTEGUI
C.C. 79.780.910 de Bogotá
T.P. 114.908

COLO-13043-0841-0004-0
CBA\CBA\ID-288255\WPC13043
No. M-2016-288255\CBA

Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

DIRECCIÓN DE NUEVAS CREACIONES MODIFICACIONES Y/O CORRECCIONES

1. **Identificación del Tramite**

☐ **Error material** ☒ **Modificación a una Solicitud en trámite**

☒ Patente de Invención

☐ Patente de Modelo de Utilidad

☐ Registro de Diseño Industrial

☐ Esquema de Trazado de Circuitos Integrados

2. **Datos del solicitante**

Persona Natural ☐ Persona Jurídica ☒

BASF AGROCHEMICAL PRODUCTS B.V.

Nombre o denominación / Nombre o razón social completa

Nombre representante legal

Tipo de empresa Micro ☐ Pequeña ☐ Mediana ☐ Otra ☐

Documento de identificación: ☐ C.C. ☐ C.E. ☐ NIT ☐ Otro Número_____

Nacionalidad/País de constitución	Dirección y domicilio del titular	Ciudad
Países Bajos	Groningensingel 1, NL-6835 EA Arnhem, Países Bajos	Arnhem
Dirección electrónica	**No. Fax**	**Número telefónico**
clientes@cavelier.com	2 11 86 50	3 47 36 11

☐ **Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico**

3. **Datos del apoderado:**

Apellidos y Nombre	**Documento de identificación**	**Tarjeta profesional**
RINCÓN USCÁTEGUI ANDRÉS	C.C. 79.780.910	T.P.A. 114.908

Dirección y domicilio		**Ciudad**
Carrera 4ª No. 72 –35		Bogotá
Dirección electrónica	**No. Fax**	**Número telefónico**
cavelier@cavelier.com	2 11 86 50	3 47 36 11
En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general		

4. Datos del trámite

Corrección:

Expediente 16.028.064

Aparece

Debe ser

Modificación:

☒ **Modificación al capítulo reivindicatorio.**
Presento nuevo capítulo reivindicatorio compuesto por 12 reivindicaciones con el fin de superar las objeciones hechas por Su Despacho a la presente solicitud de patente y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas.
Respetuosamente manifiesto que a pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada.

5. Anexos

☒ Comprobante de pago de la tasa para la presentación de la corrección y/o modificación por $164.000.

☒ Comprobante de pago por 2 reivindicaciones adicionales a la décima por $80.000.

☐ Poder, si fuere el caso.

☒ Documento que contiene las modificaciones o correcciones.

6. Firma	
Nombre y apellidos **ANDRÉS RINCÓN USCÁTEGUI**	Firma
C.C. 79.780.910 de Bogotá	Tarjeta Profesional 114.908

CBA\SDC\ID-448711\WPC13043

REIVINDICACIONES MODIFICADAS

1. Una Acetil-Coenzima A carboxilasa (ACCasa) plastídica de una planta de arroz CARACTERIZADA PORQUE posee la secuencia de aminoácidos correspondiente a SEQ ID NO:2 y tiene una sustitución de isoleucina a leucina en la posición del aminoácido correspondiente a la posición 1781 de la ACCasa plastídica de *Alopecurus myosuroides (Am)* en SEQ ID NO:2, donde dicha ACCasa plastídica, cuando es expresada en una planta, confiere a dicha planta una tolerancia a 100 g ai/ha de cicloxidim, de haloxifop y de tepraloxidim.

2. La ACCasa plastídica de la reivindicación 1, CARACTERIZADA PORQUE dicha ACCasa plastídica comprende además una sustitución de aminoácido en la posición: 1785; 1786; 1811; 1864; 2027; 2039; 2041; 2049; 2074; 2075, 2080; 2088; 2095; 2096 o 2098 correspondiente a la posición de la ACCasa plastídica de *Alopecurus myosuroides (Am)*; una duplicación de valina en la posición 2075*(Am)*; o una eliminación del aminoácido en la posición 2080*(Am)*.

3. La ACCasa plastídica de la reivindicación 2, CARACTERIZADA PORQUE comprende además:

i. una sustitución de glicina en posición 1785(Am);
ii. una sustitución de prolina en posición 1786(Am);
iii. una sustitución de asparagina en posición 1811(Am);
iv. una sustitución de glicina en posición 2039(Am);
v. una sustitución de isoleucina, leucina, valina, o fenilalanina en posición 2049(Am);
vi. una sustitución de leucina en posición 2074(*Am*);
vii. una sustitución de leucina, isoleucina, o metionina en posición 2075(*Am*);
viii. una duplicación de valina en posición 2075(*Am*);
ix. una sustitución de treonina, glicina, o lisina en posición 2078(*Am*);
x. una sustitución de ácido glutámico en posición 2080(*Am*);
xi. una sustitución de arginina, triptófano, fenilalanina, glicina, histidina, lisina, leucina, serina, treonina, o valina en posición 2088(*Am*);
xii. una sustitución de ácido glutámico en posición 2095(*Am*); o
xiii. una sustitución de alanina, histidina, prolina, serina, o glicina en posición 2098(*Am*).

4. La ACCasa de cualquiera de las reivindicaciones 1-3, CARACTERIZADA PORQUE dicha ACCasa confiere además tolerancia a un inhibidor de ACCasa seleccionado de: alloxidim, butroxidim, cletodim, cloproxidim, setoxidim, tralcoxidim, clorazifop, clodinafop, clofop, diclofop, fenoxaprop, fenoxaprop-P, fentiaprop, fluazifop, fluazifop-P, haloxifop-P, isoxapirifop, propaquizafop, quizalofop, quizalofop-P, quizalofop-P-etil, quizalofop-P-tefuril, trifop, pinoxaden o sales o ésteres aceptables para uso agrícola de los mismos, a dicha planta.

5. La ACCasa de cualquiera de las reivindicaciones 1-4, CARACTERIZADA PORQUE dicha ACCasa es tolerante a 300 g ai/ha de cicloxidim.

6. La ACCasa de cualquiera de las reivindicaciones 1-5, CARACTERIZADA PORQUE dicha ACCasa es tolerante a 120 g ai/ha de haloxifop.

7. La ACCasa de cualquiera de las reivindicaciones 1-6, CARACTERIZADA PORQUE dicha ACCasa es tolerante a 12.5 g ai/ha de tepraloxidim.

8. La ACCasa de cualquiera de las reivindicaciones 1-7, CARACTERIZADA PORQUE dicha planta es una planta de arroz.

9. La ACCasa de cualquiera de las reivindicaciones 1-8, CARACTERIZADA PORQUE dicha planta:

a. es una planta de cualquiera de las líneas OsHPHI2, OsARWI1, OsARWI3, OsARWI8, u OsHPHN1, una muestra de semilla representativa de cada línea que ha sido depositada en la American Type Culture Collection (ATCC) con el Número de Designación de Depósito para Patente PTA-10267, PTA-10568, PTA-10569, PTA-10570, o PTA-10571, respectivamente; o es un híbrido, descendiente, o un derivado obtenido por ingeniería genética de una planta de cualquiera de las líneas OsHPHI2, OsARWI1, OsARWI3, OsARWI8, u OsHPHN1, o es una planta que es la progenie de cualquiera de dichas plantas; y

b. posee las características de tolerancia a herbicidas inhibidores de ACCasa de una planta de cualquiera de las líneas OsHPHI2, OsARWI1, OsARWI3, OsARWI8, u OsHPHN1.

10. La ACCasa de cualquiera de las reivindicaciones 1-9, CARACTERIZADA PORQUE dicha planta:

a. es una planta de la línea OsHPHI2, una muestra de semilla representativa de la línea que ha sido depositada en la American Type Culture Collection (ATCC) con el Número de Designación de Depósito para Patente PTA-10267; o es un híbrido, descendiente, o un derivado obtenido por ingeniería genética de una planta de la línea OsHPHI2; o es una planta que es la progenie de la línea OsHPHI2; y

b. posee las características de tolerancia a herbicidas inhibidores de ACCasa de una planta de la línea OsHPHI2.

11. Un método para controlar malezas en un campo, CARACTERIZADO PORQUE dicho método comprende:

a. crecimiento, en un campo, de una planta que comprende la ACCasa de cualquiera de las reivindicaciones 1-10; y

b. aplicar a la planta y malezas en el campo un herbicida inhibidor de Acetil-Coenzima A carboxilasa en una cantidad que inhibe el crecimiento de una planta de tipo salvaje correspondiente;

en consecuencia controlando las malezas.

12. El método de la reivindicación 11, CARACTERIZADO PORQUE dicho herbicida inhibidor de Acetil-Coenzima A carboxilasa comprende por lo menos uno de los siguientes: alloxidim, butroxidim, cletodim, cloproxidim, cicloxidim, setoxidim, tepraloxidim, tralcoxidim, clorazifop, clodinafop, clofop, diclofop, fenoxaprop, fenoxaprop-P, fentiaprop, fluazifop, fluazifop-P, haloxifop, haloxifop-P, isoxapirifop, propaquizafop, quizalofop, quizalofop-P, quizalofop-P-etil, quizalofop-P-tefuril, trifop, y pinoxaden o sales o ésteres aceptables para uso agrícola de los mismos.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

11

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 16 - 0045052

Bogotá D.C., Mayo 10 de 2016 - 10:22:33

RECIBIDO DE : CAVELIER ABOGADOS NI 860.041.367

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
RECIBO DE CA	SIC ** RECIBO **	999999999	43565	04/05/2016	1.120.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICACIONES N.CREACION CORRECCION A SOLICITUD EN TRAMITE / MODIFICACION CAP. DESCRIPTIVO O REIVINDICATORIO	164.000.00	164.000.00
				$164.000.00

SON: **CIENTO SESENTA Y CUATRO MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-******-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 16-028064- -00001-0000
Fecha: 2016-05-16 16:27:22 Dep: 2020 DIR.NUEVASCF
Tra: 11 PATENTEIYII Eve: 378 FASENACIONALI
Act: 329 CTOINFORMACION Folios: 49

0010-13043-841-004-0

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

12

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 16 - 0045716

Bogotá D.C., Mayo 11 de 2016 - 12:27:00

RECIBIDO DE : CAVELIER ABOGADOS NI 860.041.367 RE

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
RECIBO DE CA	SIC ** RECIBO **	999999999	44112	05/05/2016	72.000.00
RECIBO DE CA	SIC ** RECIBO **	999999999	44630	06/05/2016	6.216.00
RECIBO DE CA	SIC ** RECIBO **	999999999	45060	10/05/2016	2.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
2	50005-01-01 SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INICIALES	40.000.00	80.000.00
				$80.000.00

SON: **OCHENTA MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-***-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 16-028064- -00001-0000

Fecha: 2016-05-16 16:27:22 Dep. 2020 DIR.NUEVASCF
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 329 CTOINFORMACION Folios: 49

CCO-13043-841-004-0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Application No.: US 13/973,784
Filed: August 22, 2013
Applicant: BASF Agrochemical Products, B.V.

DECLARATION OF Dale R. Carlson

I, Dale R. Carlson, the undersigned, a citizen of the United States of America and residing at 600 Sawcut Lane, Apex, North Carolina 27502, declare and state the following:

1. I hold a Bachelor of Science degree in Agronomy from the University of Missouri, and a Masters of Science degree and Ph.D. in Agronomy from the University of Minnesota.

2. I am currently employed as Senior Global Product Manager for Herbicide Tolerant Crops at BASF Plant Science, located at 26 Davis Drive, Research Triangle Park, NC. I have held this position since 2002.

3. Prior to my current position, I held a number of positions with BASF, including: Project Manager & Regulatory Scientist at BASF Corp. from 1998-2001; Marketing Manager of the Global Herbicide Marketing Group at BASF AG from 1994-1997; and Biology Research & Development Manager at BASF Corp. from 1986-1994.

4. Prior to my employment with BASF, I was Senior Research Biologist of the Plant Growth Regulator Group at Monsanto Agricultural Products Co., from 1983-1986.

5. I am co-inventor of patent application 13/973,784. I am the research scientist who jointly produced the ACCase-inhibiting herbicide tolerant rice lines disclosed in this patent application, and was involved in the research that produced all of the ACCase-inhibiting-herbicide-tolerant trait genes disclosed in the Examples thereof. At all times pertinent to the events described in this Declaration, I was employed by BASF Plant Science LP, and its predecessor in interest BASF Plant Science LLC, at the BASF site in Research Triangle Park, North Carolina.

6. On information and belief, I understand that patent application 13/973,784 is undergoing Track One Prioritized Patent Examination in the USPTO, and includes claims to rice plants and seeds and their use in methods for controlling weeds of rice fields, which rice plants and seeds exhibit tolerance to quizalofop, 100 g a.i/ha cycloxydim, and in some instances tepraloxydim and 60 g a.i/ha pinoxaden and contain a nucleic acid encoding a rice plastidic ACCase enzyme having an induced substitution of I1781(*Am*)L, including those rice plants and seeds of and descended from rice line 'HPHI2,' which was produced by the BASF Plant Science

Development group starting from germplasm of a rice line named 'Indica-1' (see Table 3 on page 103 of the present patent application).

7. I am aware that the USPTO issued a Non-Final Office action on January 12, 2015 in which the Examiner has cited against the claims of this patent application, the publication of Suzuki et al. (2008) "MNU-induced mutant pools and high performance TILLING enable finding of, any gene mutation in rice," Mol. Genet. & Genom. 279:213-223 ("Suzuki"), for its reporting a method for chemically mutagenizing rice and identifying chemically-induced mutations in the resulting rice plants.

8. In the early 2000s, we identified a need to develop mutagenized rice having a phenotype of tolerance to the herbicide, tepraloxydim. As a result, we naturally chose tepraloxydim as the screening agent for rice materials that had been mutagenized by either seed chemical mutagenesis or tissue culture mutagenesis.

9. In order to implement the seed chemical mutagenesis methodology, under my guidance and supervision, we worked together with a partner to utilize traditional chemical mutagenesis of rice seed with selection on tepraloxydim to obtain fertile rice plants exhibiting tepraloxydim tolerance, as a result of having induced a mutation in the rice plastidic ACCase. Surprisingly, after multiple attempts over three years, no fertile, tepraloxydim-tolerant rice plants were produced and, thus, the effort was abandoned.

10. Thinking that perhaps a tissue culture mutagenesis approach might succeed where seed mutagenesis had failed, we thereafter implement the tissue culture mutagenesis methodology: I along with my co-inventors, attempted to obtain tepraloxydim-tolerant rice by tissue culture mutagenesis with selection on tepraloxydim. That attempt likewise failed. We unexpectedly found that tepraloxydim was unable, in multiplicate cultures, to select for an I1781 single mutant ACCase. This was confirmed by transforming rice callus cells and maize callus cells with over-expression constructs for I1781L and I1781A single-mutant ACCase enzymes, as described in the Examples of the present patent application. As shown in Table 16 of the application, tepraloxydim consistently failed to recover any I1781 single mutants. We concluded, in hindsight, that the likely reason why the seed mutagenesis methodology failed was because – contrary to normal logic – the choice of tepraloxydim as a selection agent for obtaining tepraloxydim tolerance, was incapable of achieving that very end.

11. All statements made in this Declaration of my own knowledge are true. All statements made in this Declaration on information and belief are believed to be true.

Date: July 15, 2015

Dale R. Carlson

EN LA OFICINA DE MARCAS Y PATENTES DE ESTADOS UNIDOS

Solicitud No.: US 13/973,784
Presentada: 22 de agosto de 2013
Solicitante: BASF Agrochemical Products, B.V.

DECLARACIÓN DE DALE R. CARLSON

Yo, el suscrito Dale R. Carlson, ciudadano de Estados Unidos de América y domiciliado en 600 Sawcut Lane, Apex, North Carolina 27502, declaro y afirmo que:

1. Soy profesional en Agronomía de la Universidad de Missouri, y tengo una Maestría y Ph.D. en Agronomía de la Universidad de Minnesota.

2. Desde el año 2002 trabajo como Gerente Global de Productos para Cultivos Tolerantes a los Herbicidas en BASF Plant Science, domiciliada en 26 Davis Drive, Research Triangle Park, NC.

3. Antes de mi cargo actual, me desempeñé en diferentes cargos en BASF, incluyendo: Gerente de Proyectos & Científico Regulador en BASF Corp. en el periodo de 1998-2001; Gerente de Marketing del Grupo Global de Comercialización de Herbicidas de BASF AG en 1994-1997; y Gerente de Investigación y Desarrollo en Biología en BASF Corp. en el periodo de 1986-1994.

4. Antes de trabajar con BASF, me desempeñé como Biólogo de Investigación Sénior del Grupo Regulador de Crecimiento Vegetal en Monsanto Agricultural Products Co., en 1983-1986.

5. Soy el coinventor de la solicitud de patente 13/973,784. Soy el científico investigador que produjo en conjunto las líneas de arroz tolerantes a los herbicidas inhibidores de ACCasa divulgadas en esta solicitud de patente y estuve involucrado en la investigación que produjo todos los genes de rasgos tolerantes a los herbicidas inhibidores de ACCasa divulgados en los Ejemplos de la misma. En todos los momentos pertinentes a los hechos descritos en la presente Declaración, fui empleado por BASF Plant Science LP, y su predecesor, BASF Plant Science LLC, en las instalaciones de BASF, en Research Triangle Park, Carolina del Norte.

