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## Modeling of Glyphosate Application Timing in Glyphosate-Resistant Soybean

Ivan Sartorato, Antonio Berti, Giuseppe Zanin, and Claudio M. Dunan\*

The introduction of herbicide-resistant crops and postemergence herbicides with a wide action spectrum shifted the research focus from how to when crops should be treated. To maximize net return of herbicide applications, the evolution of weed–crop competition over time must be considered and its effects quantified. A model for predicting the yield trend in relation to weed removal time, considering emergence dynamics and density, was tested on data from glyphosate-resistant soybean grown in cropping systems in Italy and Argentina. Despite an ample variation of weed emergence dynamics and weed load in the four trials, the model satisfactorily predicted yield loss evolution. The estimated optimum time for weed control (OTWC) varied from about 18 d after soybean emergence in Argentina to 20 to 23 d in Italy, with time windows for spraying ranging from 14 to 28 d. Within these limits a single glyphosate application ensures good weed control at low cost and avoids side effects like the more probable unfavorable weed flora evolution with double applications and the presence of residues in grains. Despite the apparent simplicity of weed control based on nonselective herbicides, the study outlines that many variables have to be considered to optimize weed management, particularly for the time evolution of the infestation and, subsequently, a proper timing of herbicide application.

Nomenclature: Glyphosate; soybean, *Glycine max* (L.) Merr., 'Asgrow ASRR19', 'Asgrow XP2101R', 'Asgrow 6401'. Key words: Time density equivalent, weed emergence dynamics, weed-crop competition.

The introduction of herbicide-resistant crops (HRCs) permitted new options for weed management and the evaluation of weed control practices (Dalley et al. 2004; Mulugeta and Boerboom 2000). The efficacy of glyphosate on glyphosate-resistant crops, in particular, is quite high over a broad range of weed flora composition and development stage, although larger weeds can require a higher dosage (Krausz et al. 1996; Tharp et al. 1999). Even in some non-HRCs, however, the recent development of new herbicide families, such as imidazolinones and sulfonylureas, provided a very wide action spectrum against both broad-leaved and grass weeds, especially for POST treatments. In these crops constraints due to herbicide selectivity and efficacy are relaxed (Ateh and Harvey 1999; Reddy and Whiting 2000) and the major question becomes the identification of the best time window for herbicide application. A correct identification of the best time for weed control requires knowledge of how yield changes over time due to weed presence (Dalley et al. 2004; Gower et al. 2003) and to weed emergence dynamics (Hilgenfeld et al. 2004).

The critical period concept is the theoretical framework that helps in determining the most effective time for POST herbicide applications. The introduction of HCRs has not only greatly enabled the study of the critical period for weed control, through the use of nonselective herbicides such as glyphosate, but also created a necessity for its study (Dalley et al. 2004). As Knezevic et al. (2002) pointed out, the widespread use of POST herbicides, especially those used on HRCs, may make the critical period more useful and more frequently used.

In glufosinate-resistant corn, Hamill et al. (2000) showed that crop yield was insensitive to the shift of herbicide application from the two- to eight-leaf stages, in five out of seven experiments considered. In the remaining two trials, an early emergence of strong infestations of common lamb's

n to year and site to site. They also showed that the critical period of weed control differed for soybean grown in a no-till system as opposed to conventional tillage. This indicates that the l. optimum timing of application should vary depending on weed emergence phenology and total weed load. Therefore, a modeling approach could improve the understanding of the behavior of the crop-weed system. A dynamic approach considering the age structure of the weed population at the moment of weed control, i.e., the

quarter led to consistent losses if the treatment was delayed

from the three-leaf stage onwards. Halford et al. (2001) showed

that estimates of the critical period vary for one crop from year

weed population at the moment of weed control, i.e., the different weed competitiveness depending on weed age and density, should permit identification of the time window when weed control is most effective and profitable. Crop yield can be expressed as a function of the maximum yield of the crop kept weed-free, the weed competitive load, and time of weed emergence and removal. An optimum application time that maximizes net return of herbicide application can be identified knowing the weed population and its emergence pattern (Berti et al. 1996). Studies on critical period determination in corn, soybean, and wheat seem to indicate that the maximum yield and competitive load are strictly related to the cropping environment, while temporal parameters show less variability among different environments (Berti et al. 2008; Sattin et al. 1996). A first approach to improve the predictive capacity of the model was presented by Sartorato et al. (2001).

The main objective of this research was to develop a model for predicting the yield trend in relation to weed removal time, considering the emergence dynamics and density. The model should be able to answer the following practical questions:

- What are the impacts of time of glyphosate application, weed emergence, weed density, and floral composition on glyphosate-resistant soybean yield?
- Is there an optimum timing for glyphosate application that maximizes yield under different agroecological conditions?

