MECANISMOS DE RESISTENCIA A HERBICIDAS

Rafael De Prado

Universidad de Córdoba
España

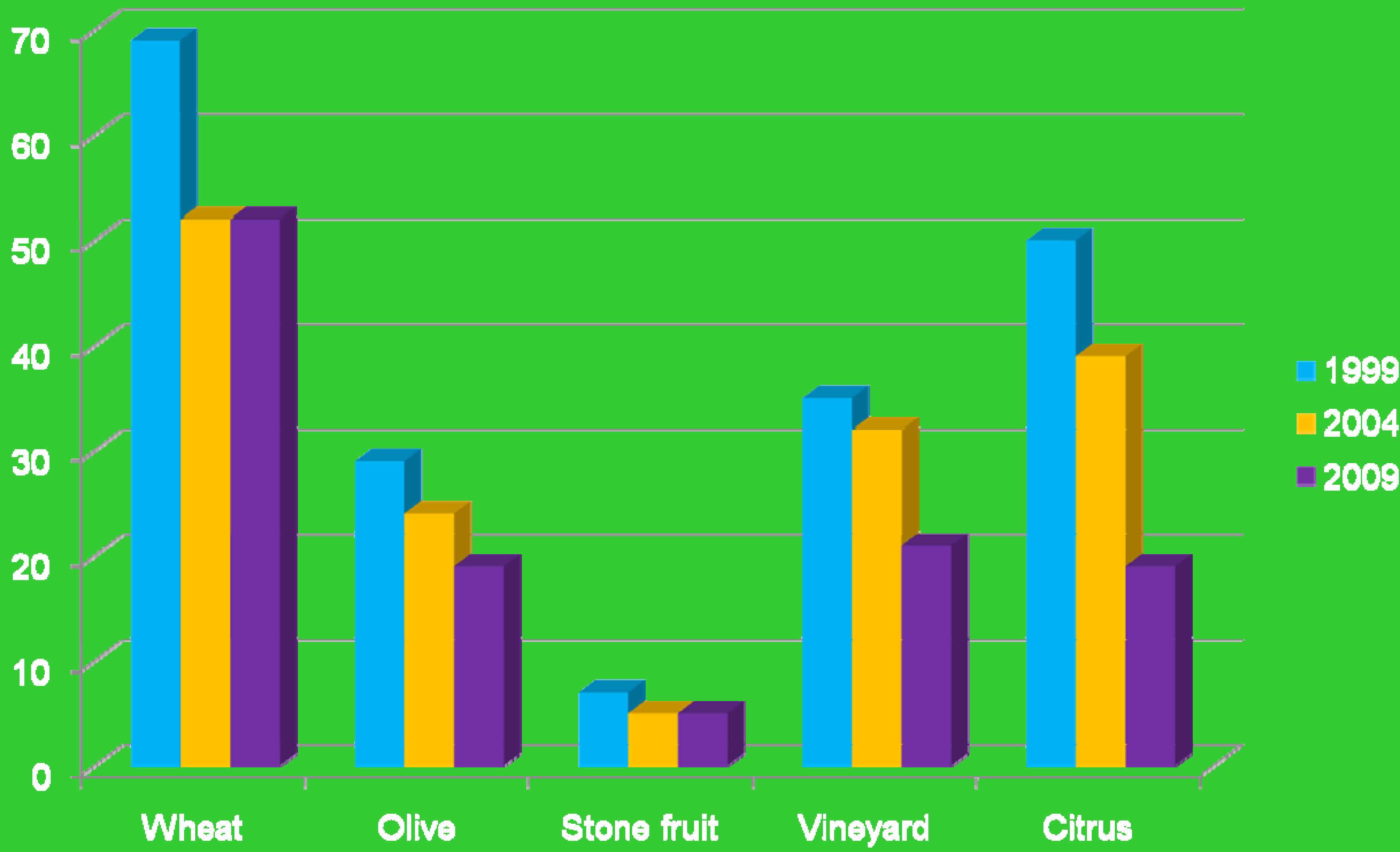
Agricultura sostenible

Aquélla en que los sistemas productivos permiten obtener beneficio continuo del uso de agua, suelo, recursos genéticos, etc., para satisfacer las necesidades actuales de la población sin destruir los recursos naturales básicos para las generaciones futuras.

Castillo, 1992.

University
of
Córdoba

Evolution of number of herbicide in wheat, olive, stone fruit, vineyard and citrus in Spain.



University of Córdoba

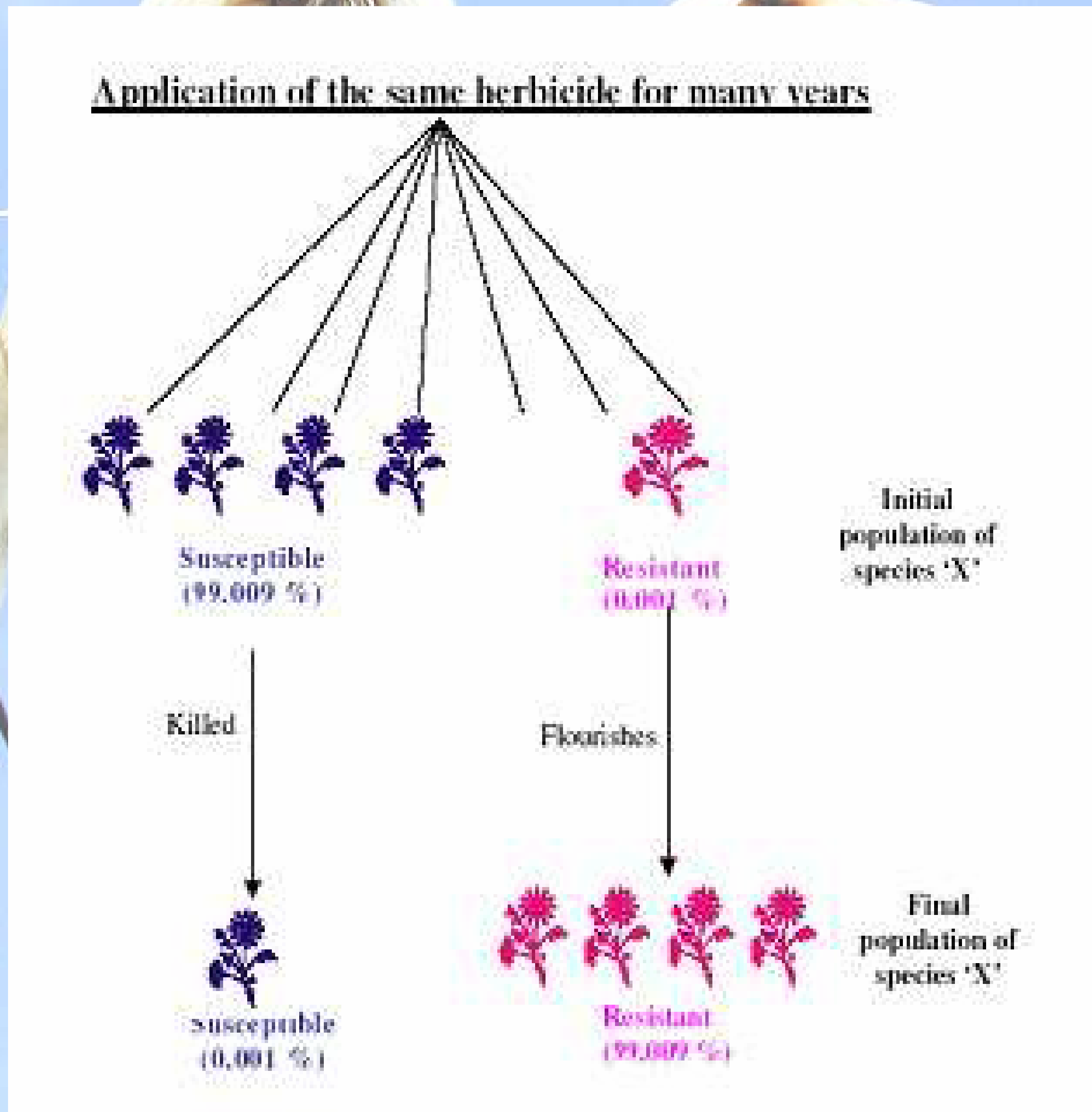
Analysis of the risk of the presence of herbicide-resistant weeds

Risk level	**Low 1**	**Medium3**	**High 5**	**Value**
1. Number of different crops in the rotation	>3	2 o 3	**1**	
2. Relation between winter crops (I) / spring crops (P)	I<P	I>P	**I**	
3. Tillage of the soil in the rotation: Tillage (L) / No Tillage (NL)	L>NL	NL>L	**NL**	
4. Mechanical control	>Twice	1 only	**None**	
5. Number of different herbicides (mode of action) for the weed control in the rotation	>3	2	**1**	
6. During how many consecutive growing seasons have you used herbicides with the same mode of action?	>3	2	**Always the same**	
7. Have you used herbicides with the same mode of action during the growing season?.	No	Once	**>Twice**	
8. Level of infestation of the plot	Low	Medium	**High**	
9. Quality of control obtained by the herbicide during the last 3 or 4 years	**Good Constant**	**Medium Variable**	**Insufficient Decreasing**	
			TOTAL	**¿?**

If the TOTAL number is under 18 Low risk
If the TOTAL number is between 18 and 32 Moderate risk
If the TOTAL number is over 32 High risk

University of Córdoba

The evolution of Herbicide Resistance (percent values are arbitrary)

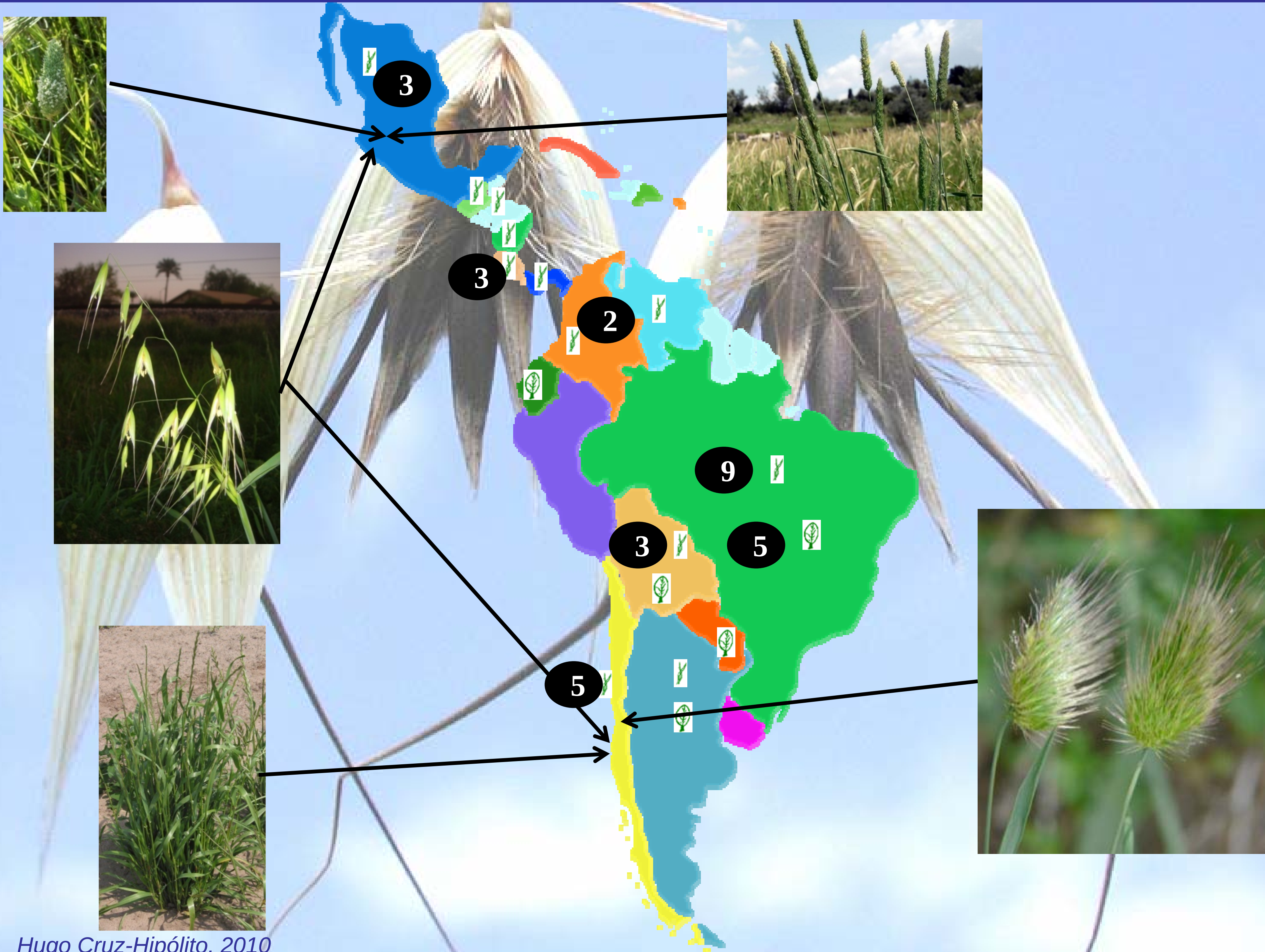


Tharayil-Santhakumar, N. 2003

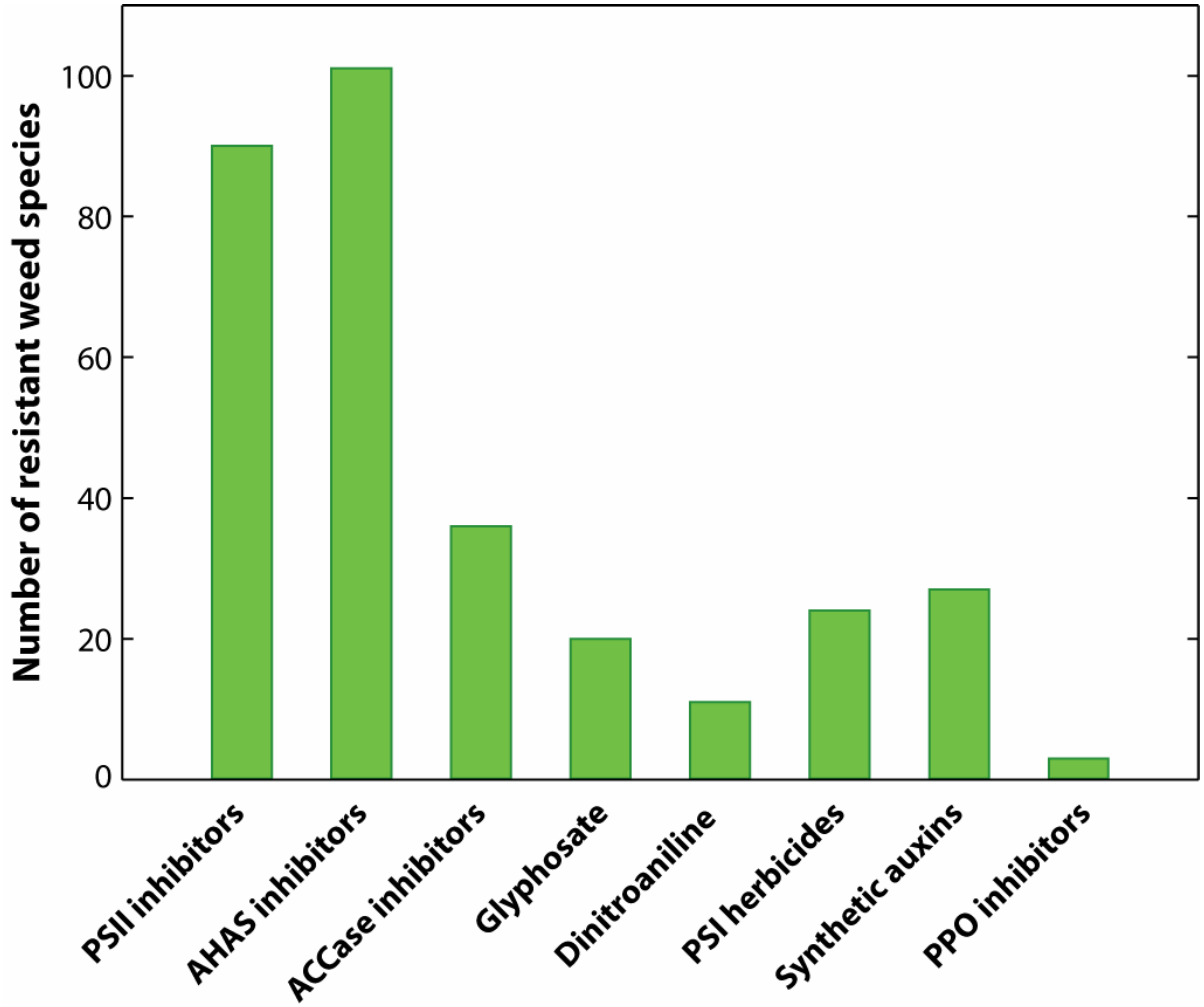
The Weed Science Society of America (WSSA) defines herbicide resistance as:

"the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type".

