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**Plant Research International B.V.**

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# 1. Introduction

The Minimum Lethal Herbicide Dose (MLHD) technology, developed by Plant Research International in the Netherlands (Ketel, 1996; Kempenaar *et al.*, 2002), is a promising decision support system allowing safe use of reduced rates of photosynthesis-inhibiting herbicides. Part of MLHD are dose recommendations differentiated for weed species and growth stage. An early detection method, based on simple and rapid measurements of photosynthetic activity, is used to evaluate the efficacy of the treatment shortly (2 to 4 days) after application. This tool permits a prompt second herbicide application in case of failure. This other part of MLHD ensures that even though minimal doses of herbicides have been used, there is a guarantee that the treatment will be successful in eliminating the weeds. Such a guarantee greatly contributed to the adoption of the MLHD methodology by certain groups of Dutch farmers, agricultural advisors and pesticide scientists.

In 2004 a project was started to introduce the MLHD technology into China. The name of the project is CMHD (www.cmhd.cn). Various parties work together in the CMHD project, which is coordinated by EARS b.v. For details on the project, see project description (Rosema, 2003). For details on MLHD, see MLHD Manual 2006 (Kempenaar, 2006, 2004). In summary, MLHD advises minimum effective doses of post emergence herbicides taking into account weed species and weed stages, and uses PPM-measurements to predict herbicide effects on weeds and crops. In Tables 1.1 and 1.2, MLHD PPM-threshold values for predicted weed control and effects on crop growth are given.

This report describes the results of calibration experiments in the CMHD project in 2005. A first series of experiments was done in 2004 (Kempenaar *et al.*, 2004). The aim of the calibration experiments in 2005 were fourfold:

1. to study PPM-time curves for different weeds and crops under greenhouse conditions in China, in particular the effect of two important grass herbicides on PPM-values,
2. to relate PPM-measurements to effects on the weeds,
3. to compare minimum effective doses in the greenhouse experiments with dose advises in MLHD tables,
4. to further build experience with MLHD technology in China.

*Table 1.1.*

| Class | Range PPM-readings | Predicted effect on weeds (efficacy) |
|---|---|---|
| 1 | < 15 | > 99 % control |
| 2 | 15-20 | > 90 % control,<br>additional treatment if crop is still 'open' |
| 3 | 20-35 | Moderate effect (growth reduction), additional treatment required |
| 4 | 35-50 | Small effect, additional treatment |
| 5 | > 50 | Hardly any effect, additional treatment |

*Table 1.2.*

| PPM-range | Predicted effect on crop |
|---|---|
| > 60 | No effect |
| 50-60 | Small effect (temporary reduction of photosynthesis by about 20 %) |
| 35-50 | Moderate effect (temporary reduction of photosynthesis by about 40 %) [1)] |
| < 35 | Large effect (temporary reduction of photosynthesis by > 40 %) [1)] |

[1)] *Attainable crop yield will be lower if the readings remain at this level for a week or more.*

# 2. Materials and Methods

## 2.1 General

Three validation/calibration experiments were carried out in 2005 at location HAAFS. Plants of different species were treated with single or mixtures of herbicides at different doses. After treatment, PPM-measurements, symptoms, plant fresh weight and plant mortality were determined. The experiments were done in a similar way as the calibration experiments in the CMHD-project in 2004 (Kempenaar *et al.* (2005)).

## 2.2 Location, plant material

The experiments were done at the research station of HAAFS in the city of Shijiazhuang, China. Single herbicides or mixtures were tested on seven plant species. Plants were grown from seeds available at HAAFS. The seeds had been collected from mature plants on arable fields near the research facility. The plants were treated when they had on 4 true leaves (4-leaf stage).

Per experiment, the following weed species were tested.
Experiment 1: *Amaranthus retroflexus, Echinochloa crus-galli, Digitaria sanguinales*
Experiment 2: *Chenopodium album, Amaranthus retroflexus, Echinochloa crus-galli*
Experiment 3: *Chenopodium album, Capsella bursa-pastors, Descurainia sophia, Bromus japonicus*

## 2.3 Plant growth conditions

The plants were grown on soil in pots with a diameter and height of 10 cm. The soil was a mixture of 95 % light clay soil plus 5 % sand (w/w). Nutrients levels in the soil were not limiting for plant growth. No extra nutrients were added during experimentation. The water content of the soil was also kept at level not limiting for plant growth. Water was added to the soil from the top on demand (visual assessment by staff of HAAFS).

Seeds were directly seeded into the soil, several seeds per pot. After emergence, plant density was thinned back to one plant per pot. Pots were placed on trays, and the trays were put in a greenhouse with pre set conditions. Growth conditions were day/night temperature 25-28/15-17, 60-85 % r.h., light 20.000 lux for experiment 1 and 2, and a day/night temperature of 20-22/10-15, 60-85 % r.h., light 20.000 lux for experiment 3. Temperature and humidity were kept between the pre set levels by an automated climate control system.

## 2.4 Herbicides

The following herbicides were tested (see Table 2.1 for content of a.i (active ingredient) in formulated products):
Experiment 1 (mixture 1): formulated atrazine / mesotrione with and without nicosulferon
Experiment 2 (mixture 2): formulated bentazone with and without quizalofop-P-ethyl
Experiment 3 (mixture 3): formulated isoproturone with and without formulated bromoxynil

*Table 2.1. Formulations of the herbicides used in experiments 1, 2 and 3.*

| Herbicide (the a.i. of formulated product) | Content of a.i. in formulated product |
|---|---|
| Atrazine | 385 g/kg |
| Bentazone | 480 g/kg |
| Mesotrione | 100 g/l |
| Nicosulfuron | 40 g/kg |
| Quizalofop-P-ethyl | 50 g/l |
| Isoproturone | 500 g/kg |
| Bromoxynil | 250 g/l |

The herbicides were tested at different doses (see Tables 2.2 – 2.4). The details are given per experiment.

Experiment 1: Atrazine dose in the mixture was varied and the mesotrione dose was kept constant. To each of these doses, nicosulfuron was added or not (see Table 2.2).

Experiment 2: Bentazone doses ranged from 0 to 2.29 kg/ha. To each of these doses, quizalofop was added or not (see Table 2.3).

Experiment 3: The herbicides isoproturone and bromoxynil were tested separately at doses ranging from 0 to 3 l or kg/ha. In addition, mixtures of these two components were tested at half the doses of the individual components (see Table 2.4).

