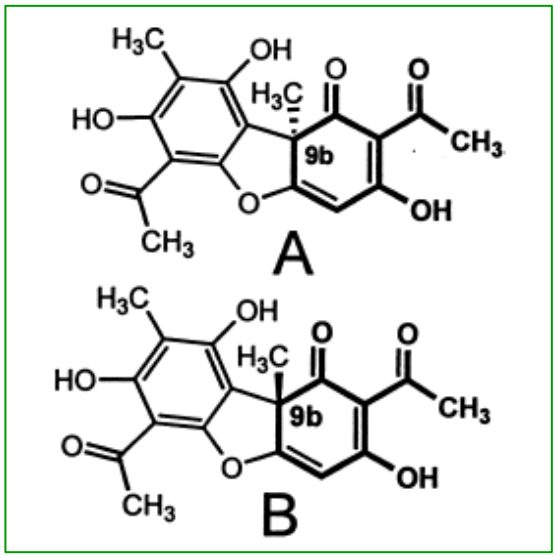
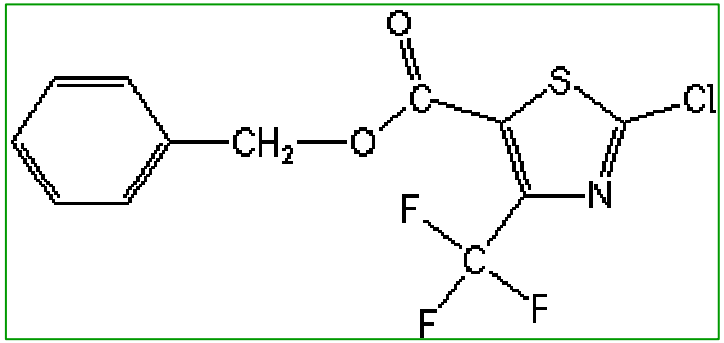
TD Herbicides

Sur la base des résultats expérimentaux ci-dessous, proposez un mode d'action pour **l'acide usnique et** le **Flurazole**, et proposez des expériences complémentaires pour tester la validité de vos hypothèses.



Romagni *et al.*, 2000



Hirase & Molin, 2001

Acide Usnique

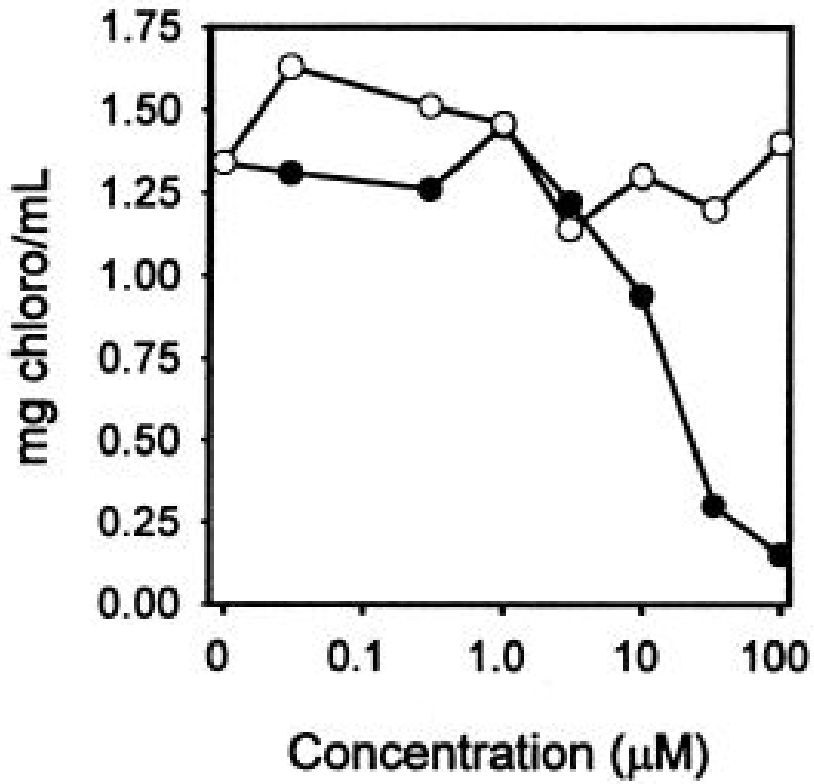
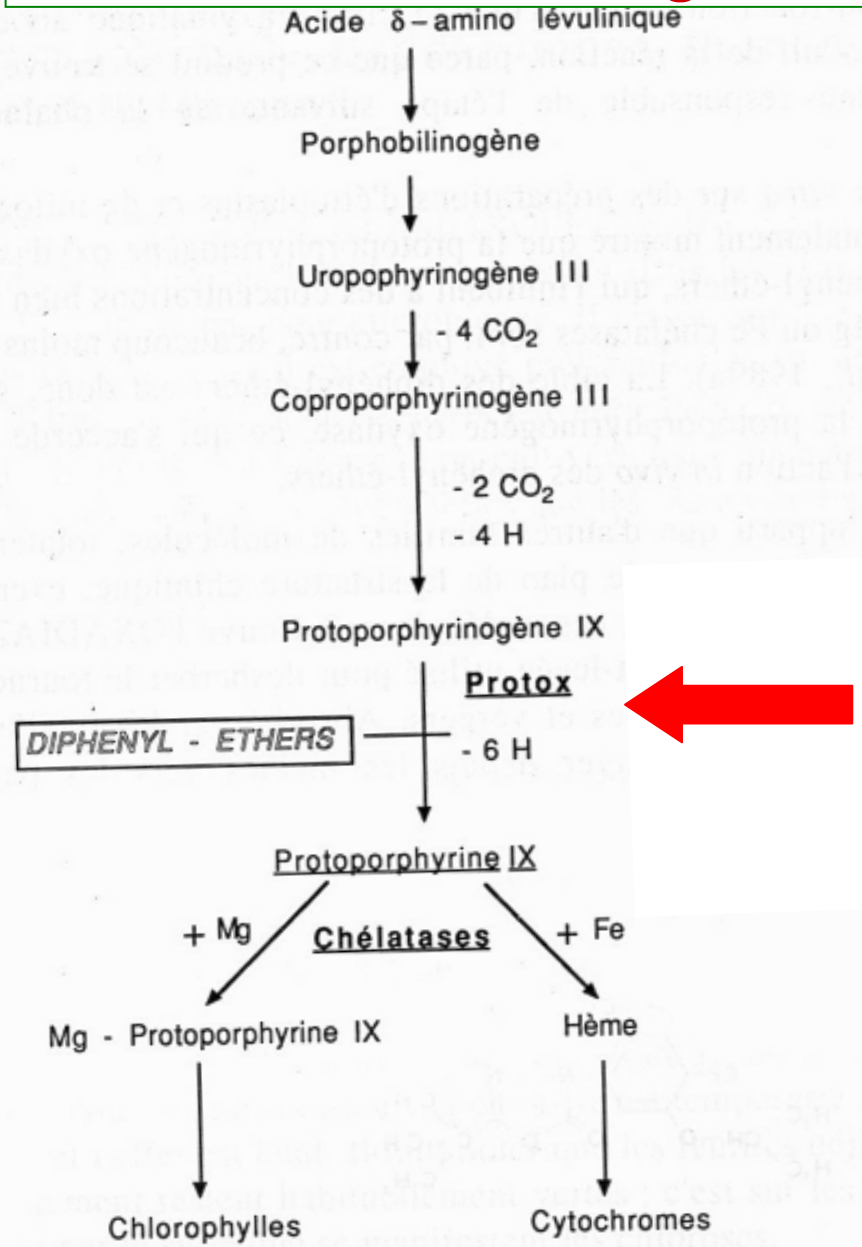
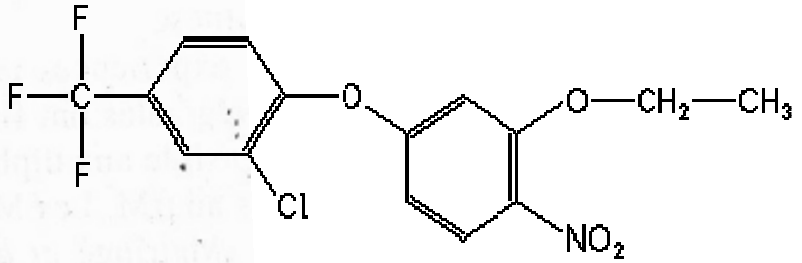


Fig. 2. Effect of (−)-usnic acid (-●-) and (+)-usnic acid (-○-) (0.03–100 µM; no data for 0.1 µM) on chlorophyll concentration in lettuce cotyledons after 6 days of growth.