Distribución de la resistencia en Latinoamérica



Hugo Cruz-Hipólito, 2010

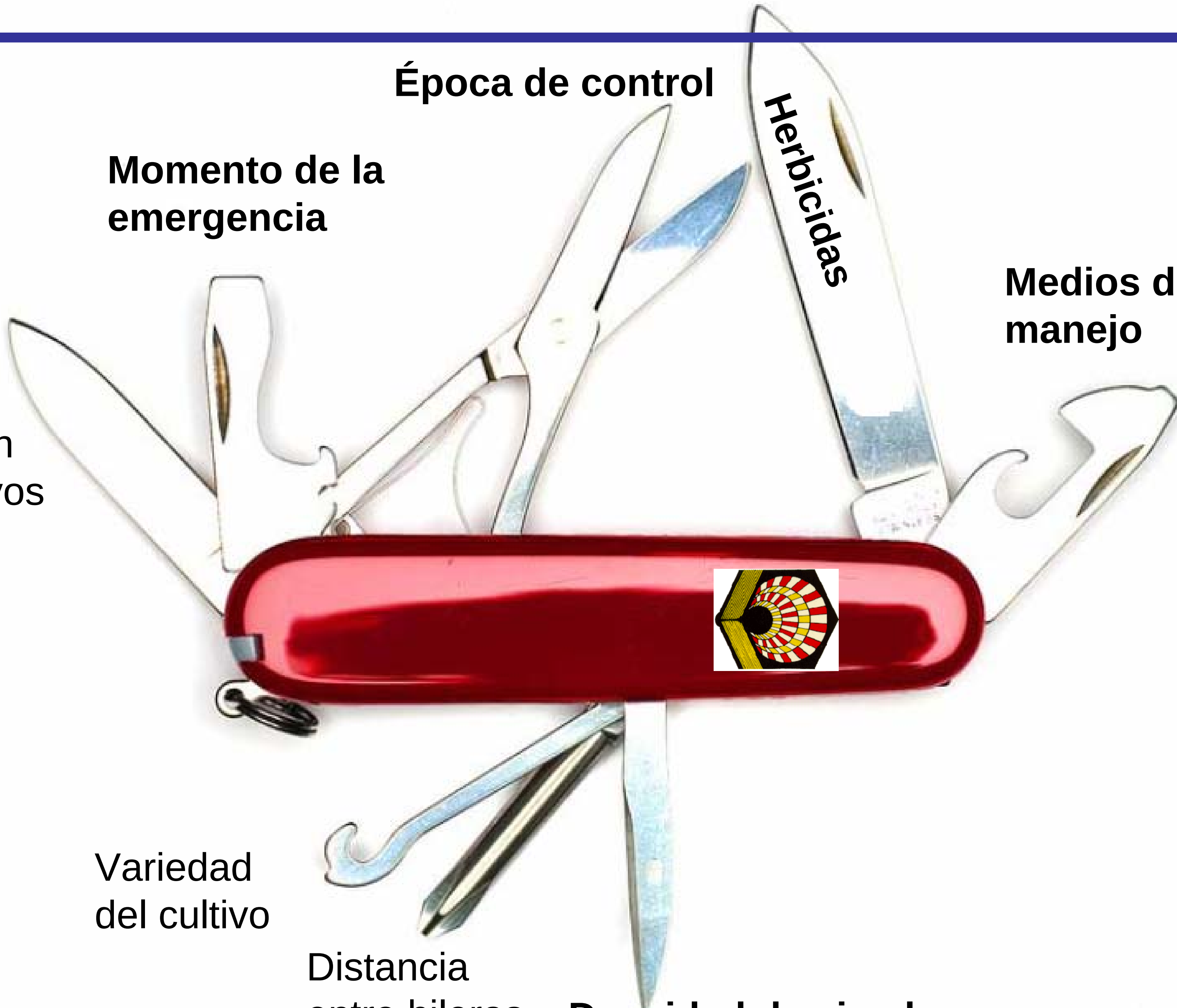


Powles SB, Yu Q. 2010.
Annu. Rev. Plant. Biol. 61:317–47

El conocimiento de los procesos biológicos responsables de la resistencia a herbicidas en una determinada mala hierba es fundamental para el diseño de una estrategia de control

A. FISCHER, 2008

Control de malas hierbas



Hugo Cruz-Hipólito, 2010

Resistencia cruzada

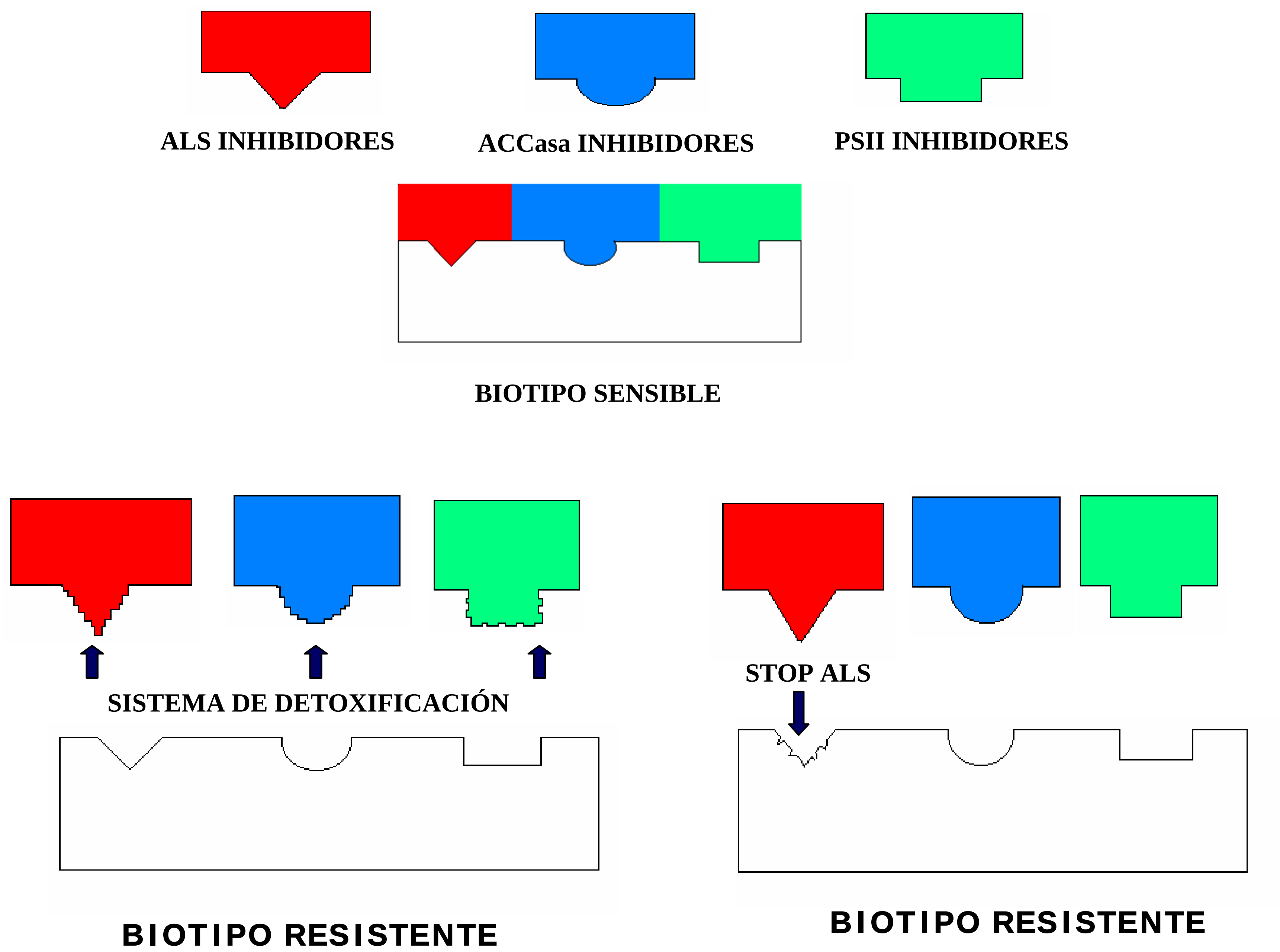


Table 2. Parameters of the Equation[a] Used To Calculate the Herbicide Dose Required for 50% Plant Injury (ED_{50}) of Resistant (R1) and Susceptible (S) Biotypes of *S. mucronatus* Using Different Herbicides

herbicide	accession	*c*	*d*	*b*	ED_{50} (g ha^{-1})	pseudo r^b	P^c	RF^d
bensulfuron-methyl	R	1.472	99.784	3.143	319.54	0.99	<0.001	1718.9
	S	2.788	100	1.376	0.1859	0.98	<0.001	
bispiribac-sodium	R	1.076	99.99	1.569	23.2	0.98	<0.0001	1.705
	S	2.471	100	1.5	13.6	0.99	<0.0001	
cyclosulfamuron	R	1.343	99.72	1.296	155.0	0.99	<0.0001	775
	S	3.293	100	2.005	0.2	0.99	<0.0001	
ethoxysulfuron	R	2.191	100	3.734	1576.8	0.99	<0.0001	59.501
	S	2.275	99.76	5.637	26.5	0.99	<0.0001	
imazamox	R	2.714	100	1.956	8.6	0.99	<0.0001	43
	S	0.976	100	4.239	0.2	0.98	<0.0001	
imazosulfuron	R	2.612	100	5.799	562.6	0.98	<0.0001	1406.5
	S	0.718	100	3.550	0.4	0.99	<0.0001	
pyrazosulfuron-ethyl	R	3.374	100	2.856	49.0	0.99	<0.0001	122.5
	S	0.020	100	3.211	0.4	0.98	<0.0001	

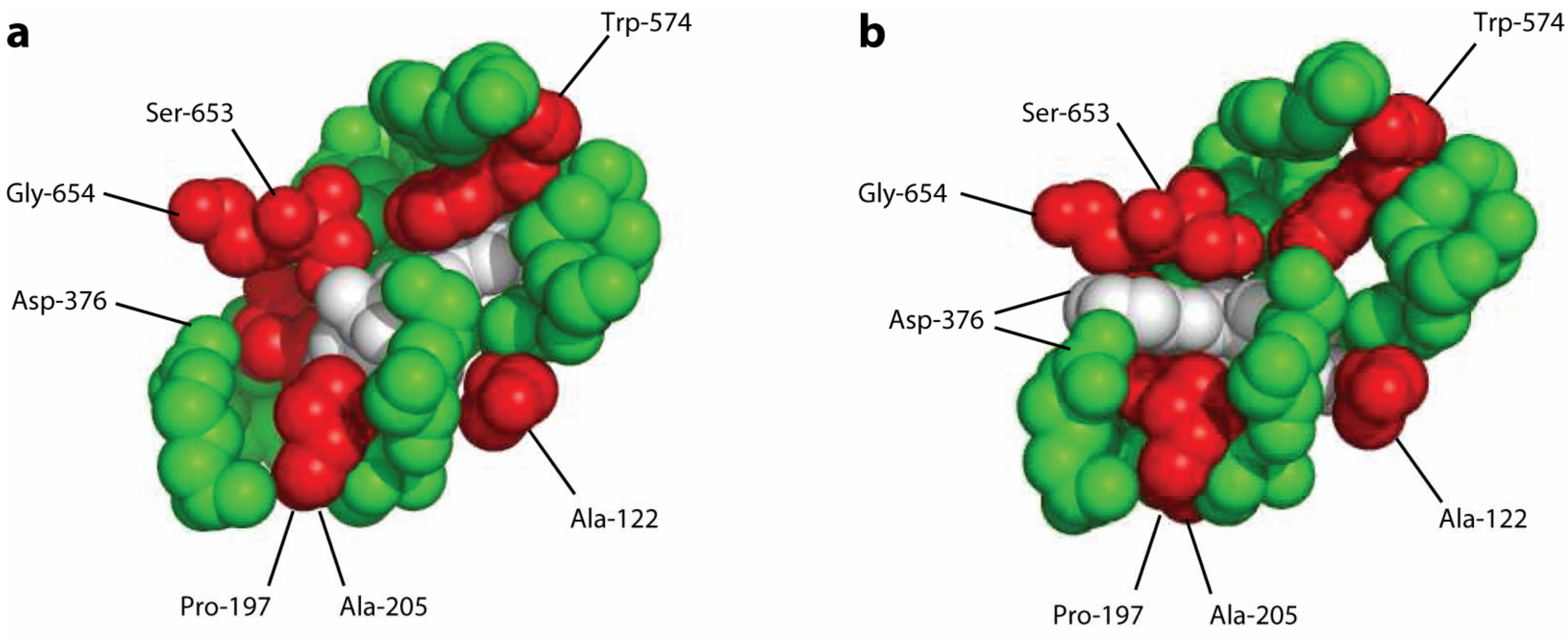
[a] Equation $Y = c + \{(d - c)/[1 + (x/g)^b]\}$, where *Y* is the percentage of plant injury, *x* (independent variable) is the herbicide rate, *c* and *d* are the lower and upper asymptotes, *b* is the slope of the line, and ED_{50} is the effective dose required for 50% plant injury. Data were pooled and fitted to nonlinear regression model. Data are means of four replicates. [b] Approximate coefficient of determination of nonlinear models with a defined intercept calculated as pseudo r^2 = 1 - (sums of squares of the regression/corrected total sums of squares). [c] Probability level of significance of the nonlinear model. [d] RF = resistant factor = ED_{50} of resistant/ED_{50} of susceptible biotype.