*Table 2.2. Experiment 1: doses herbicide mixture of formulated atrazine and mesotrione, with/without nicosulfuron (kg or l/ha)) applied on Amaranthus retroflexus, Echinochloa crus-galli and Digitaria sanguinales.*

| Treatment | | Atrazine | Mesotrione | Nicosulfuron |
|---|---|---|---|---|
| 0 | | 0 | 0 | |
| 0 | + | 0 | 0 | 0.75 |
| 1 | | 0.1 | 0.1 | |
| 1 | + | 0.1 | 0.1 | 0.75 |
| 2 | | 0.45 | 0.1 | |
| 2 | + | 0.45 | 0.1 | 0.75 |
| 3 | | 0.94 | 0.1 | |
| 3 | + | 0.94 | 0.1 | 0.75 |
| 4 | | 1.69 | 0.1 | |
| 4 | + | 1.69 | 0.1 | 0.75 |
| 5 | | 2.86 | 0.1 | |
| 5 | + | 2.86 | 0.1 | 0.75 |

*Table 2.3. Experiment 2: doses formulated bentazone with/ without quizalofop applied on weed species Chenopodium album, Amaranthus retroflexus, and Echinochloa crus-galli (kg or l/ha)).*

| Treatment | | Bentazone | Quizalofop |
|---|---|---|---|
| 0 | | 0.00 | 0.0 |
| 0 | + | 0.00 | 0.5 |
| 1 | | 0.16 | 0.0 |
| 1 | + | 0.16 | 0.5 |
| 2 | | 0.42 | 0.0 |
| 2 | + | 0.42 | 0.5 |
| 3 | | 0.89 | 0.0 |
| 3 | + | 0.89 | 0.5 |
| 4 | | 1.56 | 0.0 |
| 4 | + | 1.56 | 0.5 |
| 5 | | 2.29 | 0.0 |
| 5 | + | 2.29 | 0.5 |

*Tabel 2.4. Experiment3: doses formulated isoproturone and bromoxynil and the mixtures applied on weed species Chenopodium album, Capsella bursa-pastors, Descurainia sophia and Bromus japonicus (kg or l/ha).*

| Treatment | | Isoproturone | Bromoxynil |
|---|---|---|---|
| 1 | | | |
| 2 | I | 0.30 | |
| 2 | B | | 0.30 |
| 2 | IB | 0.15 | 0.15 |
| 3 | I | 0.50 | |
| 3 | B | | 0.50 |
| 3 | IB | 0.25 | 0.25 |
| 4 | I | 1.00 | |
| 4 | B | | 1.00 |
| 4 | IB | 0.50 | 0.50 |
| 5 | I | 2.00 | |
| 5 | B | | 2.00 |
| 5 | IB | 1.00 | 1.00 |
| 6 | I | 3.00 | |
| 6 | B | | 3.00 |
| 6 | IB | 1.50 | 1.50 |

## 2.5 Experimental setup

Three experiments were done. The experimental unit in an experiment is a plant in a pot. Experimental treatments were randomly divided over the plants. There were 8 replicates per treatment. The experimental design was a randomized block design. (To be confirmed by ICAMA/HAAFS).

## 2.6 Herbicide application and conditions

The herbicides were sprayed on the plants with a moving nozzle sprayer (see picture below). Plants were placed on the table. The sprayer was set to release a spray volume of 500 l/ha. After spraying plants were placed near the sprayer to dry for half an hour, and then returned to the greenhouse.

*Figure 2.1. Moving nozzle sprayer at research facility of HAAFS.*

## 2.7 Observations

Observations were done before treatment (before spraying) and up to circa 20 days after treatment.

The plants were observed by staff of HAAFS every 2 to 5 days up to final harvest. Important plant stages were recorded based on visual assessment (germination, emergence, seedling, 1-leaf stage, 2-leaf, 3-leaf, 4-leaf, 5-leaf, 6-leaf, 7-leaf stage, etc. A leaf was seen as a true leaf when it was longer than 1 cm long or when it had reached > 50 % of its final size.

At the time of spraying, fresh weight of 8 untreated plants per species was determined, development stage was noted and PPM-measurements on 8 untreated plants per species were done.

After treatment, PPM-measurements were done every day up to 6 days after spraying. The measurements were done in a dark room (an environment shielded from direct sunlight. Light intensity was circa 20 lux). Leaf measurements were done on the youngest measurable leaf. Plants were placed in the measurement room half an hour before the measurement.

Herbicide effects (symptoms, damage) was recorded and noted in qualitative terms (no symptoms, effects of herbicides visible (wittering, chlorosis, necrosis, wilting and mortality) every 3-5 days.

At the end of the experiment (final harvest), circa 20 days after spraying, fresh weight of the plants was determined. Plants were cut just above soil level and weighed per pot. If plants had died, the weight of the dead material was weighed per pot. Also at final harvest, the number of dead plants per treatment per species was determined.

Digital pictures were taken from plants at important growth stages (day of treatment, final harvest).

## 2.8 Data collection and analyses

All individual data on PPM, fresh weight and mortality were put in a Microsoft EXCEL spread sheet by staff of HAAFS. This data base was used for analyses.

A first evaluation of the data base was done in April 2006. Staff of HAAFS and ICAMA prepared a large number of PPM-time curves and regression analyses (PPM-versus growth reduction), and presented this information at the CMHD progress meting in April 2006 (Wang *et al.*, 2005). Plant Research International continued the analyses after a final data check in April and May 2006. The data set was concluded to be complete in June 2006.

Linear regressions were done with the Analysis ToolPak of Microsoft EXCEL. Doses that caused 90 % growth reduction of weeds in experiments could not be estimated with standard procedure S-curve fitting. Most data sets did not allow proper S-curve fitting with the a Genstat Statistical program. Instead, data were plotted in graphs, and $ED_{90}$ were estimated by eye by intra or extrapolation.

# 3. Results and Discussion

The complete data base (a Microsoft Excel spread sheet) of the three validation/calibration experiments described in this report is available at ICAMA CABET, HAAFS and Plant Research International. The data presented in Chapter 3 is a selection from the data set, to answer three research questions:

1. what is the dynamics of PPM-values over time for important weeds,
2. which date of PPM-measurements after treatment gives the best prediction of growth reduction,
3. a comparison of the minimum effective doses ED90 in the greenhouse experiments with MLHD doses advises from MLHD tables.

These questions are addressed in paragraphs 3.1, 3.2 and 3.3, respectively.

## 3.1 PPM-time curves of treated plants

In Figures 3.1 to 3.4 PPM-time curves for some weed species and herbicide treatments are shown. The data presented show PPM-dynamics over time for typical herbicide - weed interactions. The dynamics can be explained by type of herbicide (a photosynthesis inhibitor or not) and sensitivity of the weed to the herbicide(s). They are in line with earlier observations in the CMHD project in 2004 (Kempenaar *et al.*, 2005).