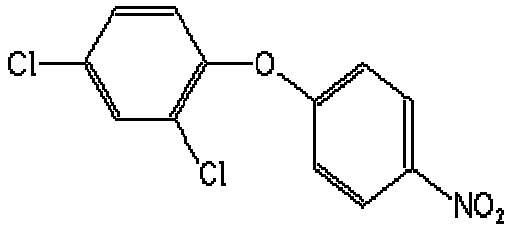
Romagni *et al.*, 2000

Herbicides / Pigments chloroplastiques

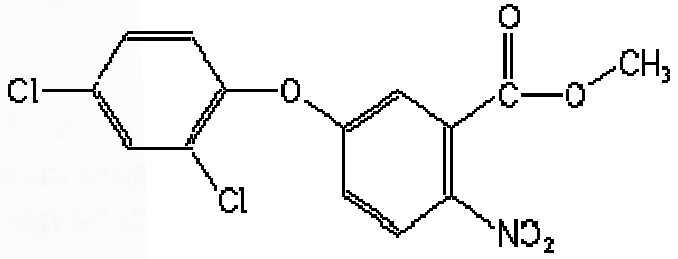




Oxyfluorfene

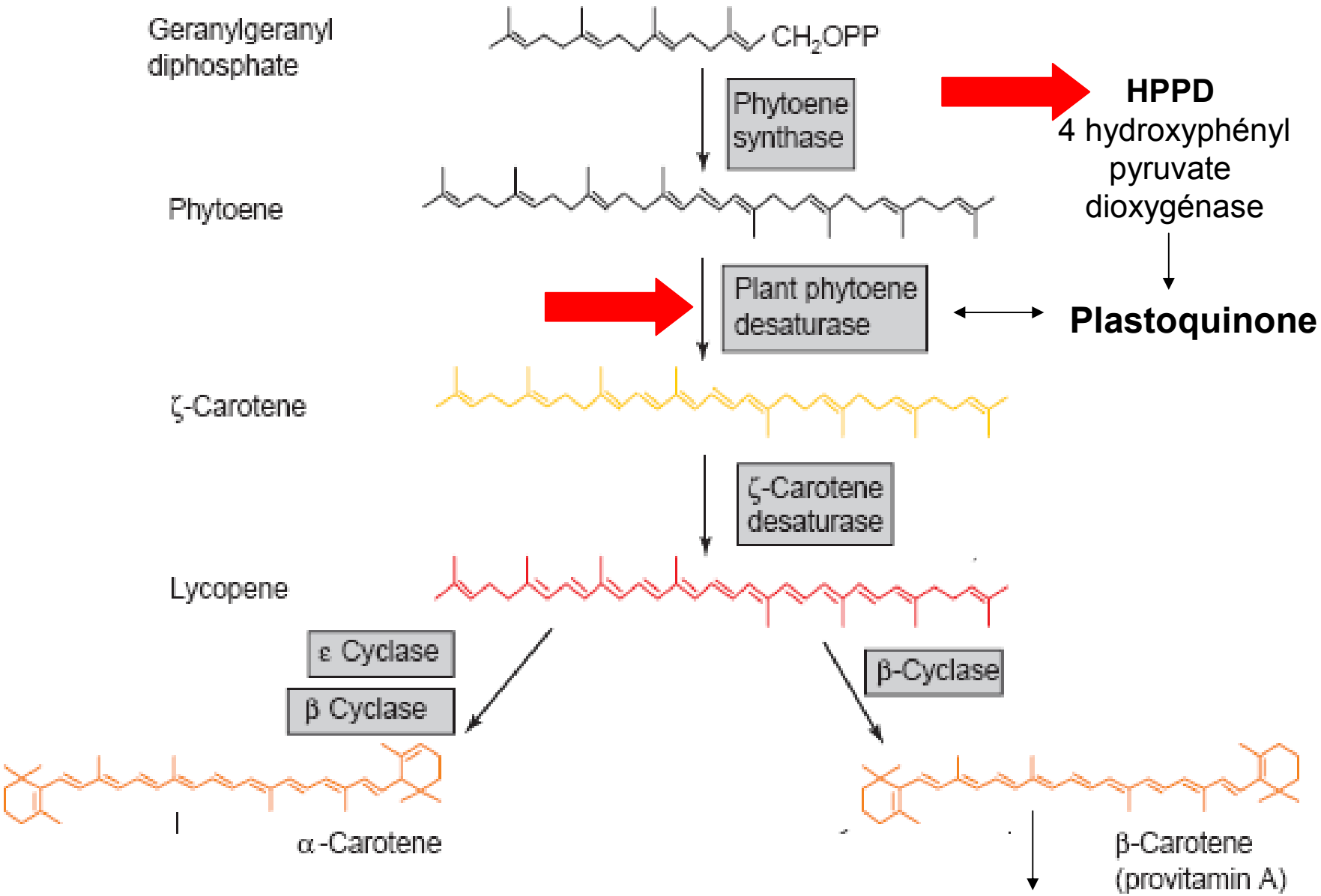


Nitrofene



Bifenox

Herbicides / Pigments chloroplastiques



Guliano et al., 2000

Xanthophylles

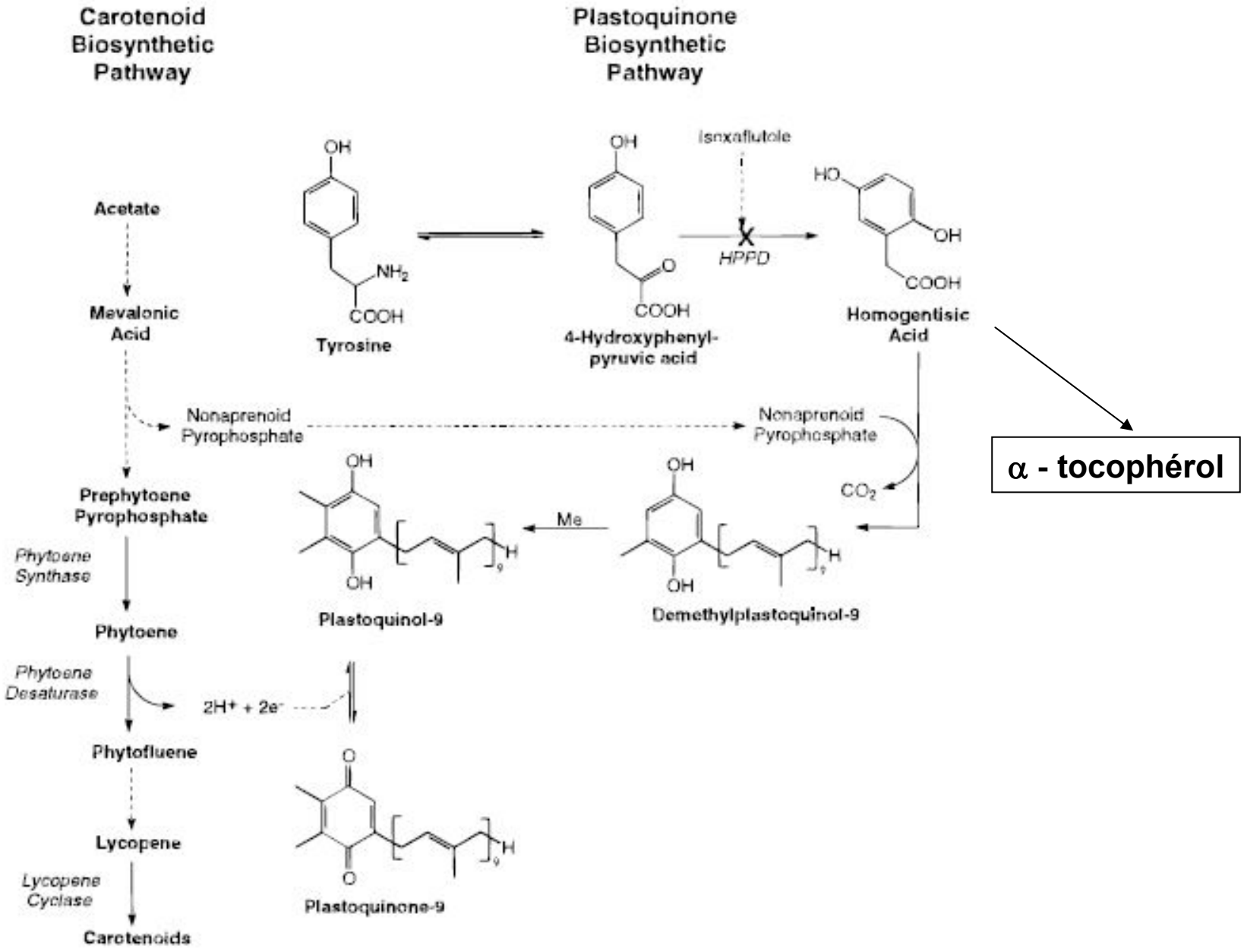


FIG. 6. The integration of the carotenoid and plastoquinone biosynthetic pathways.

Pallett *et al.* (1998)

Herbicide Resistance Action Committee
Classification of herbicides

(Cf Autre système de classification **WSSA** "Weed Science Society of America »)

http://www.plantprotection.org/HRAC/ **http://www.wssa.net/**

A (1) / Inhibition **ACCase**	**H (10)** / Inhibition **Glutamine Synthase**
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G (9) / inhibition **EPSP Synthase**	**R,S,Z (17, 25 à 27)** / mode d'action inconnu

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Acide Usnique

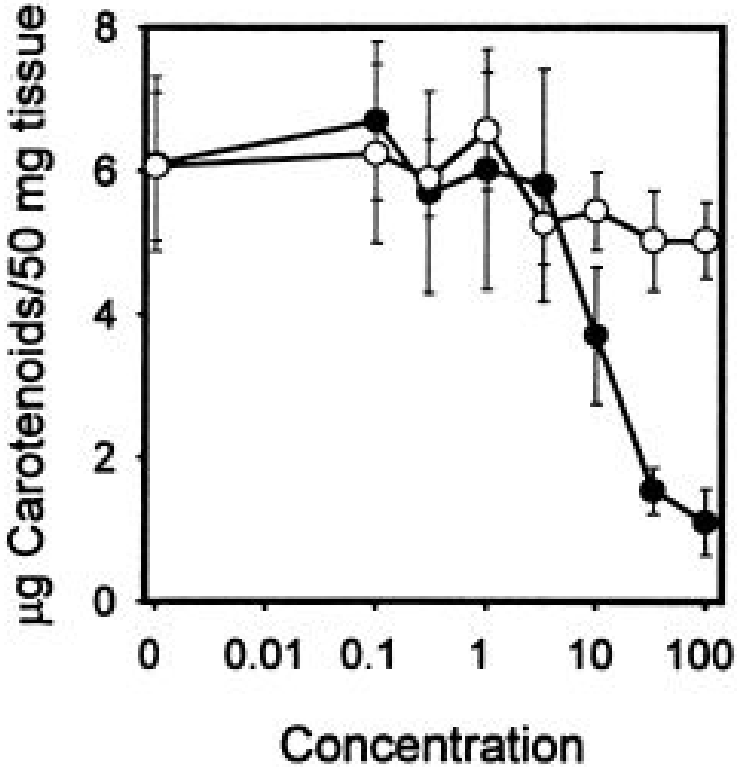

Fig. 4. Effect of (−)-usnic acid (-●-) and (+)-usnic acid (-○-) (0.1–100 µM) on carotenoid concentration in lettuce cotyledons after 6 days of growth.

Romagni *et al.*, 2000

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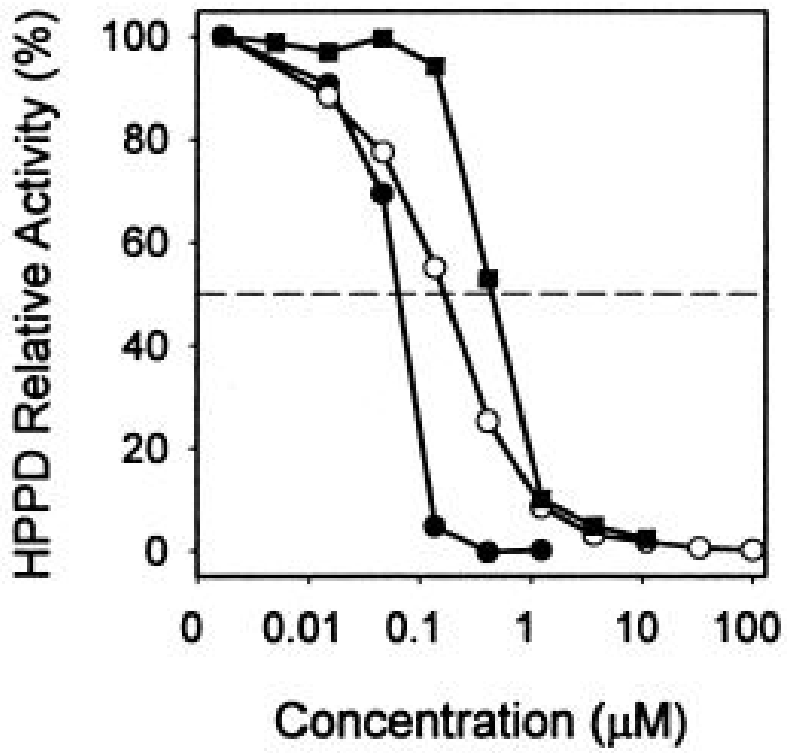

Fig. 5. Effect of (−)-usnic acid (-●-) and (+)-usnic acid (-○-) (0.01–100 µM) on activity of HPPD. The activity of the herbicide sulcotrione was added for comparison (-■-).

Romagni *et al.*, 2000

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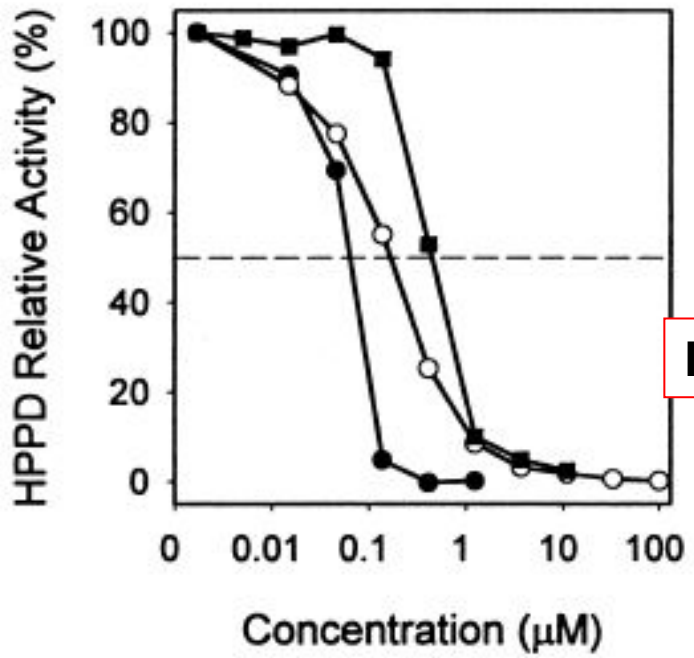

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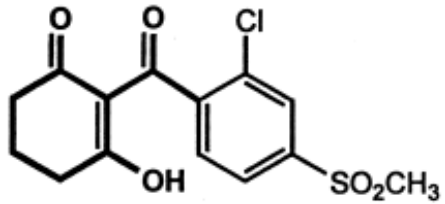
I50 (–) usnic acid = 70nM

I50 (+) usnic acid = 0,7µM



Sulcotrione

FEBS 24022

FEBS Letters 480 (2000) 301–305

The phytotoxic lichen metabolite, usnic acid, is a potent inhibitor of plant *p*-hydroxyphenylpyruvate dioxygenase

Joanne G. Romagni[a,*], Giovanni Meazza[b], N.P. Dhammika Nanayakkara[c], Franck E. Dayan[a]

[a] *USDA/ARS, Natural Products Utilization Research Unit, P.O. Box 8048, University of Mississippi, University, Lafayette Co, MS 38677, USA*
[b] *Isagro Ricerca Srl, via G. Fauser, 4, 28100 Novara, Italy*
[c] *National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Lafayette Co, MS 38677, USA*

Romagni *et al.*, 2000

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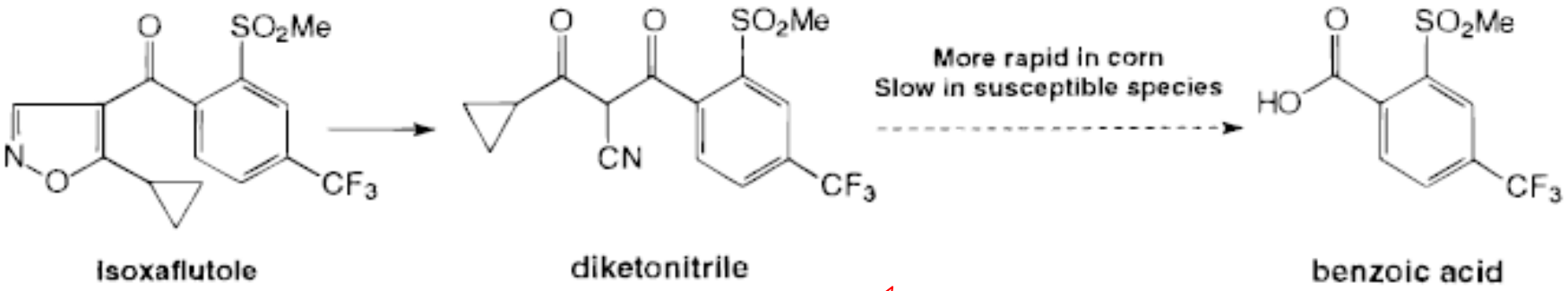
Herbicides / Pigments chloroplastiques

