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Efficacy and foliar absorption of flupyrsulfuron-methyl and prosulfocarb applied alone or in mixture on *Lolium multiflorum* and wheat

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Abstract – Mixtures of flupyrsulfuron-methyl and prosulfocarb used to control *Lolium multiflorum* Lam. in wheat (*Triticum aestivum* L.) were studied using bioassays on whole plants and foliar penetration experiments. In bioassays, flupyrsulfuron-methyl and prosulfocarb were applied at 6–7 doses, alone or in mixture at the 1:400, 1:40 and 1:4 ratios (wt/wt) respectively. Dose-response curves were fitted using a logistic model and joint action was evaluated by means of the isobol method. In bioassays conducted in the greenhouse with *L. multiflorum*, the joint action of mixtures exhibited additivity of dose at all ratios of active ingredients and levels of responses. A bioassay conducted in a growth chamber with the 1:400 mixture showed an antagonistic effect, but at doses well below those used in weed control. A bioassay conducted on wheat in the greenhouse also indicated that the effect of the 1:400 mixture followed additivity. Hence, the presence of flupyrsulfuron-methyl in the mixtures did not seem to affect the activity of prosulfocarb on *L. multiflorum* or vice versa. Prosulfocarb penetrated to some extent into wheat leaves (30% of the applied dose after 72 h) but barely into *L. multiflorum* (3% after 72 h). Flupyrsulfuron-methyl applied alone penetrated to a low extent in *L. multiflorum* and wheat (3% and 1% after 72 h, respectively). Its penetration was increased in the presence of prosulfocarb (18% and 45% after 72 h on *L. multiflorum* and wheat, respectively). It was concluded that although prosulfocarb increased the foliar penetration of flupyrsulfuron-methyl, this did not affect the efficacy of the latter on whole plants.

joint action / flupyrsulfuron-methyl / prosulfocarb / *Lolium multiflorum*

Résumé – Efficacité et absorption foliaire du flupyrsulfuron-méthyle et du prosulfocarbe appliqués séparément ou en mélange sur *Lolium multiflorum* et sur blé. Des mélanges de flupyrsulfuron-méthyle et de prosulfocarbe conçus pour améliorer la lutte contre *Lolium multiflorum* Lam. dans le blé (*Triticum aestivum* L.) ont été étudiés grâce à des essais biologiques sur plantes entières et à des études de pénétration foliaire. Dans les essais biologiques, le flupyrsulfuron-méthyle et le prosulfocarbe étaient appliqués à 6–7 doses, seuls ou en mélange dans les rapports 1:400, 1:40 et 1:4 (p/p). Les courbes de réponse étaient ajustées au moyen de modèles logistiques et l'action conjuguée était évaluée

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par la méthode des isoboles. Dans les essais biologiques conduits en serre sur *L. multiflorum*, l'action conjuguée des mélanges montrait une additivité de doses, quel que soit le rapport des matières actives et quel que soit le niveau de réponse. Un essai biologique conduit en chambre climatisée avec le mélange 1:400 a montré un effet antagoniste, mais à doses nettement inférieures à celles utilisées pour le désherbage. Un essai biologique conduit sur blé en serre a lui aussi montré que l'effet du mélange 1:400 suivait l'additivité. Par conséquent, la présence de flupyrsulfuron-méthyle dans les mélanges ne semblait pas affecter l'activité du prosulfocarbe sur *L. multiflorum*. La pénétration foliaire du prosulfocarbe était limitée chez le blé (30 % de la dose appliquée après 72 h) et faible chez *L. multiflorum* (3 % après 72 h). Le flupyrsulfuron-méhtyle appliqué seul pénétrait peu chez *L. multiflorum* et chez le blé (respectivement 3 % et 1 % après 72 h). Sa pénétration était augmentée en présence de prosulfocarbe (respectivement 18 % et 45 % après 72 h). En conclusion, bien que le prosulfocarbe augmentait la pénétration foliaire du flupyrsulfuron, cet effet ne se traduisait pas par une augmentation de l'efficacité sur plantes entières.

action conjuguée / flupyrsulfuron-méthyle / prosulfocarbe / *Lolium multiflorum*

1. Introduction

Flupyrsulfuron-methyl is a sulfonylurea herbicide used in cereals [2, 19]. It controls broad-leaved weeds such as *Galium aparine* L., *Matricaria* spp. and *Sinapis arvensis* L. In addition, unlike most other sulfonylurea herbicides, it is active against some *Graminaceae*, for example *Alopecurus myosuroides* (Huds.), *Apera spica-venti* (P. Beauv.) and *Poa trivialis* L. However, *Lolium multiflorum* L. was moderately sensitive to flupyrsulfuron at the two/three-leaf stage and hardly sensitive at tillering [6].