6. A mi leal saber y entender, tengo conocimiento de que la solicitud de patente 13/973,784 actualmente se encuentra en proceso de Examen de Patente Prioritario tipo "Track One" ante la Oficina de Marcas y Patentes de Estados Unidos (USPTO), y que incluye reivindicaciones sobre plantas y semillas de arroz y su uso en métodos para controlar la maleza en los arrozales, en donde dichas plantas y semillas de arroz exhiben tolerancia al quizalofop, 100 g a.i/ha cicloxidim, y en algunos casos a tepraloxidim y 60 g a.i/ha pinoxaden y que contienen un ácido nucleico que codifica una enzima ACCasa plastídica de arroz con una sustitución inducida de I1781(*Am*)L, incluyendo las plantas y semillas de arroz de y descendientes de la línea de arroz 'HPHI2,' producida por el grupo de Desarrollo de Ciencias Vegetales de BASF a partir del germoplasma de una línea de

arroz denominada 'Indica-1' (véase la Tabla 3 en la página 103 de la presente solicitud de patente).

7. Tengo conocimiento de que la USPTO emitió una Acción Oficial no definitiva el 12 de enero de 2015, en la que el Examinador citó contra las reivindicaciones de esta solicitud de patente la publicación de Suzuki et al. (2008) "MNU-induced mutant pools and high performance TILLING enable finding of, any gene mutation in rice," Mol. Genet. & Genom. 279:213-223 ("Suzuki"), por su reporte de un método para mutagenizar químicamente el arroz e identificar mutaciones químicamente inducidas en las plantas de arroz resultantes.

8. A principios de la década del 2000, nosotros identificamos la necesidad de desarrollar arroz mutagenizado con un fenotipo de tolerancia al herbicida tepraloxidim. Como resultado, naturalmente escogimos al tepraloxidim como el agente depurador para los materiales de arroz mutagenizados ya sea por mutagénesis química de semillas o por mutagénesis de cultivo de tejidos.

9. Con el fin de implementar la metodología de mutagénesis química de semillas, bajo mi dirección y supervisión, trabajamos con un compañero para utilizar la mutagénesis química tradicional de las semillas de arroz con selección en tepraloxidim para obtener plantas de arroz fértiles que exhibieran tolerancia al tepraloxidim como resultado de haber inducido una mutación en la ACCasa plastídica de arroz. Sorprendentemente, luego de múltiples intentos a lo largo de tres años, no se produjo ninguna planta de arroz fértil tolerante al tepraloxidim, y por lo tanto se abandonaron los esfuerzos.

10. A continuación, con la idea de que tal vez una metodología de mutagénesis de cultivo de tejidos pudiera tener éxito en donde había fallado la mutagénesis de semillas, implementamos la metodología de mutagénesis de cultivo de tejidos: mis coinventores y yo tratamos de obtener arroz tolerante al tepraloxidim mediante mutagénesis de cultivo de tejidos con selección en tepraloxidim. Este intento también falló. De manera inesperada, encontramos que el tepraloxidim no podía, en múltiples cultivos, seleccionar para una enzima ACCasa de mutación simple I1781. Esto se confirmó al transformar las células de callos de arroz y las células de callo de maíz con constructos de sobreexpresión para enzimas ACCasa de mutación simple II781L y II781A, como se describe en los Ejemplos de la presente solicitud de patente. Tal como figura en la Tabla 16 de la solicitud, el tepraloxidim fracasó consistentemente en recuperar algún mutante simple I1781. Concluimos, en retrospectiva, que la posible razón por la que la metodología de mutagénesis de semillas había fallado era porque - contrario a la lógica - la opción de tepraloxidim como un agente de selección para obtener tolerancia al tepraloxidim no pudo obtener este mismo fin.

11. Todas las afirmaciones realizadas en esta Declaración son, a mi leal saber y entender, verdaderas. Considero que todas las afirmaciones realizadas en esta Declaración son verdaderas.

Fecha: 15 de julio de 2015

[firma ilegible]
Dale R. Carlson

MAM\MAM\ID-448009\WPC13043

Molecular Bases for Sensitivity to Acetyl-Coenzyme A Carboxylase Inhibitors in Black-Grass[1]

Christophe Délye*, Xiao-Qi Zhang, Séverine Michel, Annick Matéjicek, and Stephen B. Powles

Institut National de la Recherche Agronomique, Unité Mixte de Recherche, Biologie et Gestion des Adventices, F–21065 Dijon cedex, France (C.D., S.M., A.M.); and Western Australia Herbicide Resistance Initiative, School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, Crawley, Western Australia 6009, Australia (X.-Q.Z., S.B.P.)

In grasses, residues homologous to residues Ile-1,781 and Ile-2,041 in the carboxyl-transferase (CT) domain of the chloroplastic acetyl-coenzyme A (CoA) carboxylase (ACCase) from the grass weed black-grass (*Alopecurus myosuroides* [Huds.]) are critical determinants for sensitivity to two classes of ACCase inhibitors, aryloxyphenoxypropionates (APPs) and cyclohexanediones. Using natural mutants of black-grass, we demonstrated through a molecular, biological, and biochemical approach that residues Trp-2,027, Asp-2,078, and Gly-2,096 are also involved in sensitivity to ACCase inhibitors. In addition, residues Trp-2,027 and Asp-2,078 are very likely involved in CT activity. Using three-dimensional modeling, we found that the side chains of the five residues are adjacent, located at the surface of the inside of the cavity of the CT active site, in the vicinity of the binding site for APPs. Residues 1,781 and 2,078 are involved in sensitivity to both APPs and cyclohexanediones, whereas residues 2,027, 2,041, and 2,096 are involved in sensitivity to APPs only. This suggests that the binding sites for these two classes of compounds are overlapping, although distinct. Comparison of three-dimensional models for black-grass wild-type and mutant CTs and for CTs from organisms with contrasted sensitivity to ACCase inhibitors suggested that inhibitors fitting into the cavity of the CT active site of the chloroplastic ACCase from grasses to reach their active sites may be tight. The three-dimensional shape of this cavity is thus likely of high importance for the efficacy of ACCase inhibitors.

Acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) is a biotinylated enzyme that catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA. This is a two-step, reversible reaction, consisting of the ATP-dependent carboxylation of the biotin group on the carboxyl carrier domain by the biotin-carboxylase activity, followed by the transfer of the carboxyl group from biotin to acetyl-CoA by the carboxyl-transferase (CT) activity (for review, see Nikolau et al., 2003). In eukaryotes and prokaryotes, ACCase is a key enzyme in fatty acid biosynthesis (Harwood, 1988). In plants, ACCase activity is not only involved in primary fatty acid biosynthesis, which is located in chloroplasts and mitochondria (Focke et al., 2003; Nikolau et al., 2003), but also in the formation of long-chain fatty acids and flavonoids and in malonylation, which occurs in the cytosol (Sasaki et al., 1995; Post-Beittenmiller, 1996). Two ACCase isoforms are thus found in plant cytosol and organites, respectively (Sasaki et al., 1995; Konishi et al., 1996), with the chloroplastic isoform accounting for more than 80% of the total ACCase activity (Herbert et al., 1996). The cytosolic ACCase isoform in all plants studied so far is a homodimeric enzyme, as it is in all eukaryotes (Nikolau et al., 2003). Each monomer carries all ACCase functional domains on a single polypeptide. Homomeric ACCases thus share a high degree of conservation between eukaryotes (Nikolau et al., 2003). In most plant species, the chloroplastic ACCase is a heteromeric enzyme consisting of three nuclear-encoded subunits and one subunit encoded by a chloroplastic gene (Konishi et al., 1996). However, in the Poaceae (grasses), the chloroplastic ACCase is a homodimeric enzyme (Konishi et al., 1996) encoded by a nuclear gene distinct from that coding for the cytosolic ACCase isoform (Gornicki et al., 1994, 1997; Podkowinski et al., 1996).

Aryloxyphenoxypropionates (APPs) and cyclohexanediones (CHDs) are two chemical classes of molecules that selectively inhibit homomeric, chloroplastic ACCase from grasses (Rendina et al., 1990; Burton et al., 1991), which makes them postemergent herbicides used worldwide to control grass weeds. APPs and CHDs are reversible (Gronwald, 1991), mutually exclusive (Rendina et al., 1990) inhibitors of the CT reaction. Heteromeric, chloroplastic ACCase is insensitive to CHDs and APPs (Alban et al., 1994). The highly different structure of heteromeric ACCase likely accounts for this insensitivity. Surprisingly, homomeric, cytosolic ACCases are less sensitive by several orders of magnitude than the rather similar homomeric, chloroplastic ACCases from the Poaceae (e.g. Egli et al., 1993; Herbert et al., 1996; Evenson et al., 1997; Joachimiak et al., 1997; Price et al., 2003). Although the structure of the yeast (*Saccharomyces*

[1] This work was supported by the Département Santé des Plantes et Environnement of the Institut National de la Recherche Agronomique.

* Corresponding author; e-mail delye@dijon.inra.fr; fax 33–380–693–262.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.104.046144.

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

cerevisiae) ACCase CT domain in complex with APPs has been solved (Zhang et al., 2004), the molecular bases for those differences in sensitivity to APPs and CHDs remain largely unknown.

To identify the determinants for sensitivity to ACCase-inhibiting herbicides, grass mutants with altered sensitivity due to mutation(s) within the target enzyme are of great interest. Since their introduction to world agriculture in the 1980s, APPs and CHDs have been widely used to control a variety of grass weeds. As a consequence, weed plants that are resistant to these herbicides have been reported in 33 species so far, with altered ACCase being a widespread resistance mechanism (see the International Survey of Herbicide Resistant Weeds homepage at www.weedscience.com). One of the best-studied weeds is black-grass (*Alopecurus myosuroides* [Huds.]), a major grass weed in winter crops in Europe. Analysis of resistant ACCase mutants in black-grass revealed that an Ile-1,781-Leu substitution confers resistance to some, but not all, APPs and CHDs, and that an Ile-2,041-Asn substitution confers resistance to APPs but not to CHDs (Délye et al., 2002a, 2002b, 2003). Both substitutions are located within the CT domain of homomeric, chloroplastic ACCase. Similar findings were obtained in mutant plants from the grass weeds *Lolium rigidum* (Gaud.), *Setaria viridis* (L. Beauv.), and *Avena fatua* (L.) for the Ile-to-Leu substitution (Zhang and Devine, 2000; Zagnitko et al., 2001; Christoffers et al., 2002; Délye et al., 2002b, 2002c), and from *L. rigidum* for the Ile-to-Asn substitution (Délye et al., 2003). Herein we demonstrate that three other residues, located within black-grass ACCase CT domain at positions 2,027, 2,078, and 2,096 are also crucial for sensitivity to APP and/or CHD inhibitors. Using protein modeling, we discuss the structural consequences of the five amino acid substitutions listed above for ACCase-inhibitor interactions.

RESULTS

In the following, the reference sequence for ACCase is EMBL accession AJ310767 (black-grass chloroplastic ACCase). Unless otherwise stated, all nucleotide and amino acid positions referred to hereafter correspond to those in this sequence.

Sensitivity to APPs and CHDs and Amino Acid Substitutions within the ACCase CT Domain

Among the 16 black-grass seedlings resistant to ACCase inhibitors that were used for sequencing experiments, 11 contained 2 identical ACCase alleles. A total of 21 sequences were thus obtained for analysis. Here we only considered the black-grass ACCase coding sequence for analysis. After discarding noncoding intron and 3′ sequences, the 21 sequences were aligned. A total of 25 single-nucleotide polymorphisms (SNPs), consisting of 21 synonymous and 4 nonsynonymous changes, were identified. The four nonsynonymous changes were a Trp-2,027-Cys, an Arg-2,078-Gly, a Gly-2,096-Ala, and a Lys-2,264-Arg substitution. The 21 sequences comprised a total of 10 haplotypes, 7 of which contained nonsynonymous SNPs. Eight of the 10 haplotypes found herein were not observed in a previous study (Délye et al., 2003; EMBL accession ALIGN_000483). They displayed one nonsynonymous and six synonymous SNPs that were not found previously. While the nonsynonymous substitution at position 2,027 was never reported, each of the amino acid replacements at positions 2,078 and 2,096 was previously observed in a single haplotype present in one haloxyfop-resistant, in one fenoxaprop-resistant, and in one fenoxaprop-sensitive seedling, respectively (Délye et al., 2003). Amino acid substitution at position 2,264 was previously observed in four haplotypes mostly found in herbicide-sensitive seedlings (Délye et al., 2003).

The three seedlings in population 00-016 consisted of one clodinafop-resistant and two fenoxaprop-resistant seedlings. They contained four haplotypes. Two haplotypes displayed a Trp-to-Cys change caused by a G-to-T transversion at the first position in codon 2,027. Each seedling contained at least one Cys-2,027 haplotype. One haplotype present in a single seedling contained a Lys-to-Arg change caused by a G-to-C transversion at the second position in codon 2,264 that is located outside the ACCase CT domain. Allele-specific PCR assays enabling the identification of the codons present at positions 2,027, 2,078, and 2,096 were used to genotype a total of 500 seedlings from populations 00-016 and from the derived, purified population Cys-2027-F1 (Table I). The presence of Leu-1,781 ACCase alleles that confer resistance to cycloxydim and fenoxaprop and of Asn-2,041 ACCase alleles that confer resistance to the three APPs used was also assessed in these populations using previously described allele-specific PCR assays (Délye et al., 2002a, 2003). Cys-2,027 ACCase alleles exclusively occurred in about 60% of the seedlings in population 00-016. All seedlings containing one or two Cys-2,027 allele(s) were resistant to the three APPs used and sensitive to the two CHDs. Association of the presence of Cys-2,027 allele(s) with resistance to APPs was supported by Fisher's exact test (Table I). Results from the purified population Cys-2,027-F1 further supported this association, with 100% of the seedlings in this population being resistant to the three APPs and sensitive to the two CHDs.

The 10 seedlings in population 00-049 consisted of 1 clodinafop-resistant, 6 cycloxydim-resistant, and 3 fenoxaprop-resistant seedlings. They contained three haplotypes, one of which displayed an Asp-to-Gly change caused by an A-to-G transition at the first position in codon 2,078. This haplotype was present in all 10 seedlings. No other nonsynonymous mutation was recorded among the 10 seedlings. A total of 500 seedlings from populations 00-049 and from the derived purified population Gly-2,078-F1 were

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

Table I. *Herbicide sensitivity of and mutant chloroplastic ACCase alleles detected in seedlings from four field and two purified black-grass populations*

Population	Mutant ACCase Allele(s) Detected	CHD Herbicides				APP Herbicides					
		Cycloxydim		Clethodim		Fenoxaprop		Clodinafop		Haloxyfop	
		R[a]	S[a]	R	S	R	S	R	S	R	S
00-017[b]	None	0	50	0	50	0	50	0	50	0	50
00-016	None	0	20	0	18	12	7	0	21	0	17
	Cys-2,027[c]	0	30	0	32	31	0	29	0	33	0
P-value (Cys-2,027)[d]		1.0		1.0		5.10^{-4}		0.0		0.0	
00-049	None	0	3	0	4	0	2	1	1	0	3
	Leu-1,781[c]	32	0	0	30	32	0	18	13	0	33
	Gly-2,078[c]	4	0	6	0	7	0	5	0	5	0
	Leu-1,781 + Gly-2,078[f]	11	0	10	0	9	0	12	0	9	0
P-value (Gly-2,078)[d]		0.0286		2.10^{-4}		0.0278		0.1053		0.0015	
00-099	None	0	5	0	4	0	6	0	6	0	5
	Leu-1,781[c]	1	0	0	2	1	0	0	0	0	2
	Asn-2,041[c]	0	5	0	6	4	0	6	0	6	0
	Ala-2,096[c]	0	29	0	31	28 + 3[e]	0	28 + 2[e]	0	26 + 2[e]	0
	Leu-1,781 + Asn-2,041[f]	1	0	0	0	0	0	1	0	1	0
	Leu-1,781 + Ala-2,096[f]	2	0	0	1	1	0	2	0	1	0
	Asn-2,041 + Ala-2,096[f]	0	7	0	6	7	0	5	0	7	0
P-value (Ala-2,096)[d]		1.0		1.0		0.0		0.0		0.0	
Cys-2,027-F1[b,g]	Cys-2,027[h]	0	50	0	50	50	0	50	0	50	0
Gly-2,078-F1[b,g]	Gly-2,078[h]	50	0	50	0	50	0	50	0	50	0
Ala-2,096-F1[b,g]	Ala-2,096[i]	0	50	0	50	50	0	50	0	50	0

[a]S, Sensitive; R, resistant. [b]Population used for ACCase enzyme assay. [c]One or two allele(s) mutated at the same codon were detected. [d]Fisher's exact test for the association of the presence of the mutant ACCase allele with resistance to each herbicide. [e]Resistant plants with a green first leaf of less than 1 cm in length. [f]A single copy of each mutant allele was detected. [g]Purified population (see "Materials and Methods"). [h]Two alleles mutated at the same codon were detected.

genotyped as above (Table I). Gly-2,078 ACCase alleles exclusively occurred in about 30% of the seedlings in population 00-049. However, 65% of the seedlings containing a Gly-2,078 allele also contained a Leu-1,781 allele (Table I). The few seedlings containing only one or two Gly-2,078 allele(s) were all resistant across the range of ACCase inhibitors tested. Although Leu-1,781 ACCase alleles did not confer resistance to clethodim, clodinafop, and haloxyfop, all seedlings containing one Leu-1,781 and one Gly-2,078 allele were resistant to these inhibitors (Table I). Association of the presence of Gly-2,078 allele(s) with resistance to all herbicides but clodinafop was supported by Fisher's exact test (Table I). Results from the purified population Gly-2,078-F1 supported this association, with 100% of the seedlings in this population being resistant to each of the five herbicides used, including clodinafop.