Herbicides inhibiteurs de la synthèse des caroténoïdes

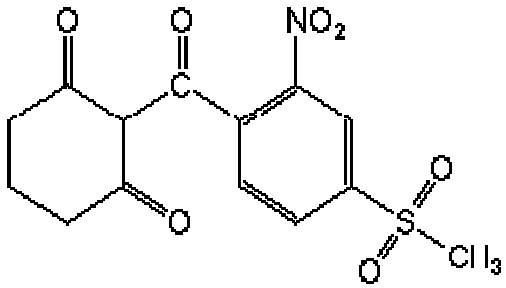
Métabolisation ⇨ Activation
Proherbicide ⇨ Herbicide



Isoxaflutole — diketonitrile — benzoic acid

(cyclopropylisoxazoles)

⇨ HPPD



Mésotrione
(benzoylcyclohexanedione)

Isoxaflutole / Pallett *et al.* (1998)

TABLE 2

Identity of ^{14}C-Labeled Products in the Shoot Extracts of Corn, *Ipomoea* spp., and *A. theophrasti* Following a 1-Day Exposure of Roots to [*phenyl*- U -^{14}C] Isoxaflutole (56,620 dpm ml^{-1} Equivalent to 0.5 µg ml^{-1}) via the Hydroponic Medium

	Corn **R**		*Ipomoea* sp **RS**		*A. theophrasti* **S**	
	1 day	1 + 6 days[a]	1 day	1 + 6 days	1 day	1 + 6 days
^{14}C extracted[b]						
% Total ^{14}C in shoot tissue	95	87	92	85	94	91
µg equivalent g^{-1} shoot	3.46	3.03	2.47	2.21	1.32	1.62
% Distribution of metabolites in shoots						
Diketonitrile	80	29	77	57	89	82
Benzoic acid	13	59	12	31	0	12
Unidentified	7	12	11	12	11	6

[a] After a 1-day exposure period the roots were transferred to fresh untreated medium and the plants were grown for a further 6 days period.

[b] Each value is the mean of three replicates. Each replicate consisted of nine shoots from each species. The results are expressed as microgram equivalent of isoxaflutole or DKN per gram fresh wt of tissue (113240 dpm is equivalent to 1 µg of isoxaflutole). The DKN and benzoic acid in the extracts were separated by radio-TLC and identified by cochromatography with the standards.

La sensibilité à l'isoxaflutole est corrélée avec le taux de DKN dans les parties aériennes

Métabolisation ≠ Détoxication !!

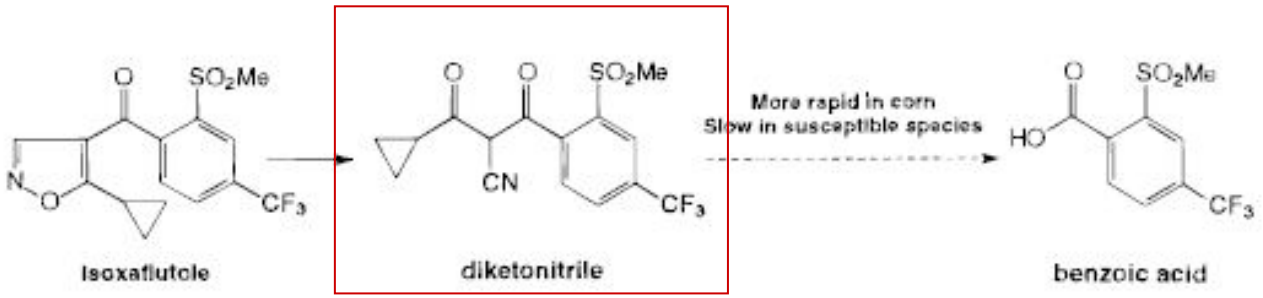


FIG. 2. The degradation of isoxaflutole in plants.

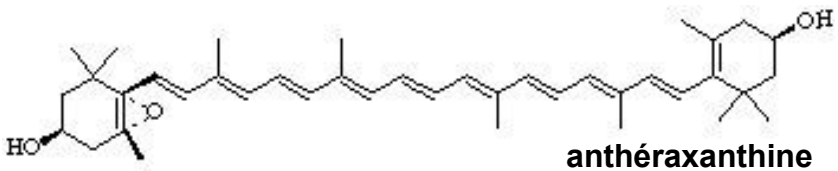
Isoxaflutole / Pallett *et al.* (1998)

TABLE 4
Pigment Analysis of *E. crus-galli* Leaves Following Postemergence Treatment with Isoxaflutole and Diflufenican

	Leaf pigment content (μg g^{-1} fresh wt)		
	Untreated	Isoxaflutole (32 g ha^{-1})	Diflufenican (125 g ha^{-1})
β-Carotene	78.5	4.7	17.8
Violaxanthin	57.7	4.6	7.0
Antheraxanthin	8.0	1.6	2.5
Lutein	102.0	12.9	27.2
Neoxanthins[a]	22.8	2.6	5.7
Phytoenes[a]	0.0	7.5	31.6
Chlorophyll a	1157.8	84.6	281.7
Chlorophyll b	252.5	26.0	63.9

Note. After 4 days, the leaves were excised and extracted as described by Barry and Pallett (10). The carotenoid and chlorophyll profiles of the leaves were determined by HPLC (11). Data are means of four replicates.

[a] Mixture of *cis*- and *trans*- isomers.

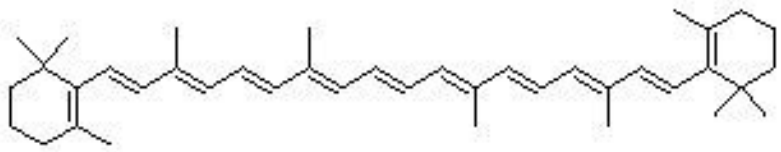


anthéraxanthine

μg.g^{-1} FW	Témoin	Isoxaflutole 32 g.ha^{-1}	Diflufénican 125 g.ha^{-1}
Phytoène	0	7,5	31,6
β Carotène	78,5	4,7 (6 %) ***Dim 94%***	17,8
Xanthophylles	190,5	21,7 (11,4%) ***Dim 88,6%***	42,4
Chlorophylles	1410,3	110,6 (7,8%) ***Dim 92,2%***	345,6

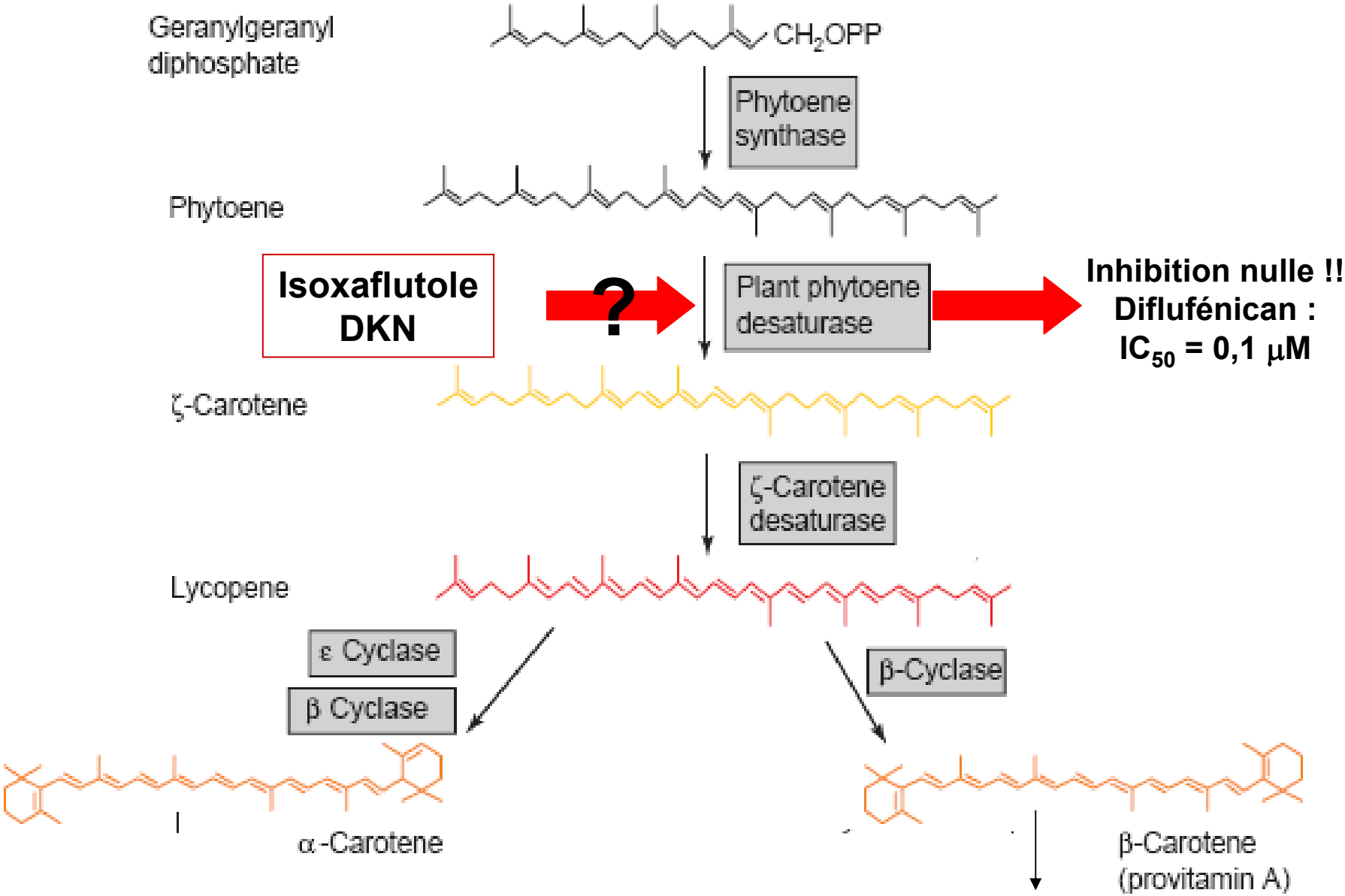
⇨Accumulation phytoène = substrat de l'enzyme Phytoène désaturase (PDS), indétectable dans témoin


phytoène


β carotène

Herbicides / Pigments chloroplastiques

Herbicides inhibiteurs de la synthèse des caroténoïdes



Guliano et al., 2000

Xanthophylles

Isoxaflutole / Pallett *et al.* (1998)

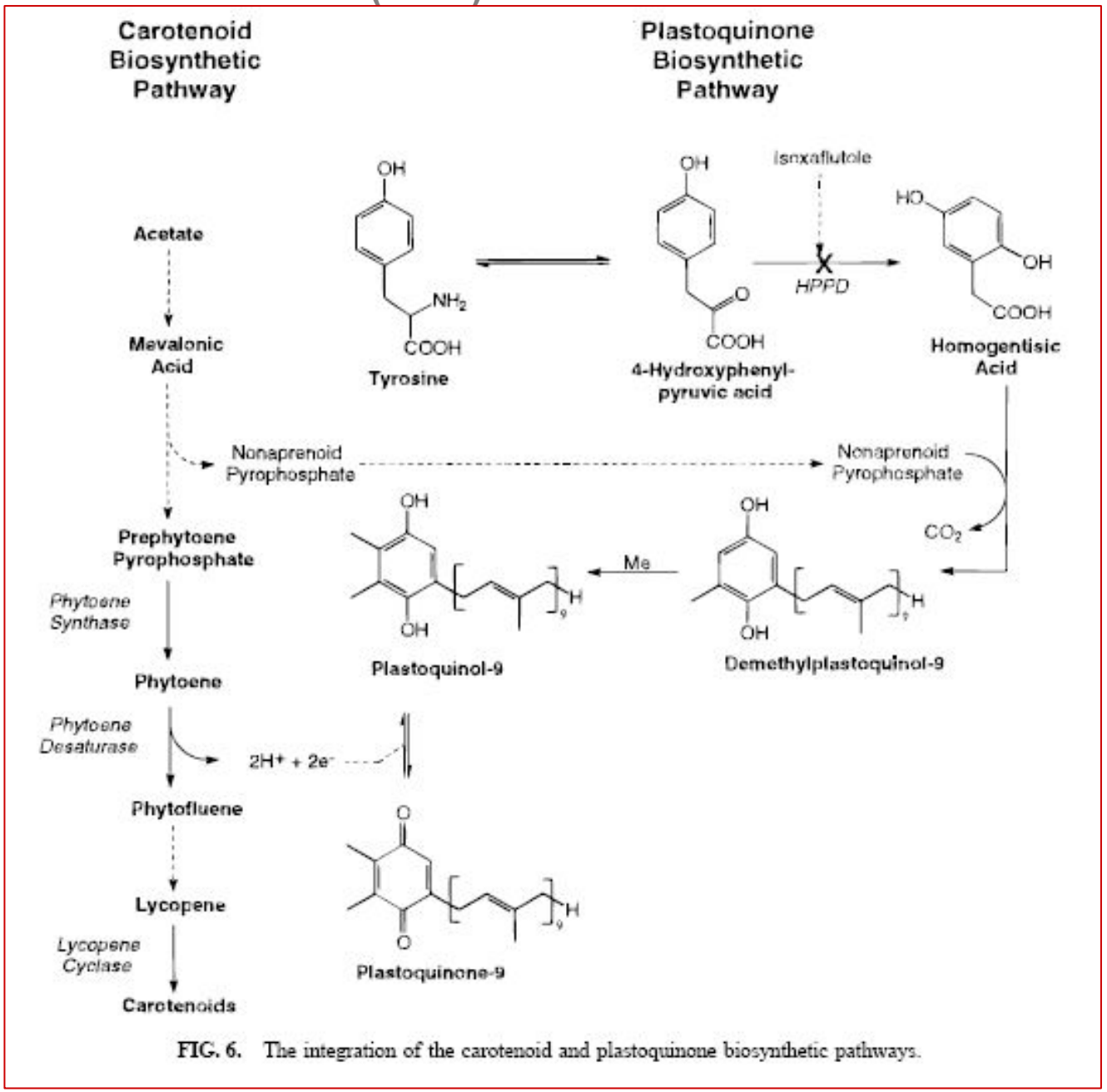


FIG. 6. The integration of the carotenoid and plastoquinone biosynthetic pathways.