Experiment 1. Figure 3.1 is split into 3.1a where the data of treatments without nicosulfuron are shown, and 3.1b for treatments with this grass herbicide. *Echinogloa crus-galli*, the weed species in Figure 3.1, is considered to be sensitive for the herbicide mixture atrazine plus mesotrione. When treated with this mixture, the plants (youngest measurable leaf) showed a large decrease in PPM-values shortly after treatment. PPM-values remained low (ca 20) 2 to 6 days after treatment. Atrazine is a photosynthesis inhibitor. Mesotrione is not classified as a photosynthesis inhibitor, but this herbicide indirectly affects photosynthesis shortly after treatment (within some days). The addition of nicosulfuron to atrazine and mesotrione had no significant effect on PPM-values (compare 3.1a and 3.1b). Nicosulfuron is not a photosynthesis inhibitor.
A dose response PPM-effect was not observed for the data in Figure 3.1. At the lowest dose tested, which was 0.1 atrazine + 0.1 mesotrione, PPM-values were already much reduced by the herbicide, and high levels of growth reduction (circa 90 % fresh weight reduction) and plant mortality were observed for all herbicide doses.
The levels of the PPM-time curves of *Digitaria* in experiment 1 were a little higher than those of *Echinogloa*. *Digitaria* is considered to be less sensitive to atrazine plus mesotrione than *Echinogloa*. The levels of the PPM-time curves of *Amaranthus* (a sensitive species in experiment 1) were comparable than to those of *Echinogloa*.

Experiment 2. Figures 3.2 and 3.3 show two more typical PPM-time curves for a sensitive (3.2) and an insensitive herbicide – weed interaction. *Amaranthus* is considered to be sensitive to the photosynthesis inhibitor bentazone when plants do not have more than 4 leaves. In this case, PPM-values were much reduced shortly after treatment. However, *Amaranthus* is not sensitive to Quizalofop (no photosynthesis inhibitor), and this herbicide had no effect on PPM-values. *Echinogloa* is not sensitive for bentazone, but sensitive for Quizalofop. PPM-values were not affected by herbicides for this weed species in experiment 2. Comparison of Figures 3.3 and 3.1 further illustrates the effect of the type of herbicide weed interaction on dynamics of PPM-over time.

Experiment 3. In Figure 3.4 shows a last example of effect of type of herbicide – weed interaction on dynamics of PPM-values over time. In this case data of *Capsella bursa-pastoris* are shown. This weed is more sensitive to bromoxynil than to isoproturone. The mixture of the 2 herbicides was also quite effective on the weed.
PPM-values in experiment 3 were a bit higher than expected. PPM-values were between 30 and 40 while strong effects (mortality) of weed plants was observed. We have no clear explanation for this. One explanation is that two species (*Capsella* and *Descurania*) have a relatively small leaves, and too small leaves may give higher values. However, this should not have been the case with *Chenopodium* plants.
*Bromus* was the least sensitive weed species in experiment 3. The reduction of PPM-values for this weed was the smallest, and in line with expectations.

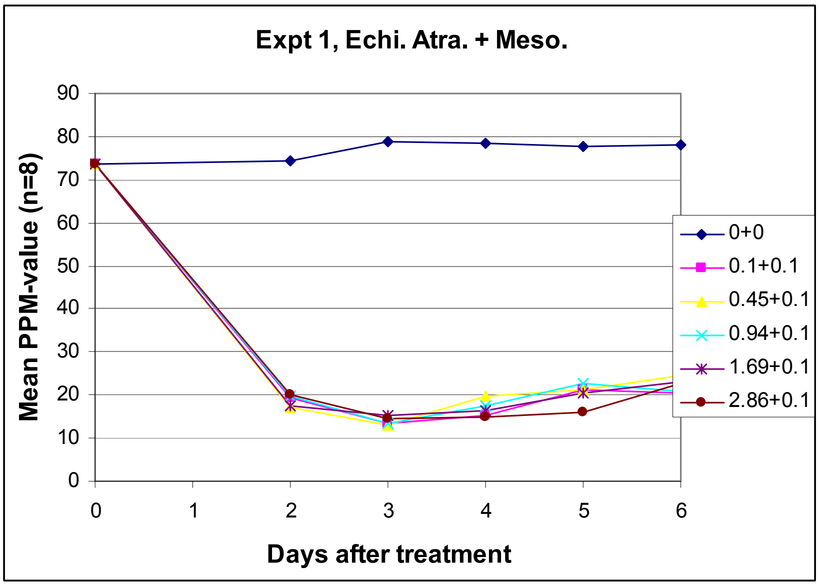


*Figure 3.1a.* *Data from experiment 1. PPM-time curves of Echinochloa plants treated with formulated atrazine and mesotrione (doses in kg or L product/ha).*

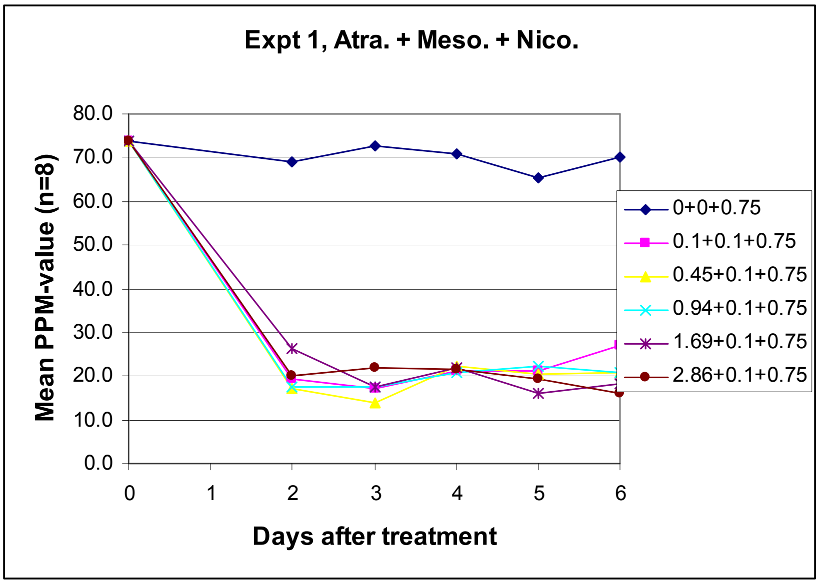


*Figure 3.1b.* *Data from experiment 2. PPM-time curves of Echinochloa plants treated with formulated atrazine and mesotrione plus nicosulfuron (doses in kg or L product/ha).*