This information would permit the most profitable weed management strategies to be defined for glyphosate-resistant soybean.

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Table 1. General and agronomic information on trials.

Year	Country	Cultivar	Sowing date	Emergence date	Harvest date
1997	Italy	Asgrow ASRR19	May 7	May 17	September 22
1998	Italy	Asgrow XP2101R	May 11	May 22	September 28
1999	Italy	Asgrow XP2101R	May 4	May 16	September 30
1997/1998	Argentina	Asgrow 6401	December 20, 1997	January 5, 1998	June 19, 1998

#### **Materials and Methods**

**Field Experiments.** Four field experiments, three in Italy and one in Argentina, were conducted on Asgrow<sup>1</sup> glyphosateresistant soybean (Table 1). In Italy the trials were carried out at the Experimental Farm of Padova University in Legnaro (north-eastern Italy, 45°21′N, 11°58′E) during 1997, 1998, and 1999, on a loamy soil. In Argentina the trial was carried out during the 1997/1998 growing season in a commercial field in the Province of Santa Fé (32°42′S, 62°07′W) on a sandy loam soil.

At Legnaro glyphosate-resistant soybean was sown in a conventional tillage system at a density of 40 plants  $m^{-2}$ , with 45-cm row spacing and six rows per plot. The plot surface was 16.2 m<sup>2</sup> (2.7 by 6 m). The experimental layout was a completely randomized design without replication during 1997 (with the exception of the untreated check, replicated three times) and a complete randomized block with three replications in 1998 and 1999. The harvested area for yield evaluation was 10.8 m<sup>2</sup> (1.8 by 6.0). Time removal experiments, using glyphosate as a removal tool, were performed on a natural weed population: the removal time comprised single and double applications of glyphosate. In 1997, single glyphosate applications were done at unifoliate stage (5 d after emergence [DAE]), at 1, 2, 3, 4, and 5 trifoliate stages (9, 13, 17, 21, and 29 DAE, respectively) and at the beginning of flowering (37 DAE). Eight double applications were also performed, with the two sprayings 1 or 2 wk apart (5 + 13, 5 + 21, 13 + 21, 13 + 29, 21 + 29,21 + 37, 29 + 37, and 29 + 45 DAE).

In 1998 the single applications were done at about the same growth stages as in 1997, corresponding to 4, 8, 18, 27, 34, and 42 DAE and at canopy closure (49 DAE). Four double applications were considered (4 + 18, 8 + 27, 18 + 34, and 34 + 49 DAE).

The experiments in 1999 followed the same plan as the preceding year, with single applications at 2, 8, 12, 18, 24, 29, and 33 DAE and double applications at 2 + 12, 8 + 18, 12 + 24, and 24 + 33 DAE. All the experiments included an untreated check.

At Santa Fé the experiment was performed on soybean planted as a second crop after wheat. The organization of the Santa Fé trial (tillage system, seed density, plot size, layout, and number of replicates) was similar to that of the trial conducted in Italy during 1998, except for the row spacing, which was 70 cm. The treatments compared were five single sprayings, at stages unifoliate (5 DAE), two trifoliate (13 DAE), five trifoliate (21 DAE), beginning of flowering (37 DAE), and canopy closure (45 DAE), four double applications (5 + 21, 13 + 37, 21 + 37, and 21 + 45 DAE), plus an untreated check. Further information on the trials is given in Table 1.

In all the experiments, glyphosate application times were then transformed into cumulated growing degree-days (GDDs) of soybean according to the equation: Growing Degree Days = [(Tmax + Tmin)/2] - 10 [1]

Where *Tmax* is the maximum daily temperature, *Tmin* is the minimum daily temperature, and 10 is the base temperature assumed for soybean growth; if *Tmin* was below 10 C, *Tmin* was taken as 10 C to avoid negative values.

*Glyphosate Applications.* Glyphosate isopropylamine salt was applied in all trials at a rate of 720 g ai ha<sup>-1</sup> (Roundup Bioflow, Monsanto<sup>2</sup>, 360 g L<sup>-1</sup> of glyphosate). The treatments were applied with a hand-cart plot sprayer equipped with flat fan Teejet (Spraying Systems<sup>3</sup>) 11003 nozzles and calibrated to deliver 385 L ha<sup>-1</sup> of water at a pressure of 200 kPa. No cultivation or hand weeding were performed.