Isoxaflutole / Pallett *et al.* (1998)

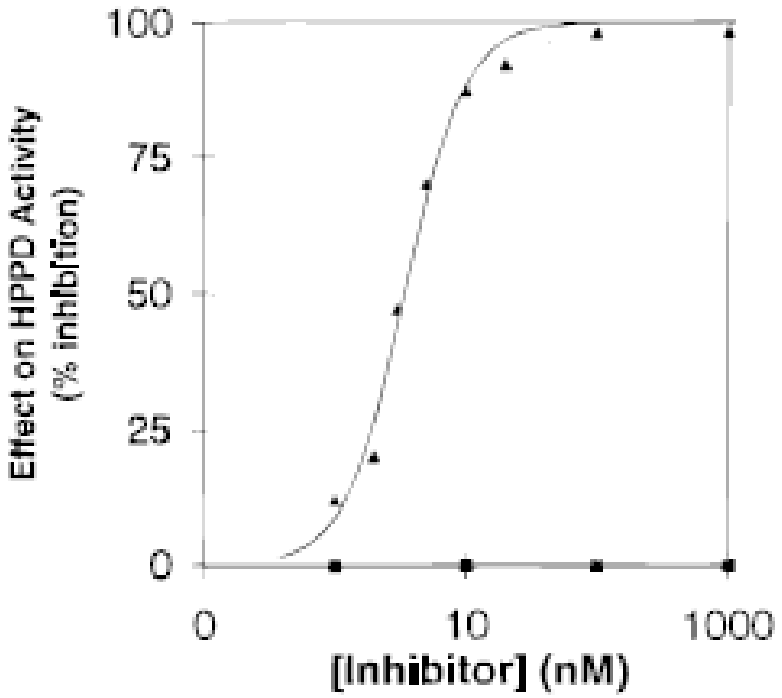


FIG. 4. The inhibition of carrot cell HPPD activity isoxaflutole (■) and by the diketonitrile derivative of isoxaflutole (▲). Enzyme activity was determined by measuring the accumulation of the product of the reaction, homogentisic acid, by its UV absorption after separation of reaction constituents by reverse-phase HPLC (9).

Test HPPD in vitro
⇨Effet nul de l'isoxaflutole
⇨DKN : IC_{50} = 5 nM

Isoxaflutole / Pallett *et al.* (1998)

Analyse extraits lipidiques foliaires après traitement par Isoxaflutole
⇨Effets sur teneur en α-tocophérol et en plastoquinone antérieurs à ceux sur teneurs en caroténoïdes
⇨enzyme-cible = HPPD ?

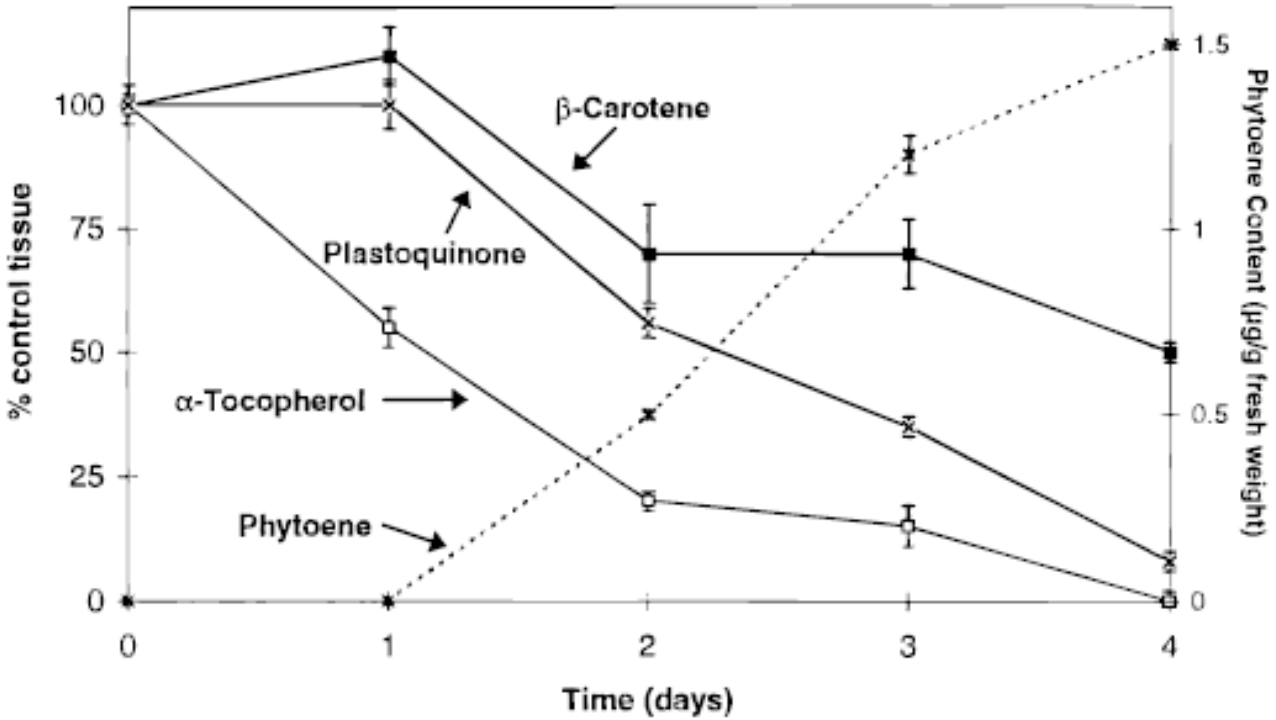
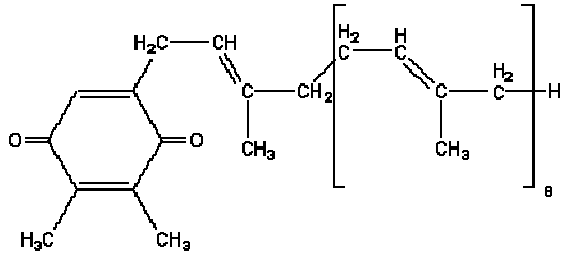
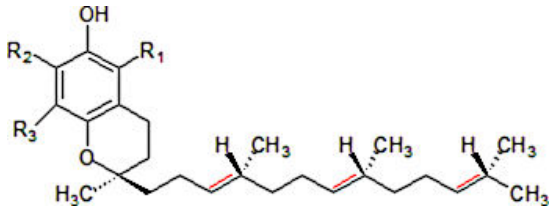


FIG. 3. The content of plastoquinone (x), α-tocopherol (□), β-carotene (■), and phytoene (✳) in extracts of *B. kaber* seedlings. These levels were determined by UV-visible HPLC, 0–4 days following a postemergence treatment with 63 g ha^{-1} isoxaflutole. The levels of plastoquinone, α-tocopherol, β-carotene, and phytoene in control plants at 0 days were 6.6, 4.0, 3.7, and 0.0 µ g^{-1} fresh wt, respectively, determined by comparison with authentic standards.



plastoquinonec



α– tocophérol (R=CH3)

Isoxaflutole / Pallett *et al.* (1998)

TABLE 5
The Reversal of the Herbicidal Activity of Isoxaflutole

Isoxaflutole (ng ml^{-1})	% Bleaching of *Brassica* seedlings Lactone of homogentisic acid (μg ml^{-1})			
	0	3	10	30
0	0	10	20	0
0.5	90	90	50	0
2.0	100	100	70	10
20.0	100	100	80	40

Note. A range of isoxaflutole and the lactone of homogentisic acid concentrations was added to the solid agar growth medium. Data represent assessment of seedlings 7 days after germination and are means of two replicates.

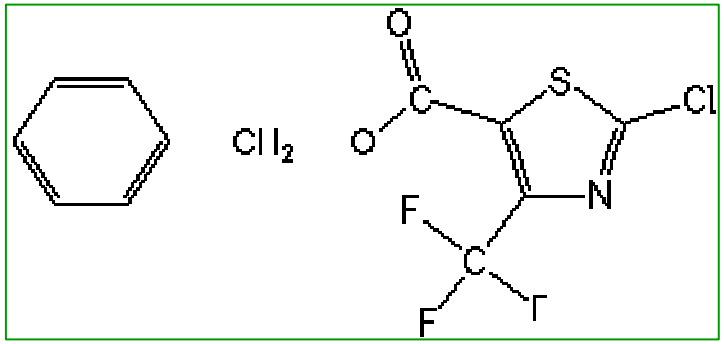
Réversion des effets phytotoxiques par addition d'acide homogentisique = produit de la réaction catalysée par HPPD

Les résultats présentés ci-dessous concernent les effets d'un traitement de 7 jours par un herbicide, le **Norflurazon, sur la teneur en pigments chloroplastiques (valeurs exprimées en** mg / g poids frais) de plantules de Blé.

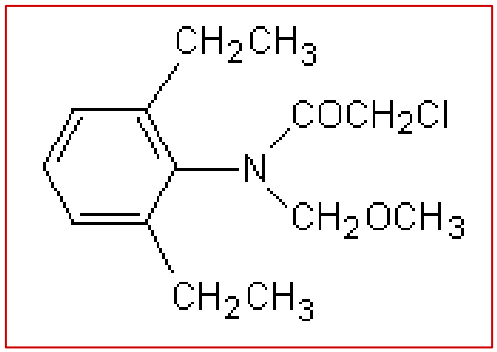
Traitement	**Intensité lumineuse**	**Chlorophylles**	**β-Carotène**	**Phytoène**
Contrôle	10 lux	394 +/- 6	21 +/- 2	0
	16 klux	801 +/- 13	23 +/- 3	0
Norflurazon	10 lux	130 +/- 5	< 1	72 +/- 4
10^{-4} M	16 klux	21 +/- 3	< 1	24 +/- 3

TD Herbicides

Sur la base des résultats expérimentaux ci-dessous, proposez un mode d'action pour **l'acide usnique et** le **Flurazole**, et proposez des expériences complémentaires pour tester la validité de vos hypothèses.



Hirase & Molin, 2001

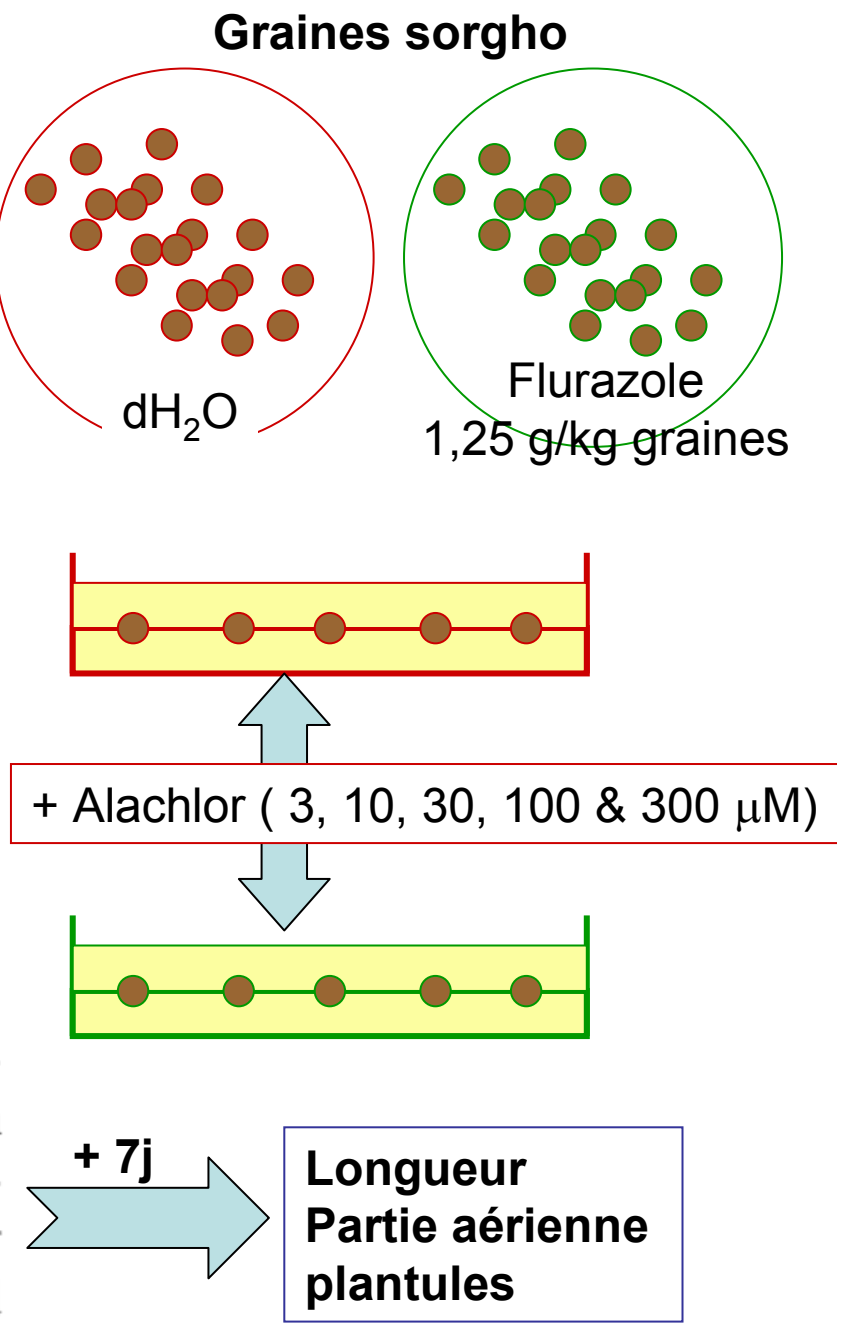


Alachlore : herbicide (chloroacétanilides)