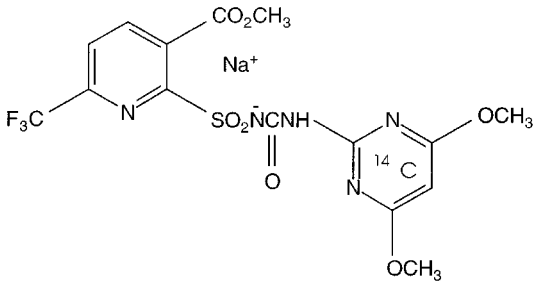
In agricultural practice, flupyrsulfuron-methyl is associated with other active ingredients to broaden its spectrum of efficacy. For instance, it is marketed as mixtures with metsulfuron-methyl or with carfentrazone-ethyl. These mixtures are intended to widen flupyrsulfuron-methyl's spectrum on broad-leaved weeds. However, there is also a need to extend the efficacy spectrum of flupyrsulfuron on *Graminaceae*, for example in the northern part of France where *L. multiflorum* is a significant weed. To this aim, prosulfocarb, a thiocarbamate herbicide, can be tank-mixed with flupyrsulfuron-methyl. Prosulfocarb controls *L. multiflorum* in wheat when applied pre-emergence [9] and also when applied post-emergence, provided that the two-leaf stage of the weeds is not exceeded [1]. It indicates that the predominant route of prosulfocarb entry into weeds is via the soil, although some foliar penetration cannot be excluded.

Since flupyrsulfuron-methyl has some efficacy on *L. multiflorum*, the prosulfocarb dose can be reduced in the mixture. The objective of the present study was to assess the joint-action of mixtures containing flupyrsulfuron-methyl and prosulfocarb and applied post-emergence to *L. multiflorum* and wheat. Concurrently, the foliar penetration of flupyrsulfuron-methyl and prosulfocarb into *L. multiflorum* and wheat leaves was studied to see whether any effects observed on whole plants could be explained by differences in foliar uptake.

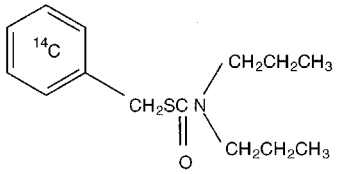
2. Materials and methods

2.1. Herbicides

The commercial preparations of flupyrsulfuron-methyl (Lexus 50 WG ®, 50% a.i. (wt/wt), DuPont de Nemours), and prosulfocarb (Defi ®, 800 g a.i.·L^{-1}, Sopra) were used in all experiments. In the flupyrsulfuron-methyl:prosulfocarb mixtures, the ratio of the active ingredients were 1:400, 1:40 and 1:4 (w/w). [^{14}C]flupyrsulfuron-methyl (0.7067 GBq·$mmol^{-1}$, DuPont de Nemours) and [^{14}C]prosulfocarb (1.73 Gbq·$mmol^{-1}$, Zeneca Agrochemicals) were uniformly labelled on the pyrimidin ring and on the phenyl ring, respectively (Fig.1). Their radiochemical purity was higher than 98%.



[^{14}C] flupyrsulfuron-methyl



[14C] prosulfocarb

Figure 1. Formula of radioactive flupyrsulfuron-methyl and prosulfocarb.

2.2. Bioassays

2.2.1. Growth conditions and treatments

Seeds of *Lolium multiflorum* L. and winter wheat (*Triticum aestivum* L.) cv. Fidel were sown in 1.5 L pots filled with 350 g of a mixture of sand, clay-loam soil and peat (1:1:1; v/v/v). Germination and cultivation of plants were done in a greenhouse for experiments 1, 3 and 4, and in a growth chamber for experiment 2. The mean temperature of the greenhouse was set at 17 °C and the relative humidity ranged from 50 to 95%. Additional light gave a 16 h photoperiod. The temperature of the growth chamber was 18 / 11 °C (light / dark) with a day lenght of 16 h. Light was produced by fluorescent tubes delivering 220 µmol photons·m^{-2}·s^{-1} PAR. Plants were watered daily with a nutrient solution containing all necessary macro and micro nutrients. The plants of *L. multiflorum* and wheat were thinned to 10 per pot before treatment.