The three seedlings in population 00-099 consisted of one fenoxaprop-resistant and two clodinafop-resistant seedlings. They contained three haplotypes, all displaying a Gly-to-Ala change caused by a G-to-C transversion at the second position in codon 2,096. No other nonsynonymous mutation was recorded among the three seedlings. A total of 500 seedlings from populations 00-099 and from the derived, purified population Ala-2,096-F1 were genotyped as above (Table I). Ala-2,096 ACCase alleles exclusively occurred in about 75% of the seedlings in population 00-099. This population also contained Leu-1,781 and Asn-2,041 alleles. Most seedlings containing Ala-2,096 alleles did not contain another mutant allele (Table I). These seedlings were always sensitive to the two CHDs used. Most seedlings containing Ala-2,096 ACCase alleles were resistant to the three APPs. However, several such seedlings were first considered sensitive to fenoxaprop (three seedlings), clodinafop (two seedlings), or haloxyfop (two seedlings) because their first leaf was less than 1 cm in length (Letouzé and Gasquez, 1999). Herbicide bioassay with the three APPs was thus repeated for populations 00-099 and 00-017. Closer observation prior to genotyping revealed that the first leaf of seedlings containing Ala-2,096 ACCase allele(s) was always fully green and alive, even when it was less than 1 cm in length. In contrast, the first leaf of sensitive seedlings not containing mutant ACCase alleles in population 00-017 and 00-099 was always softened and bleached. We consequently considered that seedlings with a green first leaf of less than 1 cm were herbicide resistant (Table I). All seedlings containing one or two Ala-2,096 ACCase allele(s) were thus sensitive to the two CHDs used and resistant to the three APPs. Association of the presence of Ala-2,096 allele(s) with resistance to APPs was supported by Fisher's exact test (Table I). Results from the purified population Ala-2,096-F1 further supported this association, with 100%

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

of the seedlings in this population being resistant to the three APPs and sensitive to the two CHDs (Table I).

As previously (Délye et al., 2003), we found that seedlings not containing any of the five mutant ACCase alleles identified to date were resistant to fenoxaprop (12 seedlings) or clodinafop (one seedling; Table I). This was consistent with a previous demonstration that other mechanisms, such as enhanced herbicide metabolism, may also account for resistance to ACCase inhibitors in black-grass (e.g. Letouzé and Gasquez, 2003).

ACCase Inhibition by APPs and CHDs

ACCase activity measured in vitro without the presence of inhibitors was always 2 times lower in extracts from the resistant black-grass-purified populations, Cys-2,027-F1 and Gly-2,078-F1, than in extracts from the sensitive population, 00-017. It was also moderately lower in extracts from the resistant population, Ala-2,096-F1, than in extracts from population 00-017 (data not shown). The action of four APPs and two CHDs upon enzymatic activity of wild-type Cys-2,027, Gly-2,078, and Ala-2,096 ACCases is shown in Figure 1. A high level of resistance to the four APPs assayed is observed for all three mutant ACCase alleles (Fig. 1). Herbicide doses required to inhibit 50% ACCase activity (I_{50}) values were 10 to 125 times higher than those obtained for the wild-type ACCase (Table II). Cys-2,027 and Ala-2,096 ACCase alleles displayed inhibition patterns similar to that of the wild-type ACCase allele for the two CHDs assayed (Fig. 1), although the I_{50} values obtained were slightly higher for both mutant alleles (Table II). In contrast, the Gly-2,078 ACCase allele displayed a high level of resistance to the two CHDs (Fig. 1), with I_{50} values for clethodim and cycloxydim 36 and 83.5 times higher than those obtained for the wild-type ACCase allele, respectively (Table II). These results fully supported the association of the Trp-2,027-Cys and Gly-2,096-Ala

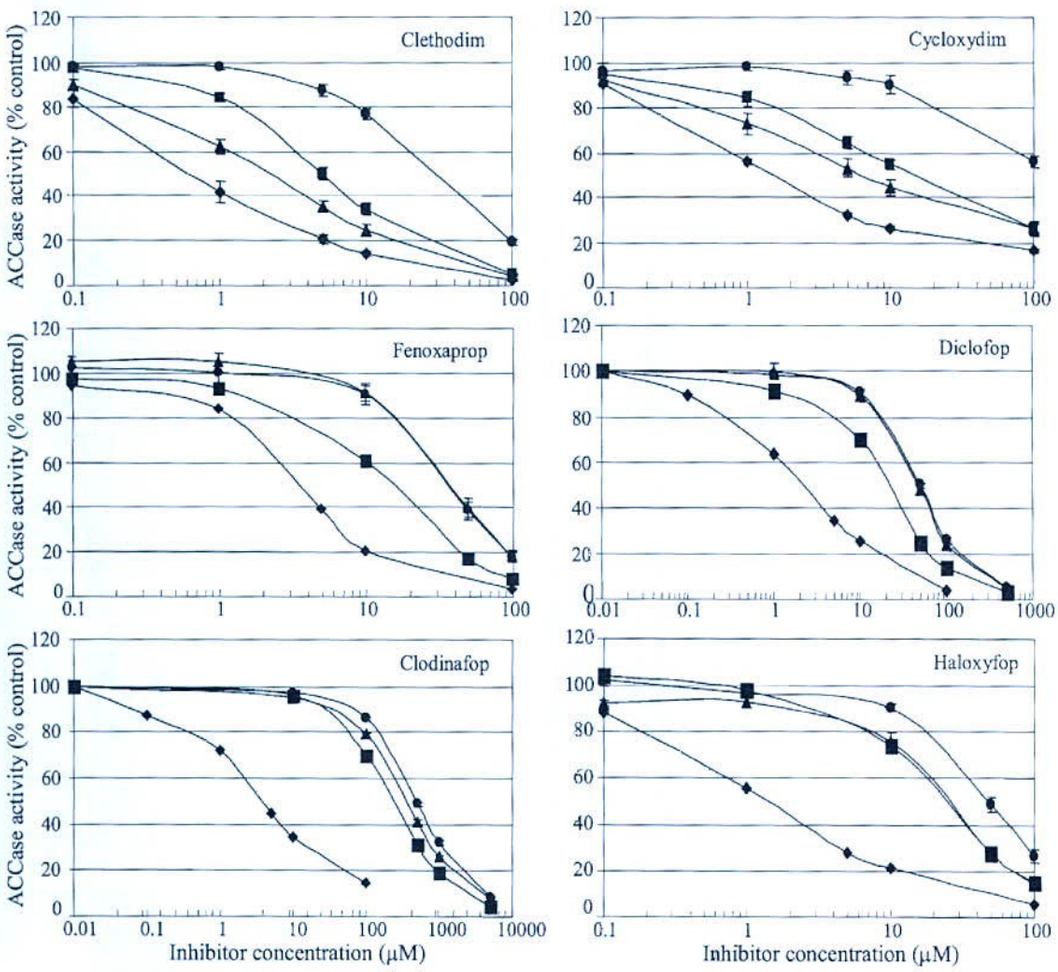


Figure 1. Inhibition of ACCase activity of wild-type (population 00-017; ♦), Cys-2,027 (population Cys-2,027-F1; ▲), Gly-2,078 (population Gly-2,078-F1; ●), and Ala-2,096 (population Ala-2,096-F1; ■) ACCase alleles by clethodim and cycloxydim (CHDs) and by fenoxaprop, diclofop, clodinafop, and haloxyfop (APPs). Averages of two independent experiments are shown with error bars. ACCase activity is expressed as a percentage of ACCase activity without inhibitor for each allele.

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

Table II. *I_{50} values obtained for wild-type, Cys-2,027, Gly-2,078, and Ala-2,096 ACCase alleles*

Values are mean ± SE of two independent experiments.

Inhibitor	00-017 (S)	Cys-2,027-F1 (R)		Gly-2,078-F1 (R)		Ala-2,096-F1 (R)	
	I_{50}	I_{50}	R:S I_{50} Ratio	I_{50}	R:S I_{50} Ratio	I_{50}	R:S I_{50} Ratio
	μM	μM		μM		μM	
Cycloxydim (CHD)	1.6 ± 0.1	7.8 ± 2.9	5.0	133.8 ± 6.7	83.5	14.7 ± 2.5	9.0
Clethodim (CHD)	0.8 ± 0.2	2.2 ± 0.4	3.0	29.0 ± 4.6	36.0	5.2 ± 0.5	6.5
Fenoxaprop (APP)	0.7 ± 0.2	37.0 ± 4.8	53.0	37.1 ± 3.7	53.0	14.3 ± 1.0	20.5
Clodinafop (APP)	4.0 ± 1.1	361.8 ± 25.9	90.5	501.4 ± 50.7	125.5	230.1 ± 2.3	57.5
Haloxyfop (APP)	1.4 ± 0.1	26.6 ± 3.0	19.0	48.7 ± 4.6	35.0	22.2 ± 0.1	16.0
Diclofop (APP)	2.2 ± 0.5	46.9 ± 3.5	21.5	50.1 ± 2.3	23.0	21.8 ± 0.2	10.0

substitutions with resistance to APPs, and of the Asp-2,078-Gly substitution with resistance to all APPs and CHDs assayed found using allele-specific PCR in black-grass (Table I). We thus concluded that Trp-2,027-Cys and Gly-2,096-Ala substitutions confer resistance to APPs but not to CHDs, while the Asp-2,078-Gly substitution confers resistance to all APPs and CHDs assayed.

Black-Grass ACCase Modeling

For a detailed evaluation of the effects of amino acid substitutions at positions 1,781, 2,027, 2,041, 2,078, and 2,096 in black-grass ACCase upon herbicide binding, we reconstructed two types of three-dimensional models of homodimeric ACCase. The first type used a model built into a 2.5-Å density map obtained by electron crystallography of the yeast free ACCase CT domain (Protein Data Bank [PDB] accession 1UYT; Zhang et al., 2004) as a template. The second type used a model built into a 2.8-Å density map obtained by electron crystallography of the yeast ACCase CT domain in complex with the APP haloxyfop (PDB accession 1UYS; Zhang et al., 2004) as a template. Alignments of the primary amino acid sequences of chains B and C in PDB accessions 1UYT and 1UYS with black-grass ACCase CT domain (residues Leu-1,639 to Leu-2,204 in EMBL accession AJ310767) were visually checked for inconsistency. The sequence identity between the black-grass CT domain and the template sequences was 55.3%, which was sufficient to achieve a satisfactory model (Schwede et al., 2003). In the following, B or C after an amino acid position number refers to the residue included in the ACCase CT monomer modeled after the B or C chain in PDB accession 1UYT or 1UYS, respectively.

The free ACCase CT dimer consisted of the head-to-tail arrangement of two monomers. The two active sites of the CT dimer were located in cavities at the interface of the dimer, with each monomer equally contributing to the active-site shape (Zhang et al., 2003). Because of the symmetry of the ACCase CT dimer, we will consider only one of the two active sites in the following. In the yeast CT domain, the binding site for the APPs haloxyfop and diclofop is located within the cavity of the active site. APP molecules interact with the side chains of residues Ala-1,627C, Leu-1,705C, Gly-1,734C, Ile-1,735C, Tyr-1,738C, Trp-1,924B, Phe-1,956C, Val-1,967C, Ile-1,974C, Gly-1,997C, Gly-1,998C, and Val-2,002C (Zhang et al., 2004), which are homologous to residues Ala-1,707C, Ile-1,781C, Gly-1,810C, Ile-1,811C, Tyr-1,814C, Trp-1,999B, Phe-2,030C, Ile-2,041C, Ile-2,048C, Gly-2,071C, Gly-2,072C, and Val-2,076C, respectively, in black-grass ACCase. Aligning the 31 full CT sequences available in the databases showed that all but three of the residues in the binding site for APPs were absolutely conserved. Residue 1,999 was either Trp or Leu (1 sequence out of 31). Only residues 1,781 (Ile/Leu) and 2,041 (Ile/Val/Met) that are involved in sensitivity to APPs and/or CHDs (Zhang and Devine, 2000; Zagnitko et al., 2001; Christoffers et al., 2002; Délye et al., 2002b, 2002c, 2003) showed appreciable variation between the available CT sequences. Because of the dimer structure of the CT domain, the side chains in residues 1,781C, 2,027B, 2,041B, 2,078B, and 2,096B were adjacent in the models for black-grass. All five residues were located at the dimer interface, deep inside the active-site cavity (Fig. 2). Residues Ile-1,781C and Ile-2,041B were homologous to residues Leu-1,705C and Val-1,967B, respectively, which belong to the binding site of APPs in yeast CT (Zhang et al., 2004). The side chains in residues Trp-2,027B and Asp-2,078B were located close to those in residues Val-2,076B and Trp-1,999B, respectively, in the binding site for APPs, while the side chain in residue Gly-2,096B was more remote (Fig. 2B).

The structural changes caused in the free ACCase CT domain by amino acid replacements at positions 1,781, 2,027, 2,041, 2,078, and 2,096 are illustrated in Figure 2. The Ile-1,781C-Leu change resulted in the aliphatic side chain that is oriented toward the inside of the CT active site in the Ile-1,781C residue being replaced with an aliphatic side chain protruding toward the opening of the cavity in residue Leu-1,781C (Fig. 2A). In yeast CT, a Leu residue is present at position 1,781. Its side chain favors herbicide binding via van der Waals interactions with the methyl group in APPs (Zhang et al., 2004). This is the case in the model for CT-herbicide complexes computed for the black-grass mutant, Leu-1,781 ACCase, but not in that computed for the wild type, Ile-1,781 ACCase. The change at position 2,027B caused the replacement of a

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

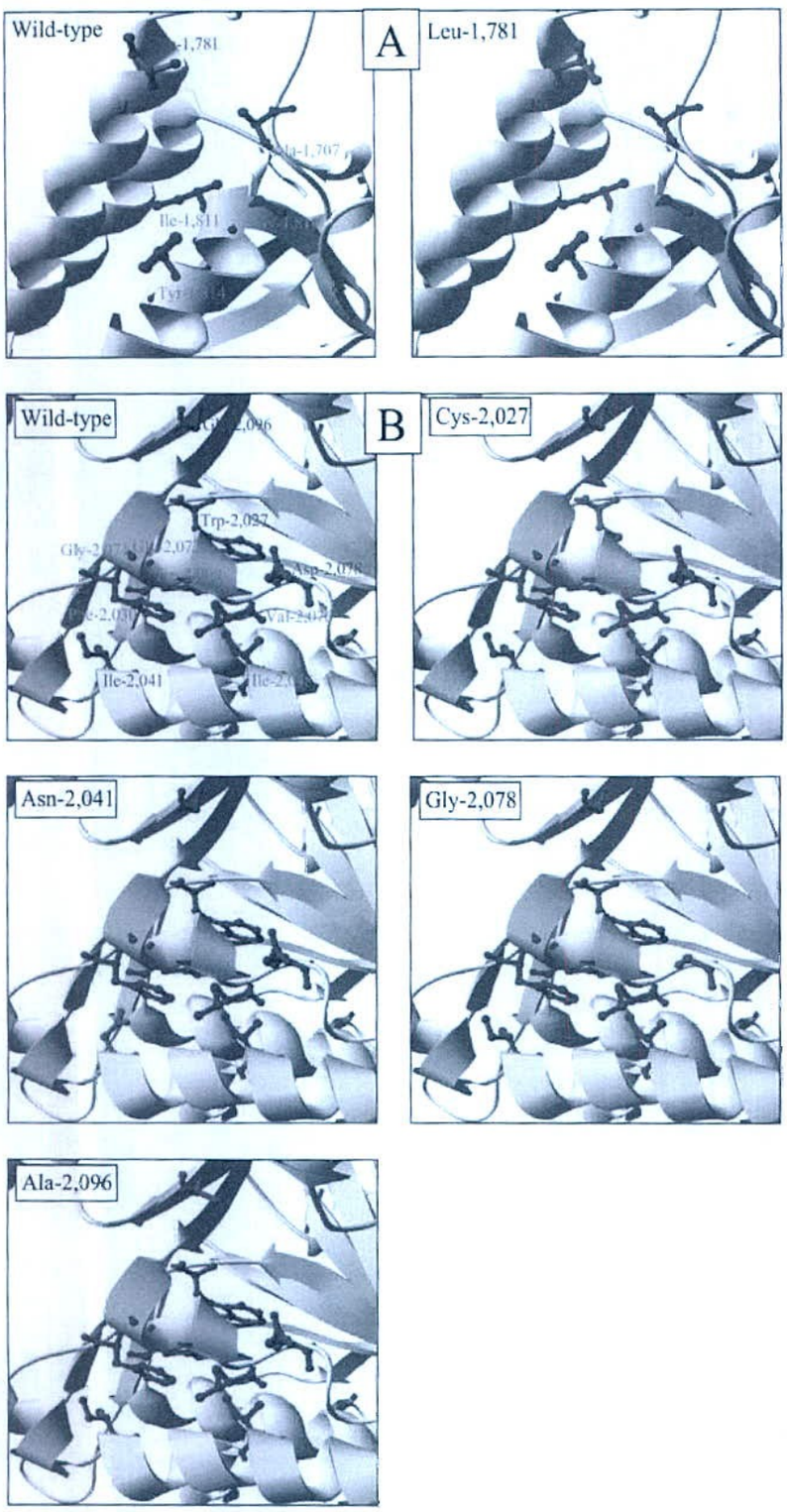


Figure 2. Ribbon diagram views of the inside of the CT active-site cavity in a three-dimensional model for black-grass chloroplastic ACCase. Side-chain colors: Green, APP-binding site; magenta, variable amino acid, residue present in wild-type black-grass ACCase; blue, variable amino acid, residue present in mutant black-grass ACCase. Hydrogen atoms and side chains not discussed in the text are not shown for clarity. A, Views of the inside of the active-site cavity part formed by monomer C in Ile-1,781 and Leu-1,781 ACCase alleles. Monomer B is not shown. B, Views of the inside of the active-site cavity part formed by monomer B in wild-type ACCase allele after a 180° rotation with respect to the views of monomer C. Monomer C is not shown.

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

mostly buried aromatic cycle in the model for the Trp-2,027 ACCase with a buried thiol group in the model for the mutant, Cys-2,027 ACCase (Fig. 2B). The change at position 2,078 caused the carboxyl group that strongly protruded within the active-site cavity of CT in the model for the Asp-2,078 ACCase to be fully removed in the model for the mutant, Gly-2,078 ACCase (Fig. 2B). The Gly-2,096B-Ala substitution resulted in the occurrence of an extra methyl group protruding within the active-site cavity of CT in the model for the mutant, Ala-2,096 ACCase (Fig. 2B). None of these three changes directly interfered with herbicide binding in models for black-grass CT-herbicide complexes (data not shown).