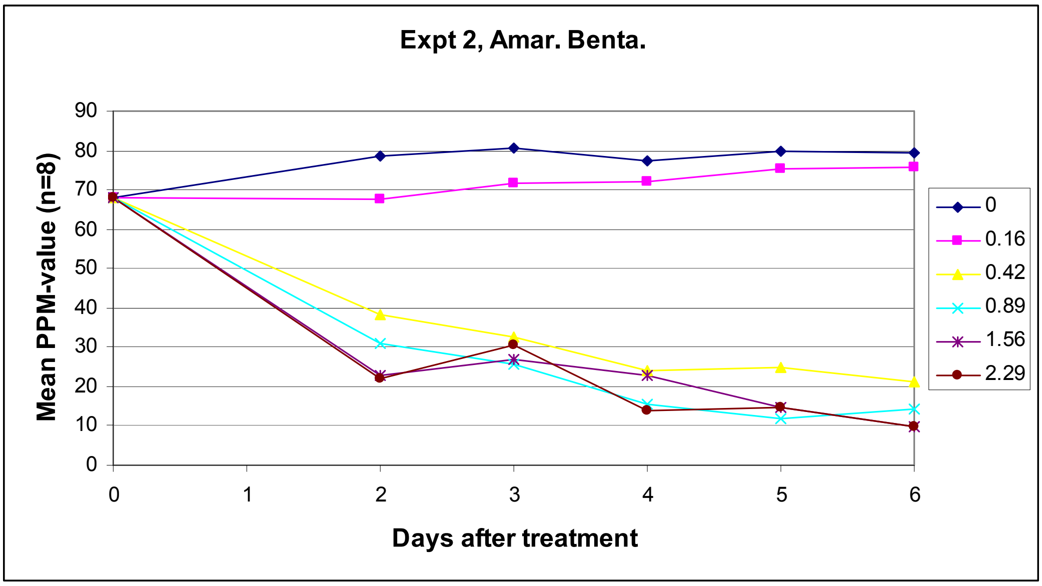


*Figure 3.2a.* *Data from experiment 2. PPM-time curves of Amaranthus plants treated with formulated bentazone (doses in kg product/ha).*

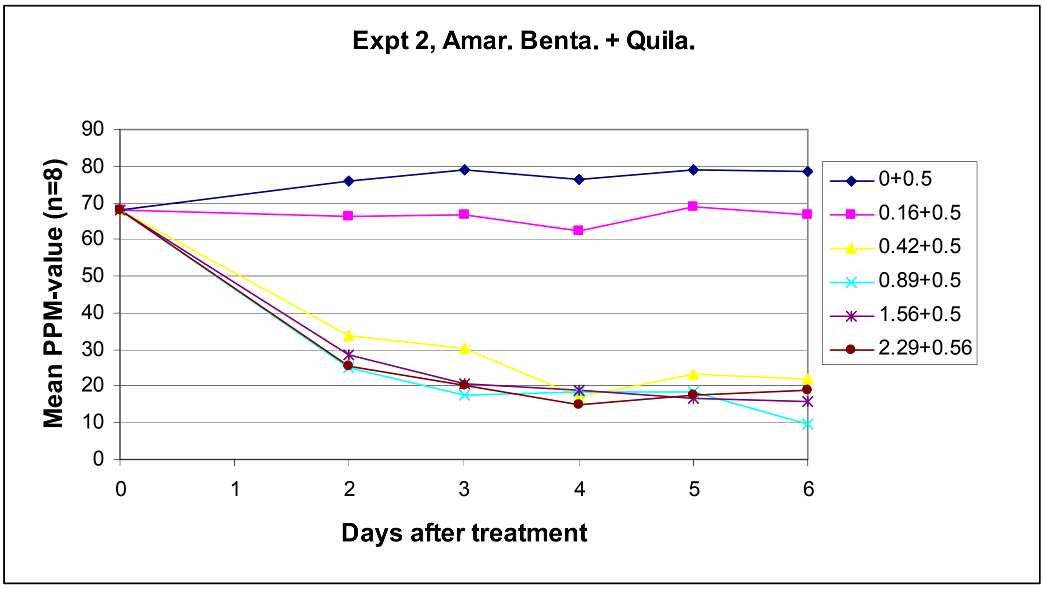


*Figure 3.2b.* *Data from experiment 2. PPM-time curves of Amaranthus plants treated with formulated bentazone plus Quizalofop (doses in kg or Lproduct/ha).*

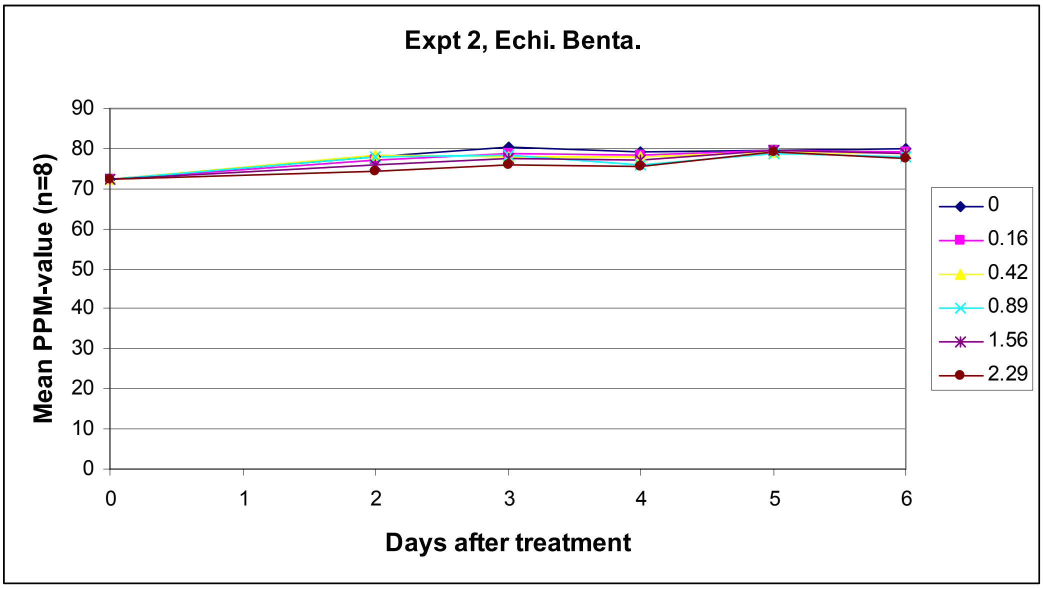


*Figure 3.3a.* *Data from experiment 2. PPM-time curves of Echinochloa plants treated with formulated bentazone (doses in kg product/ha).*