→ *Inhibition synthèse lipides*

Effect of Safeners on Alachlor Injury

Vermiculite (2 cm deep, coarse; Strong-Lite Products Corp.) was placed on the bottom of plastic trays (3 × 4 × 5 cm) and saturated with deionized water. Five sorghum seeds, either control or safener-treated, were placed on the vermiculite surface and covered with 2 cm deep vermiculite. At the initial watering, 16 ml of the deionized water for the safener-treated control or the aqueous solutions of alachlor were applied on the vermiculite surface by pipettes. Safener-treated control was prepared for each compound and for each dosage. The containers were placed in a growth chamber under 16 h photoperiod (150 μE/s·m^2) at 25°C and watered (deionized water) as necessary. Shoot length was measured 7 days after planting, and percentage shoot length was calculated compared to safener-treated control in each compound and in each dosage. Each treatment consisted of three replicates with three shoot length measurements per replicate, and the experiment was repeated three times.



Flurazole

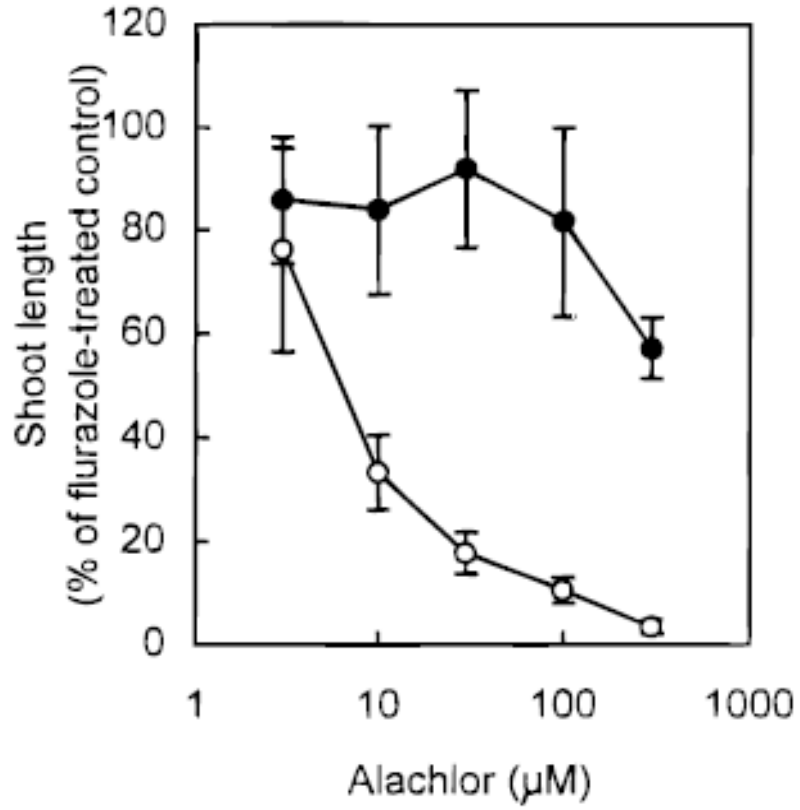


FIG. 1. Effect of flurazole seed treatment on growth inhibition by alachlor in sorghum. Vertical bars represent standard error. ●, Flurazole, 1.25 g/kg seed; ○, control.

Hirase & Molin, 2001

Flurazole

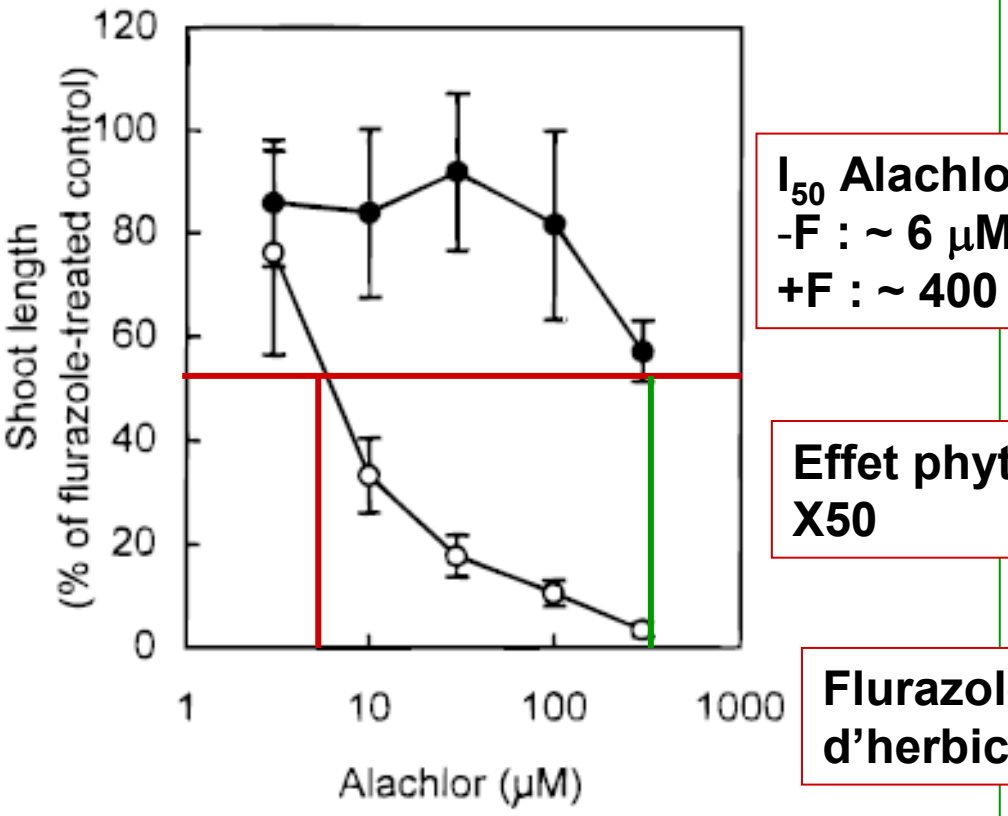
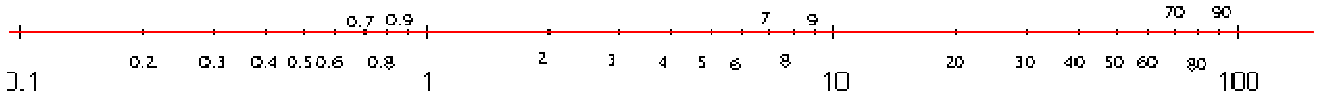


FIG. 1. Effect of flurazole seed treatment on growth inhibition by alachlor in sorghum. Vertical bars represent standard error. ●, Flurazole, 1.25 g/kg seed; ○, control.

I_{50} Alachlor/croissance :
-F : ~ 6 µM
+F : ~ 400 µM

Effet phytoprotecteur
X50

Flurazole = Antidote d'herbicide ?



Hirase & Molin, 2001

Cystéine synthase

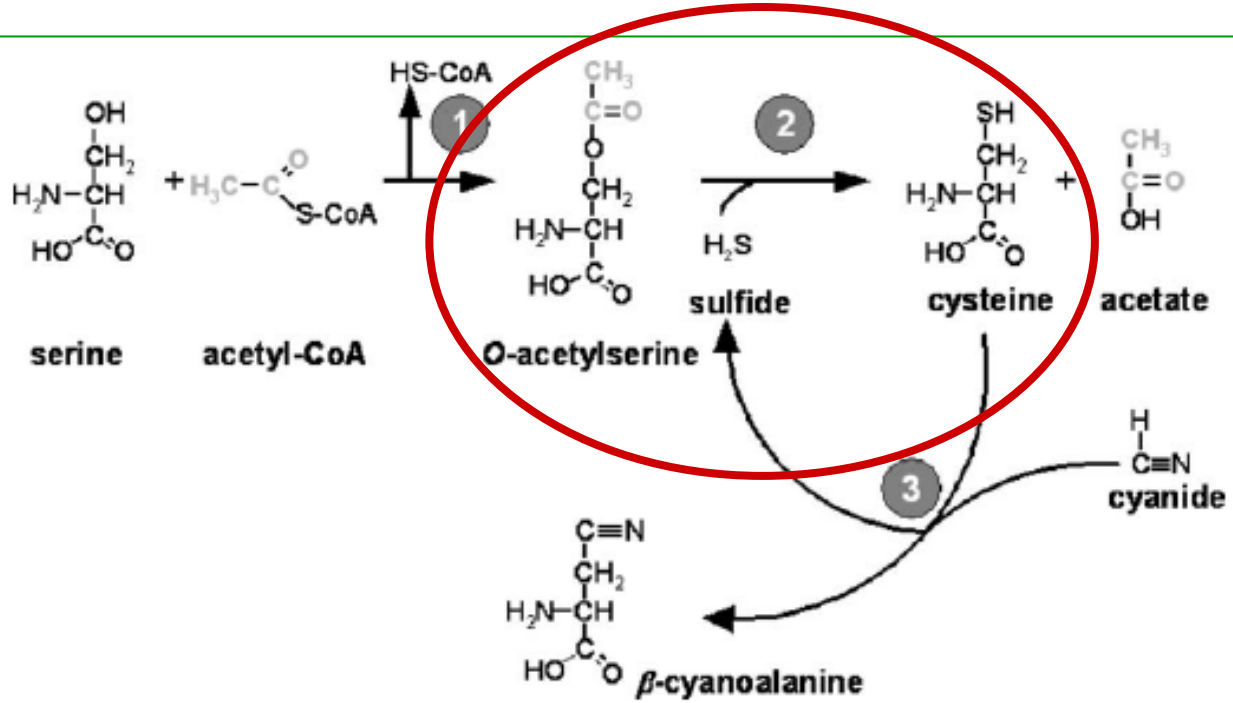


Figure 1. Chemical equation of cysteine synthesis and β-cyanoalanine formation in higher plants. Synthesis of cysteine is a two step process that is catalyzed by serine acetyltransferase (1) and *O*-acetylserine (thiol) lyase (2). The fate and behavior of the acetyl-moiety during cysteine synthesis is indicated in gray. β-cyanoalanine synthase (3) is assumed to detoxify cyanide in mitochondria using cysteine as an acceptor for cyanide.

Wirtz & Hell, 2006

CS Assay

Shoots of sorghum were weighed and homogenized with mortars and pestles on ice at approximately 4°C in 0.1 M phosphate buffer, pH 7.8, containing 1 mM dithiothreitol and 0.2% insoluble polyvinylpolypyrrolidone. The volume of the buffer was 40 ml per 1 g tissue. The homogenate was clarified by centrifugation at 15,000 *g* at 4°C for 30 min. The resulting supernatant was used for the CS assay. The CS assay (26–28) was performed in a final volume of 1 ml containing 50 mM phosphate buffer, pH 7.8, less than 0.02 mg protein, 5 mM OAS, 1 mM Na_2S, 1 mM dithiothreitol, 0.025 mM pyridoxal-5′-phosphate. The substrates were added to the enzyme to initiate the reaction, and assay test tubes were sealed with rubber caps. When the *in vitro* effect of flurazole on CS activity was examined, CS from untreated shoots was used.

Flurazole

TABLE 1
Effect of Flurazole on Extractable Cysteine Synthase Activity in Shoots of Sorghum

Time after planting (h)	Specific activity (μmol/min/mg protein)			Total activity (μmol/min/g fresh weight)		
	Flurazole[a]	Control	Ratio (flurazole/control)	Flurazole[a]	Control	Ratio (flurazole/control)
48	4.07	2.89	1.41	96.6	55.2	1.75
72	5.85	5.20	1.13	62.0	44.4	1.40
96	6.05	5.79	1.04	40.0	33.4	1.20
LSD (0.05)	—0.43—			—8.13—		

[a] Sorghum seeds were treated with flurazole (1.25 g/kg seed) dissolved in methanol before planting.