*Weed Flora Assessment.* In the Legnaro experiments, weed emergence dynamics were monitored within quadrats of 600 cm<sup>2</sup> (wire frames of 12 by 50 cm), placed on the control plot (untreated check) and on the latest treated plots (e.g., the plots treated at 42 and 49 DAE in 1998). On each plot, four frames were located in the interrow for a total of 20 quadrats in 1997 (corresponding to a monitored area of  $1.2 \text{ m}^2$ ) and 32 quadrats in 1998 and 1999 (monitored area  $1.92 \text{ m}^2$ ). Counts were made every 2 to 3 d, starting from crop sowing until canopy closure; the total number of counts was 13, 15, and 18 in 1997, 1998, and 1999, respectively.

In Santa Fé the weed emergence dynamics were monitored on the control plots, counting the new seedlings in the central  $1 \text{ m}^2$  area of each plot weekly for a total of seven assessments.

Assessment of Weed Control Efficacy. Treatment efficacy was visually assessed at harvest, attributing an index to each weed species: rating was based on an arithmetical scale, with 0 equal to no weed presence and 10 equal to completely weeded area. In the experiments conducted at Legnaro, the percentage of weed covered area was also estimated; it included weeds emerged after treatment or that had escaped the treatment, because in a phenological phase not controlled by the herbicide or because covered by the leaves of the crop or other weeds.

The kill rate (K) was also evaluated on the main species at Legnaro: at least 100 plants per plot were marked with a tooth-pick before glyphosate application and surviving plants 15 d after the glyphosate treatment were counted.

*Climatic Pattern.* At Legnaro the temperatures during the soybean growing cycle were higher than the average 30-yr trend, particularly in the summer of 1998. Rainfall was within the mean in 1997 and 1999, while summer rainfall was lower than average in 1998 (143 mm against the 30-yr average of 245 mm). However, the presence of a superficial groundwater table contributed towards limiting the water stress to the crop. At Santa Fé lower temperatures and higher rainfall resulted in a longer growing season than in Italy (Table 2).

Table 2. Meteorological parameters from sowing to harvest in the different "location by year" combinations.

	(	Cumulative GDDs	a
Trial	Average air temperature (C)	from sowing (degree days)	Cumulative rainfall (mm)
Legnaro 1997	22.4	1,665	298.2
Legnaro 1998	22.8	1,709	252.4
Legnaro 1999	22.5	1,790	336.4
Santa Fé 1997/1998	18.6	1,594	514.6

<sup>a</sup> GDD, growing degree-day.

**Modeling Approach.** The model considered in this study is based on the equation developed by Sattin et al. (1992) and subsequently refined to directly express the initial slope of the yield loss ( $Y_L$ )-weed density (D) relationship as a function of time of emergence (*te*) and removal (*tr*) (Berti et al. 2008):

$$Y_L = iD / \left[ e^{(c \cdot te)} \left( 1 + e^{(d - f \cdot tr)} \right) + (iD/a) \right]$$

$$[2]$$

The five parameters of this equation can be divided into a group of parameters related to weed competitiveness (*i* and *a*) and a group related to time of emergence and removal (*c*, *d*, and *f*), hereafter indicated as "time parameters."

To cope with mixed weed infestation, characterized by an observed emergence pattern, the density equivalent (*Deq*; Berti and Zanin 1994) and the time density equivalent (*TDE*) approach (Berti et al. 1996) were considered.

*Deq* is defined as the density of a hypothetical weed, with parameters i and a both equal to 1, which causes the same damage as the considered weed at the observed density. The whole weed infestation can be divided into daily emergence cohorts, each one characterized by a time of emergence and a *Deq*. The *Deq* of each cohort can then be transformed into its *TDE*. This is defined as the density of plants, emerged with the crop and competing until harvest, which determines the same yield loss as that caused by a group of weeds with a given density, time of emergence, and time of removal.

Under these assumptions, the sum of *TDEs*, called total time density equivalent (*TTDE*), is then related to yield loss as follows:

$$Y_L = TTDE/(1 + TTDE)$$
[3]

Having no detailed information on the competitiveness of the different weed species for each experiment, the TTDE in the absence of weed control ( $TTDE_0$ ) can be calculated from the yield observed in the untreated checks ( $Y_W$ ) and that observed in weed-free plots ( $Y_{WF}$ ). The experiments did not include weed-free plots, so  $Y_{WF}$  was estimated from the double POST plots, assuming an  $Y_{WF}$  5% higher than their mean.