The substitution at position 2,041 caused the hydrophobic, aliphatic side chain that protruded toward the opening of the CT active-site cavity in the model for Ile-2,041 ACCase to be replaced with a polar side chain containing an amide group oriented toward the inside of the cavity in the model for the mutant, Asn-2,041 ACCase (Fig. 2B). In the model for black-grass Asn-2041B ACCase in complex with APPs, this change caused a major clash that was not compatible with herbicide binding (data not shown). The clash involved residue Phe-2,030B that is crucial for herbicide binding in yeast CT (Zhang et al., 2004) and caused a heavy twisting of a CT section encompassing residues 2,035B to 2,042B.

Comparison of ACCase CT Domain Three-Dimensional Structures

Herein we assessed black-grass ACCase sensitivity to APPs and CHDs using both a whole-seedling-based bioassay and an ACCase enzyme assay. The most suitable way of comparing ACCase sensitivity to inhibitors between different organisms is by an enzyme assay. In the literature, there are a total of 10 organisms, including 5 grasses, 1 dicotyledonous plant, 1 alga, 1 fungus, 1 mammal, and 1 protozoan, for which both enzyme sensitivity studies and CT protein sequences are available (Table III). From enzyme studies, it appeared that the chloroplastic, homomeric ACCase from grasses is highly sensitive to APPs and CHDs, while cytosolic ACCases are far less sensitive (i.e. tolerant) to these compounds (Table III). For each ACCase isoform in Table III,

Table III. *Sensitivity to ACCase inhibitors of and amino acids present at specific positions in ACCase from 10 eukaryotic organisms*

I_{50} values correspond to APP or CHD concentrations inhibiting 50% ACCase activity.

Organism	Accession No.	Range of I_{50} Values		Amino Acid Present at Position									References for I_{50} Values
		APPs	CHDs	1,781	2,027	2,037	2,041	2,078	2,079	2,080	2,088	2,096	
		μM											
Black-grass cp[a]	AJ310767	0.06–4.0	0.7–9.0	Ile	Trp	Leu	Ile	Asp	Ser	Lys	Cys	Gly	Price et al. (2004); Menéndez and De Prado (1999); This work
Rye-grass cp[a]	AF359515, AJ519781	0.2–0.6	4.2	.[b]	.	.	.	.	.	.	.	.	Evenson et al. (1997); Christopher and Holtum (1998); Kuk et al. (2000)
Wheat cp[a]	AF029895	0.2–2.0	<10.0	.	.	.	.	.	.	.	Phe	.	Gornicki and Haselkorn (1993); Konishi and Sasaki (1994); Joachimiak et al. (1997)
Maize cp[a]	U19183	0.04–10.0	10.0	.	.	.	.	.	.	.	.	.	Egli et al. (1993); Herbert et al. (1996); Price et al. (2003)
Green foxtail cp[a]	AF294805	0.08–4.4	0.3–7.7	.	.	.	.	.	.	.	.	.	Marles et al. (1993); Shukla et al. (1997)
Black-grass cy[a]	AJ632096	35.0	NA[c]	Leu	.	.	.	.	.	.	Met	.	Menéndez and De Prado (1999)
Wheat cy[a]	U39321	250.0	250.0–>500.0	Leu	.	.	.	.	.	.	Met	.	Joachimiak et al. (1997)
Pea cy[a]	L25042[d]	7.0–150.0	>1,000.0	Leu	.	.	.	.	.	Arg	Met	.	Alban et al. (1994); Konishi and Sasaki (1994); Herbert et al. (1996); Christopher and Holtum (1998)
C. cryptica	L20784	>100.0	23.0	Leu	.	Met	.	.	Pro	Thr	Met	.	Roessler (1990)
T. gondii cp[a]	AF157612	5.0	>400.0	Leu	.	Met	.	.	Pro	Thr	Met	.	Jelenska et al. (2002)
T. gondii cy[a]	AF330145	>400.0	>400.0	Leu	.	Met	.	.	.	Arg	Met	.	Jelenska et al. (2002)
Yeast	Z71631	>500.0	>500.0	Leu	.	Met	Val	.	Pro	Thr	Met	Ala	Joachimiak et al. (1997)
Rat (β isoform)	AB004329	NA[c]	18.0–>100.0	Leu	.	Met	Met	.	.	Ser	Met	.	Seng et al. (2003)

[a]cp, Plastidic; cy, cytosolic ACCase isoform. [b]Dots, Residues identical to those in black-grass chloroplastic ACCase [c]NA, None available. [d]Sequence from alfalfa (see text).

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

three-dimensional models were reconstructed as described above. No obvious differences could be observed between species when comparing models for CT-herbicide complexes, which may be due to models being computed from yeast-tolerant ACCase. We therefore focused upon models for free CT domains reconstructed after PDB accession 1UYT to search for differences in the three-dimensional shape of the active-site cavity that may interfere with herbicides accessing their binding site. Two models corresponding to the plastidic and cytosolic isoforms were thus built for black-grass, wheat (*Triticum aestivum*), and *Toxoplasma gondii*. One model was built for each of the other seven organisms. No ACCase sequence from pea was available in the databases. However, because a very high conservation was observed between the CT sequences from the two legume plants available in the databases, i.e. alfalfa (*Medicago sativa*) and soybean (*Glycine max*; 92.9% amino acid identity), we used the sequence from alfalfa as a model for pea ACCase.

To assess the variability of the residues identified as potentially important for the shape of the active-site cavity, all 31 complete sequences of homomeric ACCase CT domains available in databases were aligned (data not shown). The three-dimensional shape of the active-site cavity was highly conserved between organisms (data not shown). In particular, all three-dimensional models for chloroplastic ACCases exhibited near-identical active-site three-dimensional structures (data not shown). The only structural difference between ACCase isoforms sensitive or tolerant to herbicides that could be identified in the region of the active site modeled after chain C in PDB accession 1UYT was due to the presence of a Leu-1,781 residue in all sequences but those from chloroplastic ACCases from grasses that contained an Ile-1,781 residue (Table III; see Fig. 2 for an illustration).

Five variable side chains, belonging to residues 2,037, 2,041, 2,079, 2,080, and 2,088, were observed in the region of the active site modeled after chain B in PDB accession 1UYT. Figure 3 portrays representative models. Residue Leu-2,037, the side chain of which was located near residue 2,041 in the herbicide-binding site, was conserved between all known CT sequences from higher plants. This residue was Met in all other known sequences. Residue 2,041 in the binding site for APPs was Ile in all known sequences from higher plants. It was either Ile or Val in all other sequences but that from rat (Met). The side chains in residues at positions 2,079B, 2,080B, and 2,088B were located close to that in residue Asp-2,078B. Consensus at position 2,079 between the 31 known CT sequences was Ser/Pro/Ala. Among the 10 organisms used in the modeling study, a Ser residue is found in all CT sequences from higher plants, in the sequence from rat, and in that from *T. gondii* cytosolic ACCase (Table III), while the sequences for the other organisms contained a Pro-2,079 residue. The residue at position 2,080 was variable enough between the 31 CT sequences, with consensus at this position being Lys/Arg/Thr/Ser/Gln/Ala/Leu. A Lys-2,080 residue occurred in all CT sequences from grasses (Table III), while the sequences from other organisms contained an Arg, a Thr, or a Ser residue at this position (Table III). Residue at position 2,088 was fairly conserved, being most frequently Cys or Met. A Phe-2,088 or a Thr-2,088 residue was found in 1 out of the 31 available CT sequences. All chloroplastic homomeric ACCases but that from wheat (Phe-2,088) contained a Cys-2,088 residue (Table III). The sequences from the five other ACCases in Table III contained a Met-2,088B residue.

The main differences in three-dimensional structure observed between models for ACCase isoforms sensitive or tolerant to APPs and CHDs resulted from the combination of residues present at positions 1,781C, 2,037B, 2,041B, 2,079B, 2,080B, and 2,088B in the various CT sequences (see Fig. 3 for an illustration). These combinations altered the distribution of polar groups at the surface of the bottom of the cavity of the CT active site, the level of obstruction of the opening of the cavity by hydrophobic chains, and the shape of the cavity within or at the vicinity of the binding site for APPs.

DISCUSSION

APPs and CHDs have long been known to interfere at the level of the ACCase CT domain (Rendina et al., 1990). Only recently has the three-dimensional structure of the ACCase CT domain in complex with the APPs haloxyfop and diclofop been determined (Zhang et al., 2004). This structure, however, has been determined from yeast ACCase, which is tolerant to APPs and CHDs (Joachimiak et al., 1997). Yeast ACCase contains a Leu and a Val residue at positions homologous to Ile-1,781 and Ile-2,041, respectively, in black-grass wild-type ACCase (Table III). In grasses, the Ile-1,781-Leu change confers resistance to diclofop but not to haloxyfop, while the Ile-2,041-Val substitution may confer resistance to haloxyfop (Délye et al., 2002a, 2002b, 2003). Furthermore, the yeast CT sequence, like the black-grass mutant Ala-2,096 ACCase that is resistant to APPs, contains an Ala residue at a position homologous to Gly-2,096 in black-grass wild-type ACCase (Table III). Because of this, the details of the interaction of sensitive, chloroplastic ACCases from grasses with APP inhibitors cannot be fully understood from yeast models. No data are available regarding the binding mode of CHD inhibitors to ACCase. Previous studies with grass weed mutants with altered sensitivity to ACCase inhibitors identified two Ile residues at positions 1,781 and 2,041 within the CT domain of chloroplastic, homomeric ACCase that are critical for interaction with APP and/or CHD herbicides (Zhang and Devine, 2000; Zagnitko et al., 2001; Christoffers et al., 2002; Délye et al., 2002a, 2002b, 2002c, 2003). Herein, we found that three more residues (Trp-2,027, Asp-2,078, and Gly-2,096) are also associated with sensitivity to ACCase inhibitors in black-grass. ACCase

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

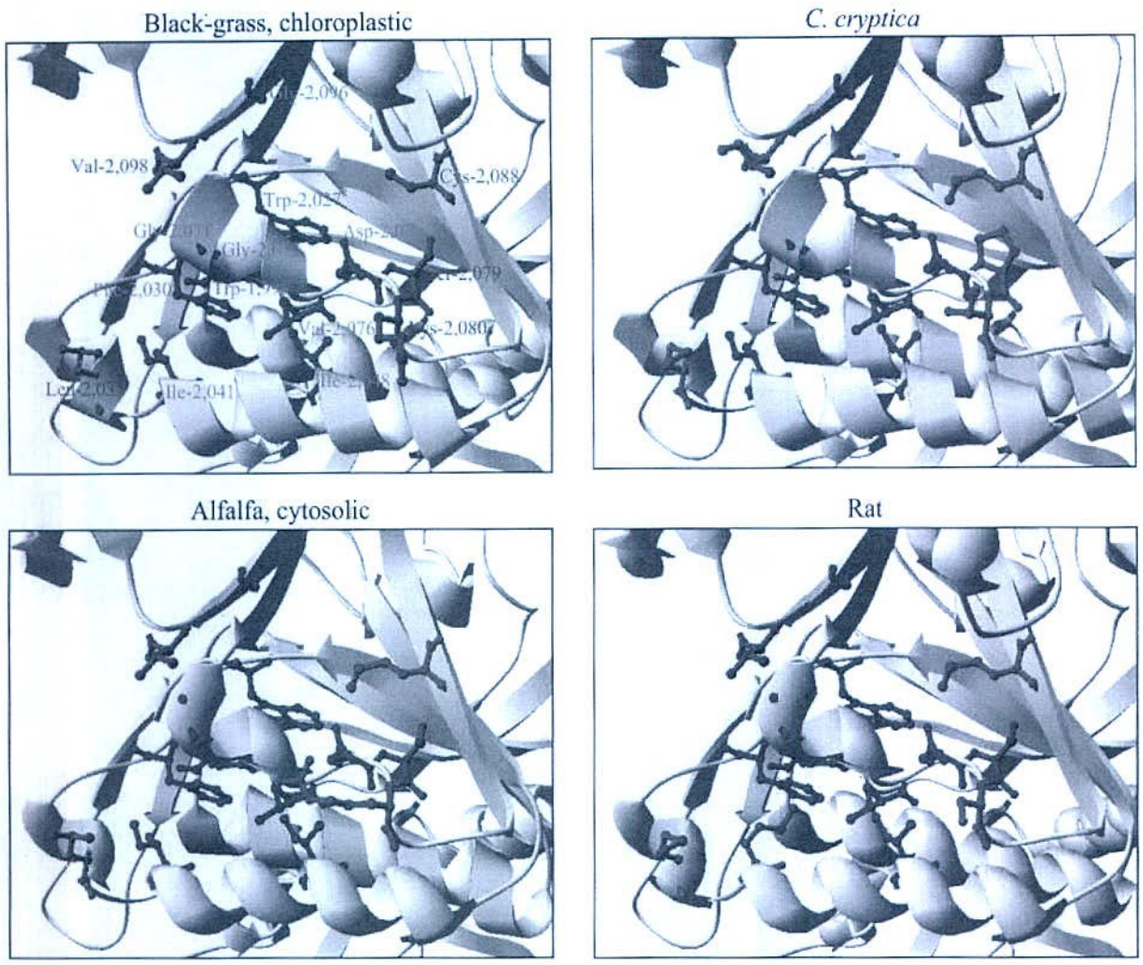


Figure 3. Ribbon diagram views of the CT active-site cavity on monomer B in three-dimensional models for black-grass chloroplastic, *C. cryptica*, alfalfa cytosolic, and rat (D) ACCases. Side-chain colors: Green, APP-binding site and variable amino acid identified in black-grass ACCase; magenta, amino acid variable between organisms; blue, amino acid variable between organisms, residue differing from that present in black-grass at this position (see Table III). Hydrogen atoms and side chains not discussed in the text are not shown for clarity. Monomer C is not shown.

enzyme assays and three-dimensional model analyses clearly show that these three residues are direct causes for altered sensitivity to ACCase inhibitors. This conclusion is further strengthened by the preliminary report of Trp-2,027-Cys and Asp-2,078-Gly substitutions as candidate herbicide mutations in wild oat (Christoffers et al., 2000).

Residue Trp-2,027 is absolutely conserved in all 31 homomeric ACCase sequences known so far. Residue Gly-2,096 is conserved in all ACCase sequences but those from yeasts (Ala-2,096, as in a black-grass mutant, resistant ACCase). Residues Trp-2,027 and Gly-2,096, like residue Ile-2,041, are both involved in sensitivity to the four APPs, but not to the two CHDs assayed (Tables I and II; Délye et al., 2003). In contrast, residue Asp-2,078 that is conserved in 30 out of the 31 known ACCase sequences (Val in the sequence from soybean) is the only one identified so far that is involved in sensitivity to all APPs and CHDs assayed (Tables I and II). In particular, the Asp-2,078-Gly substitution is the first reported mutation, to our knowledge, conferring a high level of resistance to the highly effective CHD clethodim (Table II). APPs and CHDs are reversible, competitive inhibitors of acetyl-CoA binding to the CT active site (Herbert et al., 1996; Zhang et al., 2003). The molecules of APPs and CHDs consist of a hydrophobic carbon skeleton with polar substituents (developed formulas are available at www.weedscience.com). Their bioactivity is limited by rather strict structural requirements (Webb et al., 2000; Turner and Pernich, 2002). The structure-activity relationship and the mode of binding to ACCase are poorly understood for CHDs (Webb et al., 2000). Because residues 1,781 and 2,078 are involved in sensitivity to both APPs and CHDs, and because residues 2,027, 2,041, and 2,096 are involved in sensitivity to APPs only, it is clear that binding sites for both inhibitor classes are largely overlapping, but not

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

identical, in grasses. The side chain in residue Asp-2,078B is located at the bottom of the cavity of the CT active site, suggesting that CHDs, like APPs, bind deep inside the CT active-site cavity to inhibit ACCase CT activity.

From the three-dimensional models of black-grass CT-herbicide complexes, only the Ile-2,041B-Asn substitution directly interfered with herbicide binding, very likely because of a change in the conformation of the APP-binding site (Fig. 2B). The Gly-2,096B-Ala substitution very likely did not interfere with APP binding per se. Occurrence of an extra methyl group protruding within the cavity of the CT active site would either hamper access of APP molecules to their binding site and/or interfere with the allosteric changes in CT that are necessary for proper APP binding (Zhang et al., 2004). In the models reconstructed from yeast CT-herbicide complexes, the Trp-2,027B-Cys and the Asp-2,078B-Gly substitutions, which both occur at the bottom of the cavity of the CT active site, did not interfere with APP binding. However, they resulted in a number of small allosteric changes in the three-dimensional shape of the cavity that may not accurately be modeled from yeast. The heavily reduced activity observed for Cys-2,027 and Gly-2,078 ACCases supports the hypothesis of alteration of the shape of the active site, suggesting that changes at positions 2,027 and 2,078 might interfere with herbicide action by hampering access to their binding site. The reason why the Ile-1,781C-Leu substitution causes resistance to some APPs and CHDs cannot be directly deduced from three-dimensional models derived from the structure of yeast CT-APP complexes. In yeast, the residue homologous to residue Ile-1,781 is a Leu residue, the side chain of which contributes to increasing the stability of the CT-APP complex through van der Waals interactions with the R-methyl group in APPs (Zhang et al., 2004). These van der Waals interactions do not exist in black-grass wild-type Ile-1,781 ACCase. From the yeast model, the Ile-1,781C-Leu substitution should result either in no alteration in sensitivity to haloxyfop and diclofop or in increased sensitivity of black-grass ACCase to both compounds. In fact, Leu-1,781 ACCase is sensitive to haloxyfop and resistant to diclofop (Délye et al., 2002b), thus illustrating the limits of using the three-dimensional structure of the yeast CT-APP complex as a template to model the black-grass CT-APP complex.