Plants were treated at the two-leaf stage using an indoor track sprayer comprising a movable boom equipped with two flat-fan nozzles (Albuz 110°) delivering 200 L·ha^{-1} at 300 kPa. The commercial preparations of the herbicides and their mixtures were applied at seven doses distributed geometrically. Minimum and maximum doses applied to *L. multiflorum* were 0.085 and 156 g a.i.·ha^{-1} for flupyrsulfuron-methyl, 6.53 and 12 000 g a.i.·ha^{-1} for prosulfocarb, 7.54 and 31 350 g a.i.·ha^{-1} for the 1:400 mixture, 1.74 and 3 198 g a.i.·ha^{-1} for the 1:40 mixture and 0.21 and 390 g a.i.·ha^{-1} for the 1:4 mixture. On wheat, the doses of flupyrsulfuron-methyl ranged from 0.085 to 156 g a.i.·ha^{-1}, the doses of prosulfocarb from 15.23 to 62 400 g a.i.·ha^{-1} and the doses of the 1:400 mixture from 7.64 to 31 278 g a.i.·ha^{-1}. Treatments within experiments were replicated three times and the controls were replicated six times. Treated plants and control were randomized in the growth chamber except for the control plants which were not let near the highest doses of prosulfocarb. All the plants were harvested 15–18 days after treatment. An observation corresponded to the dry biomass of 10 shoots per pot.

2.2.2. Statistical analysis of whole-plant bioassays

The results of an experiment comprising different treatments were analyzed with the technique of non-linear regression as described previously [5]. Briefly, dose-response curves for all treatments were simultaneously fitted using a sequence of log-logistic models devopped by Streibig [16] and Kudsk et al. [10].

$$U_{ij} = C + \frac{D - C}{1 + \exp\left[b_i\left(\log\left(x_{ij}\right) - \log\left(ED_{50i}\right)\right)\right]} \quad (1)$$

where U_{ij} denotes the dry matter at the jth dose of the ith herbicide treatment; D and C denote the upper and lower limits of dry matter at zero and at very large doses of active ingredients; ED_{50i} denotes the dose of herbicide i required to reduce

dry matter by half between the upper and lower limits; and b_i is proportional to the slope of curve around ED_{50i}. The acceptance of model 1 was given by the non-significance at the $P = 0.05$ level of the F-test of the lack-of-fit. It was based on the residual mean square of the regression and the error mean square of a variance analysis according to Seefeldt et al. [15], and by graphical analysis of the distribution of residuals [17]. A second step tried to reduce the number of parameters estimated by model 1. It was assessed to determine whether response curves could be considered as similar. A model 2 with four common parameters was put for the response curves submitted to comparison:

$$U_{ij} = C + \frac{D - C}{1 + \exp\left[b\left(\log\left(x_{ij}\right) - \log\left(ED_{50}\right)\right)\right]} \cdot \quad (2)$$

Model 2 was tested versus model 1. The dose giving a 80% biomass reduction could be intregrated into the log-logistic model [11]. For instance, model 1 became:

$$U_{ij} = C + \frac{D - C}{1 + \exp\left[b_i\left(\log\left(x_{ij}\right) - \log\left(ED_{80i}\right)\right) + \log\left(4\right)b_i\right]} \cdot \quad (3)$$

Fittings were made after transformation of data to stabilize variance. A Transform-Both-Sides method was used:

$$h(y, \lambda) = h\left(f_i(z), \lambda\right) + \sigma\varepsilon \quad (4)$$

with h *(*y, λ*)* given by

$$h\left(y, \lambda\right) = \frac{y^{\lambda} - 1}{\lambda} \quad (5)$$

where f_i (z) is the response model (Eqs. (1)–(3)), λ is the exponent of a power transformation in equation (5) suggested by Box and Cox [4], σ is the standard deviation and ε the residuals, corresponding to different observations and are assumed to follow the standard normal distribution.

The joint action of mixtures was evaluated according to the graphic method of isobols described by Morse [13] and Gessner [8]. For each product applied alone and all their possible mixtures, an isobol represents doses which produce the same effect on the basis of biologically equivalent doses. An isobol calculated according to this principle (Additive Dose Model) is a straight line at each level of effect if there is the additivity. Concave or convex isobols reveal synergism or antagonism, respectively. The confidence intervals for the ED_i of herbicides applied alone (shown on the axes of the figures of isobols) were calculated at the $P = 0.025$ level. Thus confidence area relative to the isobols corresponded to the $P = 0.05$ level.

2.3. Foliar absorption of ^{14}C-flupyrsulfuron-methyl and ^{14}C-prosulfocarb

L. multiflorum was sown in a mixture of sand and clay-loam soil (1:2; v/v). The plants were grown in a climatic chamber at 18/12 °C (light/dark), 16 h photoperiod (fluorescent light delivering 190 µmol photons·m^{-2}·s^{-1} PAR). Wheat seeds were germinated on moist filter paper in Petri dishes in the dark at 17 °C during 72 h. The seedlings were then planted in the same mixture and grown under the same conditions as *L. multiflorum.*

Radiolabelled herbicides were solubilized in acetone. The desired radioactivity of the herbicide under study was deposited at the bottom of a vial. After evaporation of the acetone under a nitrogen flux, the commercial herbicide preparation was added and the vial was stirred so as to dissolve the radio-labelled herbicide. The final radioactivity concentration was 92.5 Bq·µL^{-1}. The herbicide concentrations corresponded to treatments made at application volume of 150 L·ha^{-1} and delivering 50 g a.i.·ha^{-1} for flupyrsulfuron methyl and 1070 g a.i.·ha^{-1} for prosulfocarb. In the mixture, the flupyrsulfuron:prosulfocarb ratio was 1:400 (wt/wt) and it was applied at two dose rates, namely 400 and 1500 g a.i.·ha^{-1}.