Table 5. Parameters of the Equation[a] Used To Calculate the Herbicide Concentration Required for 50% Reduction of the ALS activiy (I_{50}) of Resistant (R) and Susceptible (S) Biotypes of *S. mucronatus*

herbicide	accession	*c*	*d*	*b*	I_{50}	pseudo r^{2} [b]	*P*[c]	RF[d]
bensulfuron-methyl	R	3.711	98.463	2.044	1163.0	0.975	<0.0001	161.527
	S	1.043	100.00	1.2036	7.2	0.984	<0.0001	
bispiribac-sodium	R	8.073	99.94	1.525	157.0	0.988	<0.0001	5.322
	S	1.253	98.743	1.819	29.5	0.985	<0.0001	
cyclosulfamuron	R	7.719	99.718	1.982	3.2	0.982	<0.0001	106.666
	S	0.423	100.00	1.194	0.03	0.99	<0.0001	
ethoxysulfuron	R	1.879	97.812	2.328	7.3	0.96	<0.0001	24.333
	S	0.368	97.563	2.149	0.3	0.967	<0.0001	
imazamox	R	1.169	97.911	1.875	1105.7	0.96	<0.0001	10.925
	S	1.562	98.492	1.171	101.2	0.981	<0.0001	
imazosulfuron	R	1.655	99.729	1.725	162.2	0.982	<0.0001	147.454
	S	1.21	99.267	1.923	1.1	0.978	<0.0001	
pyrazosulfuron-ethyl	R	5.768	97.242	1.645	149.5	0.964	<0.0001	6.826
	S	1.482	99.59	1.49	21.9	0.989	<0.0001	

[a] Equation $Y = c + \{(d - c)/[1 + (x/g)^{b}]\}$, where *Y* is the percentage of plant injury, *x* (independent variable) is the herbicide concentration, *c* and *d* are the lower and upper asymptotes, *b* is the slope of the line, and I_{50} is the effective dose required for 50% reduction of ALS activity. Data were pooled and fitted to nonlinear regression model. Data are means of four replicates. [b] Approximate coefficient of determination of nonlinear models with a defined intercept calculated as pseudo $r^{2} = 1 -$ (sums of squares of the regression/corrected total sums of squares). [c] Probability level of significance of the nonlinear model. [d] RF = resistant factor = I_{50} of resistant /I_{50} of susceptible biotype.

Simulation model of *Arabidopsis* AHAS structure in complex with the SU herbicide chlorsulfuron (*a*) or the IMI herbicide imazaquin (*b*). The herbicides are colored white; the residues that have evolved resistance substitutions are colored red. Note that the SU herbicide is bound deeper and closer to and has more contact with the catalytic site than does the IMI herbicide. The perspective of the images is that the atoms of the herbicides at the bottom left are those that are at the entrance of the channel, and those at the top right are inside the channel, leading to the catalytic site. (S. Friesen & S. Powles, unpublished data.)



Powles SB, Yu Q. 2010.
Annu. Rev. Plant. Biol. 61:317–47

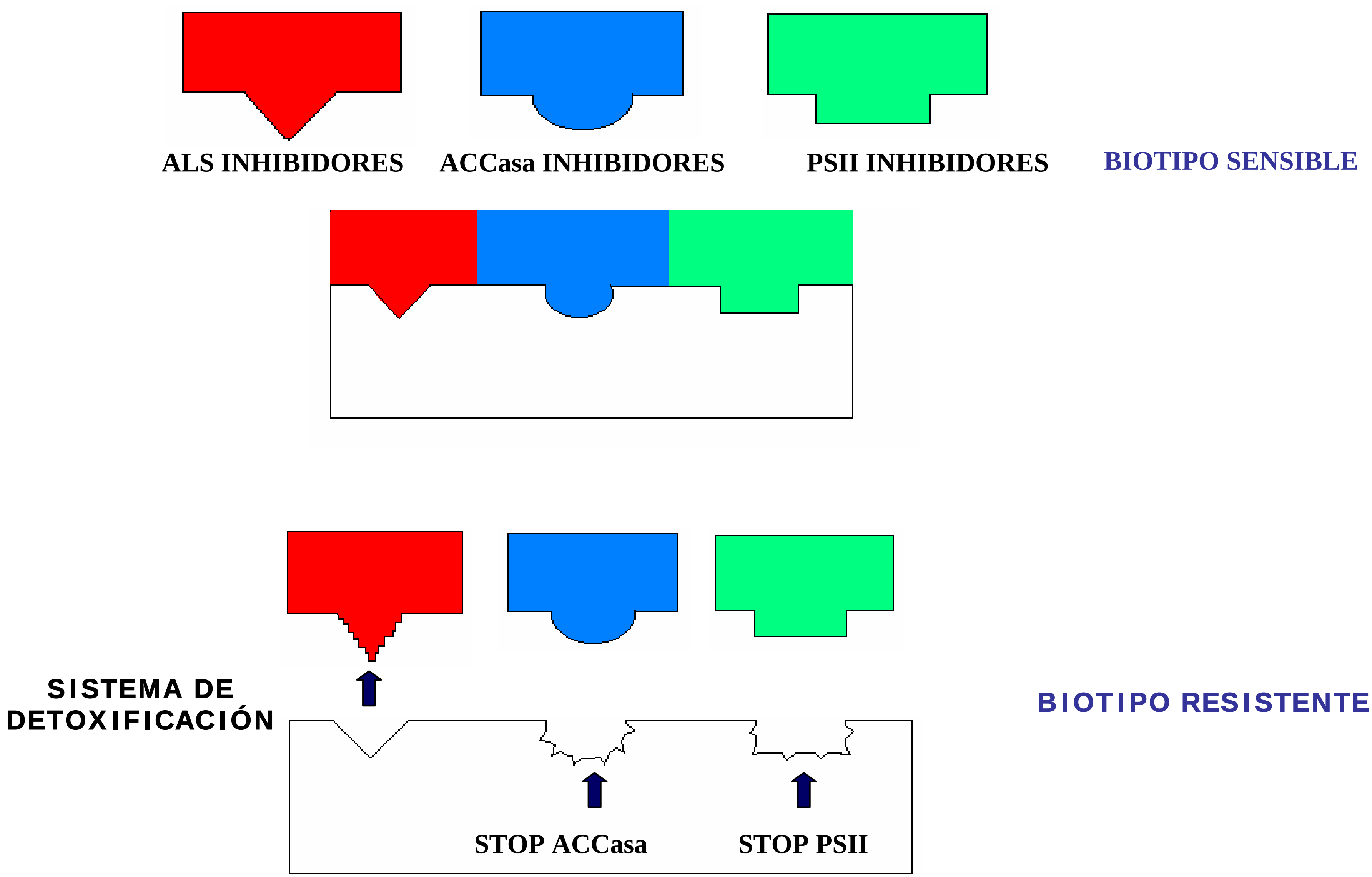
ALS amino acid substitutions that confer herbicide resistance.

AARN	SCR	Weed Species	SU(2)	IMI(2)	PTB(2)	TP(2)	SCT(2)	Year(3)
Pro 197*	His	*Lactuca serriola*	R	r	S	r	ND	1992
	His	*Papaver rhoeas*	R	S	ND	r	ND	2004
	His	*Scirpus juncoides var. ohwianus*	R	S	S	ND	ND	2007
	His	*Scirpus mucronatus*	R	R	R	R	R	2009

AARN =Amino Acid Residue and Number
SCR=Substitution conferring resistance

SU = Sulfonylureas
IMI = Imidazolinones
PTB= Pyrimidinylthiobenzoates
TP= Triazolopyrimidines
SCT=Sulfonylaminocarbonyltriazolinone

Resistencia múltiple



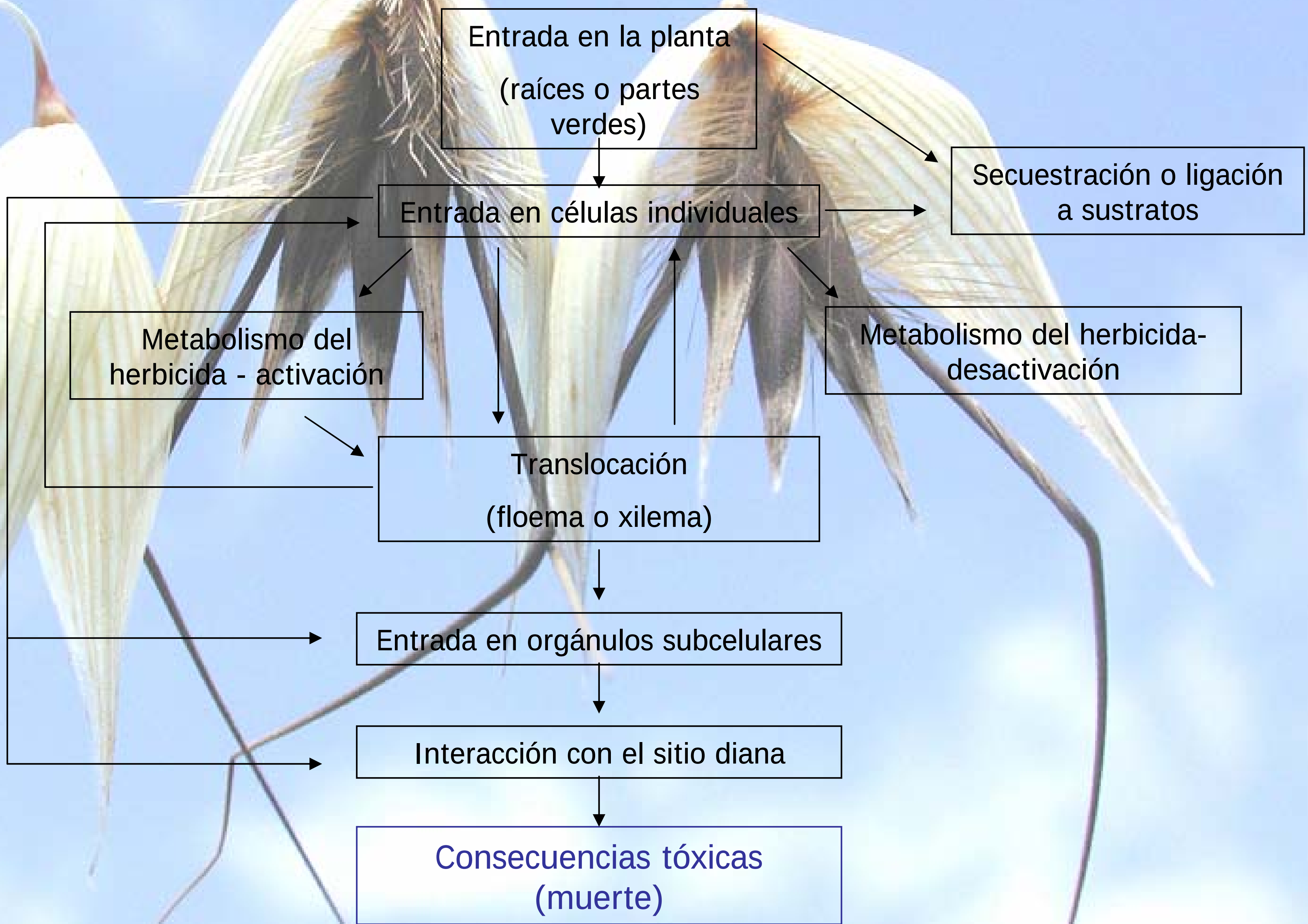
Resistencia de *Lolium rigidum* a diferentes grupos de herbicidas

Inhibidores PS II y PS I	Inhibidores ALS	Inhibidores ACCAsa	Inhibidores Mitosis	Inhibidores EPSPS	Otros modos de acción
s-Triazinas Ureas sustituidas Triazinonas	Sulfonilureas Imidazolinonas	APPs DIMs	Dinitroanilinas Carbamatos	Glicina	Cloroacetamidas Isoxazoles 1-4 triazoles

S. Powles *et al*., 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *L. rigidum* biotype. **Planta**: 225: 499-513.

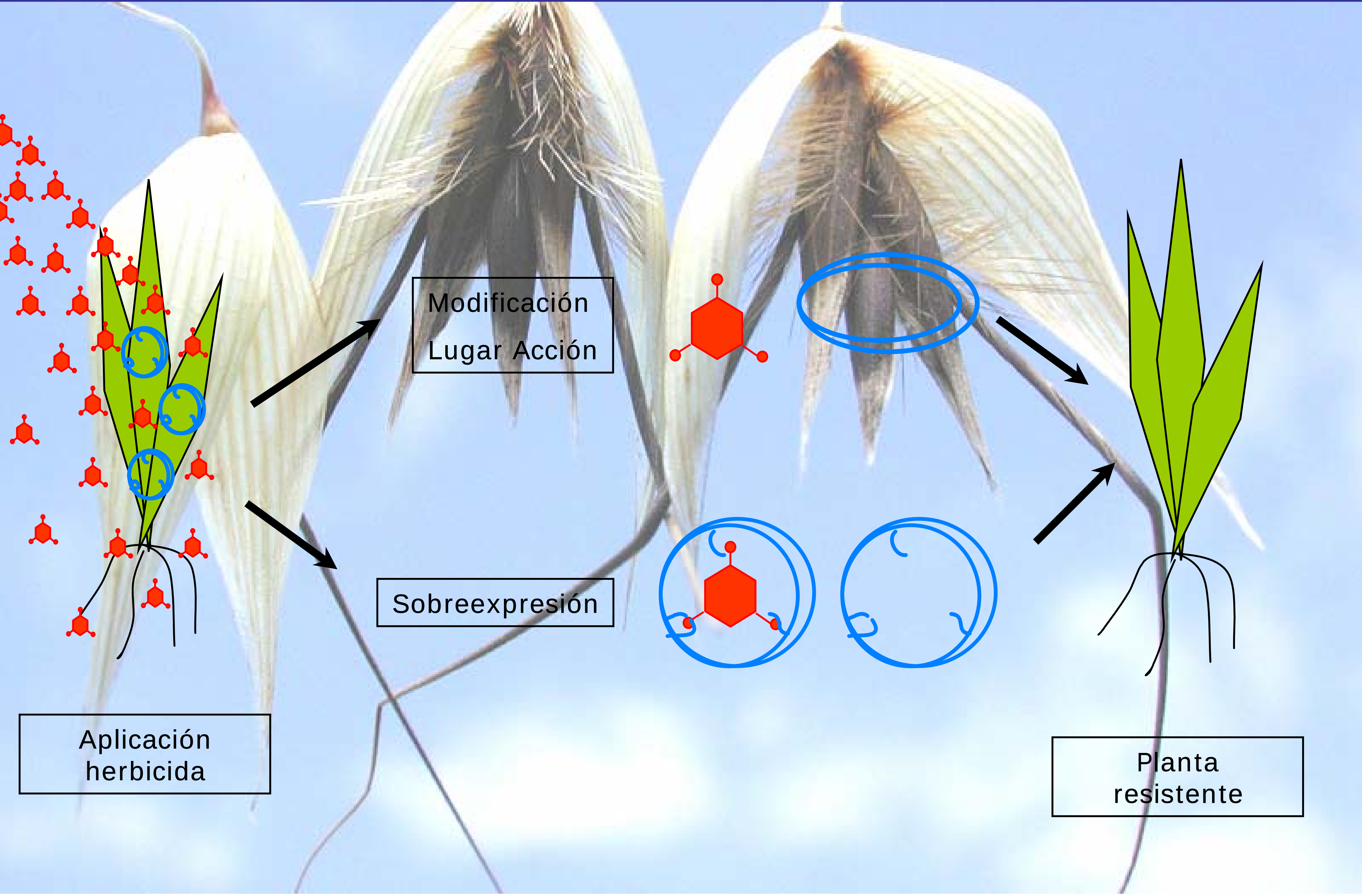
S. Powles *et al*., 2009. Distinct non-target site mechanisms endow resistance to glyphosate, ACCase and ALS-inhibiting herbicides in multiple herbicide-resistant *L. rigidum*. **Planta**: 230: 713-723.