The yield loss in the untreated checks is then:

$$Y_L = (Y_{WF} - Y_W) / Y_{WF}$$

$$[4]$$

From equation 3 and 4, it is then possible to obtain  $TTDE_0$  as a function of  $Y_W$  and  $Y_{WF}$ :

$$TTDE_0 = (Y_{WF} - Y_W) / Y_W$$
<sup>[5]</sup>

Considering a POST with a given efficacy, the weed population can be divided into three groups (Table 3): (1) weeds that emerge before the POST and are killed by the treatment; (2) weeds that survive the treatment; and (3) weeds that emerge after the treatment.

Cohorts emerging before the treatment are then split into two subcohorts, one representing the individuals killed by the treatment, the second being composed of the plants surviving weed control. Both subcohorts have the same te (time of emergence of the whole cohort), but their tr differ, with the first subcohort having a tr equal to treatment time and the second one a tr equal to harvest time (th). Instead, cohorts emerging after the treatment are left unchanged.

Considering two POSTs, the weed population can be divided into six groups: (1) weeds that emerge before the first POST and are controlled by this treatment; (2) weeds that emerge before the first POST but are controlled by the second; (3) weeds that emerge before the first POST and escape both treatments; (4) weeds that emerge between the first and second POST and are controlled by the latter; (5) weeds that emerge between the two POSTs and are not controlled; and (6) weeds that emerge after the second treatment.

As for a single POST, depending on the time of treatment, each cohort can be divided into subcohorts, each one characterized by a *Deq*, a *te*, and a *tr*.

For subcohorts remaining until harvest, the TDE can be computed as follows:

$$TDE = Deq \cdot e^{-c \cdot te}$$
 [6]

For each subcohort controlled by a POST, thus having a *tr* different from harvest time, the *TDE* is computed as follows:

$$TDE = Deq / \left[ e^{c \cdot te} \left( 1 + e^{(d - f \cdot tr)} \right) \right]$$
[7]

For each POST (or combination of treatments), a *TTDE* can then be computed as the sum of *TDEs* of the whole set of

Table 3. Subdivision of the cohorts into subcohorts, depending on their emergence time in respect to the moment of application of weed control. te, time of emergence of the cohort; twc, time of weed control; th, harvest time; K, kill rate of the weed control treatment; and Deq, equivalent density of the i-th cohort.

Subcohort	Condition	Time of removal	Equivalent density of the subcohort
Single POST			
Weeds that emerge before the POST and are killed by the treatment	te < twc	twc	$Deq_i \cdot K$
Weeds that survive the treatment	te < twc	th	$\text{Deq}_{i} \cdot (1-\text{K})$
Weeds that emerge after the treatment	$te \ge twc$	th	Deqi
Double POST			
Weeds that emerge before the first POST and are controlled by this treatment	$te < twc_1$	twc <sub>1</sub>	Deq <sub>i</sub> · K
Weeds that emerge before the first POST but are controlled by the second	$te < twc_1$	twc <sub>2</sub>	$Deq_i \cdot (1-K) \cdot K$
Weeds that emerge before the first POST and escape both treatments	$te < twc_1$	th	$Deq_i \cdot (1-K)^2$
Weeds that emerge between the first and second POST and are controlled by the latter	$twc_1 < te \le twc_2$	twc <sub>2</sub>	$Deq_i \cdot K$
Weeds that emerge between the two POSTs and are not controlled	$twc_1 < te \le twc_2$	th	$Deq_i \cdot (1-K)$
Weeds that emerge after the second treatment	$te \ge twc_2$	th	Deqi

Table 4. Details of weed flora density and composition in untreated control plots in Legnaro and Santa Fé trials.

	Legnaro			Santa Fé
	1997	1998	1999	1997/1998
Emerged weeds (pl m <sup>-2</sup> )	246.7	130.7	422.9	158.7
Number of species	16	21	19	4
Time from crop sowing to 50% of total weed emergence (days)	9	18	14	29
Time from crop sowing to 80% of total weed emergence (days)	30	24	26	45
Species				
Abutilon theophrasti Medik.	0.3	5.2	4.4	0.0
Amaranthus sp. L.	5.4	15.9	22.8	73.9
Chenopodium album L.	5.1	6.4	2.7	18.1
Digitaria sanguinalis (L.) Scop.	23.0	1.6	6.7	0.0
Echinochloa crus-galli (L.) Beauv.	25.0	1.6	34.1	4.6
Matricaria recutita L.	18.6	2.0	3.8	0.0
Mercurialis annua L.	0.0	6.8	0.0	0.0
Plantago major L.	0.7	5.6	3.7	0.0
Portulaca oleracea L.	12.2	22.7	12.6	0.0
Solanum nigrum L.	1.7	15.1	1.2	0.0
Other species	8.1	17.1	8.0	3.4

subcohorts, expressing the competitive load caused by weeds escaping weed control and those remaining in the field until treatment time.