Five droplets of approximately 0.8 µL of the preparations were deposited with a microsyringue on the upper third of the abaxial surface of the first leaf of wheat or *L. multiflorum* at the 2/3-leaf stage (four replicates). Absorption was determined 0, 3,

9, 27 and 72 h after treatment. For the times 3 and 9 h, the plants were kept under continuous light at a constant temperature of 18 °C. For the times 27 and 72 h, the plants were placed under the growth conditions described above.

At the pre-selected times, the treated area of each leaf was washed with 0.5 mL methanol. Radioactivity was determined by scintillation counting after evaporation of the methanol under a nitrogen flux. The washing efficiency of dried deposits of flupyrsulfuron-methyl and prosulfocarb was checked on glass slides of similar dimensions as the leaves. The treated leaf, the rest of aerial parts and the roots were combusted in an oxidizer for radioactivity assessment. Preparations of ^{14}C-prosulfocarb were also deposited following the same procedure onto glass slides. The latter were placed under the same controlled conditions as for foliar penetration experiments. The amount of prosulfocarb remaining on the glass slides was determined by washing the glass slides with 0.5 mL methanol immediately after deposition, after drying of the deposits (5–10 minutes) and 27 h after the deposit. The amount of radiolabel deposited on the leaves was checked for each preparation and each plant species by combusting a treated leaf excised immediately after deposition (three replicates).

3. Results

3.1. Bioassays

The data of experiments 1 to 4 could be fitted at least with model 1 which assumed upper and lower limits common to the three or four response curves of the same experiment (F-tests not significant, Tab. I). The estimated values of upper limits were roughly similar to the biomass of control (not shown). Model 2 described well the data relative to prosulfocarb and the 1:400 mixture in experiment 4 (Tab. I).

3.1.1. Experiment 1 (Lolium multiflorum)

Flupyrsulfuron-methyl produced a 10% inhibition of growth at 0.3 g·ha^{-1} and about 87% at 156 g·ha^{-1} (Fig. 2). This maximal dose used

Table I. Estimated parameters of regressions. Figures in brackets are confidence intervals at the $P = 0.05$ level.

Experiment	Plant species	a.i.	D	C	b	ED_{50}	ED_{80}	$F_{calculated}$ vs. F_{table}
1	*Lolium multiflorum* (greenhouse)	flupyrsulfuron-Me*	1005 (29)	129 (17)	0.64 (0.12)	8 (3)	73 (31)	1.61 vs. 1.84
		prosulfocarb			1.48 (0.35)	219(42)	566 (136)	
		1:400 mixture			2.07 (0.51)	214 (41)	418 (82)	
2	*Lolium multiflorum* (growth chamber)	flupyrsulfuron-Me*	1959 (47)	213 (23)	1.20 (0.27)	33 (8)	104 (21)	1.32 vs. 1.94
		prosulfocarb			2.19 (0.30)	127 (16)	239 (30)	
		1:400 mixture			2.19 (0.30)	227 (30)	427 (55)	
3	*Lolium multiflorum* (greenhouse)	flupyrsulfuron-Me*	634 (39)	138 (11)	0.98 (0.29)	12 (6)	52 (20)	1.59 vs. 1.82
		prosulfocarb			2.21 (1.03)	203 (52)	380 (94)	
		1:40 mixture			1.75 (0.64)	165 (49)	366 (98)	
		1:4 mixture			0.86 (0.24)	27 (13)	136 (53)	
4	wheat (greenhouse)	flupyrsulfuron-Me*	3492 (105)	559 (331)	0.58 (0.35)	-	-	1.04 vs. 1.91
		prosulfocarb			1.06 (0.31)	5781 (1989)	-	
		1:400 mixture			1.06 (0.31)	5781 (1989)	-	

*Flupyrsulfuron-Me is the shortened form of flupyrsulfuron-methyl.