⇒Le Flurazole provoque une augmentation de l'activité Cys Synthase en particulier lors des phases initiales de la croissance des plantules de Sorgho

Hirase & Molin, 2001

Flurazole

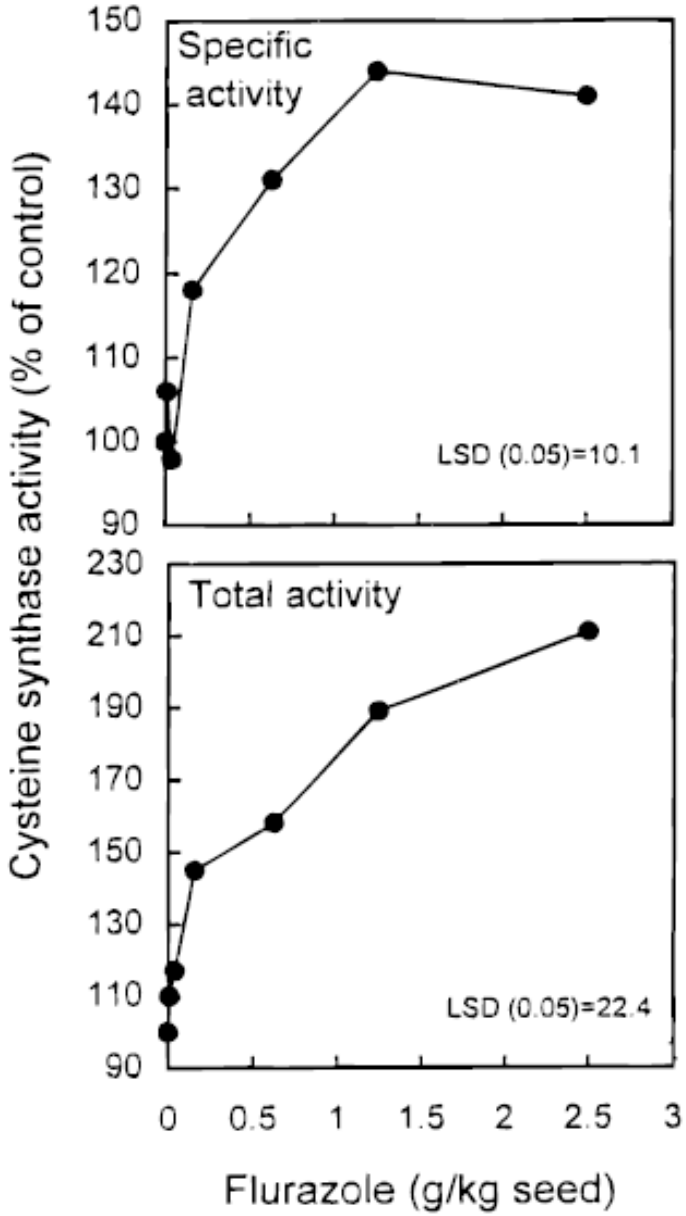


FIG. 2. Effect of flurazole dosage on extractable cysteine synthase activity in sorghum shoots. Cysteine synthase activity was measured 48 h after planting.

Hirase & Molin, 2001

Flurazole

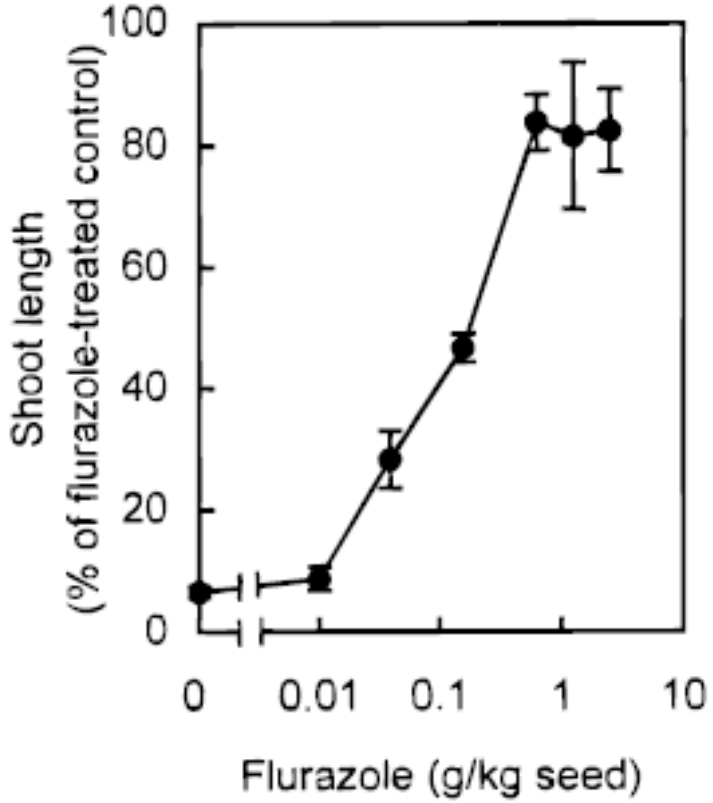


FIG. 3. Effect of flurazole dosage on growth inhibition by alachlor in sorghum. Alachlor (100 μM) was applied in all the treatments in this figure. Flurazole was treated on sorghum seeds. Vertical bars represent standard error.

Hirase & Molin, 2001

Flurazole

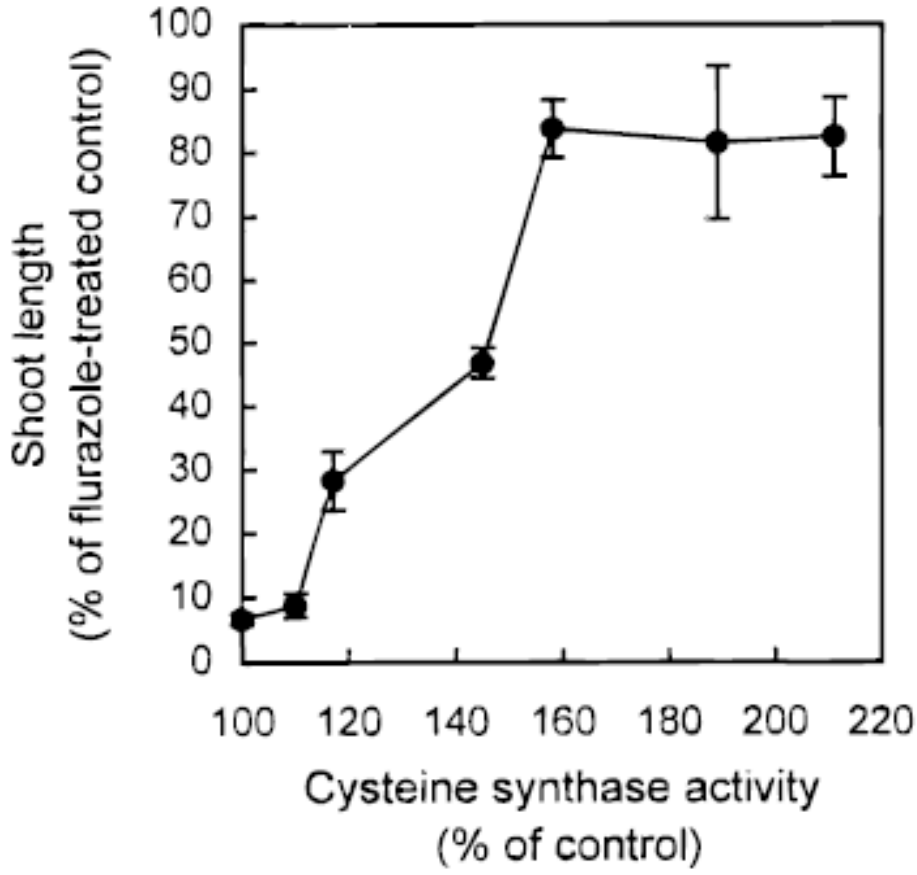


FIG. 4. Relationship between increase in extractable CS activity and growth recovery in sorghum. Shoot length in Fig. 3 was plotted against total activity of CS in Fig. 2. Vertical bars represent standard error.

Hirase & Molin, 2001

Flurazole

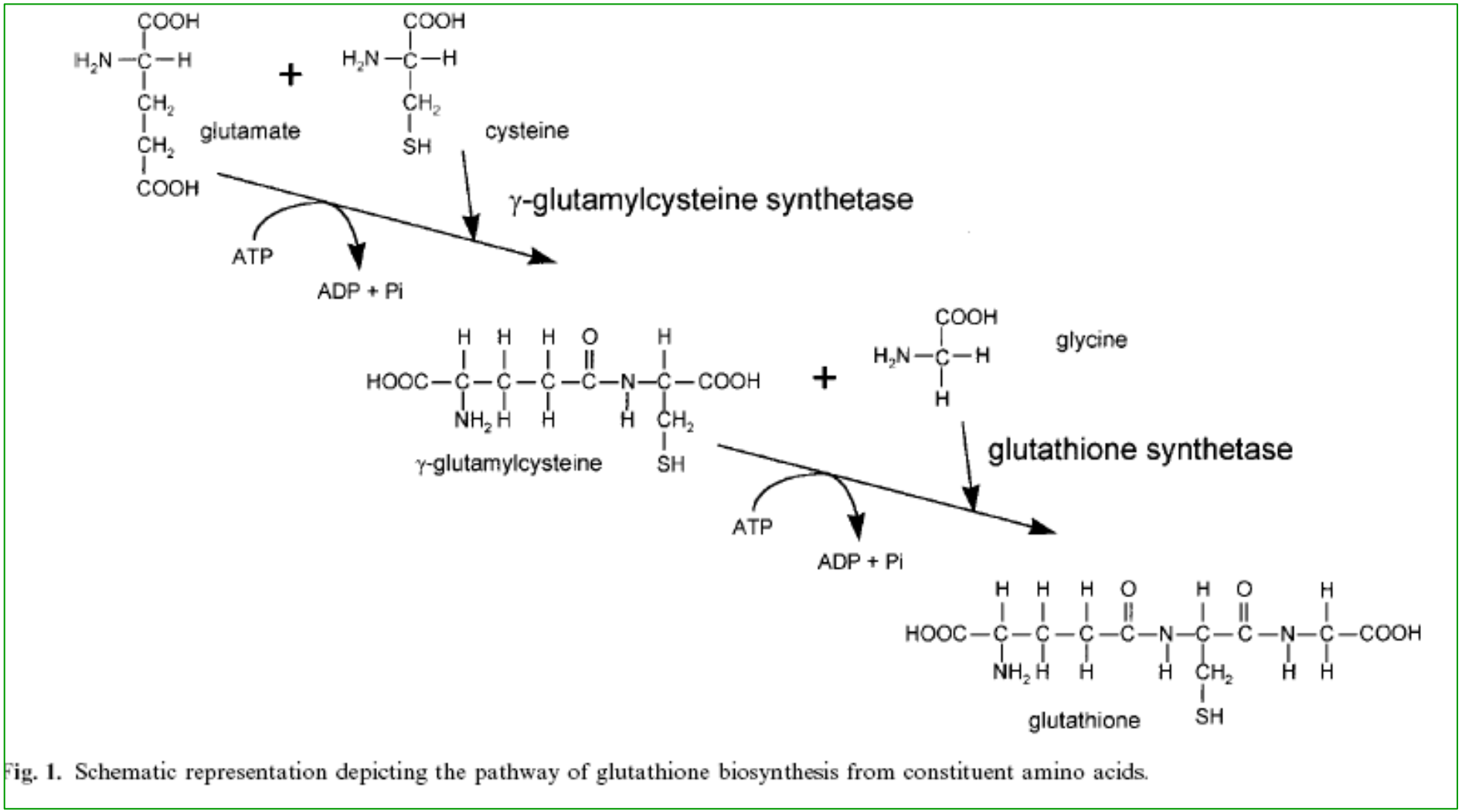


Fig. 1. Schematic representation depicting the pathway of glutathione biosynthesis from constituent amino acids.

Noctor et al., 1998

Flurazole

TABLE 3
Effect of Safeners on Shoot Length of Alachlor-Treated[a] Sorghum

Compound	Dosage (g/kg seed)	Shoot length (% of safener-treated control)[b]
Fluxofenim	0.1	73.2 ± 5.3
Fluxofenim	0.4	84.0 ± 6.8
Naphthalic anhydride	0.625	83.4 ± 7.4
Naphthalic anhydride	2.5	86.2 ± 7.6
Benoxacor	0.625	78.8 ± 4.2
Benoxacor	2.5	92.2 ± 2.7
Dichlormid	0.625	37.9 ± 6.9
Dichlormid	2.5	33.3 ± 4.8
Control (alachlor only)	0	16.8 ± 4.7

[a] Alachlor was applied as 30 μM aqueous solution containing 0.5% methanol (v/v) on the vermiculite surface at the time of initial watering in all the treatments shown in this table.

[b] Values are means ± standard error.