Herbicidas



Hugo Cruz-Hipólito

Mecanismos de resistencia (TSR)



Resistencia de sitio de acción

Pérdida de afinidad por el sitio de acción

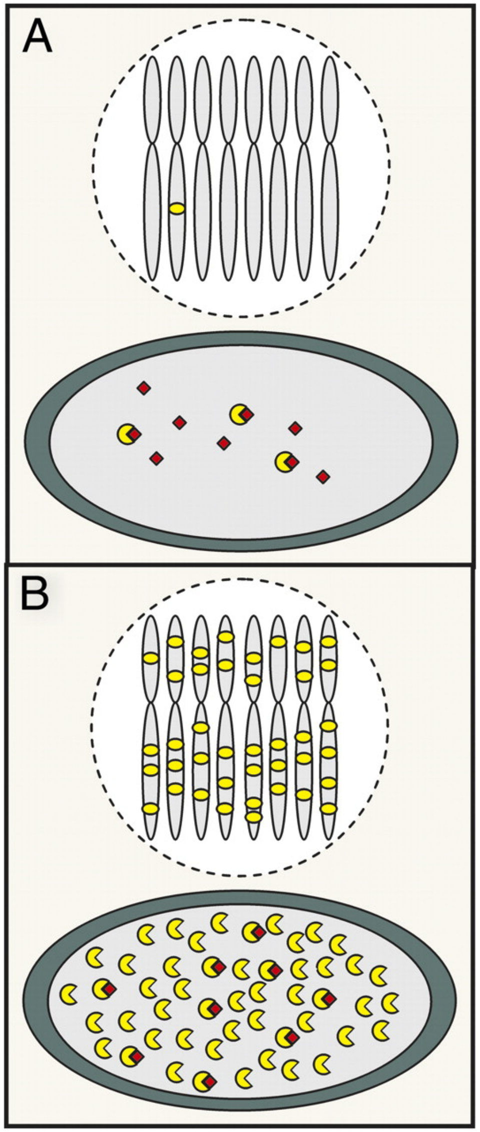
Avena fatua. Recolectada en Chile

Herbicida	Accesión	ED_{50}	FR	I_{50}	FR
Fenoxaprop	S	38,82		0,90	
	R	201,79	5,20	2,71	3,01
Diclofop	S	37,76		35,19	
	R	208,14	4,04	108,21	3,07
Cletodim	S	38,78		3,81	
	R	201,24	5,19	8,69	2,28
Cicloxidim	S	38,72		7,83	
	R	201,18	5,20	19,76	2,52

Table 3 Resistance-endowing plastidic ACCase CT domain amino acid substitutions in field-evolved resistant grass weed species

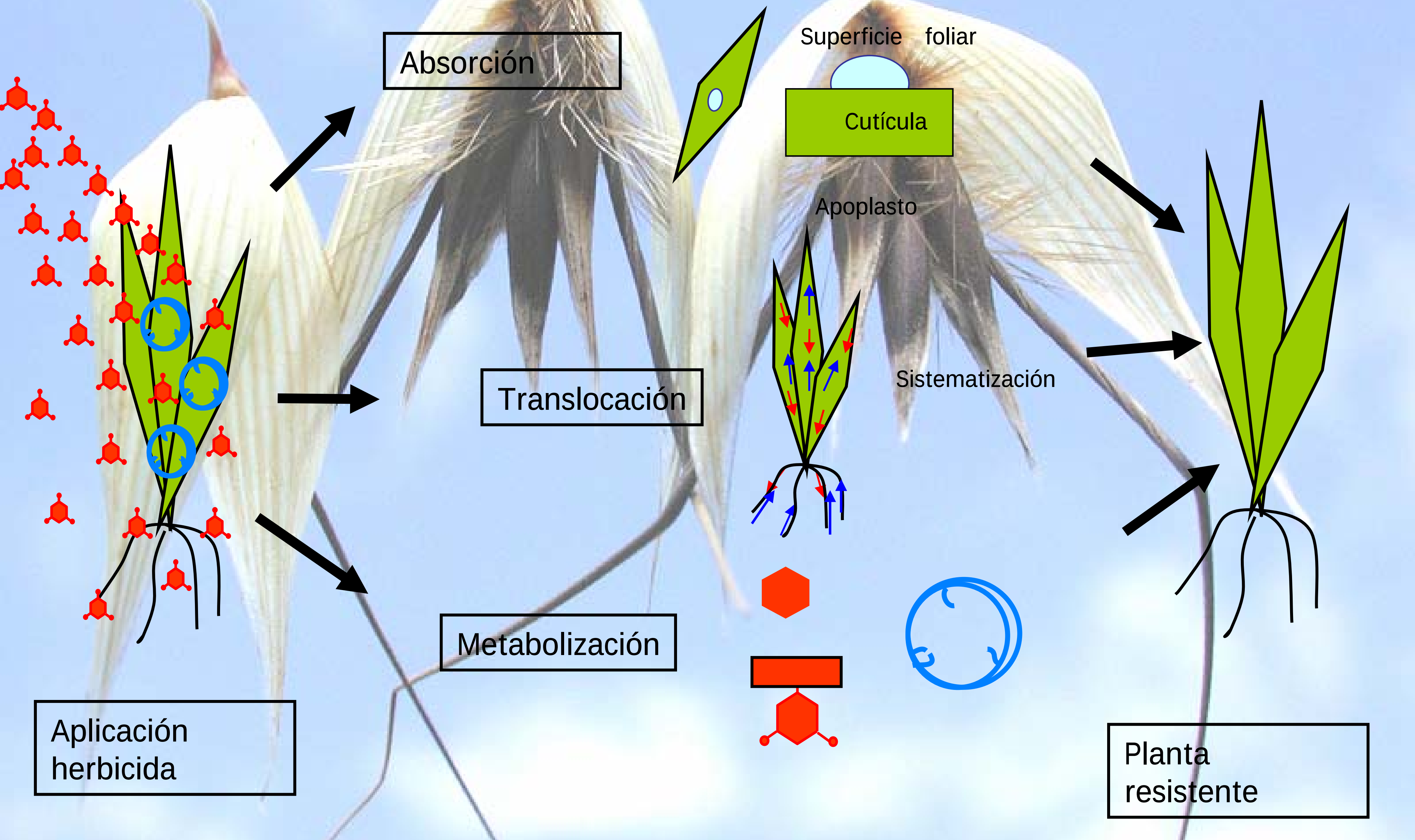
Amino acid substitution	Grass weed species	Resistance spectrum APP	CHD	PPZ	References
Ile-1781-Leu	*Alopecurus myosuroides*	R	R	R	Petit et al., 2010
	Avena fatua	R	R	r	Christoffers y Pederson, 2007
	A. sterilis	R	R	?	Liu et al., 2007
	Lolium multiflorum	?	R	?	White et al., 2005
	L. rigidum	R	R	R	Yu et al., 2007
	Setaria viridis	R	R	?	
Trp-1999-Cys	*A. sterilis*	R/S	S	?	Liu, 2007
Trp-2027-Cys	*A. myosuroides*	R	S	R	Petit et al., 2010
	A. sterilis	R/r	r	?	Petit et al., 2010
	L. rigidum	?	r	?	Yu et al., 2007
Ile-2041-Asn	*A. myosuroides*	R	S	r	Petit et al., 2010
	A. sterilis	R	r	?	Petit et al., 2010
	Phalaris paradoxa	?	?	?	Hochberg et al., 2009
	L. rigidum	R	r/S	?	Yu et al., 2007
Ile-2041-Val	*L. rigidum*	S/R	S	?	
Asp-2078-Gly	*A. myosuroides*	R	R	R	Petit et al., 2010
	A. fatua	R	R	R	Cruz-Hipolito 2010
	A. sterilis	R	R	?	Petit et al., 2010
	L. multiflorium	R	R	R	Kaundun, 2010
	L. rigidum	R	R	R	Yu et al., 2007
	P. paradoxa	R	R	R	Hochberg et al., 2009
Cys-2088-Arg	*L. rigidum*	R	R	R	Yu et al., 2007
Gly-2096-Ala	*A. myosuroides*	R	r/S	S	Petit et al., 2010

Glyphosate resistance resulting from gene amplification.



Powles S B PNAS 2010;107:955-956

Mecanismos de resistencia (NTSR)



Hugo Cruz-Hipólito

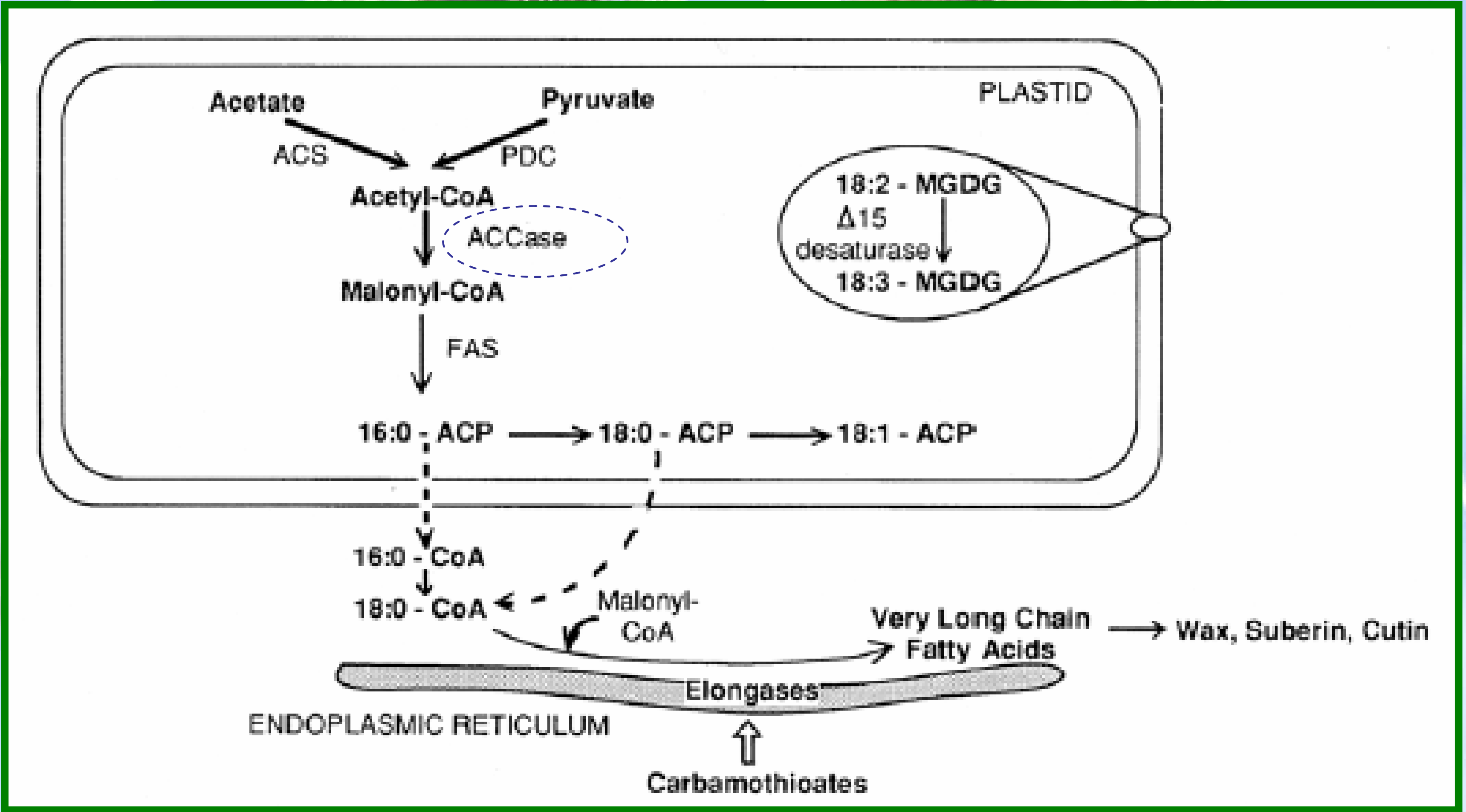
Resistencia fuera del sitio de acción
Metabolización a especies no tóxicas

Tabla 2. Resumen de las tres fases del metabolismo de plaguicidas (Adaptado de SHIMABUKURO, 1985; DE PRADO *et al.*, 2004)

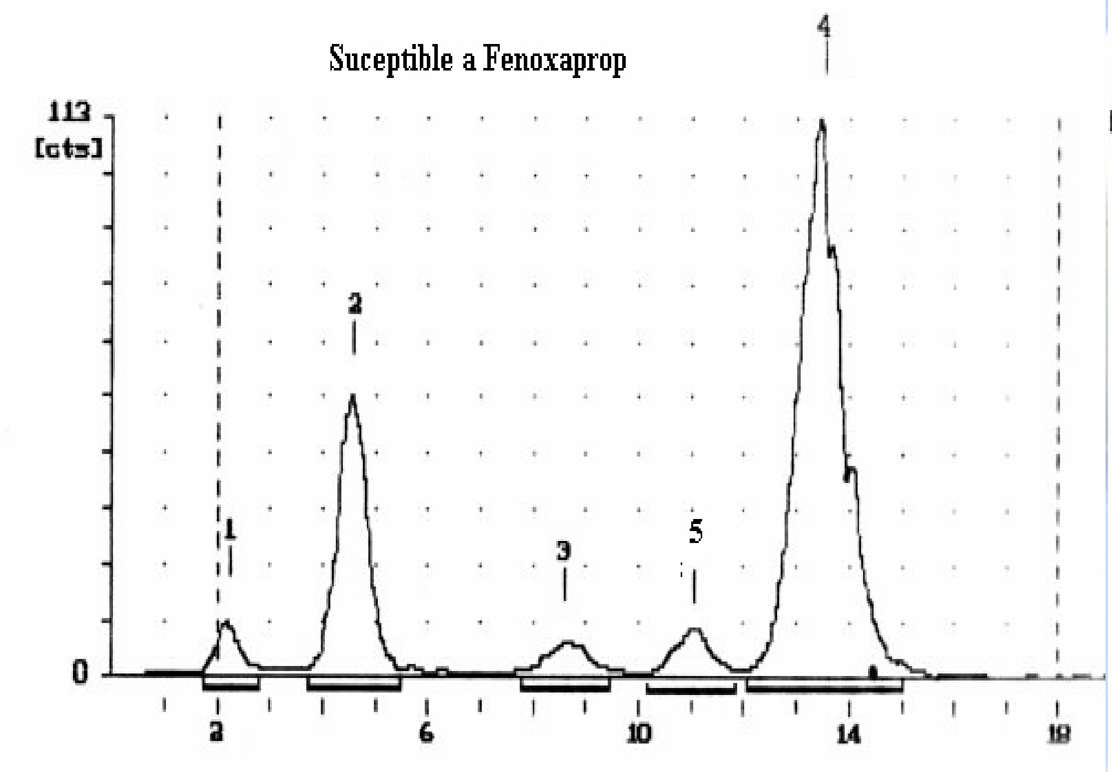
Características	**Propiedades iniciales**	**Fase I**	**Fase II**	**Fase III**
Reacciones	Compuesto inicial	Oxidación, hidrólisis, reducción	Conjugación	Conjugación secundaria o incorporación a biopolímeros
Solubilidad	Lipofílico	Anfofílico	Hidrofílico	Hidrofílico o insoluble
Fototoxicidad	Tóxico	Modificado o menos tóxico	Muy reducida o no tóxico	No tóxico
Movilidad	Selectiva	Modificada o reducida	Limitada o inmóvil	Inmóvil

Acetil Coenzima A carboxilasa (ACCasa)

- ACCasa, acetil coenzima A carboxilasa.
- Cataliza el primer paso en la biosíntesis de ácidos grasos.