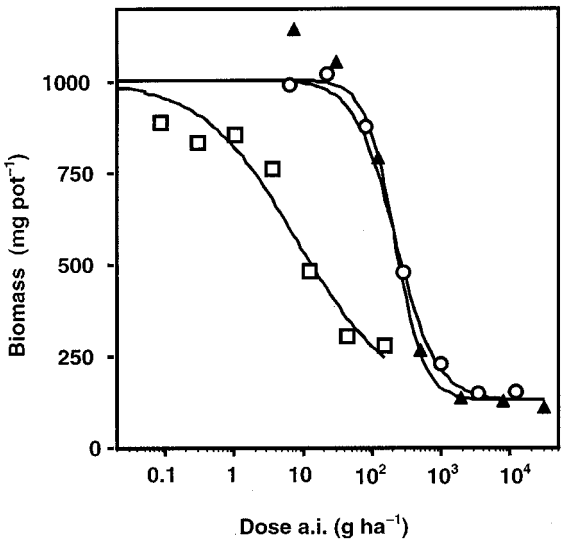


Figure 2. Mean biomass from experiment 1 and predicted response curves of *Lolium multiflorum* plotted against dose of flupyrsulfuron-methyl (squares), prosulfocarb (circles), and the mixture of flupyrsulfuron-methyl and prosulfocarb at the 1:400 ratio (triangles).

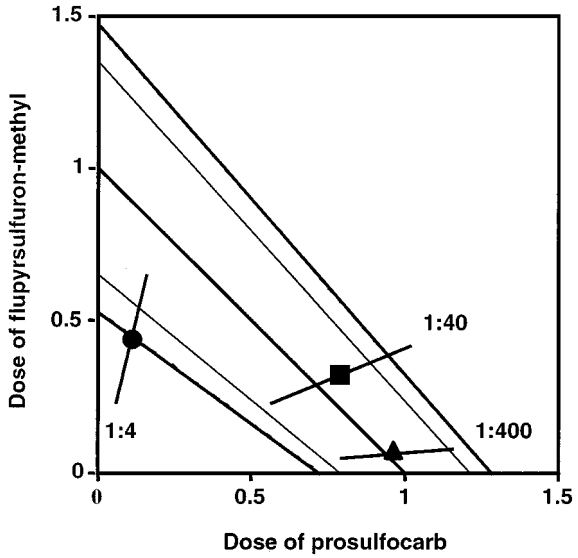


Figure 3. Location of mixtures ED_{50} relative to ED_{50} isobole from experiments 1 and 3. The axes have been scaled so that ED_{50}s of flupyrsulfuron-methyl and prosulfocarb are unity. Bold line is the ED_{50} isobole. Dotted lines delimit confidence area at $P = 0.05$ for 1:400 (fine lines), 1:40 and 1:4 (bold lines) mixtures. Symbols correspond to 1:400 (triangles), 1:40 (squares) and 1:4 (circles) mixtures. Bars indicate confidence intervals at $P = 0.05$.

corresponded to the solubility of the sulfonylurea in water (0.6 g·L^{-1}) and a spraying volume of 220 L·ha^{-1}. It slowly killed plants in this experiment. Symptoms produced by prosulfocarb and the 1:400 mixture were chlorosis, necrosis and death of plants sprayed with doses equal or higher than 2000 g·ha^{-1}. The relative potency of flupyrsulfuron-methyl relatively to prosulfocarb, i.e. the ratio of doses giving the same effect, varied to a great extent according to the level of efficacy, namely 192, 73, 27 and 8 for the ED_{10}, ED_{25}, ED_{50} and ED_{80}, respectively. Although the slope of the 1:400 mixture was asymptotically different from prosulfocarb, their ED_{50} and ED_{80} were not significantly different (Tab. I). The 1:400 mixture exhibited a joint action which followed the additivity defined by isobols at both levels ED_{50} and ED_{80} (Figs. 3 and 4).

3.1.2. Experiment 2 (Lolium multiflorum)

The herbicide treatments used in this experiment were similar to those of experiment 1 except that experiment 2 was conducted in a growth chamber