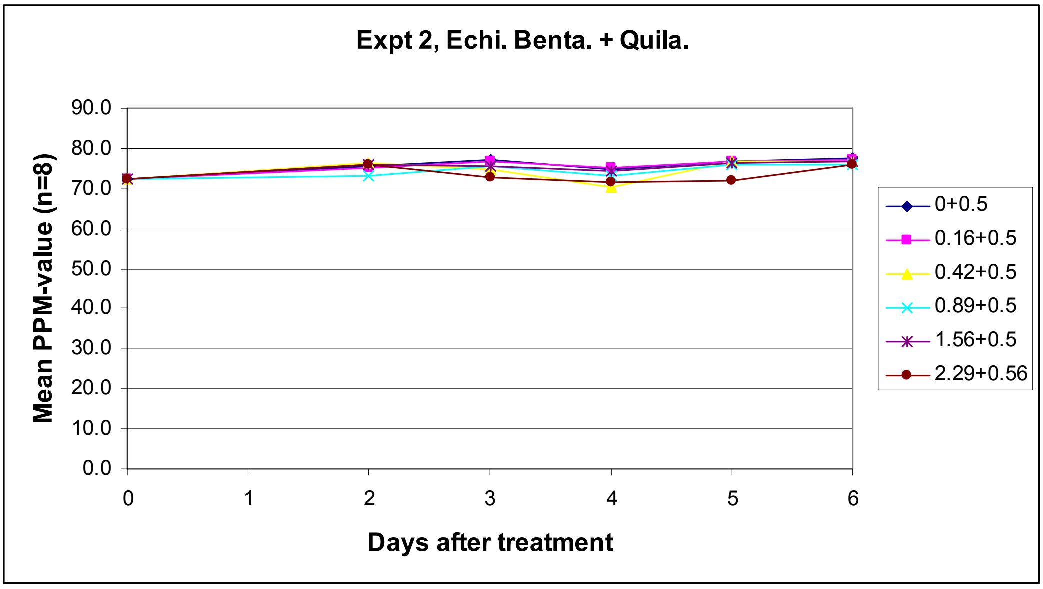


*Figure 3.3b.* *Data from experiment 2. PPM-time curves of Echinochloa plants treated with formulated bentazone plus Quizalofop (doses in kg product/ha for each herbicide).*

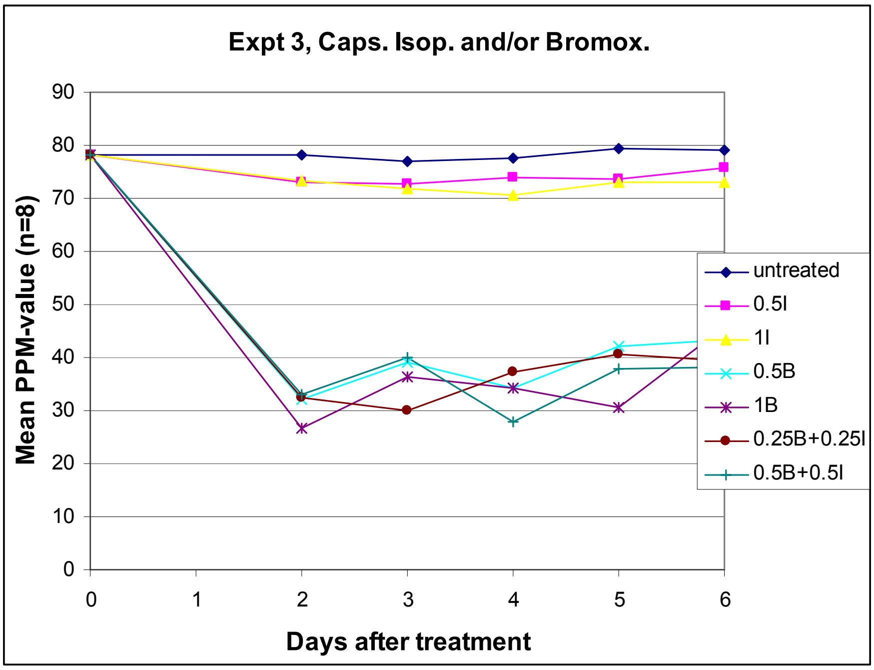


*Figure 3.4.* *Data from experiment 3. PPM-time curves of Capsella plants treated with formulated isoproturone (I), bromoxynil (B), or a mixture of the herbicides (IB) (doses in kg product/ha).*

## 3.2 Prediction of growth of treated weeds by PPM

Linear regression analyses were done to determine if and how well growth reduction of the weeds could be explained by PPM-measurements. In other words, can PPM-values shortly after treatment predict growth reduction at the end of the experiment. Regression analyses were done for PPM-data from 2, 3, 4, 5 and 6 days after treatment.

Table 3.1 shows correlation coefficients ($R^2$) of the regression analyses of PPM-measurements versus % growth reduction 14 days after treatment. In most situations (ca 80 %), good significant correlations ($R^2 > 0.8$) were observed between PPM-measurements and % growth reduction. In the cases of bentazone/*Echinogloa* with and without quizalofop correlations were moderate ($R^2$ circa 0.5 – 0.7) to poor ($R^2 < 0.5$) for all observation dates (see also Figures 3.3). In the case of bentazone, the moderate to poor correlation is explained by the type of herbicide interaction (small effects of the herbicide on both PPM-and growth, *Echinogloa* is not sensitive to the herbicide). In the case of bentazone plus quizalofop, the poor correlation is explained by the small effect of the herbicide mixture on PPM, and the large effect of the herbicide on growth of the plant (*Echinogloa* is sensitive for to herbicide mixture). A few more treatments showed moderate to poor correlations. Moderate to poor correlations were observed in experiment 3 (isoproturone and bromoxynil) for little sensitive herbicide-weed interactions and for observation date 6 (when detoxification of herbicide(s) in the plant may have occurred).

From Table 3.1 we can conclude that there was little effect of observation date on PPM-values on the correlation with growth between PPM-and growth reduction (see averages per date in Table 3.1). There was a trend that day 6 showed less good correlations than the other dates. From this follows that PPM-measurements on 2, 3, 4 and 5 can best be used to predict growth effects of herbicides on weeds later on the growth period. Because observation date had little effect, we use only data of one observation date (day 2) in further analyses of the relation between PPM-and growth reduction.