Metabolismo de ^{14}C-fenoxaprop-p-etil 24 HDT en *L. multiflorum*. Absorción foliar.



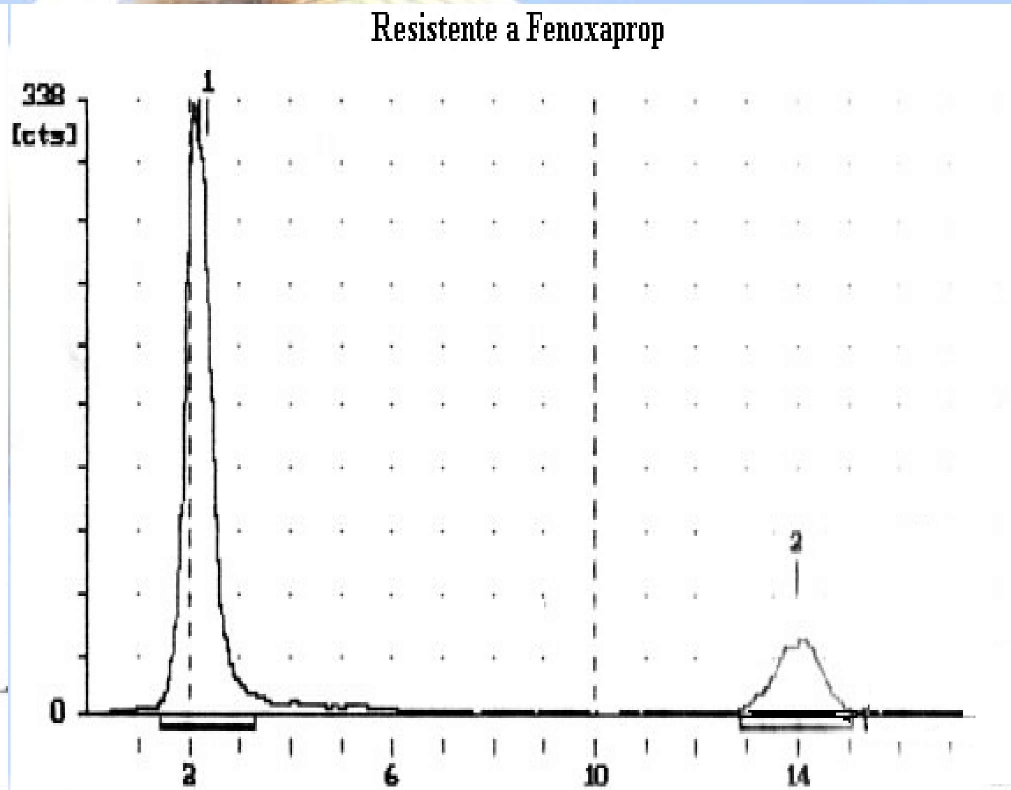
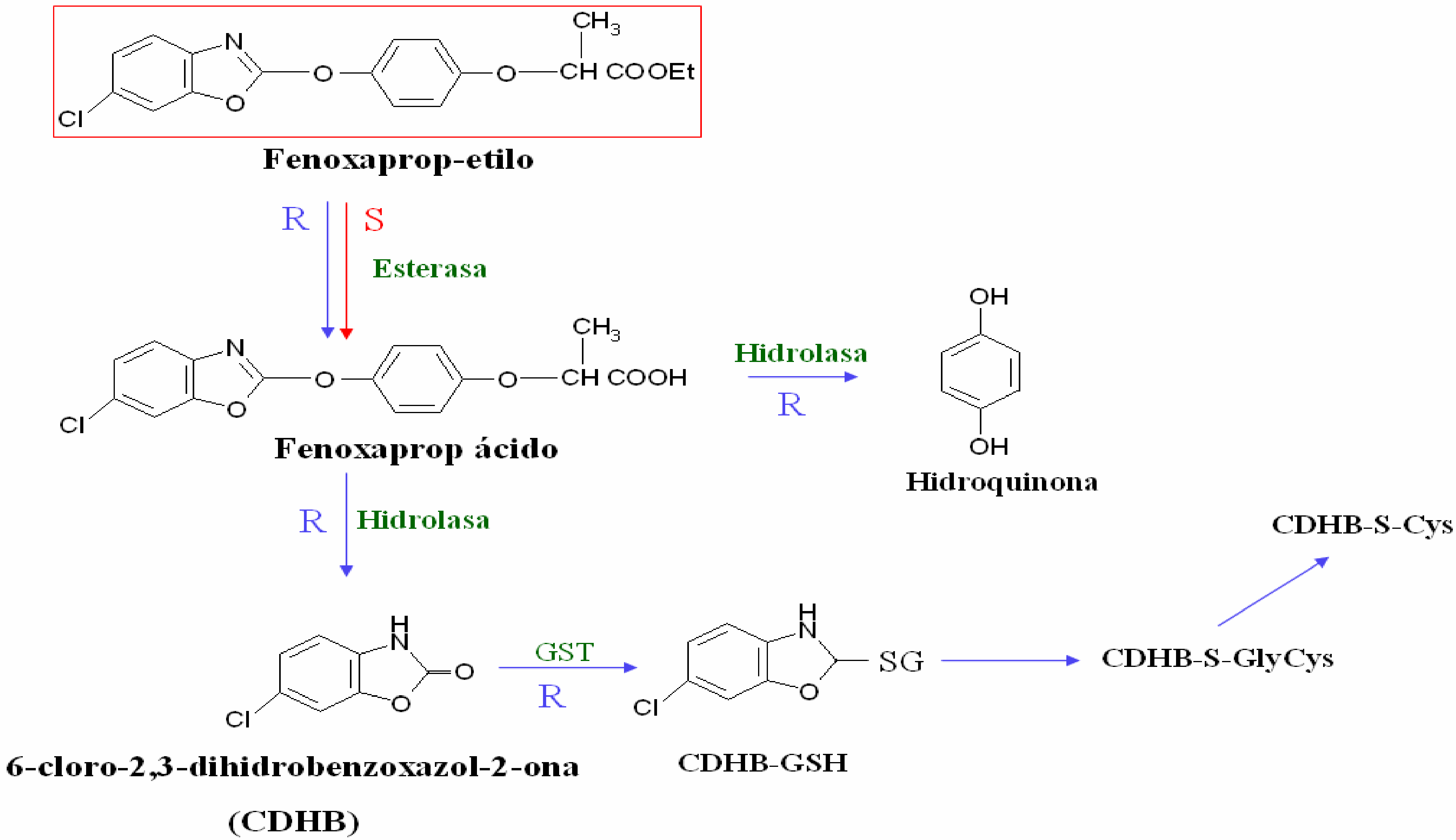


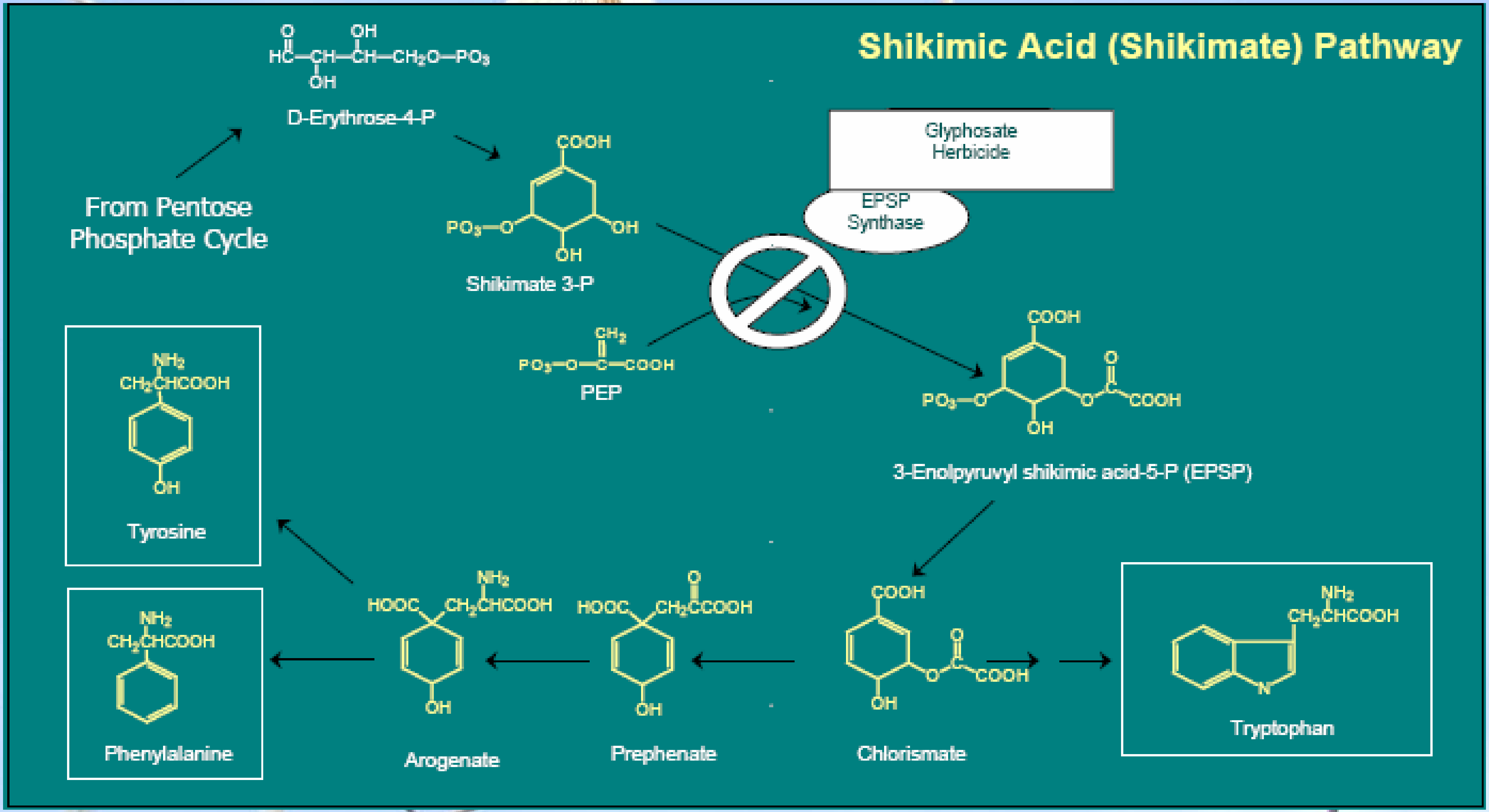
Figura 5. Ruta metabolica de detoxificación de fenoxaprop-etilo en *Loliun multiflorum*.



Resistencia fuera del sitio de acción

Reducción de la concentración de herbicida en el sitio de acción

S R


Shikimic Acid (Shikimate) Pathway
D-Erythrose-4-P
From Pentose Phosphate Cycle
Shikimate 3-P
Glyphosate Herbicide
EPSP Synthase
PEP
3-Enolpyruvyl shikimic acid-5-P (EPSP)
Tyrosine
Phenylalanine
Arogenate
Prephenate
Chlorismate
Tryptophan

Especies de malezas que incrementan su grado de infestación en soja transgénica resistente a glifosato en Argentina[1].

Nombre científico
Anoda cristata (L.) Schltdl.
Artemisia annua L.
Clematis montevidensis Spreng
Commelina erecta L.
Conyza bonariensis (L.) Cronq.[2]
Convolvulus arvensis L.
Eleusine indica (L.) Gaertn[3]
Hybanthus parviflorus (L.f.) Baill.
Ipomoea purpurea (L.) Roth and other species
Iresine diffusa Humb and Bonpl.
Oenothera indecora Cambess
Parietaria debilis G. Foster
Petunia axillaris (Lam.) Britton, Sterns & Pogg.
Rumex crispus L.
Sida rhombifolia L.
Solanum chacoense Bitter.
Spharalcea bonariensis (Cav.) Griseb.
Trifolium repens L.
Verbena bonaerensis L.
Viola arvensis Murr
Wedelia glauca Oct. Hoffmann

[1] Recopilado de Valverde B. y Gressel J. , 2006.

[2] Reportada como resistente a glifosato en Brasil, España y Sudáfrica.

[3] Reportada como resistente a glifosato en Malasia, Taiwán y Bolivia.

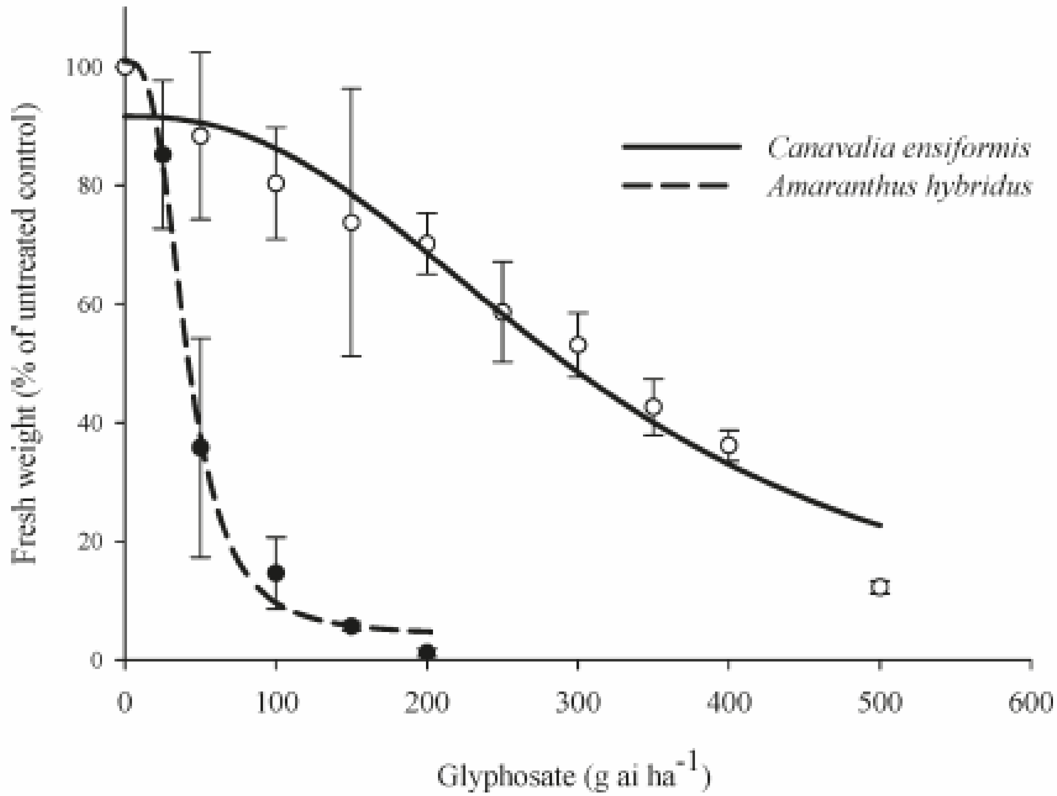


Figure 1. Dose−response assays of *C. ensiformis* (○) and *A. hybridus* (●). The plant fresh weight was determined 21 DAT, and data are expressed as percentage of the untreated control; each point is the mean ± standard errors (SE) of three experiments.