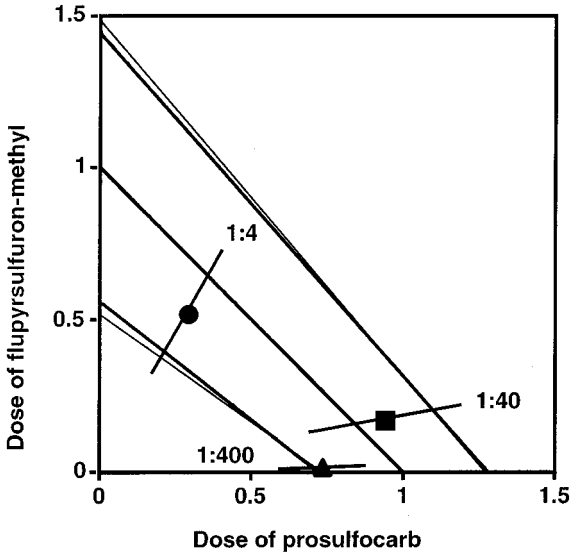


Figure 4. Location of mixtures ED_{80} relative to ED_{80} isobole from experiments 1 and 3. The axes have been scaled so that ED_{80}s of flupyrsulfuron-methyl and prosulfocarb are unity. Bold line is the ED_{80} isobole. Dotted lines delimit confidence area at $P = 0.05$ for 1:400 (fine lines), 1:40 and 1:4 (bold lines) mixtures. Symbols correspond to 1:400 (triangles), 1:40 (squares) and 1:4 (circles) mixtures. Bars indicate confidence intervals at $P = 0.05$.

where the highest temperature never exceeded 18 °C during the photoperiod (Tab. I). The plants cultivated under these conditions showed a stronger growth than those in experiment 1. The complete killing of plants were recorded with the highest doses of prosulfocarb and the 1:400 mixture, but not with flupyrsulfuron-methyl which only induced a reduction of growth. Statistics indicated that the response curves relative to prosulfocarb and the 1:400 mixture were parallel. Since the ED_{50} and ED_{80}, of the 1:400 mixture were significantly higher than prosulfocarb, the 1:400 mixture exhibited an antagonism which was confirmed by the method of isobols (not shown). Nevertheless, the difference in effects between prosulfocarb and the 1:400 mixture was less than 3% when the doses were equal or higher than 1000 g·ha^{-1}.

3.1.3. Experiment 3 (Lolium multiflorum)

The highest doses of flupyrsulfuron-methyl did not induce the death of plants. The higher the proportion of flupyrsulfuron-methyl in the herbicide mixtures, the lower the doses which produced the same effect (Fig. 5). The joint actions of mixtures followed additivity at both levels ED_{50} and ED_{80}, (Figs. 3 and 4).

3.1.4. Experiment 4 (wheat)

Flupyrsulfuron-methyl, prosulfocarb and the 1:400 mixture were applied to wheat grown in a greenhouse. The doses used allowed almost complete response curves to be recorded with prosulfocarb and the 1:400 mixture since the highest doses were lethal. However this was not true with flupyrsulfuron-methyl (Fig. 6). On the basis of the test of similarity, the response curves of prosulfocarb and the 1:400 mixture were not significantly different (Tab. I).

The expected doses giving 10% inhibition were 12 ± 12 and 722 ± 379 g·ha^{-1} for flupyrsulfuron-methyl and prosulfocarb or the 1:400 mixture, respectively. The highest dose of flupyrsulfuron-methyl (156 g·ha^{-1}) exhibited about 33% inhibition. It is likely that the mixture followed additivity since the ratio of active ingredients being 1:400 in the mixture, the additivity curve was expected to be close to the response curve of prosulfocarb.

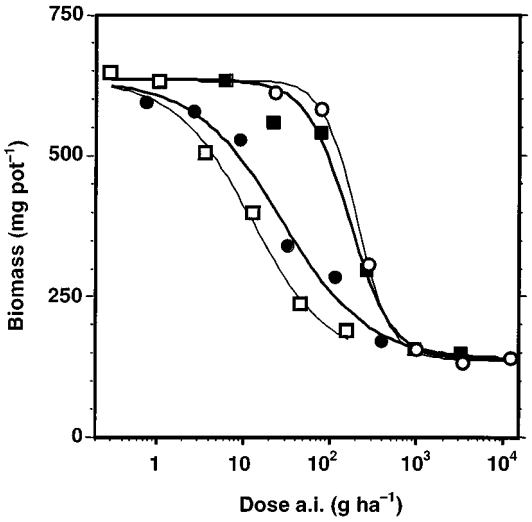


Figure 5. Mean biomass from experiment 3 and predicted response curves of *Lolium multiflorum* plotted against dose of flupyrsulfuron-methyl (open squares), prosulfocarb (open circles), and the mixtures of flupyrsulfuron-methyl and prosulfocarb at the 1:40 (closed squares) and 1:4 (closed circles) ratios.