In the three-dimensional models for the five grass species analyzed (Table III), the molecular surface of the region around the APP-binding site is absolutely conserved. However, substantial variation in I_{50} values are observed within the APP and CHD inhibitor classes, even within a given grass species (Tables II and III). This, and the heavy structural requirements for herbicidal activity observed for APPs and CHDs (Webb et al., 2000; Turner and Pernich, 2002), suggests that herbicide fitting into the cavity of the chloroplastic ACCase CT domain active site from grasses to reach their binding site(s) is extremely tight, and that some herbicide molecules fit better than others in the active-site cavity. Tight fitting is also likely the reason why such a subtle change as the Ile-1,781-Leu substitution, which may be considered as highly conservative, has a drastic effect upon the sensitivity of ACCase to the APPs fenoxaprop and diclofop and to the CHD cycloxydim, while it does not significantly alter sensitivity to the APPs clodinafop and haloxyfop, nor to the CHD clethodim (Zhang and Devine, 2000; Zagnitko et al., 2001; Christoffers et al., 2002; Délye et al., 2002a, 2002b, 2002c). Until the details of the binding of each inhibitor molecule within the cavity of the chloroplastic ACCase CT active site from grasses are fully understood, the cross-resistance pattern associated with specific structural changes will remain unpredictable.

All three-dimensional models for the ACCase CT domain from ACCase isoforms tolerant to APPs and CHDs (Table III) display both obstructing, hydrophobic side chains at the opening of the active-site cavity, differences in the shape of the bottom of this cavity, and differences in the distribution of polar groups at the bottom of the cavity when compared to three-dimensional models for chloroplastic ACCases from grasses (Fig. 3). Considering a similar binding mode for APPs and CHDs, these differences likely reduce the ability of the inhibitor molecules to reach their respective binding sites, thus making these ACCase isoforms tolerant to APPs and CHDs. Differences at the level of the binding site for APPs itself, because of variation at positions 1,781 in all tolerant ACCases, and at positions 2,096 and/or 2,041 in yeast and rat ACCases, are likely an additional reason for tolerance to ACCase inhibitors. In this respect, it can be proposed that the considerable differences in sensitivity to the APP inhibitors haloxyfop and clodinafop observed between the ACCase from the alga *Cyclotella cryptica* and the apicoplast ACCase from the protozoan *T. gondii* (Table III; Roessler, 1990; Jelenska et al., 2002), which display identical residues at the nine positions discussed above (Table III), might be attributed to the presence of a Leu-1,999 residue and/or an Ile-2,098 residue in the sequence from *C. cryptica*. All 30 other ACCase sequences known so far display a Trp-1,999 and a Val-2,098 residue. The Trp-1,999 residue belongs to the binding site for APPs. In three-dimensional models, the side chain in residue Ile-2,098B protrudes close to the opening of the active-site cavity, possibly impeding the access of APPs (Fig. 3).

Homomeric ACCase isoforms sensitive (chloroplastic) or tolerant (cytosolic) to APPs or CHDs have been shown to have similar catalytic properties (Herbert et al., 1996; Price et al., 2003). Mutant, chloroplastic ACCases with an Ile-1,781-Leu substitution did not display altered catalytic activity (Marles et al., 1993; Shukla et al., 1997). Similarly, mutant chloroplastic ACCases with an Ile-2,041-Asn or a Gly-2,096-Ala substitution displayed an activity close to that of wild-type ACCase (Délye et al., 2003). Residues 1,781, 2,041, and 2,096 are all located close to the

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

opening of the cavity of CT active site, in the hydrophobic part of the cavity (Fig. 2). Unaltered activity of ACCase alleles mutated at these positions suggests that these residues do not play a major role in CT activity. In contrast, mutant chloroplastic ACCases with a Trp-2,027-Cys or an Asp-2,078-Gly substitution displayed a strongly reduced ACCase activity (about one-half the activity of the wild-type enzyme). This suggests that these residues that are located in the polar area at the bottom of the CT active-site cavity are involved in CT catalytic activity. This is consistent with the polar acetyl-thioester group in acetyl-CoA being inserted inside the cavity (Zhang et al., 2003, 2004), and with residues Trp-2,027 and Asp-2,078 being conserved in 31 and 30 out of the 31 known ACCase CT sequences, respectively.

This raises the question of whether there is a fitness penalty associated with black-grass mutant chloroplastic ACCase alleles. All black-grass mutant ACCase alleles discussed herein have been selected for in black-grass field populations by two APPs, fenoxaprop and clodinafop. As discussed before, the cross-resistance pattern associated with a given mutation is unpredictable. The cross-resistance to other APPs and/or CHDs observed for each mutant allele is thus a collateral effect of the selection. Black-grass plants containing mutant ACCase alleles are capable of surviving and spreading in the field under agronomic conditions. It may be that reduced ACCase activity does not significantly hamper the fitness of the whole plant. In this respect, a reduction of 70% in ACCase activity was shown to enable normal growth in yeast (Zagnitko et al., 2001), which suggests that ACCase activity might be overabundant enough in an organism to tolerate a significant reduction in enzyme efficiency without deleterious consequences. Black-grass populations, such as populations 00-049 or 00-099 that contain several mutant ACCase alleles (Table I), are potentially of interest for conducting in situ fitness cost studies. Allele-specific PCR assays developed in this work and in previous studies (Délye et al., 2002b, 2003) would then be of great use in assessing the evolution of the respective allele frequencies.

This work raised the number of residues within the black-grass homomeric ACCase CT domain that proved to be involved in sensitivity to APPs and/or CHDs to five. Among these residues, polar residues Trp-2,027 and Asp-2,078 also likely play a role in the CT reaction. Although the cavity of the CT active site is constituted almost equally by two distinct regions of the CT domain, we found that one proved determinant for herbicide sensitivity in grasses (residue 1,781) occurred in a region of highly conserved three-dimensional structure, while the other four were clustered in a more variable region encompassing amino acid positions 2,027 to 2,096. It is possible that additional, yet unknown, mutations exist there that confer altered sensitivity to ACCase inhibitors. Characterizing field or laboratory mutants at these positions will certainly improve our understanding of the mechanism of both the CT reaction and the inhibitory action of APPs and CHDs.

MATERIALS AND METHODS

Plant Material and Chloroplastic ACCase CT Domain Sequencing

We investigated three black-grass (*Alopecurus myosuroides* [Huds.]) populations (Table I) collected in France in 2000. Preliminary herbicide and genotyping assays revealed that, in these populations, the frequency of herbicide-resistant plants was considerably higher than the sum of frequencies of plants containing Leu-1,781 or Asn-2,041 ACCase alleles, which we expected was due to the presence of novel resistant ACCase allele(s). Resistance to 3 APPs (fenoxaprop, clodinafop, and haloxyfop) and 2 CHDs (cycloxydim and clethodim) was assessed using 50 seedlings per population and per herbicide as described elsewhere (Letouzé and Gasquez, 1999). The concentrations discriminating herbicide-resistant from herbicide-sensitive seedlings were 6 μM for clethodim and as described for other herbicides (Délye et al., 2002b). All seedlings were subsequently genotyped using previously described allele-specific PCR assays enabling the identification of amino acids present at positions 1,781 (Délye et al., 2002a) and 2,041 (Délye et al., 2003).

A total of 16 herbicide-resistant seedlings not containing Leu-1,781 or Asn-2,041 ACCase alleles were selected for sequencing experiments. They consisted of one clethodim-resistant, six cycloxydim-resistant, and three fenoxaprop-resistant seedlings from population 00-049, one clodinafop-resistant and two fenoxaprop-resistant seedlings from population 00-016, and one fenoxaprop-resistant and two clodinafop-resistant seedlings from population 00-099. DNA extraction and PCR amplification of a DNA fragment, including nucleotide positions 4,368 to 7,329 in the black-grass chloroplastic ACCase coding sequence (EMBL accession AJ310767; Délye et al., 2002a) with a proofreading polymerase, was as described (Délye et al., 2003). The amplified DNA fragment encompassed the entire CT domain of black-grass ACCase. The amplicon obtained from at least five independent PCRs pooled was purified using a Nucleospin Extract kit (Macherey-Nagel, Duren, Germany) and directly sequenced on both strands using gene-specific primers. When seedlings contained two different ACCase alleles, the amplicon was cloned in plasmid pDrive (Qiagen, Valencia, CA), and three different DNA inserts were sequenced for each ACCase allele. All differences observed after aligning the sequences were visually rechecked from chromatograms. Sequence assembling and alignments were performed using BioEdit software (Hall, 1999) and Multalin software (Corpet, 1988), respectively.

Bidirectional Allele-Specific PCR

Two four-primer bidirectional allele-specific PCR assays (Délye et al., 2002c) were designed to identify the codons present at positions 2,078 and 2,096. Primers VG2078-For: GCCTGGGTCGTGATTGG and VD2078-Rev: CGATCTGGGTTTATCTTGCTAT were designed to specifically prime ACCase sequences containing G or A at nucleotide position 6,389, respectively. Primers VA2096-For: CTGAGAGGACTGCAAAGGC and VG2096-Rev: CCTTGAGGTTCGAGAACATTAC were designed to specifically prime ACCase sequences containing C or G at nucleotide position 6,443, respectively. Primers VG2078-For and VD2078-Rev were used together with primers ACVII11: CTGCAAACATTGGTGGACCTCTTCCTATTAC and ACVII11R: CAGTCGGTGCTTCCTGCTGCAGCTG at a final concentration of 0.2 μM for each of the four primers. Primers VA2096-For and VG2096-Rev were used together with primers ACVII11 and ACVII11R at a final concentration of 0.2 μM for each of the four primers but VG2096-Rev (0.4 μM). Primers were designed to generate up to three distinct sizes of amplicons in a PCR, depending on the ACCase alleles present within one plant. Primer pair ACVII11/ACVII11R yielded a 1,082-bp fragment. Primer pairs ACVII11/VD2078-Rev and VG2078-For/ACVII11R yielded a 596- and a 524-bp fragment, respectively. Primer pairs ACVII11/VG2096-Rev and VA2096-For/ACVII11R yielded a 650- and a 472-bp fragment, respectively.

Development of a four-primer bidirectional allele-specific PCR was not possible for codon 2,027, possibly because of DNA secondary structure. We thus used two three-primer PCR assays for genotyping at codon 2,027. Primers VC2027-For: TCTGTTCATACTTGCTAACTGT and VW2027-For:

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

CTGTTCATACTTGCTAACTGG were designed to specifically prime ACCase sequences containing T or G at nucleotide position 6,237, respectively. Each primer was used together with primers ACVII11 and ACVII11R at a final concentration of 0.2 μM for each of the three primers. Up to two distinct sizes of amplicons were expected in a PCR, depending on the ACCase alleles present within one plant. Primer pairs VC2027-For/ACVII11R and VW2027-For/ACVII11R yielded a 681- and a 680-bp fragment, respectively.

All PCR mixes were as described (Délye et al., 2002a). For all primer combinations, the cycling program consisted of one denaturation step of 30 s at 95°C, followed by 37 cycles of 10 s at 95°C, 15 s at 62°C, and 30 s at 72°C.

Inhibition of Black-Grass ACCase Activity by APPs and CHDs

For enzyme studies, three purified populations were obtained by enabling cross-pollination between six black-grass plants that each contained two ACCase alleles with the same mutant codon. Each of the Cys-2,027-F1, Gly-2,078-F1, and Ala-2,096-F1 populations was thus derived from six plants containing two Cys-2,027, two Gly-2,078, or two Ala-2,096 ACCase alleles, respectively. The plants used for pairings were selected from populations 00-016 (Cys-2,027 allele), 00-049 (Gly-2,078 allele), and 00-099 (Ala-2,096 allele), respectively, using the allele-specific PCR assays described above. Sequencing the ACCase CT domain in these 18 plants showed no other amino acid change when compared to the CT domain from sensitive plants.

Populations 00-017, Cys-2,027-F1, Gly-2,078-F1, and Ala-2,096-F1 (Table I) were used for ACCase assays. Population 00-017 is a field-sensitive population where no mutant ACCase alleles could be detected. ACCase extraction, enzyme assay, and determination of APP and CHD inhibitory action were performed as described (Shukla et al., 1997) from three to four leaf seedlings. Assays were performed twice for each inhibitor concentration. The data were fitted to the logistic sigmoidal, three-parameter model of Sigmaplot software (SPSS Science, Chicago), and the concentrations inhibiting 50% of ACCase activity (I_{50} values) were computed as the x_0 from the equation for each inhibitor.

Protein Modeling

Homodimeric ACCase CT models were computed using the Swiss-model server (Schwede et al., 2003). For all CT sequences studied, each monomer was modeled separately using the three-dimensional structure of the free CT domain from yeast (*Saccharomyces cerevisiae*) as a template (PDB accession 1UYT; Zhang et al., 2003). To assess the consequences of the amino acid substitutions we identified in the black-grass CT domain upon the herbicide-binding site, mutant and wild-type black-grass CT domains were also modeled using the three-dimensional structure of the yeast CT domain in complex with the APP haloxyfop as a template (PDB accession 1UYS; Zhang et al., 2004). CT dimers were visualized using Deepview 3.7 (Schwede et al., 2003).

Sequence data from this article have been deposited with the EMBL/GenBank data libraries under accession numbers AJ310767 and AJ632096.

Received May 11, 2004; returned for revision July 22, 2004; accepted August 17, 2004.

LITERATURE CITED

Alban C, Baldet P, Douce R (1994) Localization and characterization of two structurally different forms of acetyl-CoA carboxylase in young pea leaves, of which one is sensitive to aryloxyphenoxypropionate herbicides. Biochem J **300:** 557–565

Burton JD, Gronwald JW, Keith RA, Somers DA, Gegenbach BG, Wyse DL (1991) Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Pestic Biochem Physiol **39:** 100–109

Christoffers MJ, Berg ML, Messersmith CG (2000) Analysis of acetyl-CoA carboxylase gene sequences from fenoxaprop-P-resistant wild oat biotypes (abstract no. 121). Proc North Cent Weed Sci Soc **55:** 67

Christoffers MJ, Berg ML, Messersmith CG (2002) An isoleucine to leucine mutation in acetyl-CoA carboxylase confers herbicide resistance in wild oat. Genome **45:** 1049–1056

Christopher JT, Holtum JAM (1998) The dicotyledonous species *Erodium moschatum* (L.) L'Hér. ex. Aiton is sensitive to haloxyfop herbicide due to herbicide-sensitive acetyl-coenzyme A carboxylase. Planta **207:** 275–279

Corpet F (1988) Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res **16:** 10881–10890

Délye C, Calmès É, Matéjicek A (2002a) SNP markers for black-grass (*Alopecurus myosuroides* Huds.) genotypes resistant to acetyl CoA carboxylase-inhibiting herbicides. Theor Appl Genet **104:** 1114–1120

Délye C, Matéjicek A, Gasquez J (2002b) PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in black-grass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Manag Sci **58:** 474–478

Délye C, Wang T, Darmency H (2002c) An isoleucine-leucine substitution in chloroplastic acetyl-Co A carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. Planta **214:** 421–427

Délye C, Zhang X-Q, Chalopin C, Michel S, Powles SB (2003) An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-CoA carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors. Plant Physiol **132:** 1716–1723

Egli MA, Gegenbach BG, Gronwald JW, Somers DA, Wyse DL (1993) Characterization of maize acetyl-coenzyme A carboxylase. Plant Physiol **101:** 499–506

Evenson KJ, Gronwald JW, Wyse DL (1997) Isoforms of acetyl-coenzyme A carboxylase in *Lolium multiflorum*. Plant Physiol Biochem **35:** 265–272

Focke M, Gieringer E, Schwan S, Jänsch L, Binder S, Braun H-P (2003) Fatty acid biosynthesis in mitochondria of grasses: malonyl-coenzyme A is generated by mitochondrial-localized acetyl-coenzyme A carboxylase. Plant Physiol **133:** 875–884

Gornicki P, Faris J, King I, Podkowinski J, Gill B, Haselkorn R (1997) Plastid-localised acetyl-CoA carboxylase of bread wheat is encoded by a single gene on each of the three ancestral chromosome sets. Proc Natl Acad Sci USA **94:** 14179–14184

Gornicki P, Haselkorn R (1993) Wheat acetyl-CoA carboxylase. Plant Mol Biol **22:** 547–552

Gornicki P, Podkowinski J, Scappino LA, DiMaio J, Ward E, Haselkorn R (1994) Wheat acetyl-coenzyme A carboxylase: cDNA and protein structure. Proc Natl Acad Sci USA **91:** 6860–6864

Gronwald JW (1991) Lipid biosynthesis inhibitors. Weed Sci **39:** 435–449

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41:** 95–98

Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol **39:** 101–138

Herbert D, Price LJ, Alban C, Dehaye L, Job D, Cole DJ, Pallett KE, Harwood JL (1996) Kinetic studies on two isoforms of acetyl-CoA carboxylase from maize leaves. Biochem J **318:** 997–1006

Jelenska J, Sirikhachornkit A, Haselkorn R, Gornicki P (2002) The carboxyltransferase activity of the apicoplast acetyl-CoA carboxylase of *Toxoplasma gondii* is the target of aryloxyphenoxypropionate inhibitors. J Biol Chem **277:** 23208–23215

Joachimiak M, Tevzadze G, Podkowinski J, Haselkorn R, Gornicki P (1997) Wheat cytosolic acetyl-CoA carboxylase complements an *ACC1* null mutation in yeast. Proc Natl Acad Sci USA **94:** 9990–9995

Konishi T, Sasaki Y (1994) Compartmentalization of two forms of acetyl-CoA carboxylase in plants and the origin of their tolerance towards herbicides. Proc Natl Acad Sci USA **91:** 3598–3601