Hirase & Molin, 2001

Flurazole

TABLE 4
Effect of Safeners on Extractable Cysteine Synthase Activity in Shoots of Sorghum

Compound	Dosage (g/kg seed)	Specific activity (μmol/min/mg protein)	Total activity (μmol/min/g fresh weight)
Fluxofenim	0.4	3.47 (107)	73.4 (136)
Naphthalic anhydride	2.5	3.55 (110)	74.4 (138)
Benoxacor	2.5	4.75 (147)	87.2 (161)
Dichlormid	2.5	4.36 (135)	65.8 (122)
Control	0	3.24 (100)	54.0 (100)
LSD (0.05)		0.33	9.6

Note. Cysteine synthase activity was measured 48 h after planting. Values in parentheses represent percentage of control.

Hirase & Molin, 2001

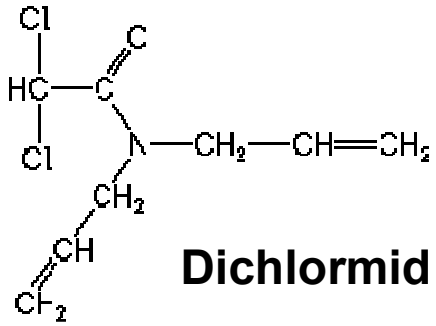
Pesticide Biochemistry and Physiology **71**, 116–123 (2001)
doi:10.1006/pest.2001.2567, available online at http://www.idealibrary.com on IDEAL®

Effect of Flurazole and Other Safeners for Chloroacetanilide Herbicides on Cysteine Synthase in Sorghum Shoots

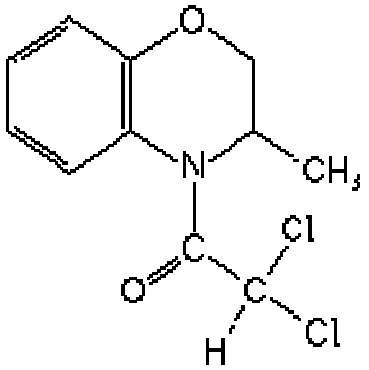
Kangetsu Hirase[1] and William T. Molin

Southern Weed Science Research Unit, USDA-ARS, P.O. Box 350, Stoneville, Mississippi 38776

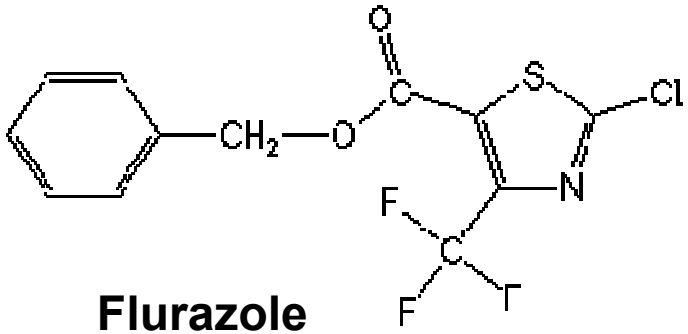
Antidotes d'Herbicides = « herbicide safeners »



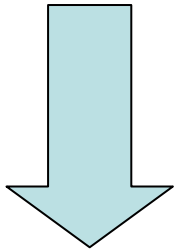
Dichlormid



Benoxacor



Flurazole



Détoxication de l'herbicide correspondant dans plante de culture

DUAL® GOLD SAFENEUR®	AV N° 9800259
Usages et doses autorisés	Désherbage du maïs et du maïs doux : 2,1 l /ha
Composition	915 g/l de S-métolachlore* + 45 g/l de bénoxacor*
Formulation	EC (concentré émulsionnable)
Classement	Xi : irritant – N - Dangereux pour l'environnement - R43 : peut entraîner une sensibilisation par contact avec la peau - R 50 : très toxique pour les organismes aquatiques - R 53 : peut entraîner des effets néfastes à long terme pour l'environnement aquatique
Mode d'action	Action multisite. Pénétration par le coléoptile et les racines des adventices provoquant l'inhibition de la germination ou l'arrêt de la croissance, provoquant la mort de la plantule.
Délai d'emploi avant récolte	Maïs = 90 jours. Maïs doux = 60 jours
Emballage	5 – 20 – 100 – 200 litres.

Métolachlore (herbicide)
=> inhibition synthèse lipide + effet antagoniste gibbérellines
Benoxacor (antidote, phytoprotecteur, safener)
=> augmentation activité GST

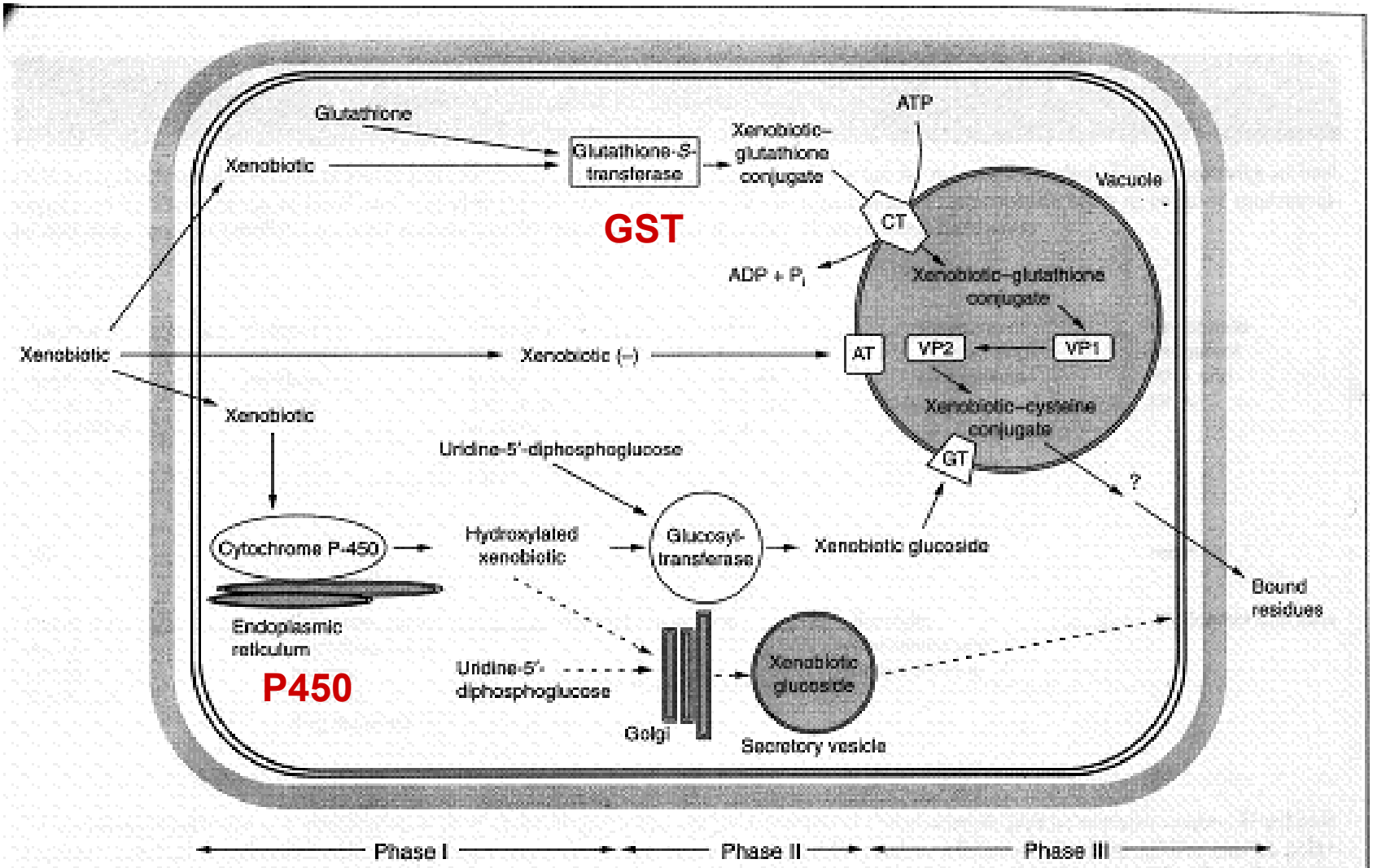


Fig. 1. The enzyme-catalysed reactions responsible for the detoxification of xenobiotics in plants are localized in or associated with several organelles and cellular compartments. The broken arrows represent a proposed pathway for the glucosylation of xenobiotics in the Golgi, followed by release of the metabolites into the apoplast via exocytosis[72]. Abbreviations: CT, glutathione-conjugate transporter; AT, ATP-dependent xenobiotic anion (taurocholate) transporter; GT, ATP-dependent glucoside-conjugate transporter; VP, vacuolar peptidase.

Coleman et al., 1997

PERGAMON

Chemosphere 48 (2002) 965–974

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Herbicide safeners: uses, limitations, metabolism, and mechanisms of action

Aqel W. Abu-Qare *, Harry J. Duncan

Department of Chemistry, Agricultural, Environmental and Analytical Chemistry, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK

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Activity of glutathione *S*-transferase toward some herbicides and its regulation by benoxacor in non-embryogenic callus and in vitro regenerated tissues of *Zea mays*

Daniele Del Buono, Maurizio Micheli, Luciano Scarponi *, Alvaro Standardi

Table 2
Extractable GST activity toward CDNB from untreated and benoxacor-treated tissues regenerated on agarized substrate and on liquid medium

	GST(CDNB) activity [nmol s^{-1} (mg of $protein^{-1}$)]
Tissue regenerated on agarized substrate	9.22 ± 0.55^{a}
Tissue regenerated on agarized substrate with benoxacor	13.25 ± 0.32^{b}
Tissue regenerated on liquid medium	10.01 ± 0.25^{a}
Tissue regenerated on liquid medium with benoxacor	12.50 ± 0.39^{b}

The data represent the means of triplicate determinations ± SD. Means within the column followed by the same letter are not significantly different at a 5% level using the *t* test of Student. The statistical analyses have been done comparing only data of activity coming from the tissues regenerated using the same substrate.

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2.4. GST extraction and purification

GST extraction was carried out according to the procedure of Cummins et al. [14]. Samples of callus and regenerated tissues (10.0 g) were powdered in liquid nitrogen using a mortar and pestle. The powders were suspended in extraction buffer (1/5, w/v), composed of 100 mM Tris–HCl (pH 7.5), containing 2 mM EDTA, 1 mM dithiothreitol, and polyvinylpolypyrrolidone (1.5%, w/v). After filtration through two layers of muslin, the homogenate was centrifuged at 15,000 rpm for 20 min and the supernatant was adjusted to 80% saturation with respect to $(NH_4)_2SO_4$ to precipitate the proteins (4 °C for 3 h). The resulting suspension was centrifuged at 15,000 rpm for 10 min and the protein pellets collected and stored at −20 °C. The pellet was dissolved in 20 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol and applied onto a Sephadex G-25 for desalting (enzyme extract).

All the extraction steps were carried out at 4 °C.

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2.5. GST(CDNB) activity and kinetic parameters

The spectrophotometric procedure described by Edwards [27] was used to determine GST activity toward 1-chloro-2,4-dinitrobenzene (CDNB) "as model substrate." The GST activity was determined by adding 25 µL of 40 mM CDNB to a solution containing 900 µL of 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 6.5), 25 µL of enzymatic extract, and 50 µL of 0.1 M GSH (pH 7.0). The amount of conjugate formed by reaction between GSH and CDNB was evaluated spectrophotometrically at 340 nm and 35 °C. From this result the amount of conjugate formed in a reaction mixture in which the enzymatic extract was substituted by the buffer (non-enzymatic reaction) was then subtracted. The GST activity was expressed as nanomoles of GSH-CDNB formed $s^{-1}\,mg^{-1}$ of protein employed for the assay.