For the *i*-th treatment, it is then possible to compute a residual yield loss as follows:

$$Y_{Li} = TTDE_i / (1 + TTDE_i)$$
<sup>[8]</sup>

**Statistical Analysis.** The datasets from 1997 and 1998 were used to calibrate the time parameters. The calibration was done on single treatment data. In a first phase, an optimization of time parameters specific for each trial was done, considering the sum of the squared deviation of the two experiments as the loss function. A second optimization considering a common set of time parameters for the two experiments was then performed. A partial F test revealed if the complex model with time parameters specific for each experiment could be reduced to the simplified one, with common time parameters.

The experiments from 1999 and the dataset from Argentina, as well as the data of 1997 and 1998 referring to double treatments, were used for validation, applying the time parameters obtained in the calibration phase.

A sensitivity analysis was done considering the effects of the three time parameters on OTWC: for a given crop and weed infestation, a treatment at OTWC gives the best achievable result, with the lower residual yield loss due to the best balance between the competitive effects of the weeds before and after the treatment, the latter due both to weeds surviving until harvest and weeds emerging after the treatment.

The analysis was performed using the simplified model on calibration data sets, evaluating the effects of a  $\pm$  10% variation of the parameters on OTWC and on maximum crop yield.

#### **Results and Discussion**

Weed Community Characteristics. Weed flora density and composition in Legnaro were highly variable among years; the lowest density occurred in 1998 with 131 plants m<sup>-2</sup> and the highest in 1999 with 423 plants m<sup>-2</sup> (Table 4). Weed emergence was slow in 1998, taking 18 d (170 GDDs) from soybean sowing to reach 50% of the final density, compared

with 9 d (78 GDDs) in 1997 and 14 d (121 GDDs) in 1999 (Figures 1 and 2, dashed lines). Weed flora structure was also variable, being balanced between monocots and dicots in 1997 and 1999, while dicots represented more than 95% of the total density in 1998; in general the most abundant species were pigweed (*Amaranthus* sp.) and purslane (*Portulaca oleracea* L.) among dicots and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.] among monocots.

The Argentina trial was mainly characterized by a strong simplification of the weed flora, with only four species dominated by pigweed; there was also very slow emergence: 29 d from soybean sowing being needed to reach 50% of the final density (Figure 2).

**Glyphosate Performance.** In all the experiments, no visible injury was observed on soybean with either single or double applications.

Figure 3 shows the weed-free area at harvest, in relation to the treatments carried out at different times. The trends are sufficiently clear in 1998 and 1999, when it was observed that the weed-free area initially increased until reaching a maximum in correspondence to the treatments carried out 18 to 24 d after crop sowing. The weed-free area then slightly decreased and reached the lowest value in correspondence to the treatments carried out about 30 to 40 d after sowing. The weeds observed at harvest on plots treated within 18 d of

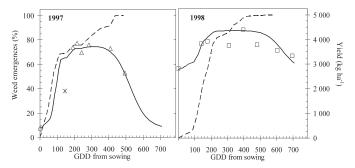


Figure 1. Weed emergence pattern (dashed line) and comparison between observed and simulated (continuous line) yields for single applications of glyphosate in the calibration dataset (Legnaro 1997 and Legnaro 1998). The \* symbol indicates an anomalous 1997 datum, not included in model parameterization.

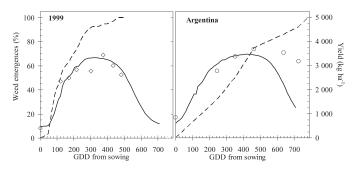


Figure 2. Weed emergence pattern (dashed line) and comparison between observed and simulated (continuous line) yields for single applications of glyphosate in the validation data set (Legnaro 1999 and Santa Fé 1997/98).

sowing were mainly those emerged after the treatment, while most of those present in the plots treated 18 to 24 d after sowing had escaped the treatment, thanks to the herbicide interception by crop foliage.

In 1997, without repetitions, data were more variable. The treatments carried out 9 and 13 d after sowing (in correspondence to 75% of emergence) assured a good control, with a final weed-free area of more than 90%. For later treatments, the weed-free area decreased, probably due to the shielding of the vegetation, with the exception of the treatment at 21 d, which showed an anomalous result, and the minimum was reached with the treatment at 37 d after sowing.

The K of glyphosate on the main species was good: K against barnyardgrass was very high (about 99%) in 1997 and 1998, while it was poor (about 46%) in 1999, when a cold period after the treatment reduced plant growth and glyphosate efficacy. Pigweed control was good (100 and 91.5% in 1997 and 1998, respectively), while 82 to 100% of purslane was killed, depending on plant stage at treatment, it being relatively less sensitive at the stage of four to five ramifications.