Figures 3.5 to 3.7 show results of linear regression analyses of PPM-measurements 2 days after treatment and % growth reduction 14 days after treatment for the three experiments. The results are presented and discussed per experiment. In all three experiments, good correlations were observed with $R^2$ in the order of 0.9.

Atrazine and mesotrione with and without nicosulfuron. In Figure 3.5, all data points of experiment 1 are shown except those for treatments that contained only nicosulfuron. The effect of nicosulfuron could not be measured with PPM while weeds were much affected by the herbicide. The data points in Figure 3.5 are clustered in two parts of the graph. In the right lower corner we find the untreated treatments data points and in the left upper corner the herbicide treatments data points. All the herbicide treatment data points are close to the 100 % growth reduction, with *Echinogloa* and *Amaranthus* data points more to the left than those of *Digitaria*. In the figure the result of the linear regression is shown ($R^2$ >0.82; y = -1.45x + 124.8). If the *Digitaria* data are excluded, the correlation improves much ($R^2$ = 0.96; y = -1.62+123.0). The regression parameters are much in line with those found in the atrazine + bentazone greenhouse experiment in 2004 at ICAMA-CABET.

Bentazone with and without quizalofop. In Figure 3.6, all data points of experiment 2 are shown except those for treatments bentzone + quizalofop and *Echinogloa* (PPM had no correlation with the effect). The data points in Figure 3.6 are spread over the PPM-range from 20 to 80 much better than in Figure 3.5, which is a good situation for regression analysis. The linear regression showed good correlation between PPM-and growth effect (statistics are given in Figure 3.6). If the data points of treatments with quizalofop are excluded, the regression results hardly changed ($R^2$ = 0.94; y = -1.72+140.2). The regression parameters are much in line with those found in the bentazone greenhouse experiment in 2004 at ICAMA-CABET.

Isoproturone and bromoxynil. In Figure 3.7, all data points of experiment 3 are shown. The data points in Figure 3.7 are spread over the PPM-range from 20 to 80 much better than in Figure 3.5, which is a good situation for regression analysis. The linear regression showed quite good correlation between PPM and growth reduction (regression parameters are given in Figure 3.7). The regression line was little affected by the herbicides. *Chenopodium* data points pulled the regression line to the right (the five data points closest to the equation in right upper comer of Figure 3.7), and resulted in a more steep slope of the regression line than expected (slope value -2.07 instead of -1.65 for isoproturone calibration experiment in 2004).

Considering all experiments, we conclude that PPM-values 2 days after treatment predicted growth reduction of greenhouse grown weed plants quite well. When PPM-values were below 20, a high level of growth reduction was observed at 14 days after herbicide application. Often plants had died by then. The observed regression parameters in the 2005 experiments were mostly comparable to those observed in the experiments in 2004. PPM-values of 20 or less predict a high level of control. PPM-values between 20 and 40 may also give large effects on growth of plants. MLHD PPM-threshold values for effects on plants under field conditions are given in Table 1.1 or MLHD manuals.

*Table 3.1. Correlation coefficient ($R^2$ values) of regressions of mean PPM-values on 2, 3, 4, 5 or 6 days after treatment and % growth reduction 14 days after treatment for the different experiments. Explanations of abbreviations of herbicides and species are given in paragraphs 2.2 and 2.4.*

| Herbicides | Species | PPM-measurement date (days after spraying) | | | | |
|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 |
| Atr/Meso | Amar | 0.98 | 0.99 | 0.99 | 1 | 1 |
| Atr/Meso/Nico | Amar | 0.90 | 0.92 | 0.91 | 0.92 | 0.90 |
| Atr/Meso | Echi | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 |
| Atr/Meso/Nico | Echi | 0.85 | 0.85 | 0.87 | 0.88 | 0.96 |
| Atr/Meso | Digi | 0.89 | 0.97 | 0.82 | 0.93 | 0.82 |
| Atr/Meso/Nico | Digi | 0.92 | 0.92 | 0.84 | 0.94 | 0.86 |
| Bent | Cheno | 0.92 | 0.97 | 0.98 | 1 | 0.98 |
| Bent/Quiza | Cheno | 0.99 | 0.96 | 0.9 | 0.89 | 0.85 |
| Bent | Amar | 0.97 | 0.97 | 0.94 | 0.95 | 0.95 |
| Bent/Quiza | Amar | 0.98 | 0.98 | 0.97 | 0.97 | 0.97 |
| Bent | Echi | 0.65 | 0.77 | 0.59 | 0.16 | 0.58 |
| Bent/Quiza | Echi | 0.18 | 0.43 | 0.02 | 0.61 | 0.16 |
| Iso | Cheno | 0.67 | 0.70 | 0.69 | 0.63 | 0.85 |
| Iso | Capse | 0.85 | 0.74 | 0.84 | 0.87 | 0.87 |
| Iso | Descu | 0.52 | 0.64 | 0.91 | 0.91 | 0.72 |
| Iso | Bromu | 0.69 | 0.26 | 0.65 | 0.93 | 0.07 |
| Bromox | Cheno | 0.81 | 0.77 | 0.77 | 0.86 | 0.90 |
| Bromox | Capse | 0.98 | 0.95 | 0.88 | 0.94 | 0.92 |
| Bromox | Descu | 0.93 | 0.96 | 0.76 | 0.97 | 0.81 |
| Bromox | Bromu | 0.61 | 0.90 | 0.80 | 0.83 | 0.61 |
| Iso/Bromox | Cheno | 0.88 | 0.97 | 0.97 | 0.98 | 0.93 |
| Iso/Bromox | Capse | 0.99 | 0.96 | 0.97 | 0.89 | 0.98 |
| Iso/Bromox | Descu | 0.97 | 0.81 | 0.90 | 0.80 | 0.46 |
| Iso/Bromox | Bromu | 0.91 | 0.90 | 0.85 | 0.84 | 0.26 |
| Average | | 0.83 | 0.85 | 0.83 | 0.86 | 0.77 |