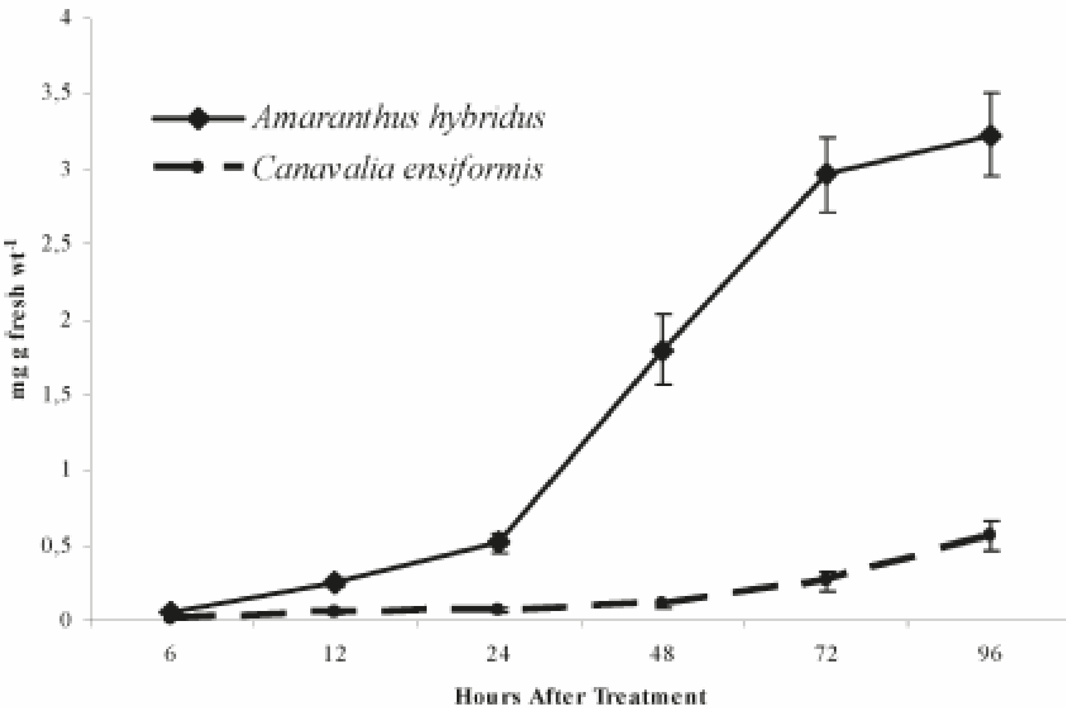


Figure 2. Shikimic acid accumulation in shoots of *A. hybridus* and *C. ensiformis* plants following the application of glyphosate at 500 g ha^{-1}. Vertical bars represent $\pm$ standard errors of the mean.

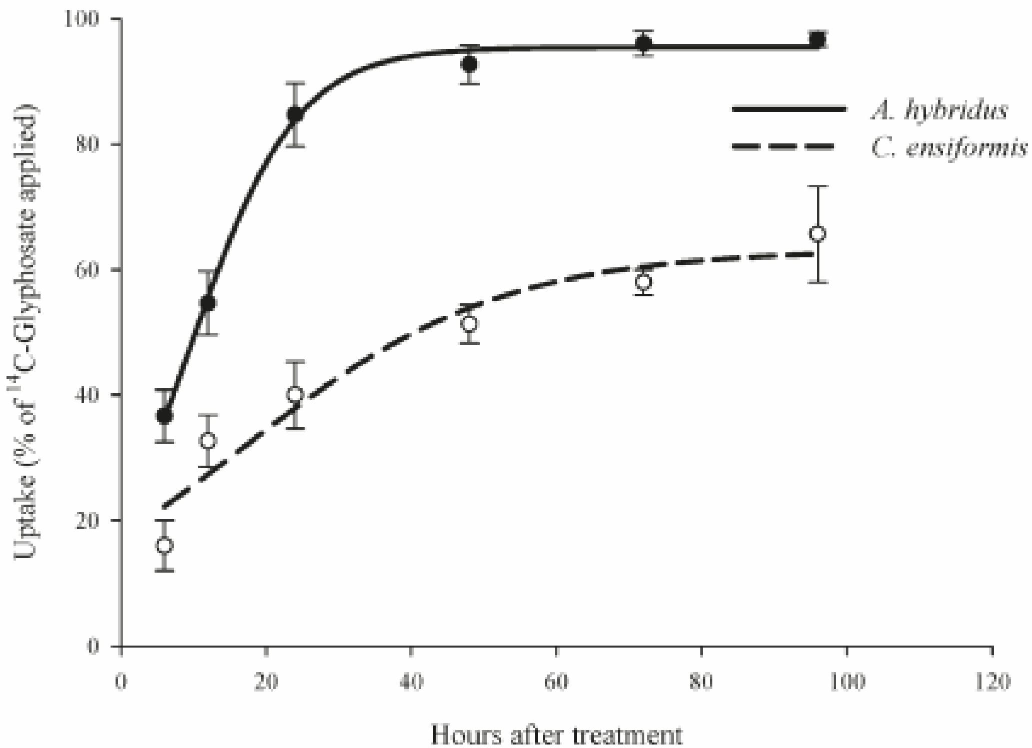
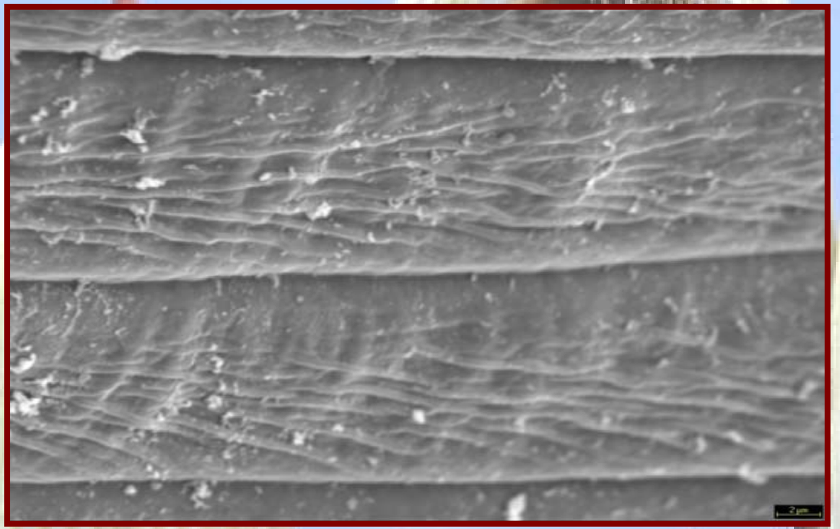
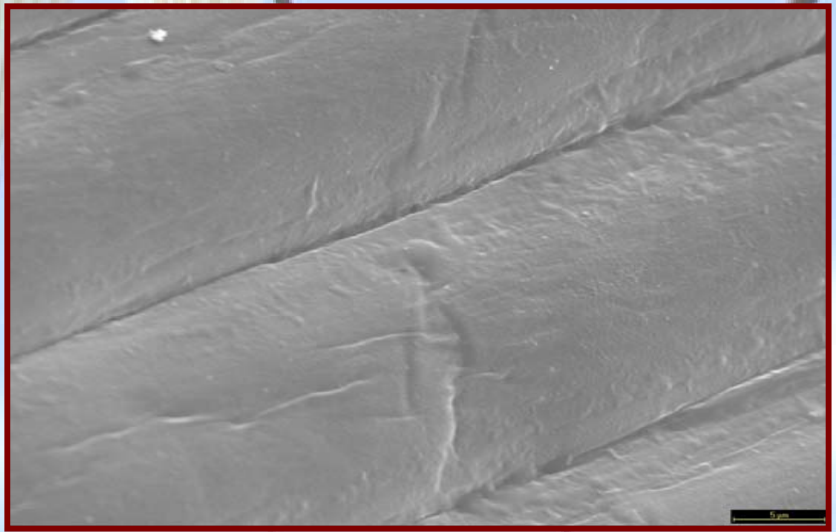


Figure 3. Foliar absorption of [^{14}C]glyphosate in *A. hybridus* and *C. ensiformis* over 96 h. Vertical bars represent ± standard errors of the means.

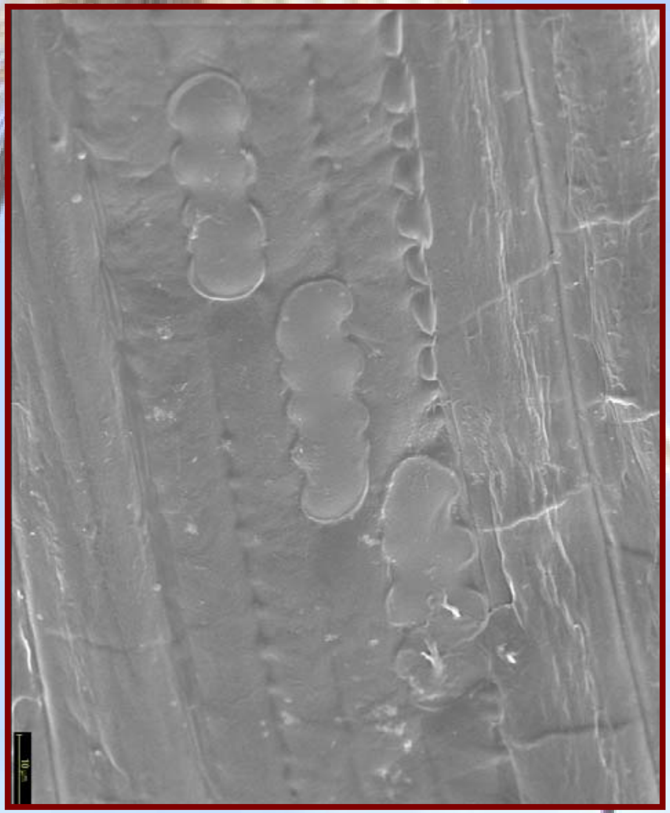
Resistente (M: 3000)



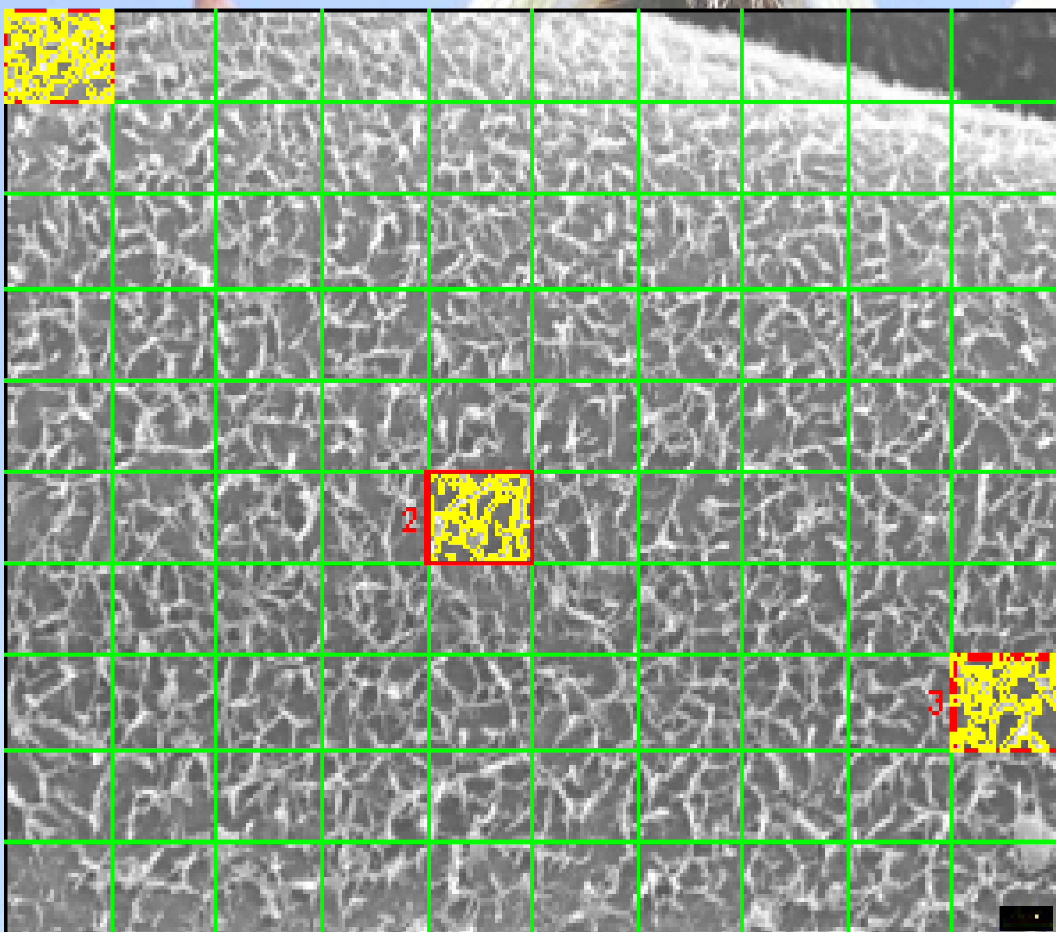
Susceptible (M: 2500)



Resistente (M: 1000)



Ceras epicuticulares



} 2µm²

Cuantificación con un CAD software

Área cubierta por ceras (%)	
S	R
42,9 (0,66)	102,6 (2,38)

Table 2. Translocation (Percent of Absorbed Radioactivity) of [^{14}C]Glyphosate in *Amaranthus hybridus* and *Canavalia ensiformis*

h after treatment	species	[^{14}C]glyphosate (% of absorbed)[a]		
		treated leaf	root	rest of plant
24	*A. hybridus*	80.00 ± 2.00 B	8.33 ± 1.53 JK	11.66 ± 2.08 IJ
	C. ensiformis	96.66 ± 3.06 A	0.00 ± 0.00 L	3.33 ± 0.58 KL
48	*A. hybridus*	64.00 ± 2.30 C	16.00 ± 1.40 HI	20.00 ± 1.80 GH
	C. ensiformis	84.00 ± 2.40 B	2.00 ± 0.20 KL	14.00 ± 0.80 HIJ
72	*A. hybridus*	40.00 ± 3.4 D	28.00 ± 4.80 EF	32.00 ± 4.30 E
	C. ensiformis	68.00 ± 2.10 C	8.00 ± 0.80 JK	24.00 ± 2.3 FG

[a] Means within a column followed by the same letter are not significantly different at the 5% level as determined by the Tukey test. Values ± standard error of the mean; 0 = nondetected.