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Dosage des protéines

kit Sigma/"microprotein determination«
(BRADFORD)

Gamme : BSA (stock 1mg/ml), 10 à 60 µg/tube
ou **extrait 10**µl & 20µl

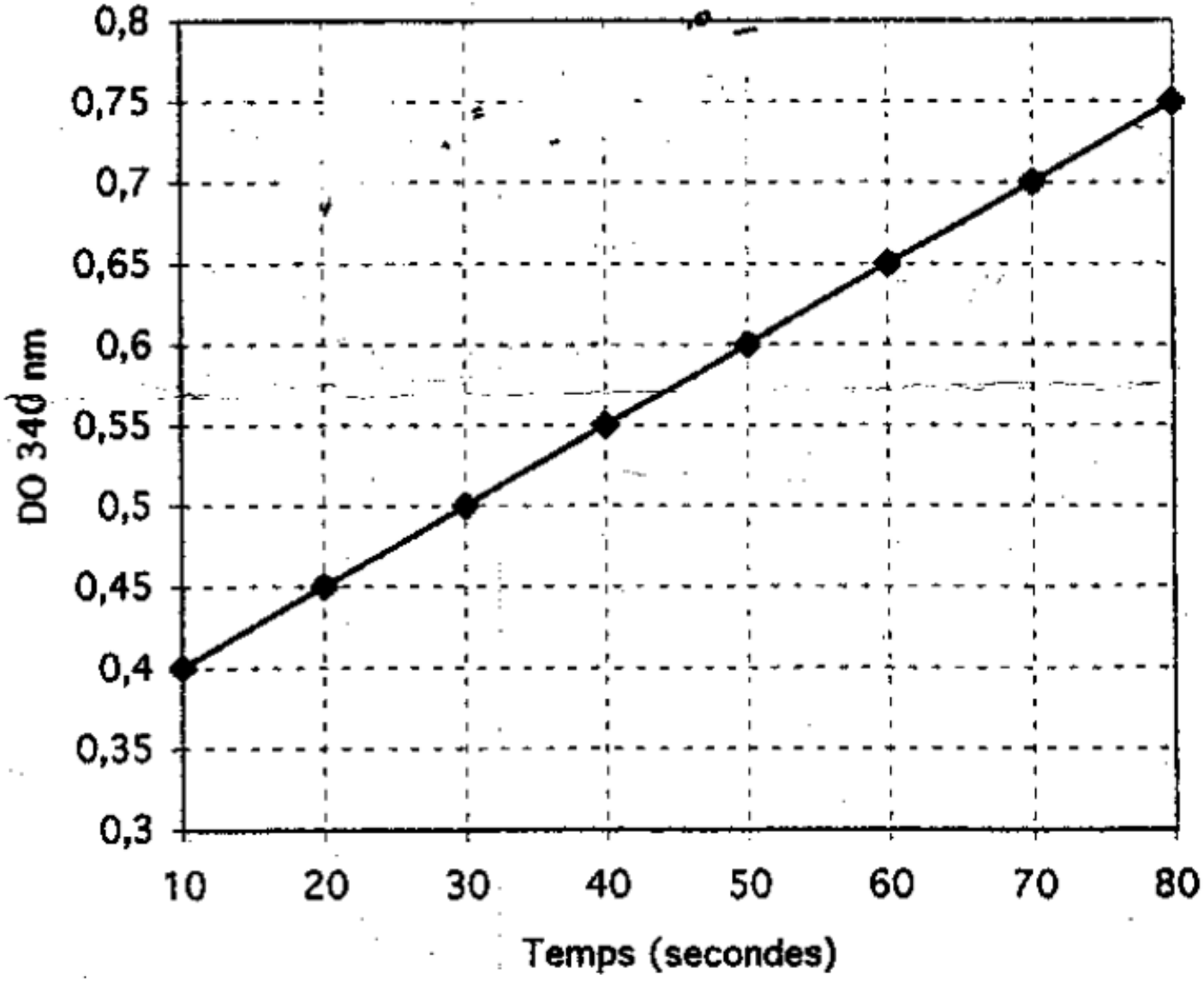
+ **dH2O** qsp 100 µl

+ 5 ml **réactif de Bradford** (dilué 5x)

+ 5 min

=> **DO 595 nm**

=> exprimer la **concentration protéique en mg protéines/ml extrait.**



Conc Prot = 1,57 mg/ml extrait

On veut déterminer l'activité **Glutathion S-Transférase (GST) dans des racines de plantules de Maïs, en suivant le protocole expérimental suivant :**

Dans une cuve de spectrophotomètre en verre (trajet optique 1 cm), on prépare le milieu réactionnel de composition : 350 µl extrait protéique
+ 50 µl CDNB 100mM
+ 2 ml tampon Phosphate 100 mM pH 6,5

Après homogénéisation, la cuve est mise en place dans le spectrophotomètre, et la réaction est initiée par addition de 100 µl de GSH 100 mM.
L'enregistrement de la DO à 340 nm en fonction du temps permet d'obtenir une droite dont la pente est de 0,005 U.DO/sec.

Questions :

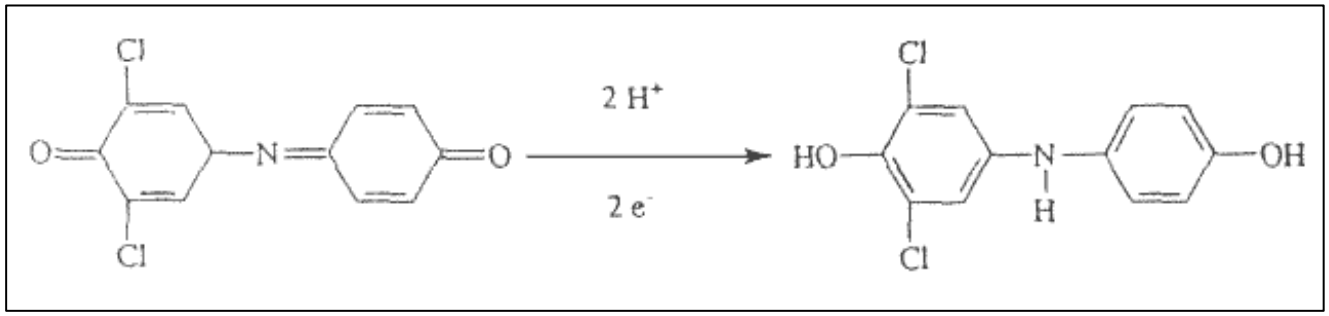
1/ Sachant que la concentration en protéines de l'extrait utilisé est de 1,2 mg / ml, exprimez l'activité GST en nmoles / min / mg protéine ($\varepsilon = 9{,}6\ mM^{-1}.\ cm^{-1}$).

2/ La même expérience est réalisée avec des racines de plantules de Maïs traitées avec un antidote d'herbicide, le BAS 145138, et l'activité GST mesurée est de 390 nmoles / min / mg protéine : ce résultat vous paraît-il logique ?

TP2 - Herbicides inhibiteurs PSII

***Objectif* : déterminer** *in vitro* le I50 de l'Atrazine sur le transport photosynthétique des électrons dans des chloroplastes d'Epinard (plante sensible) et de Maïs (plante résistante).

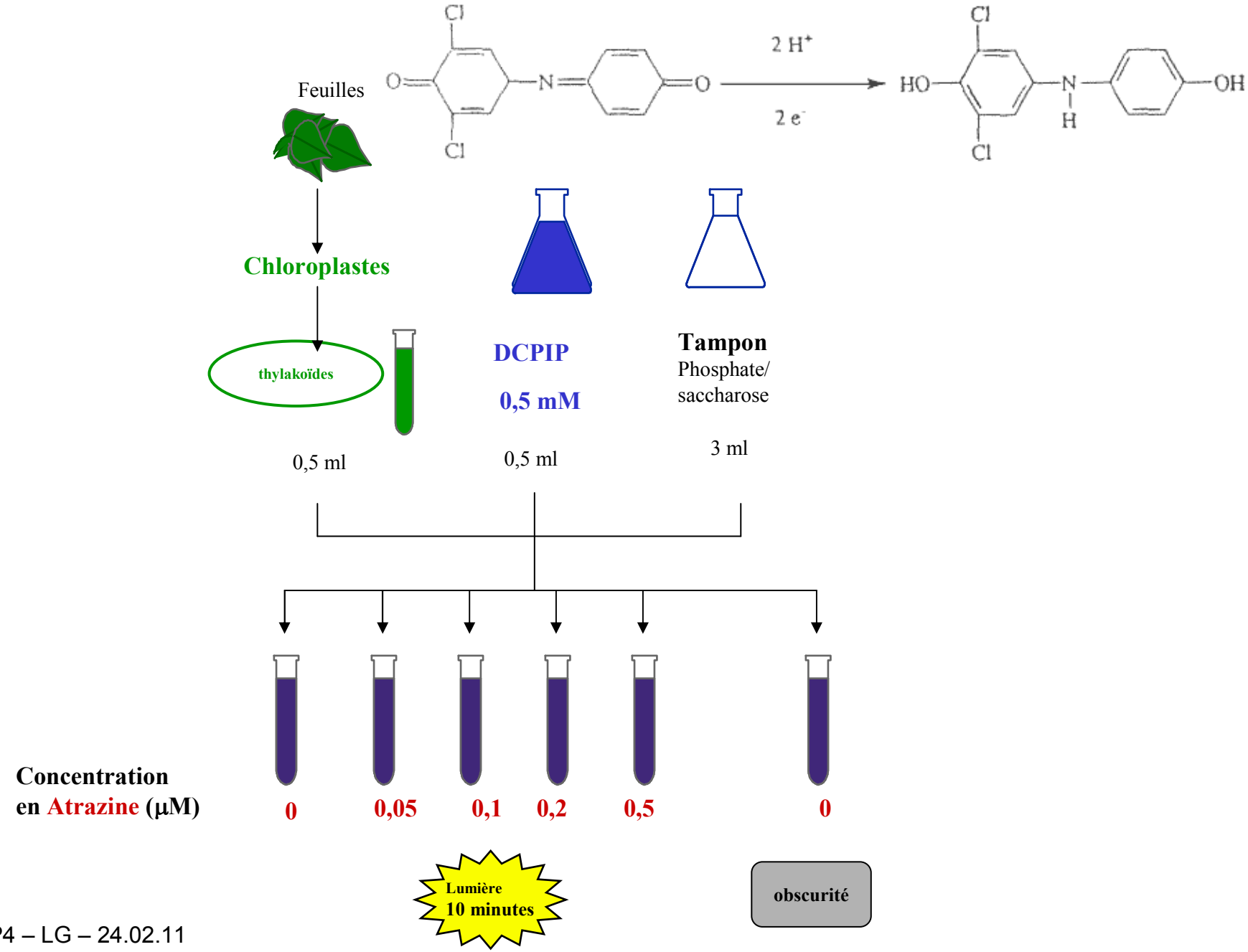
***Principe* : le transport des électrons est mesuré dans des chloroplastes par quantification spectrophotométrique de la réduction d'un accepteur d'électrons artificiel : le DCPIP (2,6-dichlorophénolindophénol)**



DCPIP oxydé, bleu
(λmax 600 nm)

DCPIP réduit, incolore

Atrazine / mesure activité *in vitro*



1/ Préparation des chloroplastes

- ***Broyage*** **dans mortier (frigo)**

Feuilles Epinard ou Maïs (3g) découpées en fines lanières (enlever nervures)

+ 6 ml Tampon d'extraction : -Tris HCl 0,01M pH 7,4

- MgCl2 0,01M
- Saccharose 0,4M

- ***Filtration*** **de l'homogénat (gaze) => tube de centrifugation (GLACE)**
- ***Centrifugation*** **5min 5000 rpm (Jouan)** 4°C
- ***Remise du culot en suspension*** **dans 3 ml de Tampon TR (pH6,5):**
  - Na2HPO4 0,2M
  - KH2PO4 0,1M
  - Saccharose 0,5M

Rq : Ne pas prendre la partie inférieure du culot (blanchâtre) constituée d'amyloplastes

⇨ Transvaser la **suspension de chloroplastes dans un tube à hémolyse, homogénéiser doucement et garder dans la glace à l'obscurité (tube entouré avec papier aluminium)**

2/ Dosage spectrophotométrique chlorophylles

100 µl suspension chloroplastes
⇨ tube eppendorf **2,2 ml**
- Centrifugation 5 min 5000 rpm 4°C
⇨ Culot + 2 ml acétone 90%
=> Vortexer
+ Centrifugation 5 min 10.000 rpm 4°C
⇨ Surnageant
⇨ DO 645 et 663 nm

Déterminer la concentration en chlorophylles (Ca+Cb) dans votre suspension de chloroplastes en mg/ml dans acétone à 90%:

Ca µg/ml = 12,7 DO_{663} - 2,69 DO_{645}
Cb µg/ml = 22,9 DO_{645} - 4,68 DO_{663}
(formules de MacKinney)

Dilution de la suspension chloroplastique dans TpR (⇨ Ca+Cb = 30 µg/ml)

3/ Mesure de l'intensité du transport d'électrons en présence de concentrations croissantes d'Atrazine

Milieu réactionnel à préparer dans tube hémolyse :
0,5 ml **suspension chloroplastes diluée (Ca+Cb = 30** µg/ml)
+ 4 ml **TR**
+ 0,5 ml **DCPIP 0,55 mM/KCl 0,01%**
+ **Atrazine (solution stock : 50mM dans méthanol)**
=> concentrations finales (µM) :
0,1/0,15/0,2/0,25/0,3/0,4/0,5/1

Rq : Ne pas oublier le **témoin sans Atrazine + témoin obscurité !**
⇨placer les tubes dans une bassine avec de l'eau pour éviter un échauffement trop important du milieu **et éclairer 10 min environ**
(le tube sans Atrazine doit être décoloré)

Déterminer la DO 600 nm et tracer la courbe de la DO à 600 nm en fonction de la concentration en Atrazine => détermination graphique du I50 (µM)

Organisation TP Pesticides et Phytoprotection – 2011

Salle de TP1, Institut de Botanique (2ème étage)

	TP1 (Atrazine)	TP2 (GST)
Master ISIE *7 étudiants*	**Mardi 29/03** 13h30-18h00	**Mercredi 30/03** 16h-19h
Parcours PE *12 étudiants*	**Jeudi 31/03** 8h30-12h30	**Vendredi 08/04** 8h30-12h30
Parcours BVI *3 étudiants*	**Lundi 04/04** 8h30-12h30	**Vendredi 15/04** 8h30-12h30
Parcours VRV *11 étudiants*		