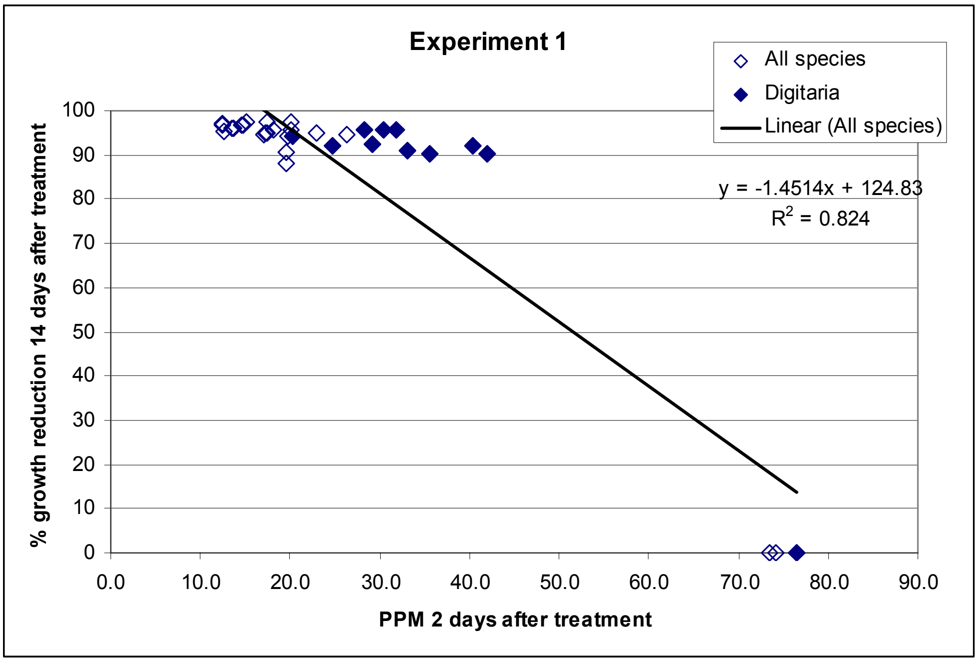


*Figure 3.5.* *PPM-values 2 days after treatment and growth reduction 14 days after treatment for the 3 weed species (*Echinogloa, Amarantus *and* Digitaria*) treated with a mixture of Atrazine and Mesotrione, with and without Nicosulfuron (details, see experiment 1).*

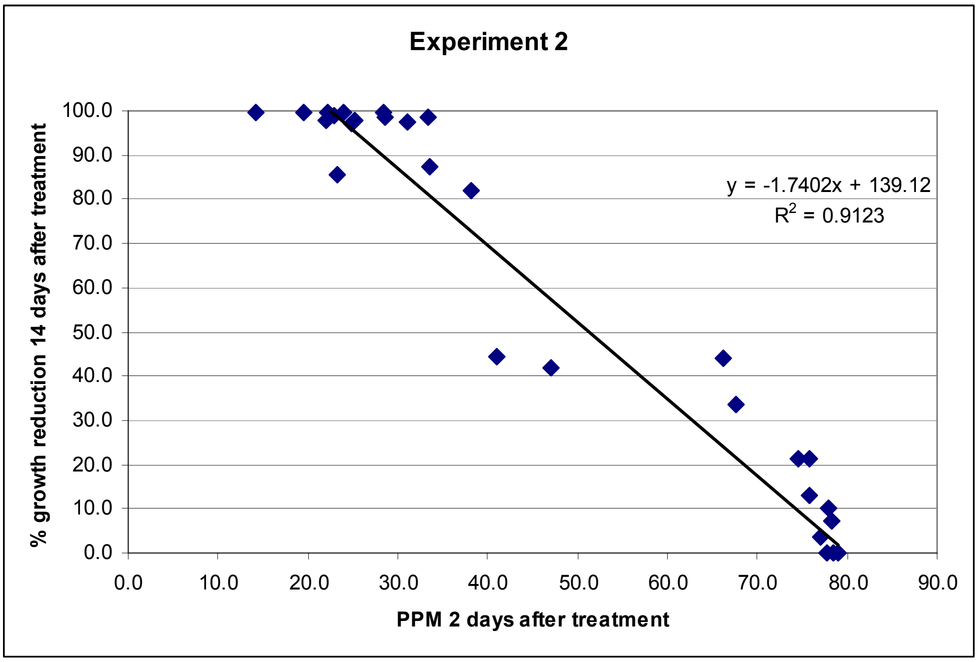


*Figure 3.6.* *PPM-values 2 days after treatment and growth reduction 14 days after treatment for the 3 weed species (*Echinogloa, Amarantus *and* Chenopodium*) treated with Bentazone with and without Quizalofop (details, see experiment 2).*

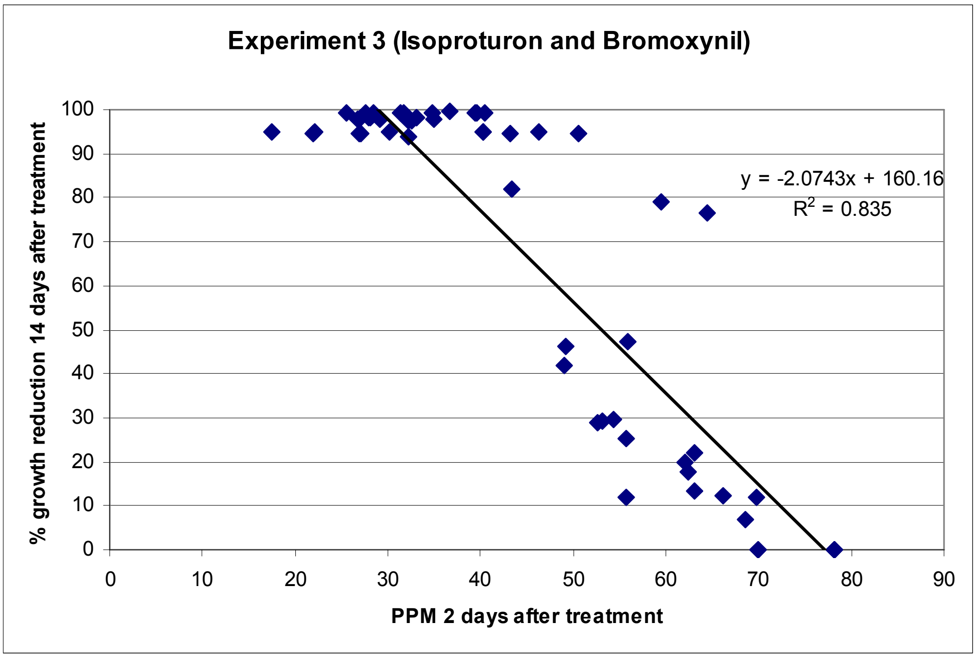


*Figure 3.7.* *PPM-values 2 days after treatment and growth reduction 14 days after treatment for the 4 weed species* (Chenopodium, Descurania, Capsella *and* Bromus) *treated with Isoproturone and/or Bromoxynil).*

## 3.3 Comparison of minimum effective doses from calibration experiments with MLHD doses

Table 3.2 contains estimates of doses that gave circa 90 % growth reduction for the different herbicide weed interactions studied in experiments 1, 2 and 3. In most cases the data sets did not allow non linear S-curve fitting. Because of this, this standard procedure S-curve fitting could not be used to calculate $ED_{90}$ values for the different herbicide-weed interactions. In stead $ED_{90}$ were estimated by eye from the data plotted in graphs. Observed $ED_{90}$ values can be used to evaluate how sensitive a weed species is for a particular herbicide or herbicide mixture. The observed $ED_{90}$ were in line with expectations (sensitive species showed smaller $ED_{90}$).