Konishi T, Shinohara K, Yamada K, Sasaki Y (1996) Acetyl-CoA carboxylase in higher plants: most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. Plant Cell Physiol **37:** 117–122

Kuk Y-I, Burgos NR, Talbert RE (2000) Cross- and multiple resistance of diclofop-resistant *Lolium* spp. Weed Sci **48:** 412–419

Letouzé A, Gasquez J (1999) A rapid reliable test for screening aryloxyphenoxypropionic acid resistance within *Alopecurus myosuroides* and *Lolium* spp populations. Weed Res **39:** 37–48

Letouzé A, Gasquez J (2003) Enhanced activity of several herbicide-degrading enzymes: a suggested mechanism responsible for multiple resistance in black-grass (*Alopecurus myosuroides* Huds.). Agronomie (Paris) **23:** 601–608

Marles MAS, Devine MD, Hall JC (1993) Herbicide resistance in *Setaria viridis* conferred by a less sensitive form of acetyl coenzyme A carboxylase. Pestic Biochem Physiol **46:** 7–14

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

Menéndez J, De Prado R (1999) Characterization of two acetyl-CoA carboxylase isoforms in diclofop-methyl-resistant and -susceptible biotypes of *Alopecurus myosuroides*. Pestic Biochem Physiol **65:** 82–89

Nikolau BJ, Ohlrogge JB, Wurtele ES (2003) Plant biotin-containing carboxylases. Arch Biochem Biophys **414:** 211–222

Podkowinski J, Sroga GE, Haselkorn R, Gornicki P (1996) Structure of a gene encoding a cytosolic acetyl-CoA carboxylase of hexaploid wheat. Proc Natl Acad Sci USA **93:** 1870–1874

Post-Beittenmiller D (1996) Biochemistry and molecular biology of wax production in plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 405–430

Price LJ, Herbert D, Cole DJ, Harwood JL (2003) Use of plant cell cultures to study graminicide effects on lipid metabolism. Phytochemistry **63:** 533–541

Price LJ, Moss SR, Cole DJ, Harwood JL (2004) Graminicide resistance in a black-grass (*Alopecurus myosuroides*) population correlates with insensitivity of acetyl-CoA carboxylase. Plant Cell Environ **27:** 15–26

Rendina AR, Craig-Kennard AC, Beaudoin JD, Breen MK (1990) Inhibition of acetyl-coenzyme A carboxylase by two classes of grass-selective herbicides. J Agric Food Chem **38:** 1282–1287

Roessler PG (1990) Purification and characterization of acetyl-CoA carboxylase from the diatom *Cyclotella cryptica*. Plant Physiol **92:** 73–78

Sasaki Y, Konishi T, Nagano Y (1995) The compartmentation of acetyl-coenzyme A carboxylase in plants. Plant Physiol **108:** 445–449

Schwede T, Kopp J, Guex N, Peitsch MC (2003) SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res **31:** 3381–3385

Seng TW, Skillman TR, Yang N, Hammond C (2003) Cyclohexanedione herbicides are inhibitors of rat heart acetyl-CoA carboxylase. Bioorg Med Chem Lett **13:** 3237–3242

Shukla A, Leach GE, Devine MD (1997) High-level resistance to sethoxydim conferred by an alteration in the target enzyme, acetyl-CoA carboxylase, in *Setaria faberi* and *Setaria viridis*. Plant Physiol Biochem **35:** 803–807

Turner JA, Pernich DJ (2002) Origin of enantiomeric selectivity in the aryloxyphenoxypropionic acid class of herbicidal acetyl coenzyme A carboxylase (ACCase) inhibitors. J Agric Food Chem **50:** 4554–4566

Webb SR, Durst GL, Pernich D, Hall JC (2000) Interaction of cyclohexanediones with acetyl coenzyme-A carboxylase and an artificial target-site antibody mimic: a comparative molecular field analysis. J Agric Food Chem **48:** 2506–2511

Zagnitko O, Jelenska J, Tevzadze G, Haselkorn R, Gornicki P (2001) An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexanedione inhibitors. Proc Natl Acad Sci USA **98:** 6617–6622

Zhang H, Tweel B, Tong L (2004) Molecular basis for the inhibition of the carboxyltransferase domain of acetyl-coenzyme-A carboxylase by haloxyfop and diclofop. Proc Natl Acad Sci USA **101:** 5910–5915

Zhang H, Yang Z, Shen Y, Tong L (2003) Crystal structure of the carboxyltransferase domain of acetyl-coenzyme A carboxylase. Science **299:** 2064–2067

Zhang XQ, Devine MD (2000) A possible point mutation of plastidic ACCase gene conferring resistance to sethoxydim in green foxtail (*Setaria viridis*) (abstract no. 81). Weed Sci Soc Am **40:** 33

Downloaded from www.plantphysiol.org on February 29, 2016 - Published by www.plant.org
Copyright © 2005 American Society of Plant Biologists. All rights reserved.

30/30

ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/273757342

Délye, C. Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci

ARTICLE *in* WEED RESEARCH · SEPTEMBER 2005

Impact Factor: 1.69 · DOI: 10.1614/WS-04-203R.1

CITATIONS
117

READS
87

1 AUTHOR:



Christophe Délye
French National Institute for Agricultural Res...
60 PUBLICATIONS 1,924 CITATIONS

SEE PROFILE

Available from: Christophe Délye
Retrieved on: 22 February 2016

Weed Science, 53:728–746. 2005

Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update

Christophe Délye
UMR Biologie et Gestion des Adventices, Institut National de la Recherche Agronomique, BP 86510, F-21065 Dijon cédex, France; delye@dijon.inra.fr

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 herbicides 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: $HCO_3^- + \text{ACC-biotin} + \text{ATP} \leftrightarrow \text{ACC-biotin-}CO_2^- + \text{ADP} + \text{Pi}$, 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 Incledon 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

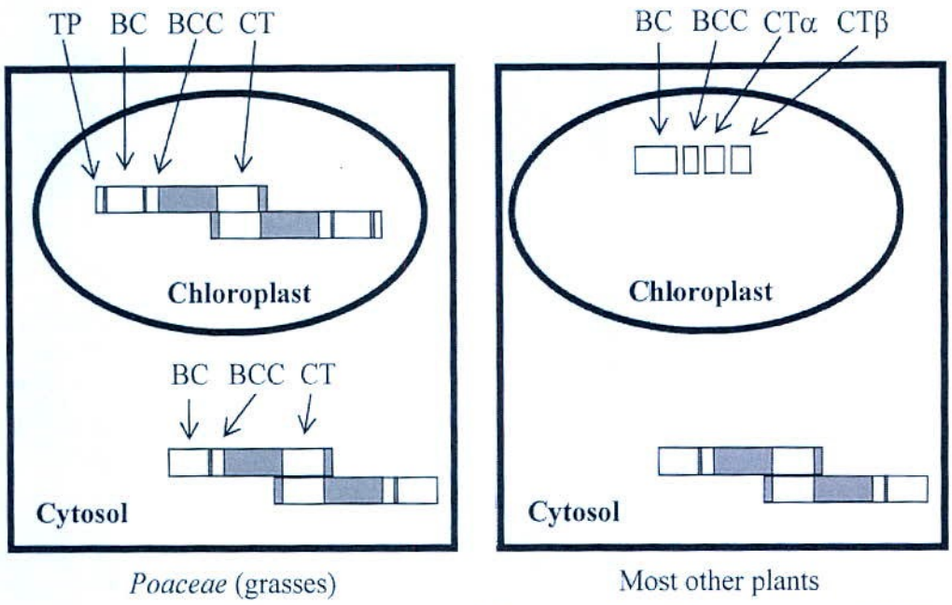


FIGURE 1. Acetyl coenzyme A carboxylase (ACC) isoforms present in plants. TP, transit peptide; ACC functional domains: BCC, biotin carboxyl-carrier; BC, biotin carboxylase; CT, carboxyl transferase. Note that the TP could be cleaved after the plastidic ACC isoform has been imported into the chloroplast.

(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 (Incledon 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-

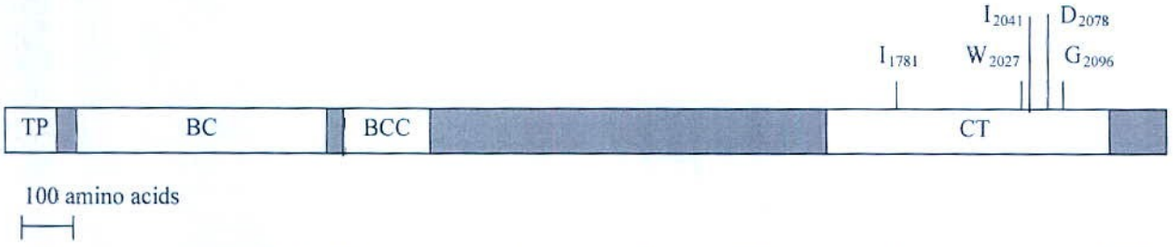


FIGURE 2. Schematic representation of a plastidic homomeric acetyl coenzyme A carboxylase (ACC) showing the three functional domains (BCC, biotin carboxyl-carrier; BC, biotin carboxylase; CT, carboxyl transferase) and the transit peptide (TP) that is absent in cytosolic ACCs. The five amino acid residues 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
Arabidopsis thaliana L.	Plastidic[a]	2375	AF062308	gDNA
A. thaliana	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 homomeric 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).

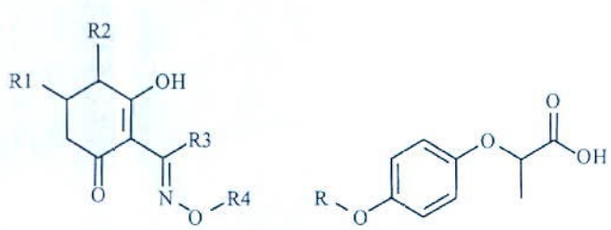


FIGURE 3. Generic structures of the aryloxyphenoxypropionate (APP, right) and cyclohexanedione (CHD, left) herbicides. Developed formulas of commercial compounds are available in Heap (2004).

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

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 (Joachimiak 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 (μM)										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	Délye et al. 2003, 2005; Menéndez and Prado 1996, 1999; Moss et al. 2003; De Prado et al. 2004
	Cytosolic	NA	35.0	NA	NA	NA	NA	NA	NA	NA	NA	Menéndez and De Prado 1999
Avena spp.	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; Maneechote et al. 1997; Shukla et al. 1997a
Digitaria ischeamum 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; Volenberg and Stoltenberg 2002a
Goosegrass	Plastidic	NA	NA	1.0	5.6	NA	NA	1.5	NA	3.8	NA	Leach et al. 1995
Hordeum leporinum Link.	Plastidic	NA	0.6	NA	10.2	1.7	NA	NA	NA	16.0	2.7	Matthews et al. 2000
Lolium 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
	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
Giant foxtail	Plastidic	NA	NA	NA	5.8	NA	NA	1.3	NA	6.2	NA	Volenberg and Stoltenberg 2002a
Green foxtail	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
	Cytosolic	NA	95.0	NA	NA	NA	NA	NA	NA	NA	NA	De Prado et al. 2004
Corn	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 (μM)										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
	Cytosolic	NA	NA	NA	NA	250.0	NA	NA	NA	> 500.0	NA	Joachimiak et al. 1997
Tall fescue	Plastidic	NA	NA	NA	NA	5.8	NA	NA	NA	6.9	NA	Stoltenberg et al. 1989
Festuca rubra L.	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. *glauca*)	Plastidic[c]	NA	NA	NA	283.0	NA	NA	NA	NA	688.0	NA	Catanzaro et al. 1993
Poa annua 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
Vulpia bromoides L. Gray	Plastidic[c]	NA	22.0	NA	73.0	19.0	NA	NA	NA	> 100.0	8.0	Yu et al. 2004
Pisum sativum 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
Cyclotella cryptica	Cytosolic	NA	NA	NA	NA	> 100.0	NA	23.0	NA	NA	NA	Roessler 1990
Toxoplasma gondii	Plastidic	5.0	NA	NA	NA	5.0	NA	NA	NA	> 400.0	NA	Jelenska et al. 2002
	Cytosolic	> 400.0	NA	NA	NA	> 400.0	NA	NA	NA	> 400.0	NA	Jelenska et al. 2002
Yeast	Cytosolic	NA	NA	NA	NA	> 500.0	NA	NA	NA	>500.0	NA	Joachimiak et al. 1997
Rattus sp.	Cytosolic	NA	NA	NA	NA	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.

36

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 (*Orysa 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 nonconserved 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/DDBJ 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 substitution 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 (Catanzaro 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]

Species[b]	Selecting herbicide[c]	R/S I_{50} ratios (RFs)										References
		APPs						CHDs				
		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
Avena sterilis ssp. *ludoviciana* Durieu	U	NA	3.7	8.2	NA	NA	NA	NA	0.8	NA	1.6	Cocker et al. 2000
Digitaria ischeamum	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
Lolium temulentum 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
Hordeum leporinum	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]									
			APPs					CHDs				
Wild-type	Resistant	Weed	Cd	Dc	Fx	Fz	Hx	Ct	Cx	Sx	Tk	References
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	*Lolium* 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	*Lolium* sp.	R	R	ND	ND	R	ND	S	ND	ND	Délye et al. 2003
	Val	*Lolium* 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
Gly_{2096}	Ala	Blackgrass	R	ND	R	ND	R	S	S	ND	ND	Délye et al. 2005

[a] Ala, alanine; APP, aryloxyphenoxypropionate; Asn, asparagine; Asp, aspartic acid; Cd, clodinafop; CHD, cyclohexanedione; Ct, clethodim; Cx, cycloxydim; Cys, cysteine; Dc, diclofop; Fx, fenoxaprop; Fz, fluazifop; 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 $NADPH_2$-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, NottsA1	NI	Photosystem II[d]	Yes (Leu_{1781})	Cocker et al. 1999; Hall et al. 1997; Moss et al. 2003
	Peldon	GST, NI	Photosystem II,[d] peroxidizing herbicides	No	Cummins et al. 1999; Hall et al. 1997
	RB	GST	No	No	Letouzé and Gazquez 2003
	RC	CYP, GST	No	No	Letouzé and Gasquez 2003
	NE Spain	CYP, GT	Photosystem II[d]	Yes	Menéndez and De Prado 1996
Wild oat	T11, Dorset	NI	NA	No	Cocker et al. 2000
Avena sterilis	NAS4	CYP	NA	Yes	Maneechote et al. 1997
Large crabgrass	Murray Bridge	NI	NA	No	Hidayat and Preston 1997
Hordeum leporinum	Arthurton	NI	NA	Yes	Matthews et al. 2000
Italian ryegrass	EssexA1, LincsA1, WiltsB1	CYP, GST	Photosystem II[d]	No	Cocker et al. 2001
Rigid ryegrass	SLR31	CYP	Acetolactate synthase[d]	Yes (Leu_{1781})	Preston and Powles 1998; Vila-Aiub et al. 2005
	VLR69	CYP, NI	No	Yes	Preston et al. 1996
Lolium spp.	Roma94, Tuscana97	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-hydroxyled 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; Maneechote 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 Leu_{1781} and Asn_{2041} 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 discriminating 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 Leu_{1781} 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 Leu_{1781} ACC alleles rather than Asn_{2041} 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 Asn_{2041} ACC alleles (Délye et al. 2004a). This hypothesis must be checked by more extensive studies. It is consistent with the Ile_{1781} 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 Cys_{2027} and Gly_{2078} 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.

Literature Cited

Alban, C., P. Baldet, and R. Douce. 1994. Localization and characterization of two structurally different forms of acetyl-CoA carboxylase in young pea leaves, of which one is sensitive to aryloxyphenoxypropionate herbicides. Biochem J. 300:557–565.

Andrews, T. S., I. N. Morrison, and G. A. Penner. 1998. Monitoring the spread of ACCase inhibitor resistance among wild oat (*Avena fatua*) patches using AFLP analysis. Weed Sci. 46:196–199.

Anonymous. 1998. "Herbicide resistance" and "herbicide tolerance" defined. Weed Technol. 12:789.

Anonymous. 2004. Herbicide resistance action committee. www.plantprotection.org/HRAC. Accessed October 11, 2004.