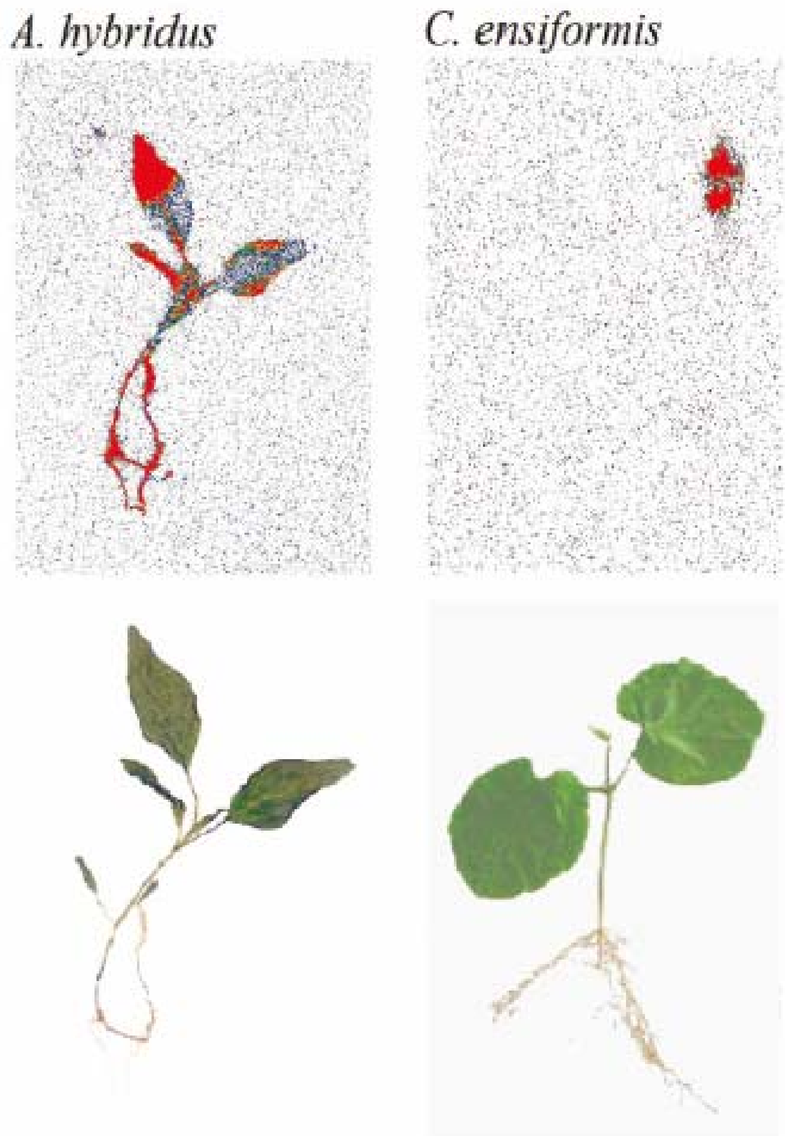


Figure 4. Phosphorimaging visualization of [^{14}C]glyphosate translocation of *A. hybridus* (left) and *C. ensiformis* (right), 24 HAT.

Resistencia fuera del sitio de acción

Resistencia asociada a procesos de secuestración o compartimentación

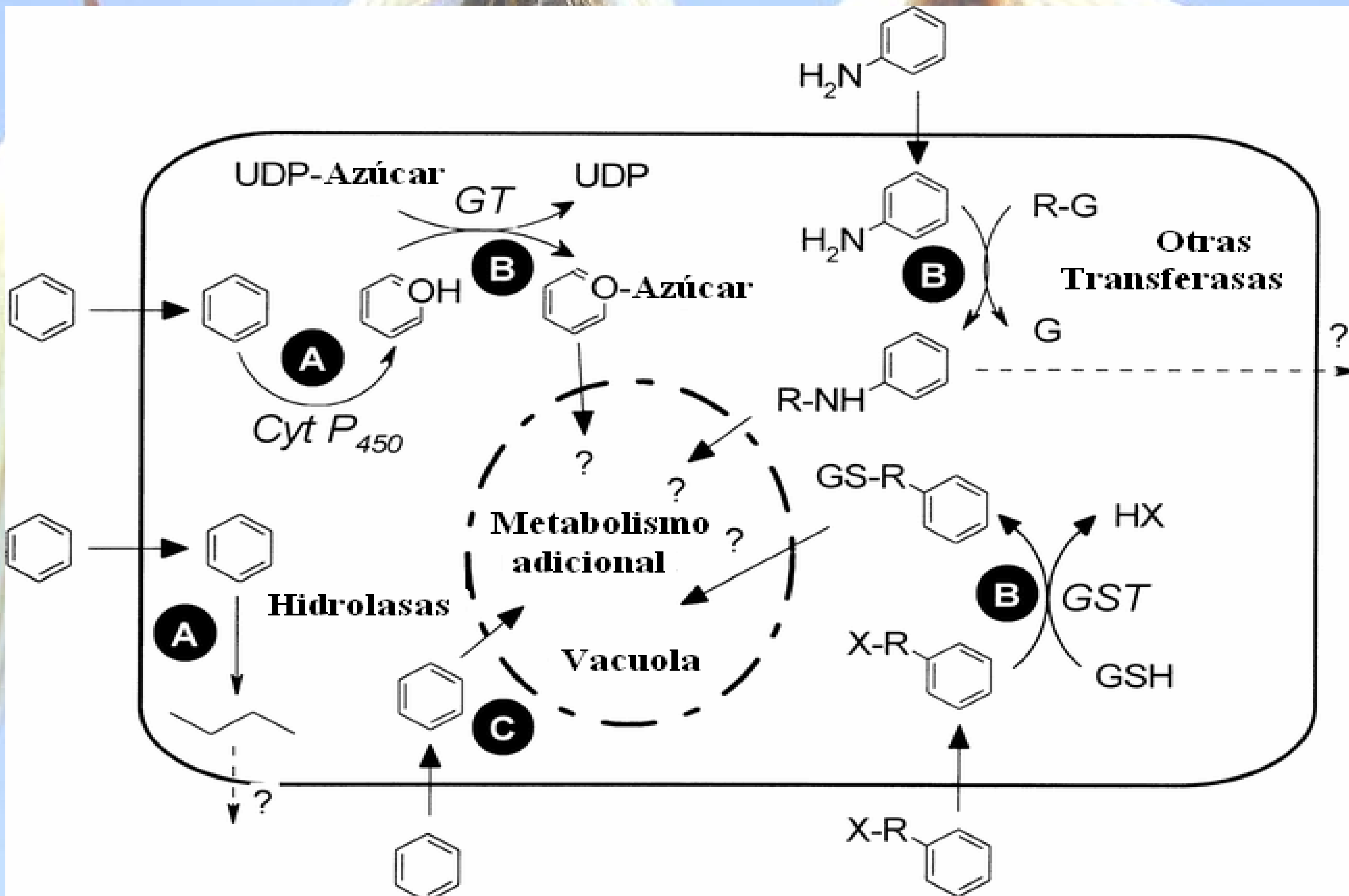


Figura 7. Rutas metabólicas de detoxificación de herbicidas en malezas (Adaptado de DE PRADO y FRANCO, 2004).


GRACIAS

Sitio de acción del isoproturón

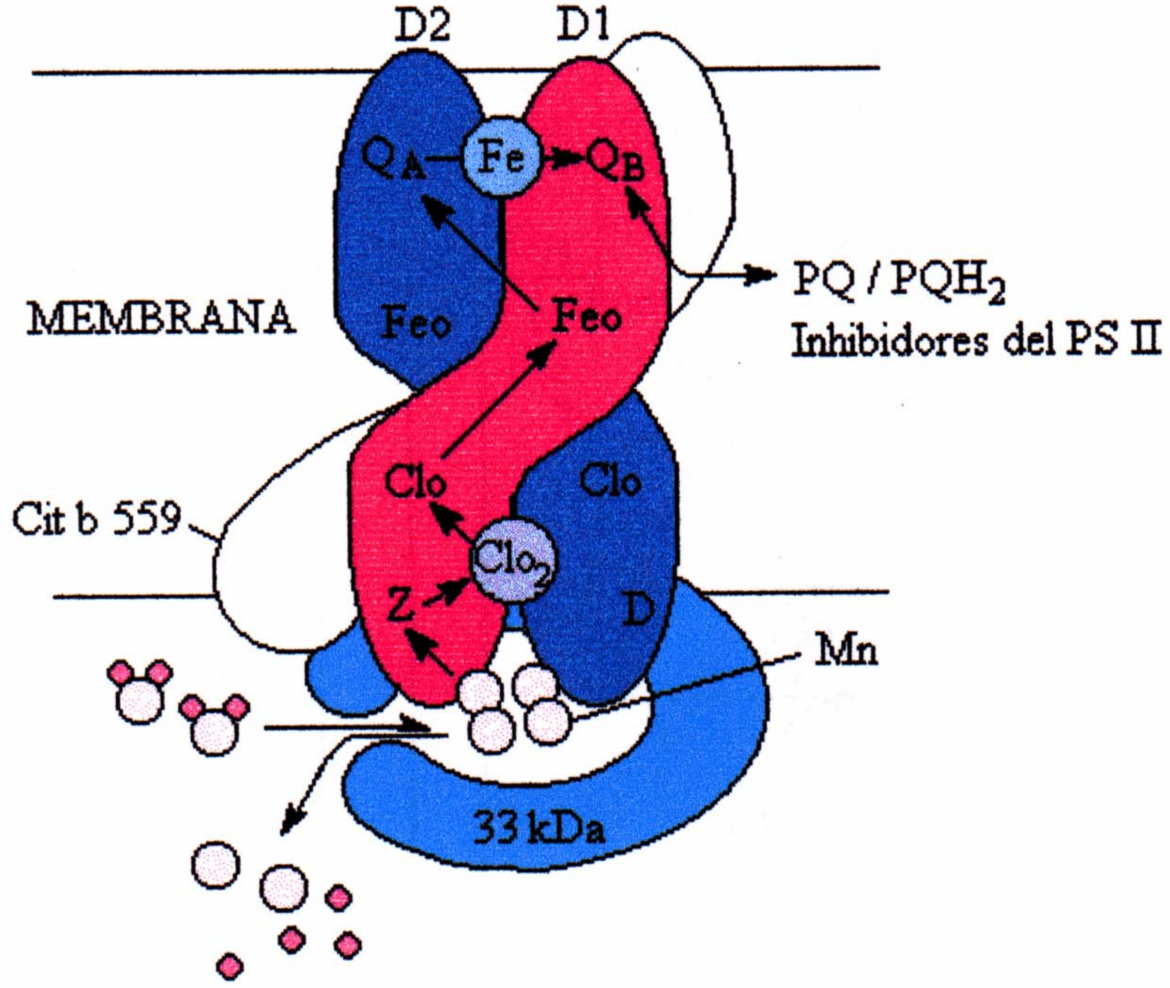
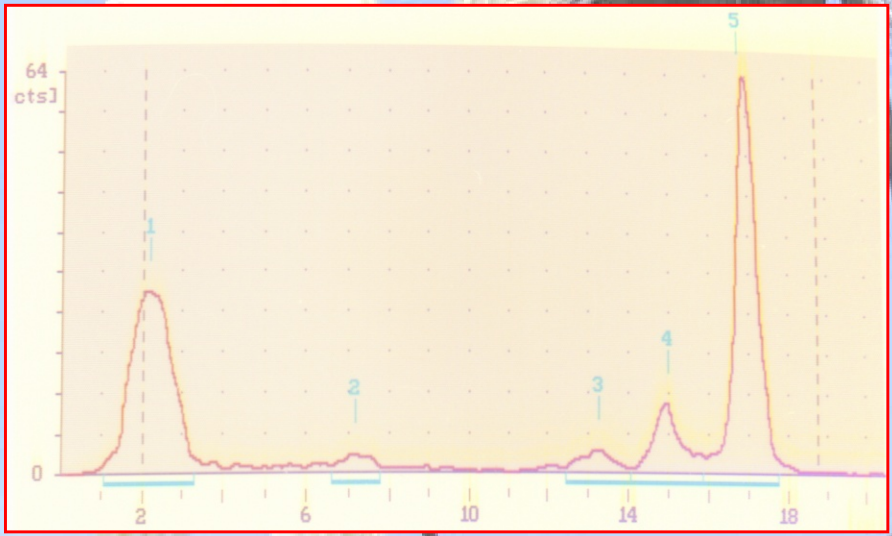
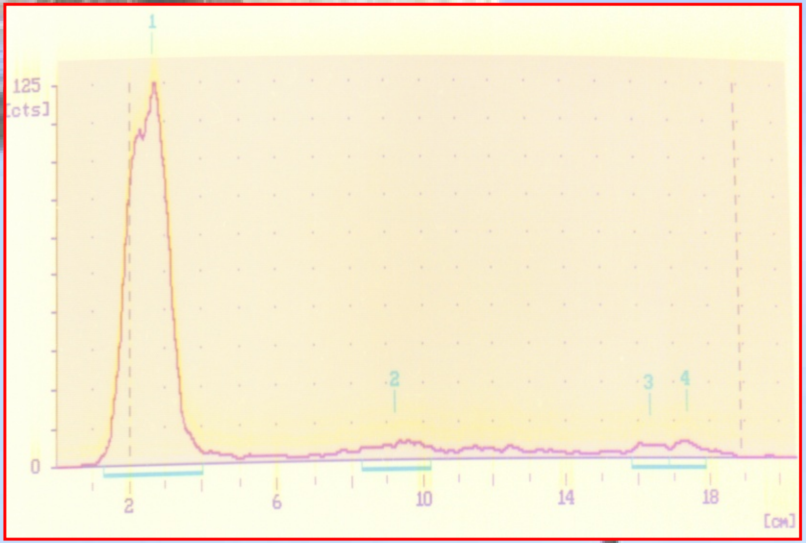


Figura 5. Modelo D1/D2 de la cadena de transporte electrónico desde el agua hasta la plastoquinona Q_B en el complejo proteínico del CR (Barber, 1987a).

Detoxificación de isoproturon en *Loliun rigidum*. Absorción radicular



S



R

De Prado et al., J. Agric. Food Chem, Vol.56, No. 6. 2005.

Efecto de ABT (70µM) sobre el metabolismo de ^{14}C-isoproturón en hojas de *Lolium rigidum* (R y S) incubados en herbicida durante 24 h y luego transferidos a solución nutritiva 24 h.

	Radioactividad (%)			
	-ABT		+ABT	
Metabolitos[a]	S	R	S	R
ISO	73,31±8,24	7,25±1,21	74,27±5,34	78,34±5,67
M-ISO	9,21±0.67	5,33±1,67	17,21±8,45	12,21±3,54
H-ISO	2,97±0,76	4,27±0.86	nd	nd
C-ISO	14,51±1,27	83,15±10,24	8,52±2,36	9,45±2,94

[a] ISO, isoproturón; M-ISO, monodesmetil-isoproturon; H-ISO, hidroxi-isoproturón; C-ISO, conjugado-isoproturón.

De Prado et al., J. Agric. Food Chem, Vol.56, No. 6. 2005.