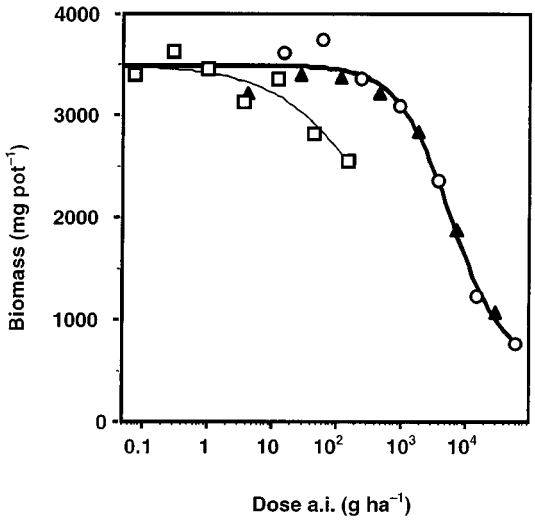


Figure 6. Mean biomass from experiment 4 and predicted response curves of wheat plotted against dose of flupyrsulfuron-methyl (squares), prosulfocarb (circles), and the mixture of flupyrsulfuron-methyl and prosulfocarb at the 1:400 ratio (triangles).

3.2. Foliar penetration of flupyrsulfuron-methyl and prosulfocarb

After 27 and 72 h, radioactivity recovery of ^{14}C-prosulfocarb was most of the time lower than 50%. Hence, its foliar absorption had to be expressed as the percentage of radioactivity found in plant after combustion, relative to deposited radioactivity. Radioactivity recovery of flupyrsulfuron-methyl was always higher than 92%. Hence, its foliar uptake could be expressed in a more precise way, namely as the percentage of radioactivity found in plant after combustion, relative to recovered radioactivity.

The foliar penetration of flupyrsulfuron-methyl into wheat was virtually nil (Fig. 7). For instance, after 72 h, it amounted to only 1% of recovered radioactivity. When deposited as a mixture with prosulfocarb, the penetration of flupyrsulfuron-methyl into wheat leaves was higher. In the presence of prosulfocarb at the 1500 g·ha^{-1} dose, it was 45% after 72 h (Fig. 7). At the 400 g·ha^{-1} dose, the influence of prosulfocarb was intermediate (15% penetration after 72 h, not shown). The foliar penetration of flupyrsulfuron-methyl into *L. multiflorum* was only 3–4% after 72 h (Fig. 7). Prosulfocarb increased uptake; after 72 h it reached 9% at the 400 g·ha^{-1} dose (not shown) and 17–18% at the 1500 g·ha^{-1} dose (Fig. 7).

After deposition on the foliar surfaces of *L. multiflorum* and wheat, significant prosulfocarb losses were observed. For instance, on wheat after 27 h they amounted to 42 ± 15% (confidence interval at the 95% level) and reached 68 ± 3% after 72 h. On *L. multiflorum*, the losses were 62 ± 16% and 91 ± 4% after 27 and 72 h, respectively. A check on glass slides showed that losses from this surface were of the same order of magnitude (not shown). The foliar penetration of prosulfocarb into wheat increased with time in a first stage and reached 25% after 9 h and then it levelled off and slowly increased from 9 to 72 h (Tab. II). Flupyrsulfuron-methyl did not influence the foliar penetration of prosulfocarb. On *L. multiflorum*, the foliar penetration of prosulfocarb remained low (around 4%

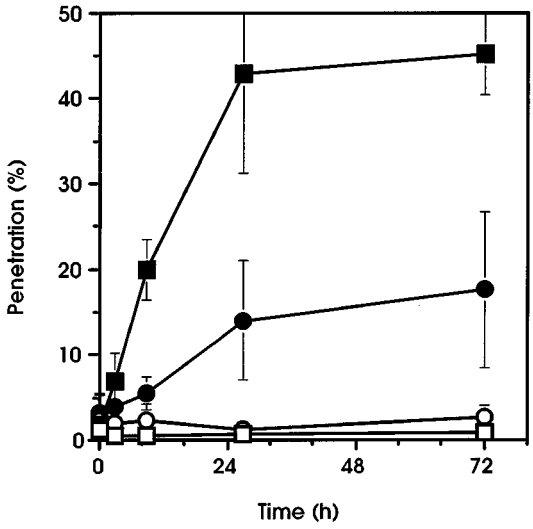


Figure 7. Time-course of the foliar penetration of flupyrsulfuron-mehtyl applied alone (open symbols) or in the presence of prosulfocarb (closed symbols) into wheat (squares) and *Lolium multiflorum* (circles). Penetration is expressed as percentage of recovered radioactivity. Bars are confidence intervals at $P = 0.05$.

Table II. Time-course of the foliar penetration into wheat and *Lolium multiflorum* of prosulfocarb applied alone or in the presence of flupyrsulfuron-methyl. Penetration is expressed as percentage of deposited radioactivity. Figures in brackets are confidence intervals at $P = 0.05$. Me = methyl.