Ashton, A. R., C.L.D. Jenkins, and P. R. Whitfeld. 1994. Molecular cloning

of two different cDNAs for maize acetyl CoA carboxylase. Plant Mol. Biol. 24:35–49.
Barnes, J. W. and L. R. Oliver. 2004. Cloransulam antagonizes annual grass control with aryloxyphenoxypropionate graminicides but not cyclohexanediones. Weed Technol. 18:763–772.
Basu, C., M. D. Halfhill, T. C. Mueller, and C. N. Stewart, Jr. 2004. Weed genomics: new tools to understand weed biology. Trends Plant Sci. 9: 391–398.
Beckie, H. J., L. M. Hall, S. Meers, J. J. Laslo, and F. C. Stevenson. 2004. Management practices influencing herbicide resistance in wild oat. Weed Technol. 18:853–859.
Beckie, H. J., I. M. Heap, R. J. Smeda, and L. M. Hall. 2000. Screening for herbicide resistance in weeds. Weed Technol. 14:428–445.
Beckie, H. J., A. G. Thomas, A. Légère, D. J. Kelner, R. C. Van Acker, and S. Meers. 1999. Nature, occurrence, and cost of herbicide-resistant wild oat (*Avena fatua*) in small-grain production areas. Weed Technol. 13:612–625.
Beckie, H. J., A. G. Thomas, and F. C. Stevenson. 2002. Survey of herbicide-resistant wild oat (*Avena fatua*) in two townships in Saskatchewan. Can. J. Plant Sci. 82:463–471.
Betts, K. J., N. J. Ehlke, D. L. Wyse, J. W. Gronwald, and D. A. Somers. 1992. Mechanism of inheritance of diclofop resistance in italian ryegrass (*Lolium multiflorum*). Weed Sci. 40:184–189.
Boutsalis, P. 2001. Syngenta Quick-Test: a rapid whole-plant test for herbicide resistance. Weed Technol. 15:257–263.
Bradley, K. W., J. Wu, K. K. Hatzios, and E. S. Hagood, Jr. 2001. The mechanism of resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in a johnsongrass biotype. Weed Sci. 49:477–484.
Bravin, F., G. Zanin, and C. Preston. 2001. Resistance to diclofop-methyl in two *Lolium* spp. populations from Italy: studies on the mechanism of resistance. Weed Res. 41:461–473.
Brazier, M., D. J. Cole, and R. Edwards. 2002. *O*-glycosyltransferase activities towards phenolic natural products and xenobiotics in wheat and herbicide-resistant and herbicide-susceptible black-grass (*Alopecurus myosuroides*). Phytochemistry 59:149–156.
Burton, J. D., J. W. Gronwald, R. A. Keith, D. A. Somers, B. G. Gegenbach, and D. L. Wyse. 1991. Kinetics of inhibition of acetyl-coenzyme A carboxylase by sethoxydim and haloxyfop. Pestic. Biochem. Physiol. 39:100–109.
Burton, J. D., J. W. Gronwald, D. A. Somers, J. A. Connelly, B. G. Gegenbach, and D. L. Wyse. 1987. Inhibition of plant acetyl-coenzyme A carboxylase by the herbicides sethoxydim and haloxyfop. Biochem. Biophys. Res. Commun. 148:1039–1044.
Catanzaro, C. J., J. D. Burton, and W. A. Skroch. 1993. Graminicide resistance of acetyl-CoA carboxylase from ornamental grasses. Pestic. Biochem. Physiol. 45:147–153.
Cavan, G., P. Biss, and S. R. Moss. 1998a. Herbicide resistance and gene flow in wild-oats (*Avena fatua* and *Avena sterilis* ssp. *ludoviciana*). Ann. Appl. Biol. 133:207–217.
Cavan, G., P. Biss, and S. R. Moss. 1998b. Localized origins of herbicide resistance in *Alopecurus myosuroides*. Weed Res. 38:239–245.
Cavan, G., J. Cussans, and S. Moss. 2000. Modelling different cultivation and herbicide strategies for their effect on herbicide resistance in *Alopecurus myosuroides*. Weed Res. 40:561–568.
Cavan, G., J. Cussans, and S. Moss. 2001. Managing the risks of herbicide resistance in wild oat. Weed Sci. 49:236–240.
Chauvel, B., J.-P. Guillemin, N. Colbach, and J. Gasquez. 2001. Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroides* Huds.). Crop Prot. 20:127–137.
Christoffers, M. J., M. L. Berg, and C. G. Messersmith. 2002. An isoleucine to leucine mutation in acetyl-CoA carboxylase confers herbicide resistance in wild oat. Genome 45:1049–1056.
Christopher, J. T. and J.A.M. Holtum. 1998. The dicotyledonous species *Erodium moschatum* (L) L'Hér. ex. Aiton is sensitive to haloxyfop herbicide due to herbicide-sensitive acetyl-coenzyme A carboxylase. Planta 207:275–279.
Christopher, J. T. and J.A.M. Holtum. 2000. Dicotyledons lacking the multisubunit form of acetyl coenzyme A carboxylase may be restricted to the family Geraniaceae. Aust. J. Plant Physiol. 27:845–850.
Cobb, A. H. and R. C. Kirkwood. 2000. Challenges for herbicide development. Pages 1–24 *in* A. H. Cobb and R. C. Kirkwood, eds. Herbicides and Their Mechanisms of Action. Sheffield, UK: Sheffield Academic Press.
Cocker, K. M., J.O.D. Coleman, A. M. Blair, J. H. Clarke, and S. R. Moss. 2000. Biochemical mechanisms of cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in populations of *Avena* spp. Weed Res. 40:323–334.
Cocker, K. M., S. R. Moss, and J.O.D. Coleman. 1999. Multiple mechanisms of resistance to fenaxoprop-P-ethyl in United Kingdom and other European populations of herbicide-resistant *Alopecurus myosuroides* (black-grass). Pestic. Biochem. Physiol. 65:169–180.
Cocker, K. M., D. S. Northcroft, J.O.D. Coleman, and S. R. Moss. 2001. Resistance to ACCase-inhibiting herbicides and isoproturon in UK populations of *Lolium multiflorum*: mechanisms of resistance and implications for control. Pest Manag. Sci. 57:587–597.
Collings, L. V., A. M. Blair, A. P. Gay, C. J. Dyer, and N. Mackay. 2003. The effect of weather factors on the performance of herbicides to control *Alopecurus myosuroides* in winter wheat. Weed Res. 43:146–153.
Cummins, I., D. J. Cole, and R. Edwards. 1999. A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. Plant J. 18:285–292.
Cummins, I. and R. Edwards. 2004. Purification and cloning of an esterase from the weed black-grass (*Alopecurus myosuroides*), which bioactivates aryloxyphenoxypropionate herbicides. Plant J. 39:894–904.
Davies, J. and J. C. Caseley. 1999. Herbicide safeners: a review. Pestic. Sci. 55:1043–1058.
Délye, C., É. Calmès, and A. Matéjicek. 2002a. SNP markers for blackgrass (*Alopecurus myosuroides* Huds.) genotypes resistant to acetyl CoA-carboxylase inhibiting herbicides. Theor. Appl. Genet. 104:1114–1120.
Délye, C., A. Matéjicek, and J. Gasquez. 2002b. PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in blackgrass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Manag. Sci. 58:474–478.
Délye, C., C. Straub, A. Matéjicek, and S. Michel. 2004a. Multiple origins for black-grass (*Alopecurus myosuroides* Huds.) target site–based resistance to herbicides inhibiting acetyl-CoA carboxylase. Pest Manag. Sci. 60:35–41.
Délye, C., C. Straub, S. Michel, and V. Le Corre. 2004b. Nucleotide variability at the acetyl-coenzyme A carboxylase gene and the signature of herbicide selection in the grass weed *Alopecurus myosuroides* (Huds.). Mol. Biol. Evol. 21:884–892.
Délye, C., T. Wang, and H. Darmency. 2002c. An isoleucine–leucine substitution in chloroplastic acetyl-Co A carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. Planta 214:421–427.
Délye, C., X.-Q. Zhang, C. Chalopin, S. Michel, and S. B. Powles. 2003. An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-CoA carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors. Plant Physiol. 132:1716–1723.
Délye, C., X.-Q. Zhang, S. Michel, A. Matéjicek, and S. B. Powles. 2005. Molecular bases for sensitivity to acetyl-coenzyme A carboxylase inhibitors in black-grass. Plant Physiol. 137:794–806.
De Prado, R., J. González-Gutiérrez, J. Menéndez, J. Gasquez, J. W. Gronwald, and R. Giménez-Espinosa. 2000. Resistance to acetyl CoA carboxylase–inhibiting herbicides in *Lolium multiflorum*. Weed Sci. 48: 311–318.
De Prado, R., M. D. Osuna, and A. J. Fisher. 2004. Resistance to ACCase inhibitor herbicides in a green foxtail (*Setaria viridis*) biotype in Europe. Weed Sci. 52:506–512.
Devine, M. D. and R. H. Shimabukuro. 1994. Resistance to acetyl coenzyme A carboxylase inhibiting herbicides. Pages 141–169 *in* S. B. Powles and J.A.M. Holtum, eds. Herbicide Resistance in Plants. Boca Raton, FL: CRC Press.
Diggle, A. J., P. B. Neve, and F. P. Smith. 2003. Herbicides used in combination can reduce the probability of herbicide resistance in finite weed populations. Weed Res. 43:371–382.
Dinelli, G., A. Bonetti, I. Marotti, M. Minelli, and P. Catizone. 2004. Characterization of Italian populations of *Lolium* spp. resistant and susceptible to diclofop by inter simple sequence repeat. Weed Sci. 52: 554–563.
Di Tomaso, J. M. 1994. Evidence against a direct membrane effect in the mechanism of action of graminicides. Weed Sci. 42:302–309.
Egli, M. A., B. G. Gegenbach, J. W. Gronwald, D. A. Somers, and D. L. Wyse. 1993. Characterization of maize acetyl-coenzyme A carboxylase. Plant Physiol. 101:499–506.
Evenson, K. J., J. W. Gronwald, and D. L. Wyse. 1997. Isoforms of acetyl-coenzyme A carboxylase in *Lolium multiflorum*. Plant Physiol. Biochem. 35:265–272.

Faris, J., A. Sirikhachornkit, R. Haselkorn, B. Gill, and P. Gornicki. 2001. Chromosome mapping and phylogenetic analysis of the cytosolic acetyl-CoA carboxylase loci in wheat. Mol. Biol. Evol. 18:1720–1733.

Focke, M., E. Gieringer, S. Schwan, L. Jänsch, S. Binder, and H.-P. Braun. 2003. Fatty acid biosynthesis in mitochondria of grasses: malonyl-coenzyme A is generated by mitochondrial-localized acetyl-coenzyme A carboxylase. Plant Physiol. 133:875–884.

Foster, D. K., P. Ward, and R. T. Hewson. 1993. Selective grass-weed control in wheat and barley based on the safener fenchlorazole-ethyl. Pages 1267–1272 *in* British Crop Protection Council, ed. Proceedings of the Brighton Crop Protection Conference—Weeds. Surrey, Great Britain, British Crop Protection Council.

Friesen, L. F., T. L. Jones, R. C. Van Hacker, and I. N. Morrison. 2000. Identification of *Avena fatua* populations resistant to imazamethabenz, flamprop and fenoxaprop-P. Weed Sci. 48:532–540.

Frova, C. 2003. The plant glutathione transferase gene family: genomic structure, functions, expression and evolution. Physiol. Plant. 119: 469–479.

Gerwick, B. C., L. A. Jackson, J. Handly, N. R. Gray, and J. W. Russell. 1988. Pre-emergence and post-emergence activities of the (*R*) and (*S*)-enantiomers of haloxyfop. Weed Sci. 36:453–456.

Gornicki, P., J. Faris, I. King, J. Podkowinski, B. Gill, and R. Haselkorn. 1997. Plastid-localised acetyl-CoA carboxylase of bread wheat is encoded by a single gene on each of the three ancestral chromosome sets. Proc. Natl. Acad. Sci. USA 94:14179–14184.

Gornicki, P. and R. Haselkorn. 1993. Wheat acetyl-CoA carboxylase. Plant Mol. Biol. 22:547–552.

Gressel, J. 1990. Need herbicide resistance have evolved? Generalizations from around the world. Pages 173–184 *in* J. W. Heap, ed. Proceedings of the 9th Australian Weeds Conference. Adelaide, Australia: Crop Science Society of South Australia.

Gronwald, J. W., C. V. Eberlein, K. J. Betts, R. J. Baerg, N. J. Ehlke, and D. L. Wyse. 1992. Mechanism of diclofop resistance in an Italian ryegrass (*Lolium multiflorum* Lam.) biotype. Pestic. Biochem. Physiol. 44:126–139.

Hadfield, S. T., A. Wilson, S. F. Kuet, and R. Mason. 1994. The metabolism of tralkoxydim in field-grown spring wheat and maize plant cell suspension culture. Pestic. Sci. 40:193–200.

Hall, L. M., S. R. Moss, and S. B. Powles. 1997. Mechanisms of resistance to aryloxyphenoxypropionate herbicides in two resistant biotypes of *Alopecurus myosuroides* (blackgrass): herbicide metabolism as a cross-resistance mechanism. Pestic. Biochem. Physiol. 57:87–98.

Harwood, J. L. 1988. Fatty acid metabolism. Annu. Rev. Plant Physiol. 39: 101–138.

Harwood, J. H., Jr., S. F. Petras, L. D. Shelly, et al. 2003. Isozyme-nonselective *N*-substituted bipiperidylcarboxamide acetyl-CoA carboxylase inhibitors reduce tissue malonyl-CoA concentrations, inhibit fatty acid synthesis, and increase fatty acid oxidation in cultured cells and in experimental animals. J. Biol. Chem. 278:37,099–37,111.

Hatzios, K. K. and N. R. Burgos. 2004. Metabolism-based herbicide resistance: regulation by safeners. Weed Sci. 52:454–467.

Heap, I. M. 2004. International survey of herbicide resistant weeds. www.weedresearch.com. Accessed December 7, 2004.

Heap, I. M. and I. N. Morrison. 1996. Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in green foxtail (*Setaria viridis*). Weed Sci. 44:25–30.

Herbert, D., D. J. Cole, K. E. Pallett, and J. L. Harwood. 1996a. Susceptibilities of different test systems from maize (*Zea mays*), *Poa annua* and *Festuca rubra* to herbicides that inhibit the enzyme acetyl-coenzyme A carboxylase. Pestic. Biochem. Physiol. 55:129–139.

Herbert, D., L. J. Price, C. Alban, L. Dehaye, D. Job, D. J. Cole, K. E. Pallett, and J. L. Harwood. 1996b. Kinetic studies on two isoforms of acetyl-CoA carboxylase from maize leaves. Biochem. J. 318:997–1006.

Hidayat, I. and C. Preston. 1997. Enhanced metabolism of fluazifop acid in a biotype of *Digitaria sanguinalis* resistant to the herbicide fluazifop-*p*-butyl. Pestic. Biochem. Physiol. 57:137–146.

Hill, B. D., E. H. Stobbe, and B. L. Jones. 1978. Hydrolysis of the herbicide benzoylprop-ethyl by wild oat esterase. Weed Res. 18:149–154.

Holtum, J.A.M., R. E. Häusler, M. D. Devine, and S. B. Powles. 1994. Recovery of transmembrane potentials in plants resistant to aryloxyphenoxypropionate herbicides: a phenomenon awaiting explanation. Weed Sci. 42:293–301.

Huang, S., A. Sirikhachornkit, J. D. Faris, X. Su, B. S. Gill, R. Haselkorn, and P. Gornicki. 2002. Phylogenetic analysis of the acetyl-CoA carboxylase and 3-phosphoglycerate kinase in wheat and other grasses. Plant Mol. Biol. 48:805–820.

Incledon, B. J. and J. C. Hall. 1997. Acetyl-coenzyme A carboxylase: quaternary structure and inhibition by graminicidal herbicides. Pestic. Biochem. Physiol. 57:255–271.

Jasieniuk, M., A. L. Brûlé-Babel, and I. N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Sci. 44:176–193.

Jeffcoat, B. and W. N. Harries. 1973. Selectivity and mode of action of ethyl (±)-2-(*N*-benzoyl-3,4-dichloroanilino)propionate in the control of *Avena fatua* in cereals. Pestic. Sci. 4:891–899.

Jelenska, J., A. Sirikhachornkit, R. Haselkorn, and P. Gornicki. 2002. The carboxyltransferase activity of the apicoplast acetyl-CoA carboxylase of *Toxoplasma gondii* is the target of aryloxyphenoxypropionate inhibitors. J. Biol. Chem. 277:23,208–23,215.

Joachimiak, M., G. Tevzadze, J. Podkowinski, R. Haselkorn, and P. Gornicki. 1997. Wheat cytosolic acetyl-CoA carboxylase complements an *ACC1* null mutation in yeast. Proc. Natl. Acad. Sci. USA 94:9990–9995.

Kibite, S., K. N. Harker, and P. D. Brown. 1995. Inheritance of resistance to diclofop-methyl and fenoxaprop-*p*-ethyl in two *Avena sativa* × *A. fatua* populations. Can. J. Plant Sci. 75:81–85.

Konishi, T. and Y. Sasaki. 1994. Compartimentalization of two forms of acetyl-CoA carboxylase in plants and the origin of their tolerance towards herbicides. Proc. Natl. Acad. Sci. USA 91:3598–3601.

Konishi, T., K. Shinohara, K. Yamada, and Y. Sasaki. 1996. Acetyl-CoA carboxylase in higher plants: most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. Plant Cell Physiol. 37:117–122.

Kreuz, K., R. Tommasini, and E. Martinoia. 1996. Old enzymes for a new job. Herbicide detoxification in plants. Plant Physiol. 111:349–353.

Kuk, Y.-I., N. R. Burgos, and R. E. Talbert. 2000. Cross- and multiple resistance of diclofop-resistant *Lolium* spp. Weed Sci. 48:412–419.

Kuk, Y.-I., J. Wu, J. F. Derr, and K. K. Hatzios. 1999. Mechanism of fenaxoprop resistance in an accession of smooth crabgrass (*Digitaria ischaemum*). Pestic. Biochem. Physiol. 64:112–123.

Leach, G. E., M. D. Devine, R. C. Kirkwood, and G. Marshall. 1995. Target enzyme-based resistance to acetyl-coenzyme A carboxylase inhibitors in *Eleusine indica*. Pestic. Biochem. Physiol. 51:129–136.

Légère, A., H. J. Beckie, F. C. Stevenson, and A. G. Thomas. 2000. Survey of management practices affecting the occurrence of wild oat (*Avena fatua*) resistance to acetyl-CoA carboxylase inhibitors. Weed Technol. 14:366–376.

Letouzé, A. and J. Gasquez. 1999. A rapid reliable test for screening aryloxyphenoxypropionic acid resistance within *Alopecurus myosuroides* and *Lolium* spp. populations. Weed Res. 39:37–48.

Letouzé, A. and J. Gasquez. 2000. A pollen test to detect ACCase target-site resistance within *Alopecurus myosuroides* populations. Weed Res. 40:151–162.

Letouzé, A. and J. Gasquez. 2001. Inheritance of fenoxaprop-P-ethyl resistance in a blackgrass (*Alopecurus myosuroides* Huds.) population. Theor. Appl. Genet. 103:288–296.