The weed control at harvest, expressed as percent of weedfree area, was consistently high for the double applications.

**Model Parameterization and Validation.** Model parameters for the first 2 yr of experiments at Legnaro are presented in Table 5. Comparing the complex model, with time parameters specific for each year, with the simplified model with common time parameters, the increase in residual sum of

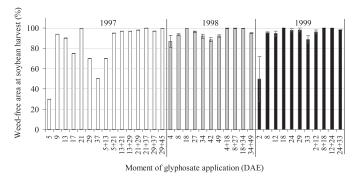


Figure 3. Weed control at harvest (Legnaro experiments), expressed as percentage of weed-free area, in relation to the glyphosate application timing (expressed as days after emergence [DAE]). Vertical bars represent the standard errors (no replications in 1997).

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Table 5. Calibration data set. Comparison between the parameters of equation 2, with specific time parameters for each trial (1997 and 1998), with common set of time parameters (1997 + 1998). Values  $\pm$  standard error of parameters; RSS, residual sum of squares; df, degrees of freedom.

	1997	1998	1997 + 1998
Weed-free Yield, Ywf (kg ha <sup>-1</sup> ) Total time density	4,200	4,400	
equivalent, <i>TTDE</i> (pl m <sup>-2</sup> )	8.10	1.00	
c	$0.0182 \pm 0.0073$	$0.0183\pm0.0081$	$0.0156 \pm 0.0019$
d	$15.0885 \pm 10.2497$	$3.2056 \pm 1.1836$	$11.1380 \pm 1.0429$
f	$0.0298 \pm 0.0252$	$0.0055 \pm 0.0027$	$0.0204 \pm 0.0025$
RSS	0.3898		0.891
df	7		10
F			3.002
p level			0.104

squares proved to be nonsignificant. The simplified model was therefore retained for further analyses. The plot of observed vs. expected values (Figure 4) shows the good agreement between these two variables in the case of a single weed control treatment (see also Figure 1). The model with the common set of time parameters was then used to forecast yields for the two remaining experiments (Legnaro 1999 and Santa Fé 1997/98). Figure 2 shows the estimated and observed soybean yield for these two experiments in the case of a single weed control treatment, while the whole set of data used for validation is reported in Figure 5. The behavior of the model was fairly good for Legnaro 1999, but an evident deviation of the estimated yields for late treatments can be observed in the Santa Fé dataset. It is worth noting that the two experiments presented a very different weed emergence pattern: in Legnaro 1999, 90% of the weeds emerged in the first 280 GDDs after sowing, while in Argentina emergences were delayed and prolonged, reaching 90% only after 720 GDDs. In the two experiments used for calibration, 90% emergence was reached after about 370 GDDs. The delayed weed emergence in the Santa Fé experiment can be related to

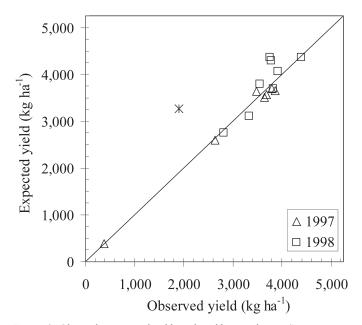


Figure 4. Observed vs. expected yields in the calibration data set (Legnaro 1997 and 1998). The \* symbol indicates an anomalous 1997 datum, not included in model parameterization.

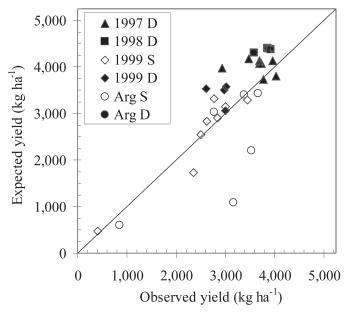


Figure 5. Observed vs. expected yields in the validation data set (all experiments, single [S] and double [D] treatments).

the wider interrow used (70 vs. 45 cm) that allowed more light to penetrate beneath the crop canopy in later stages. This encouraged late emergences, modifying the weed dynamics, as also emphasized by Knezevic et al. (2003) and by Jha et al. (2008). However, these late emerging weeds seem to exert an almost negligible effect on yield. By comparison, the Legnaro datasets show few late emerging weeds, and this may have affected the estimation of time parameters. Further studies with different emergence patterns and with different interrows would therefore be useful to ascertain whether the deviations observed for Santa Fé are due to an overestimation of the competitive effect of late emerging weeds or to some local effect in this experiment.