Experiment 1 showed high levels of control and there were little differences between species. The mixtures were quite effective on the grasses. Experiment 2 confirmed that bentazone is not effective on grasses. Experiment 3 showed e.g. that isoproturone is not effective on *Capsella* and *Bromus*. Bromoxynil has a strong effect on *Capsella*, but not on *Bromus*.

Greenhouse experiments cannot be used to determine a precise minimum effective doses for field situations. This is because weeds in the greenhouse are generally less hardened than weeds in the field. Because of this, weeds in the field generally need a higher dose to be killed than in weeds in a greenhouse. However, a comparison between minimum effective doses in the greenhouse and MLHD dose advises for field situation can be made in Table 3.2. The MLHD dose recommendations in Table 3.2 are on average higher than the $ED_{90}$ estimates of greenhouse experiments 1 and 3, and lower when than those of experiment 2 (bentazone).

*Table 3.2. Doses that gave circa 90 % growth reduction in the greenhouse experiments 14 days after treatment and dose recommendations from the MLHD Manual. In the case of mixtures (e.g. Atr/Meso) the doses for each herbicide are given.*

| Herbicides | Species | Dose (g a.i./ha) | | |
|---|---|---|---|---|
| | | ED 90% from greenhouse expt. | Dose advises from MLHD dose tables* | Comment |
| Atr/Meso | Amar | < 40/10 | 200/50 | Lowest dose > 90 % reduction |
| Atr/Meso/Nico | Amar | < 40/10/30 | 200/50/0 | Lowest dose > 90 % reduction |
| Atr/Meso | Echi | 120/10 | 300/50 | |
| Atr/Meso/Nico | Echi | 40/10/30 | 250/50/25 | |
| Atr/Meso | Digi | 40/10 | 300/50 | |
| Atr/Meso/Nico | Digi | <40/10/30 | 250/50/25 | Lowest dose > 90 % reduction |
| Bent | Cheno | 300 | 240 | |
| Bent/Quiza | Cheno | 240/50 | 200/50 | |
| Bent | Amar | 300 | 240 | |
| Bent/Quiza | Amar | 240/50 | 200/50 | |
| Bent | Echi | > 1100 | Not Recommended | Bentazone has no effect on |
| Bent/Quiza | Echi | 0/50 | 0/50 | Echinogloa (insensitive combi.) |
| Iso | Cheno | 500 | 1000 | |
| Iso | Capse | > 1500 | Not recommended | Highest dose < 90 % reduction |
| Iso | Descu | < 150 | 750 | Lowest dose > 90 % reduction |
| Iso | Bromu | > 1500 | ? | Highest dose < 90 % reduction |
| Bromox | Cheno | 100 | 200 | |
| Bromox | Capse | < 75 | 200 | Lowest dose > 90 % reduction |
| Bromox | Descu | < 75 | ? | Lowest dose > 90 % reduction |
| Bromox | Bromu | > 750 | ? | Highest dose < 90 % reduction |
| Iso/Bromox | Cheno | < 75/38 | 350/200 | Lowest dose > 90 % reduction |
| Iso/Bromox | Capse | < 75/38 | 350/200 | Lowest dose > 90 % reduction |
| Iso/Bromox | Descu | < 75/38 | 350/200 | Lowest dose > 90 % reduction |
| Iso/Bromox | Bromu | > 750/375 | ? | Highest dose < 90 % reduction |

* *For 4-leaf stage plants, favorable weather conditions and optimal boon sprayer conditions.*

# 4. Concluding summary

The following conclusions were drawn from the greenhouse experiments:

- Calibration experiments were carried out successfully at HAAFS in 2005 in the frame of the CMHD project. The experiments yield an extra data set for validation of MLHD under greenhouse conditions.
- Observed PPM-time curves for weeds treated with the photosynthesis inhibiting herbicides in the experiments were in line with expectations:
    - Sensitive weed species showed a fast and large reduction in PPM-values the first 2 to 6 days after treatment,
    - Little sensitive weed species showed lesser or no reduction in PPM-values after treatment compared to sensitive species,
    - Insensitive species did not show an effect of herbicide on PPM-values.
    - Differences in sensitivity of species can be explored / predicted with the PPM-meter.
- In the experiments, good correlations were observed between PPM-values shortly after treatment and percent growth reduction 14 days after treatment for weeds treated with one or two photosynthesis inhibiting herbicides. The smaller the PPM-value, the larger the growth reduction was. $R^2$ of linear regressions were in the order of 0.8. This confirms earlier conclusions in the project that PPM can be used to predict level of control by photosynthesis inhibiting herbicides.
- Two grass herbicides were studied: quizalofop and nicosulfuron. The use of these herbicides had no effect on PPM-values up to 6 days after treatment. The grass herbicides do not affect photosynthesis of plants during this period. The effect of quizalofop or nicosulfuron on weeds could not be predicted by PPM.
- Variation in PPM-values between plants with the same treatment were observed. Variation in PPM-values may be caused by variation in herbicide deposition on the plants, translocation in the plant, environmental conditions, plant morfology, condition of PPM-meter and way of measurement. Variation can be reduced in some situations; by doing measurements in a dark environment, by dark adaptation of leaves before measurement, by taking sufficiently large leaves (see manual) for measurements, by doing a 'destructive measurement' (take leaf to be measured from the plant) and by avoiding measurements on necrotic leaf tissue. In the calibration experiments, non destructive measurements were done (which causes extra variation).
- The MLHD-thresholds values for good levels of control (see Table 1.1) complied with the results of the calibration experiments. In experiment 3, PPM-values were a little higher then expected (PPM were around 30 while large effects on the weeds were observed). This finding in experiment 3 can be partly explained by the fact that two of the broadleaf species tested have small leaves and are quite difficult to measure non destructively.
- Minimum effective doses in the greenhouse experiments (doses that gave 90 % growth reduction) were on average a little higher than doses advises from MLHD tables in two of three experiments in 2005, and a little lower in one of three experiments.
- Experiment 3 shows that bromoxynil has some potential to control *Bromus* in wheat under Chinese conditions, though further studies are needed to control this problematic weed.

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