Letouzé, A. and J. Gasquez. 2003. Enhanced activity of several herbicide-degrading enzymes: a suggested mechanism responsible for multiple resistance in black-grass (*Alopecurus myosuroides* Huds.). Agronomie 23:601–608.

Levert, K. L. and G. L. Waldrop. 2002. A bisubstrate analog inhibitor of the carboxyltransferase component of acetyl-CoA carboxylase. Biochem. Biophys. Res. Commun. 291:1213–1217.

Mallory-Smith, C. A. and E. J. Retzinger, Jr. 2003. Revised classification of herbicides by site of action for weed resistance management strategies. Weed Technol. 17:605–619.

Maneechote, C., C. Preston, and S. B. Powles. 1997. A diclofop-methyl–resistant *Avena sterilis* biotype with a herbicide-resistant acetyl-coenzyme A carboxylase and enhanced metabolism of diclofop-methyl. Pestic. Sci. 49:105–114.

Marles, M.A.S., M. D. Devine, and J. C. Hall. 1993. Herbicide resistance in *Setaria viridis* conferred by a less sensitive form of acetyl coenzyme A carboxylase. Pestic. Biochem. Physiol. 46:7–14.

Marshall, G., R. C. Kirkwood, and G. E. Leach. 1994. Comparative studies on graminicide-resistant and susceptible biotypes of *Eleusine indica*. Weed Res. 34:177–185.

Marshall, L. C., D. A. Somers, P. D. Dotray, B. G. Gegenbach, D. L. Wyse, and J. W. Gronwald. 1992. Allelic mutations in acetyl-coenzyme A carboxylase confer herbicide tolerance in maize. Theor. Appl. Genet. 83:435–442.

Matthews, N. and S. B. Powles. 1992. Aspects of the population dynamics

of selection for herbicide resistance in *Lolium rigidum* (Gaud.). Proc. First Int. Weed Control Congr. 2:318–320.

Matthews, N., S. B. Powles, and C. Preston. 2000. Mechanisms of resistance to acetyl-coenzyme A carboxylase–inhibiting herbicides in a *Hordeum leporinum* population. Pest Manag. Sci. 56:441–447.

McFadden, J. J., D. S. Frear, and E. R. Mansager. 1989. Aryl hydroxylation of diclofop by a cytochrome P450 dependent monooxygenase from wheat. Pestic. Biochem. Physiol. 32:92–100.

Menéndez, J. and R. De Prado. 1996. Diclofop-methyl cross-resistance in a chlortoluron-resistant biotype of *Alopecurus myosuroides*. Pestic. Biochem. Physiol. 56:123–133.

Menéndez, J. and R. De Prado. 1999. Characterization of two acetyl-CoA carboxylase isoforms in diclofop-methyl-resistant and -susceptible biotypes of *Alopecurus myosuroides*. Pestic. Biochem. Physiol. 65:82–89.

Mengistu, L. W., C. G. Messersmith, and M. J. Christoffers. 2003. Diversity of herbicide resistance among wild oat sampled 36 years apart. Weed Sci. 51:764–773.

Milner, L. J., J.P.H. Reade, and A. H. Cobb. 2001. Developmental changes in gluthatione *S*-transferase activity in herbicide-resistant populations of *Alopecurus myosuroides* Huds (black-grass) in the field. Pest Manag. Sci. 57:1100–1106.

Morgan, W. G., I. P. King, S. Koch, J. A. Harper, and H. M. Thomas. 2001. Introgression of chromosomes of *Festuca arundinacea* var *glaucescens* into *Lolium multiflorum* revealed by genomic in situ hybridisation (GISH). Theor. Appl. Genet. 103:696–701.

Moss, S. R., K. M. Cocker, A. C. Brown, L. Hall, and L. M. Field. 2003. Characterisation of target-site resistance to ACCase-inhibiting herbicides in the weed *Alopecurus myosuroides* (black-grass). Pest Manag. Sci. 59:190–201.

Murray, B. G., A. L. Brûlé-Babel, and I. N. Morrison. 1996. Two distinct alleles encode for acetyl-CoA carboxylase inhibitor resistance in wild oat (*Avena fatua*). Weed Sci. 44:476–481.

Murray, B. G., I. N. Morrison, and A. L. Brûlé-Babel. 1995. Inheritance of acetyl-CoA carboxylase inhibitor resistance in wild oat (*Avena fatua*). Weed Sci. 43:233–238.

Murray, B. G., I. N. Morrison, and L. F. Friesen. 2002. Pollen-mediated gene flow in wild oat. Weed Sci. 50:321–325.

Nikolau, B. J., J. B. Ohlrogge, and E. S. Wurtele. 2003. Plant biotin-containing carboxylases. Arch. Biochem. Biophys. 414:211–222.

Nikolskaya, T., O. Zagnitko, G. Tevzadze, R. Haselkorn, and P. Gornicki. 1999. Herbicide sensitivity determinant of wheat plastid acetyl-CoA carboxylase is located in a 400-amino acid fragment of the carboxyltransferase domain. Proc. Natl. Acad. Sci. USA 96:14647–14651.

Parker, W. B., L. C. Marshall, J. D. Burton, D. A. Somers, D. L. Wyse, J. W. Gronwald, and B. G. Gegenbach. 1990. Dominant mutations causing alterations in acetyl-coenzyme A carboxylase confer tolerance to cyclohexanedione and aryloxyphenoxypropionate herbicides in maize. Proc. Natl. Acad. Sci. USA 87:7175–7179.

Peeters, N. and I. Small. 2001. Dual targeting to mitochondria and chloroplasts. Biochim. Biophys. Acta 1541:54–63.

Podkowinski, J., G. E. Sroga, R. Haselkorn, and P. Gornicki. 1996. Structure of a gene encoding a cytosolic acetyl-CoA carboxylase of hexaploid wheat. Proc. Natl. Acad. Sci. USA 93:1870–1874.

Preston, C. 2004. Herbicide resistance in weeds endowed by enhanced detoxification: complications for management. Weed Sci. 52:448–453.

Preston, C. and S. B. Powles. 1998. Amitrole inhibits diclofop metabolism and synergises diclofop-methyl in a diclofop-methyl–resistant biotype of *Lolium rigidum*. Pestic. Biochem. Physiol. 62:179–189.

Preston, C., F. J. Tardif, J. T. Christopher, and S. B. Powles. 1996. Multiple resistance to dissimilar herbicide chemistries in a biotype of *Lolium rigidum* due to enhanced activity of several herbicide degrading enzymes. Pestic. Biochem. Physiol. 54:123–134.

Price, L. J., D. Herbert, D. J. Cole, and J. L. Harwood. 2003. Use of plant cell cultures to study graminicide effects on lipid metabolism. Phytochemistry 63:533–541.

Price, L. J., S. R. Moss, D. J. Cole, and J. L. Harwood. 2004. Graminicide resistance in a blackgrass (*Alopecurus myosuroides*) population correlates with insensitivity of acetyl-CoA carboxylase. Plant Cell Environ. 27: 15–26.

Ratterman, D. M. and N. E. Balke. 1988. Herbicidal disruption of proton gradient development and maintenance by plasmalemma and tonoplast vesicles from oat root. Pestic. Biochem. Physiol. 31:221–236.

Reade, J.P.H. and A. H. Cobb. 2002. New, quick tests for herbicide resistance in black-grass (*Alopecurus myosuroides* Huds) based on increased gluthatione *S*-transferase activity and abundance. Pest Manag. Sci. 58: 26–32.

Reade, J.P.H., L. J. Milner, and A. H. Cobb. 2004. A role for gluthatione *S*-transferase in resistance to herbicides in grasses. Weed Sci. 52:468–474.

Rendina, A. R., A. C. Craig-Kennard, J. D. Beaudoin, and M. K. Breen. 1990. Inhibition of acetyl-coenzyme A carboxylase by two classes of grass-selective herbicides. J. Agric. Food Chem. 38:1282–1287.

Rendina, A. R., J. M. Felts, J. D. Beaudoin, A. C. Craig-Kennard, L. L. Look, S. L. Paraskos, and J. A. Hagenah. 1988. Kinetics characterization, stereoselectivity, and species selectivity of the inhibition of plant acetyl-CoA carboxylase by the aryloxyphenoxypropionic acid grass herbicides. Arch. Biochem. Biophys. 265:219–225.

Retrum, J. and F. Forcella. 2002. Giant foxtail (*Setaria faberi*) seedling assay for resistance to sethoxydim. Weed Technol. 16:464–466.

Richter, J. and S. B. Powles. 1993. Pollen expression of herbicide target site resistance genes in annual ryegrass (*Lolium rigidum*). Plant Physiol. 102:1037–1041.

Roessler, P. G. 1990. Purification and characterization of acetyl-CoA carboxylase from the diatom *Cyclotella cryptica*. Plant Physiol. 92:73–78.

Roux, F., J. Gasquez, and X. Reboud. 2004. The dominance of the herbicide resistance cost in several *Arabidopsis thaliana* mutant lines. Genetics 166:449–460.

Sandermann, H., Jr. 2004. Molecular ecotoxicology of plants. Trends Plant Sci. 9:406–413.

Sasaki, Y., T. Konishi, and Y. Nagano. 1995. The compartmentation of acetyl-coenzyme A carboxylase in plants. Plant Physiol. 108:445–449.

Sasaki, Y. and Y. Nagano. 2004. Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. Biosci. Biotechnol. Biochem. 68:1175–1184.

Secor, J., C. Cseke, and J. W. Owen. 1989. The discovery of the selective inhibition of acetyl-coenzyme A carboxylase activity by two classes of graminicides. Brighton Crop Prot. Conf. Weeds 3B:145–154.

Seefeldt, S. S., D. L. Hoffman, D. R. Gealy, and E. P. Fuerst. 1998. Inheritance of diclofop resistance in wild oat (*Avena fatua* L.) biotypes from the Willamette Valley of Oregon. Weed Sci. 46:170–175.

Seng, T. W., T. R. Skillman, N. Yang, and C. Hammond. 2003. Cyclohexanedione herbicides are inhibitors of rat heart acetyl-CoA carboxylase. Bioorg. Med. Chem. Lett. 13:3237–3242.

Shimabukuro, R. H., D. G. Davis, and B. L. Hoffer. 2001. The effect of diclofop-methyl and its antagonist, vitamin E, on membrane lipids in oat (*Avena sativa* L.) and leafy spurge (*Euphorbia esula* L.). Pestic. Biochem. Physiol. 69:13–26.

Shimabukuro, R. H., W. C. Walsh, and R. A. Hoerauf. 1979. Metabolism and selectivity of diclofop-methyl in wild oat and wheat. J. Agric. Food Chem. 27:615–623.

Shukla, A., S. Dupont, and M. D. Devine. 1997a. Resistance to ACCase-inhibitor herbicides in wild oat: evidence for target site–based resistance in two biotypes from Canada. Pestic. Biochem. Physiol. 57:147–155.

Shukla, A., G. E. Leach, and M. D. Devine. 1997b. High-level resistance to sethoxydim conferred by an alteration in the target enzyme, acetyl-CoA carboxylase, in *Setaria faberi* and *Setaria viridis*. Plant Physiol. Biochem. 35:803–807.

Shukla, A., C. Nycholat, M. V. Subramanian, R. J. Anderson, and M. D. Devine. 2004. Use of resistant ACCase mutants to screen for novel inhibitors against resistant and susceptible forms of ACCase from grass weeds. J. Agric. Food Chem. 52:5144–5150.

Stoltenberg, D. E., J. W. Gronwald, D. L. Wyse, J. D. Burton, D. A. Somers, and B. G. Gegenbach. 1989. Effect of sethoxydim and haloxyfop on acetyl-coenzyme A carboxylase activity in *Festuca* species. Weed Sci. 37:512–516.

Tardif, F. J., J.A.M. Holtum, and S. B. Powles. 1993. Occurrence of a herbicide-resistant acetyl-coenzyme A carboxylase mutant in annual ryegrass (*Lolium rigidum*) selected by sethoxydim. Planta 190:176–181.

Tardif, F. J. and S. B. Powles. 1994. Herbicide multiple-resistance in a *Lolium rigidum* biotype is endowed by multiple mechanisms: isolation of a subset with resistant acetyl-CoA carboxylase. Physiol. Plant. 91: 488–494.

Tardif, F. J., C. Preston, J.A.M. Holtum, and S. B. Powles. 1996. Resistance to acetyl-coenzyme A carboxylase-inhibiting herbicides endowed by a single major gene encoding a resistant target site in a biotype of *Lolium rigidum*. Aust. J. Plant Physiol. 23:15–23.

Tal, A., E. Kotoula-Syka, and B. Rubin. 2000. Seed-bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. Crop Prot. 19:467–472.

Tal, A., M. L. Romano, G. R. Stephenson, A. L. Schwan, and J. C. Hall.

1993. Glutathione conjugation: a detoxification pathway for fenoxaprop-ethyl in barley, crabgrass, oat and wheat. Pestic. Biochem. Physiol. 46:190–199.

Tal, A. and B. Rubin. 2004. Molecular characterization and inheritance of resistance to ACCase-inhibiting herbicides in *Lolium rigidum*. Pest Manag. Sci. 601013–1018.

Turner, J. A. and D. J. Pernich. 2002. Origin of enantiomeric selectivity in the aryloxyphenoxypropionic acid class of herbicidal acetyl coenzyme A carboxylase (ACCase) inhibitors. J. Agric. Food Chem. 50: 4554–4566.

Van Eerd, L. L., R. E. Hoagland, R. M. Zablotowicz, and J. C. Hall. 2003. Pesticide metabolism in plants and microorganisms. Weed Sci. 51: 472–495.

Vila-Aiub, M. M., C. M. Ghersa, and M. Carceller. 2003. Effect of the herbicide diclofop-methyl on proton extrusion from *Lolium multiflorum* seedlings differing in resistance and fungal endophyte (*Neotyphodium* sp.) infection. Physiol. Plant. 119:429–439.

Vila-Aiub, M. M., P. Neve, K. J. Steadman, and S. B. Powles. 2005. Ecological fitness of a multiple herbicide resistant *Lolium rigidum* population: dynamics of seed germination and seedling emergence of target-site vs. metabolism-based resistant and susceptible phenotypes. J. Appl. Ecol. 42:288–298.

Volenberg, D. and D. Stoltenberg. 2002a. Altered acetyl-coenzyme A carboxylase confers resistance to clethodim, fluazifop and sethoxydim in *Setaria faberi* and *Digitaria sanguinalis*. Weed Res. 42:342–350.

Volenberg, D. S. and D. E. Stoltenberg. 2002b. Giant foxtail (*Setaria faberi*) outcrossing and inheritance of resistance to acetyl-coenzyme A carboxylase inhibitors. Weed Sci. 50:622–627.

Walker, K. A., S. M. Ridley, T. Lewis, and J. L. Harwood. 1988. Fluazifop, a grass-selective herbicide which inhibits acetyl-CoA carboxylase in sensitive plant species. Biochem. J. 254:307–310.

Wang, T., and H. Darmency. 1997. Inheritance of sethoxydim resistance in foxtail millet, *Setaria italica* (L.) Beauv. Euphytica 94:69–73.

Wang, T. and H. Darmency. 1998. Cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in foxtail millet (*Setaria italica*). Pestic. Biochem. Physiol. 59:81–88.

Webb, S. R., G. L. Durst, D. Pernich, and J. C. Hall. 2000. Interaction of cyclohexanediones with acetyl coenzyme-A carboxylase and an artificial target-site antibody mimic: a comparative molecular field analysis. J. Agric. Food Chem. 48:2506–2511.

Webb, S. R. and C. J. Hall. 2002. Development and evaluation of an immunological approach for the identification of novel acetyl coenzyme A carboxylase inhibitors: assay optimization and pilote screen results. J. Agric. Food Chem. 48:1219–1228.

Werck-Reichhart, D. and R. Feyereisen. 2000. Cytochromes P450: a success story. Genome Biol. 1:3003.1–3003.9.

Werck-Reichhart, D., A. Hehn, and L. Didierjean. 2000. Cytochromes P450 for engineering herbicide tolerance. Trends Plant Sci. 5:116–123.

Wiederholt, R. J. and D. E. Stoltenberg. 1996a. Similar fitness among large crabgrass (*Digitaria sanguinalis*) accessions resistant or susceptible to acetyl-coenzyme A carboxylase inhibitors. Weed Technol. 10:42–49.

Wiederholt, R. J. and D. E. Stoltenberg. 1996b. Absence of differential fitness among giant foxtail (*Setaria faberi*) accessions resistant and susceptible to acetyl-coenzyme A carboxylase inhibitors. Weed Sci. 44: 18–24.

Yu, Q., L.J.S. Friesen, X.-Q. Zhang, and S. B. Powles. 2004. Tolerance to acetolactate synthase and acetyl-coenzyme A carboxylase inhibiting herbicides in *Vulpia bromoides* is conferred by two co-existing resistance mechanisms. Pestic. Biochem. Physiol. 78:21–30.

Zagnitko, O., J. Jelenska, G. Tevzadze, R. Haselkorn, and P. Gornicki. 2001. An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexanedione inhibitors. Proc. Natl. Acad. Sci. USA 98:6617–6622.

Zhang, H., B. Tweel, J. Li, and L. Tong. 2004a. Crystal structure of the carboxyltransferase domain of acetyl-coenzyme A carboxylase in complex with CP-640186. Structure 12:1683–1691.

Zhang, H., B. Tweel, and L. Tong. 2004b. Molecular basis for the inhibition of the carboxyltransferase domain of acetyl-coenzyme-A carboxylase by haloxyfop and diclofop. Proc. Natl. Acad. Sci. USA 101: 5910–5915.

Zhang, H., Z. Yang, Y. Shen, and L. Tong. 2003. Crystal structure of the carboxyltransferase domain of acetyl-coenzyme A carboxylase. Science 299:2064–2067.

Received December 16, 2004, and approved April 26, 2005.