With two treatments, the weed flora was almost eliminated in all the plots. In this case expected yields calculated by the model are very close to the maximum value, while the observed yields show a typical random variation as commonly observed in field plots (Figure 5).

The model showed a good capacity to predict the yield trend according to the timing of weed removal. A deviation between predicted trend and the observed data is evident in the Santa Fé dataset only with the late treatments, due to an overestimation of the competitive effect of late emerging weeds. On the other hand, the good estimation of crop yield in the first half of the curve (where the competitive effect is mainly driven by the weed cohorts with earlier emergence), shows the robustness of model even in a completely different environment.

Sensitivity Analysis. Of the three time parameters considered, c acts modifying the competitiveness of both untreated or treated weeds, while the other two (d and f) are of relevance only in the case of a time of removal different from harvest time (i.e., in the case of the application of weed control).

On the whole, the model proved its robustness, showing percentage changes of the outputs lower than or of the same order of magnitude as the variations in the parameters. The more pronounced variations were observed for the OTWC, while the estimation of maximum yield appeared very stable. This is because the relationship between time of treatment and yield loss is quite flat around OTWC. The model showed a low sensitivity to parameter c (Table 6), with a modest anticipation of OTWC in the case of its increase and a delay with its decrease. Parameters d proved to be more critical, causing a marked reduction of OTWC when d was reduced, while f shows an opposite behavior, even if the size of variations is far more limited. For both parameters the effect Ymax are very small, ranging from +0.5 to -0.9%.

*Optimum Timing for Weed Control.* Table 7 shows the OTWCs estimated by the model. OTWC ranged from 313 to 350 GDDs after sowing in the three Italian experiments, corresponding to 20 to 23 d after soybean emergence, which is from third to fourth trifoliate stage. The much higher Santa Fé value (419 GDDs after sowing) corresponds to just 18 d after crop emergence, or to the fourth trifoliate stage. The faster development of Argentinean soybean is explained by the different temperature regimes experienced by a second crop, sown at the beginning of the summer season, after wheat.

**Critical Time for Weed Removal.** This study is not based on the classical determination of the weed-free period and duration of tolerated competition, aiming to evaluate the effect of a given treatment in relation to the timing of application. Consequently, it is not possible to define a classical critical period, sensu Zimdahl (1988), but the model can estimate a critical time for weed removal (CTWR), a time window were the weed control treatment gives technical or economic results, or both, close to OTWC. Assuming a certain interval of acceptable yield loss, it is possible to identify a period when treatment gives a residual yield loss within this interval.

Considering a single POST and an acceptable yield loss level of 5% of Ymax, with high infestation (Legnaro 1997 and 1999) the time window for spraying the crop included a period of 166 to 203 GDDs, from the first trifoliate to the

Table 6. Sensitivity analysis, effects of a  $\pm$  10% variation of the time parameters (c, d, f) on optimum timing for weed control (OTWC) and on maximum yield (Ymax).

		1997 1998		98	Average		
	RSS <sup>a</sup>	OTWC (GDD)	Ymax (kg ha <sup>-1</sup> )	OTWC (GDD)	Ymax (kg ha <sup>-1</sup> )	OTWC %	Ymax %
Original parameters	0.891	313	3.73	350	4.37	0.0	0.0
c + 10%	0.909	299	3.73	341	4.37	-3.4	-0.1
c - 10%	0.915	328	3.73	358	4.36	3.5	0.0
d + 10%	1.614	328	3.77	377	4.37	6.3	0.5
d - 10%	2.199	273	3.67	322	4.36	-10.2	-0.9
f+ 10%	1.584	299	3.71	341	4.36	-3.4	-0.3
f - 10%	1.420	328	3.75	350	4.37	2.2	0.3

<sup>a</sup> RSS, residual sum of squares ; GDD, growing degree-day.

Table 7. Estimated optimum timing for weed control (OTWC) and critical time for weed removal (CTWR) for a single glyphosate application, in the four experiments considered.

	OTWC CTWR				
	(GDDs <sup>a</sup> from sowing)				
		Beginning	End		
Legnaro 1997	313	192	395		
Legnaro 1998	350	199	531		
Legnaro 1999	326	256	422		
Santa Fé 1997/1998	419	318	518		

<sup>a</sup> GDD, growing degree-day.

beginning of flowering (Table 7). With low weed density (Legnaro 1998) the time window for glyphosate treatment was longer and ranged for 332 GDDs, from the unifoliate stage to the sixth trifoliate, making the timing of the treatment less critical than in more infested fields. The beginning of the CTWR corresponded to about 70 to 75% of weed emergence with high infestations and to 38% with a low competitive load, thus the higher the weed density is the higher is the fraction of emerged weeds that should be intercepted by the treatment. During these time windows, a single glyphosate treatment could protect soybean yield.