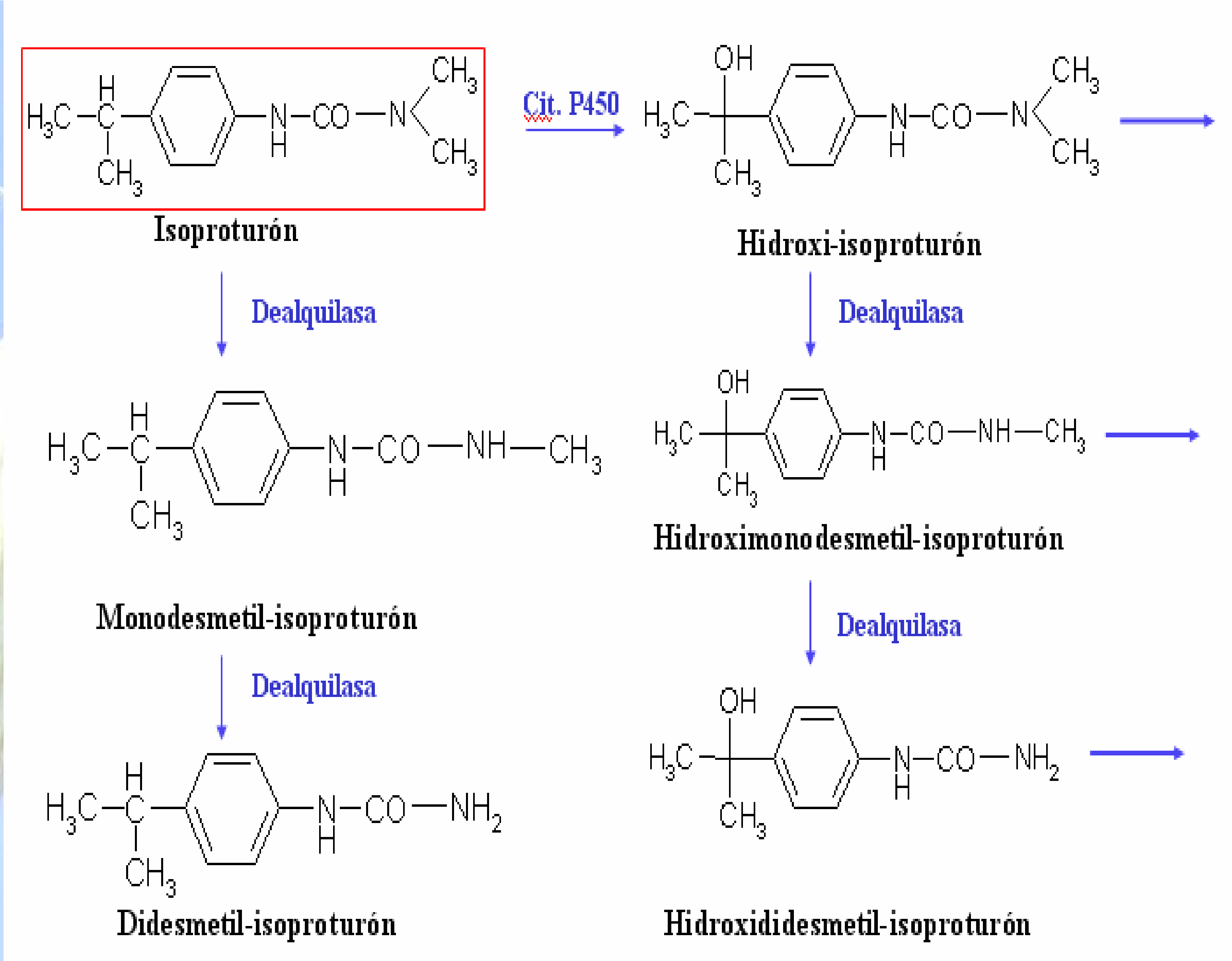
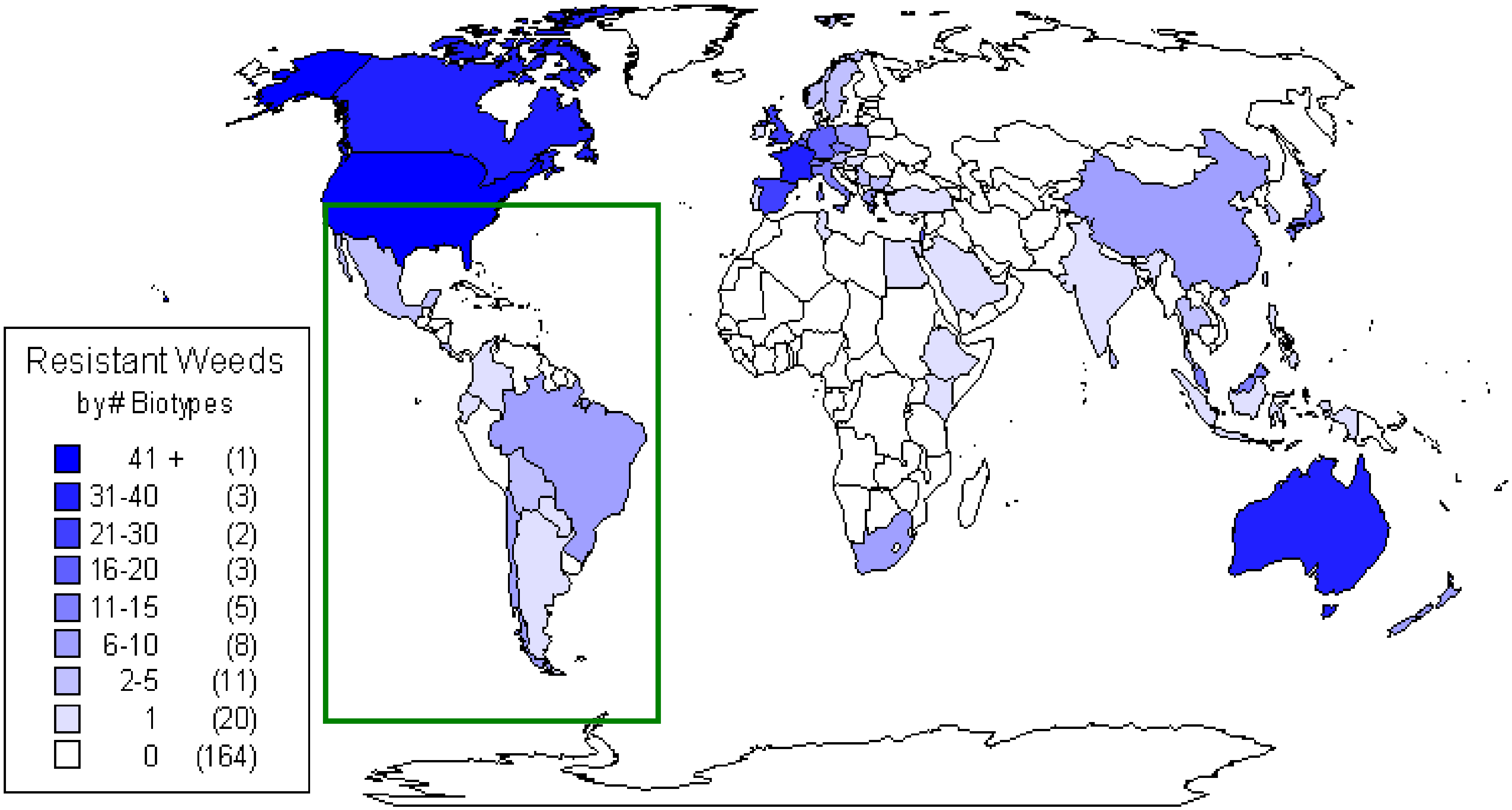


Figura 4. Ruta metabolica de detoxificación de isoproturon en *Loliun rigidum*.

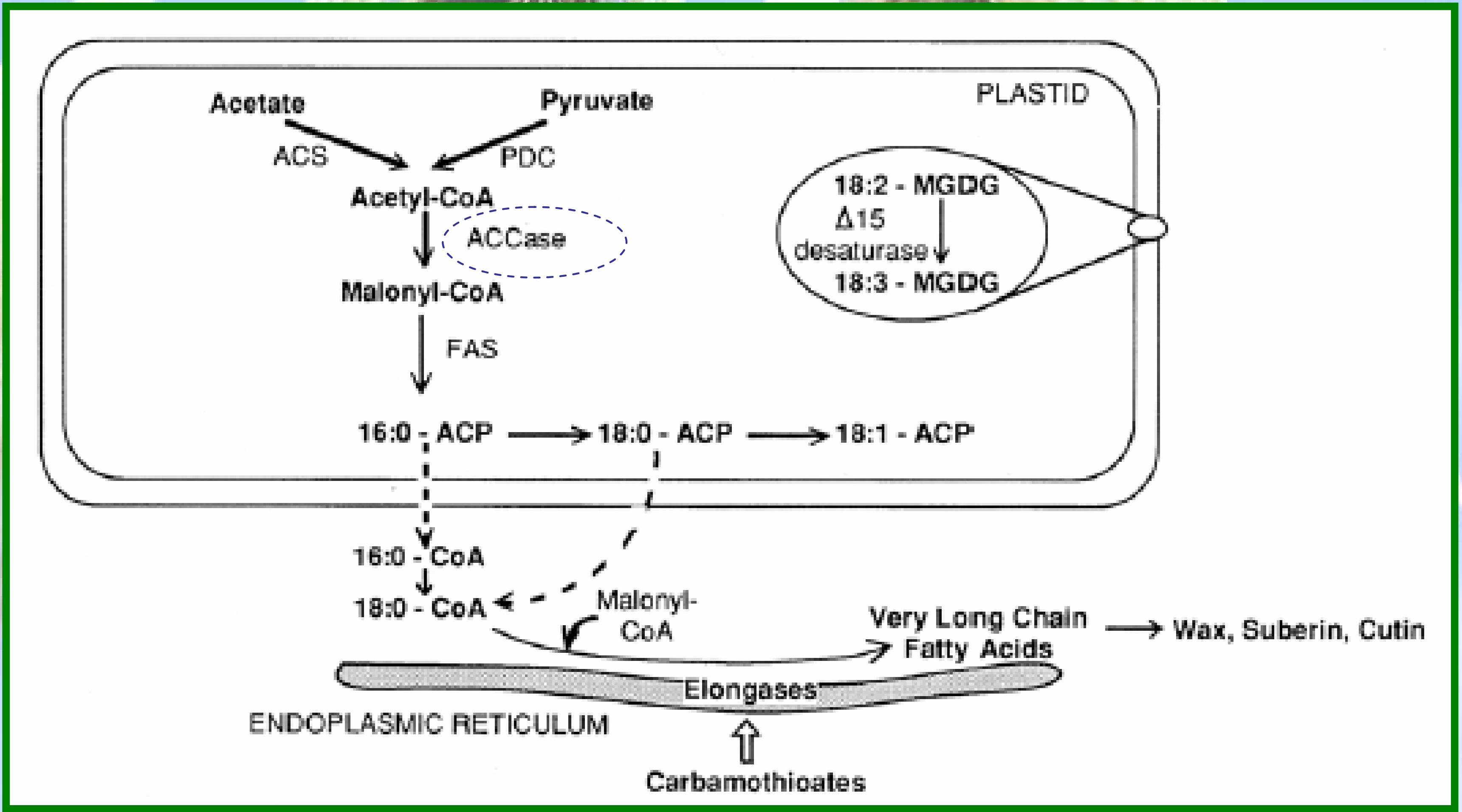
Distribution of Herbicide Resistant Biotypes



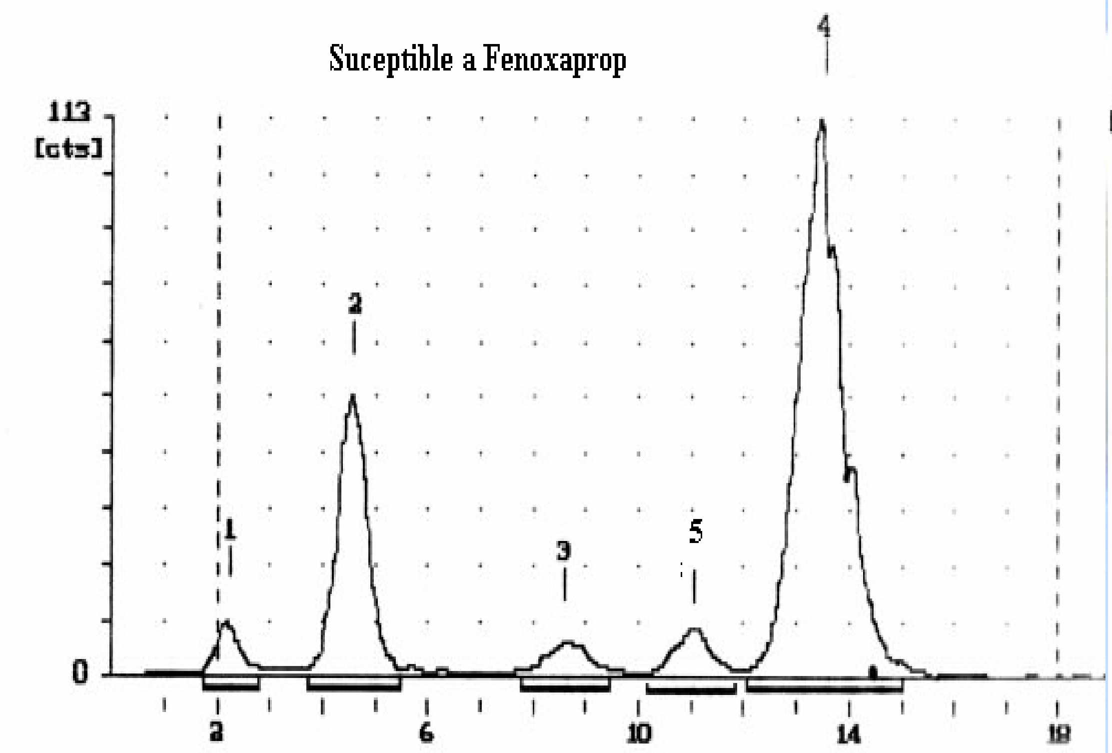
Source: Dr. Ian Heap
www.weedscience.com

Acetil Coenzima A carboxilasa (ACCasa)

- ACCasa, acetil coenzima A carboxilasa.
- Cataliza el primer paso en la biosíntesis de ácidos grasos.



Metabolismo de ^{14}C-fenoxaprop-p-etil 24 HDT en *L. multiflorum*. Absorción foliar.



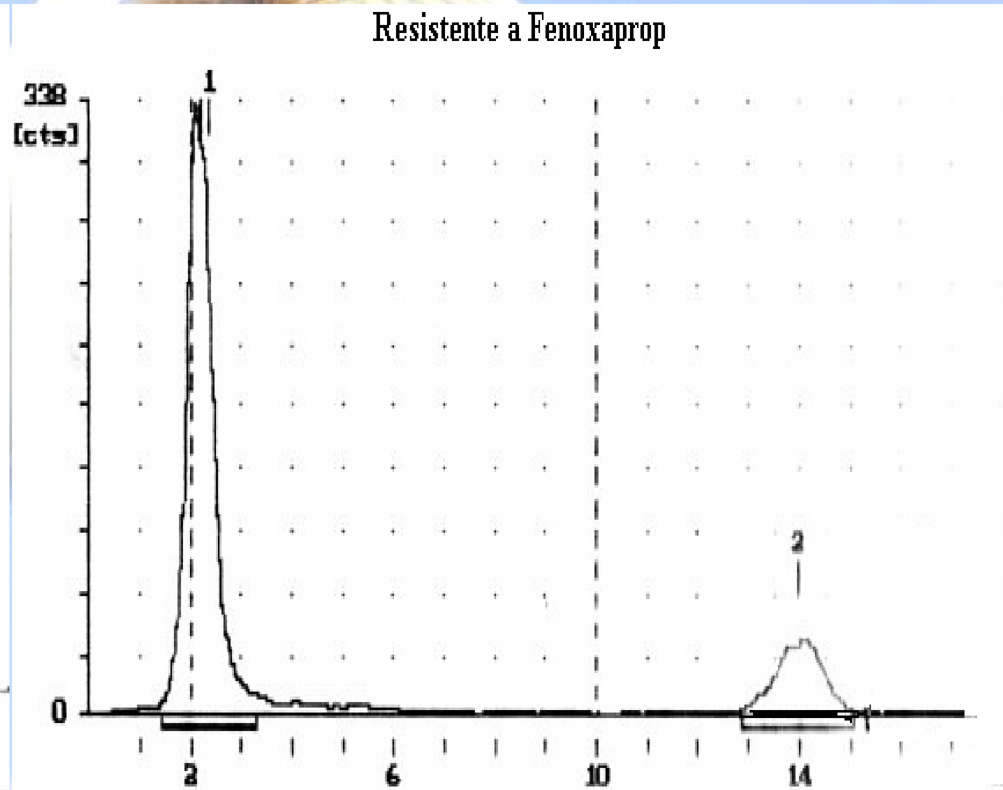


Figura 5. Ruta metabolica de detoxificación de fenoxaprop-etilo en *Loliun multiflorum*.