	Wheat		*Lolium multiflorum*	
	Prosulfocarb	+ flupyrsulfuron-Me	Prosulfocarb	+ flupyrsulfuron-Me
0 h	2.4 (1.8)	0.8 (0.6)	0.1 (0.2)	0.6 (1.0)
3 h	9.8 (5.9)	5.4 (2.7)	2.0 (2.6)	4.8 (4.3)
9 h	25.2 (6.7)	19.1 (5.9)	2.7 (1.9)	3.1 (3.4)
27 h	27.7 (5.6)	17.8 (6.7)	5.0 (3.1)	3.1 (2.5)
72 h	29.9 (13.2)	28.3 (4.7)	2.7 (1.7)	6.9 (4.2)

after 27 and 72 h) and was not affected by flupyrsulfuron-methyl (Tab. II).

4. Discussion

The bioassays conducted in the greenhouse showed that flupyrsulfuron-methyl poorly controlled *L. multiflorum*. Complete kill was recorded only in experiment 1, and only at the maximum dose of 156 g·ha^{-1}. The non-lethal ED_{80} doses were much higher than the recommended field dose (10 g·ha^{-1}) which induced only a growth inhibition of 53, 18 and 45% in experiments 1–3, respectively. Flupyrsulfuron-methyl showed the lowest efficacy in the growth chamber experiment, maybe because the plants were growing more vigourously. The results obtained under controlled conditions did not quite agree with previous results obtained in the field [3]. It is however a common observation that herbicide performance can differ greatly between experiments in the field and under controlled conditions [18].

Prosulfocarb controlled *L. multiflorum* under our experimental conditions. A 95% efficacy was obtained with 1600, 490 and 775 g a.i.·ha^{-1} in experiments 1–3, respectively. These doses were much lower than that recommended for weed control in cereals, namely 4000 g a.i.·ha^{-1} [1]. The 1:400, 1:40 and 1:4 flupyrsulfuron-methyl:prosulfocarb mixtures also controlled *L. multiflorum* in experiments 1–3. In addition, the ED_{80}s of the 1:400 mixture were strikingly similar between experiments (Tab. I). Plants were killed when mixture doses were higher than 1000 g a.i.·ha^{-1}. In the experiments made in the greenhouse, the joint action of active ingredients followed additivity. Thus, the presence of flupyrsulfuron-methyl in the mixtures did not affect the activity of prosulfocarb.

Flupyrsulfuron-methyl penetrated to a low extent into wheat leaves and its uptake into *L. multiflorum* was barely detectable. We cannot exclude that the site on the leaf where we deposited ^{14}C-flupyrsulfuron-methyl was not favourable to its foliar penetration. However, results obtained in our laboratory, for example with diclofop-methyl [14], phenmedipham [12], 2,4-D or glyphosate [7], show that herbicides with a wide variety of physicochemical properties all exhibited significant foliar uptakes under similar conditions. Therefore, foliar penetration does not seem to play an important role in the mode of action of flupyrsulfuron-methyl. The underground parts are a more likely route of entry into the weed. This is in agreement with experiments on *A. myosuroides*, which showed that the foliar uptake of flupyrsulfuron-methyl was as low as 1% after 72 h [22]. Moreover, its herbicidal efficacy was greatly limited when the ground was protected from the spray.

The foliar uptake of prosulfocarb was higher than flupyrsulfuron-methyl but was limited since it did not amount to more than 30% of the applied label. This may be due to volatilization from the leaf surface and may explain its poor efficacy after the 1–2 leaf stage of the weeds [1]. When flupyrsulfuron-methyl and prosulfocarb were deposited as mixtures onto the leaves of *L. multiflorum*, the foliar uptake of flupyrsulfuron-methyl was about five-fold higher than when deposited alone. A similar phenomenon was observed on *A. myosuroides* [22]. The increase in penetration was more pronounced on wheat since uptake was brought from 1% to 45%. The promotion of flupyrsulfuron penetration may be due to co-formulants in the formulation of prosulfocarb or even to prosulfocarb itself. However, this effect was not reflected in the bioassays since mixtures of prosulfocarb and flupyrsulfuron-methyl showed only additivity of effects and no synergy. Most often a relationship is observed between foliar penetration and efficacy of herbicides [5, 14]. However, a few reports suggest that it may not always be true [20, 21]; this clearly is the case in the present study. The following explanations can be put forward. Firstly, *L. multiflorum* may not be the best test plant to assess joint-action since it exhibits a low susceptibility to flupyrsulfuron-methyl. Secondly, in our bioassays the confidence intervals of the ED_{50}s were large, which may have concealed an eventual effect of prosulfocarb. Finally, because of the already mentionned predominance of the underground route for flupyrsulfuron-methyl penetration into *L. multiflorum*,

events occurring on the leaf surface may have little influence on the overall herbicide efficacy.

To conclude, the present study showed that mixing prosulfocarb with flupyrsulfuron-methyl widened the activity spectrum of the latter, without decreasing the efficacy of any of the components of the mixture.

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