In Santa Fé the treatment window begun very late, at 318 GDDs (in correspondence to 60% of weed emergence), and spanned for 200 GDDs, indicating an intermediate behavior of the competitive relationships in comparison with the Italian trials. The different response is probably correlated with the slow weed emergence dynamics and the different competition exerted by late emerging weeds, because of the larger soybean interrow.

With two POSTs, the treatment time window was considerably longer in all the experiments, ranging from very early treatments (70 to 100 GDDs) for the first spraying to very late applications for the second treatment, close to canopy closure (data not shown).

The model results emphasize the basic role of the dynamics of weed emergence for the optimization of weed control. A proper prediction of weed emergence dynamics can therefore be the key point for improving the use of wide spectrum POSTs in both HRCs and nonresistant crops.

This study shows that with a single treatment it is possible to obtain crop yields very close to the one obtainable with a double treatment. The time window for herbicide application ranges from about 165 GDDs for the higher densities to about 330 GDDs for lower weed loads, ranging from the first trifoliate stage to the sixth trifoliate-beginning of flowering stage. These results are in agreement with those of Mulugeta and Boerboom (2000) and correspond to the critical period identified by Van Acker et al. (1993). There is some difference with regard to the results of Swanton et al. (2000), but they were obtained on no-till soybean, planted with 76-cm furrow width. Finally, results do not support the impression (Bonny 2008) that the period when weed treatment can be applied is slightly longer in herbicide resistant soybean.

With a single treatment, the crop will have some residual infestation at harvest: even with a treatment done within the time windows for herbicide application, the plots with a single treatment had an average weed cover of 6% at harvest, against 2% cover in the double treatment plots. This can be a concern for the building up of a future infestation, but can also have a positive aspect, by contributing to maintain biodiversity and ecosystem complexity (Martinez-Ghersa et al. 2003; Sartorato and Zanin 1999).

The advantages of controlling weeds with a single glyphosate treatment are: (1) lower weed control cost; (2) mitigation of the selection pressure, slowing unfavorable weed flora evolutions (shift and resistance) and helping to maintain biodiversity (Scursoni and Satorre 2010); and (3) restricting glyphosate contamination of grains. Recent studies in Argentina have shown the possible presence of glyphosate residues on grains: higher concentrations were detected when glyphosate was sprayed several times during the crop cycle and when treatments approached the flowering stage (Arregui et al. 2004).

Glyphosate-resistant soybean as a weed management tool has provided farmers with the opportunity and flexibility to manage a broad spectrum of weeds. The main reason for the rapid increase of glyphosate-resistant soybean acreage in the United States and Argentina is probably the simplification of weed control (Freyssinet 2003; Reddy and Zablotowicz 2003). Bonny (2008) agrees with this conclusion, but she appends "at least in the short term." In effect, the more recent surveys and statistics in the United States (Gianessi 2005) show a "trend of increasing glyphosate use rates" in relation to weed flora shift: this is the first indicator of the arising of complications in weed management. POST glyphosate use associated with glyphosate-resistant crops very significantly increases risk of resistance evolution (Neve 2008) and the movement of naturally resistant weed species into glyphosateresistant crop fields (Duke 2005). In this context, the reduced selection pressure exerted by a single glyphosate treatment concurs to reduce the weed shift phenomena but, as stated by Reddy and Norsworthy (2010), diversity in weed management systems is critical to reduce weed species shifts and to maintain sustainability of glyphosate-resistant crops as an effective weed management tool.

Wilkerson et al. (2002) highlight that by definition models are simplifications of real systems and as such, they do not include all the factors influencing weed-crop interaction. Nevertheless, the availability of a model that can predict the evolution of yield in relation to weed emergence dynamics could be of great importance. Although a grower may choose a weed management program based on simplicity or effectiveness, a decision model may help in determining the cost of that simplicity.

It is possible to conclude, paraphrasing an affirmation by Clausewitz, a military strategy theorist of the 19th century, reported by Luttwak (2001), that "all is very simple in weed control of glyphosate resistant soybean, but what is really simple is not always easy."

In effect, the present study outlines that, despite the apparent simplicity of a weed control based on nonselective herbicides, also in glyphosate-resistant soybean, there are many variables to be considered to optimize the weed management, particularly for the time evolution of the weed infestation and, subsequently, for a proper timing of herbicide application.

### Source of Materials

<sup>1</sup> Asgrow Seed Co., 5926 US Highway 14, Janesville, WI 53546. <sup>2</sup> Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167.

<sup>3</sup> Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187-7901.

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