# Quantifying herbicide injuries in maize by use of remote sensing

Quantifizierung von Herbizidschäden in Mais mit Hilfe von Fernerkundung

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#### Summary

Maize breeders and plant protection companies require early information about negative side effects of herbicides on maize plants to identify cultivars which are susceptible to specific herbicide agents. Experiments were conducted in 2005 and 2006 to quantify herbicide injuries in maize. In 2005, sulfonylurea type herbicides and an untreated control were established in a susceptible and an insusceptible maize cultivar at University of Bonn, Research Station Dikopshof. Multispectral images were taken via airborne remote sensing after herbicide application. The normalized difference vegetation index (NDVI), an indicator for crop vitality, was calculated. Biomass was assessed before harvest. Yield was mapped at harvest. In 2006, 12 maize cultivars were sown in strips at Dingbuchenhof, near Erkelenz. Plots with an untreated control and plots with two doses of a sulfonylurea type herbicide were established square to the direction of the maize strips. Maize vitality was observed by visual ratings after spraying. Multispectral images were taken via airborne remote sensing and NDVI was calculated.

In 2005, corn and straw biomass was significantly reduced in the treated plots within the susceptible cultivar. NDVI values and crop yields were reduced in the plots treated with sulfonylurea type herbicides. High correlations between NDVI- and yield values were calculated. However, no differences were assessed in the insusceptible maize cultivar.

In 2006, overdoses of the sulfonylurea type herbicide caused vitality losses in almost all cultivars. Observations on crop vitality from visual ratings corresponded in many cases with the vitality values (NDVI) measured with remote sensing. Visual ratings can be influenced by individual estimation mistakes. By contrast, remote sensing enables numerically discrimination of herbicide injury in entire maize fields. This kind of measure may be helpful to accelerate the detection process of maize cultivars that are susceptible to herbicides.

Keywords: Crop vitality, digital measure, on-farm-research, sulfonylurea type herbicides

### Zusammenfassung

Maiszüchter und Hersteller von Pflanzenschutzmitteln benötigen Informationen, um die Herbizidverträglichkeit von Maissorten frühzeitig abschätzen zu können. Es wurden Feldversuche durchgeführt, um Schädigungen von Herbiziden an Mais zu detektieren und zu quantifizieren. Im Jahr 2005 wurden Sulfonylharnstoffe im Vergleich zu einer unbehandelten Kontrolle in je einer gegenüber dieser Herbizidgruppe unempfindlichen und einer empfindlichen Maissorte appliziert (Versuchsgut Dikopshof der Universität Bonn). Nach der Herbizidapplikation wurde die Versuchsfläche aus der Luft mit einer Multispektralkamera aufgenommen. Der "Normalized Difference Vegetation Index" (NDVI), ein Indikator für Vitalität der Pflanzen, wurde in allen Varianten berechnet. Vor der Ernte wurde die Biomasse bestimmt. Zur Ernte wurde der Ertrag in den Parzellen per Ertragskartierung erfasst. Im Jahr 2006 wurden 12 Maissorten in Streifen nebeneinander angelegt (Dingbuchenhof bei Erkelenz). Im rechten Winkel zur Anlage der Sorten wurden ebenfalls in Streifen zwei Dosierungen eines Herbizides aus der Gruppe der Sulfonylharnstoffe appliziert. Ein unbehandelter Kontrollstreifen wurde zum Vergleich ebenfalls quer über die Sorten angelegt. Die Vitalität der Sorten in den Varianten wurde visuell geschätzt. Gleichzeitig wurde die Versuchsanlage aus der Luft mit einer Multispektralkamera aufgenommen. Der NDVI wurde in allen Varianten berechnet.

Ergebnisse 2005: Die Biomasse war in den behandelten Parzellen der empfindlichen Sorte stark reduziert. Ebenfalls waren die NDVI-Werte sowie die Erträge in den behandelten Parzellen wesentlich niedriger als in den unbehandelten. NDVI-Werte und Erträge waren positiv miteinander korreliert. In der unempfindlichen Maissorte gab es keine Unterschiede zwischen behandelten und unbehandelten Parzellen.

Ergebnisse 2006: Überdosierungen führten zu Vitalitätsverlusten an nahezu allen getesteten Maissorten.

Visuelle Schätzungen folgten dem gleichen Trend wie die NDVI-Werte. Visuelle Bonituren sind durch individuelle Schätzfehler beeinflusst und zeitintensiv. Sensorische Bestimmung der Vitalität via Fernerkundung mit Multispektralkamera ermöglicht es dagegen, Vitalitätsverluste durch Herbizide numerisch zu erfassen, wodurch der Prozess der Identifizierung von herbizidempfindlichen Maissorten beschleunigt werden könnte.

Stichwörter: Digitale Messung, On-Farm-Experiment, Sulfonylharnstoffe, Vitalität von Pflanzen

## 1. Introduction

Herbicides may cause severe injuries in maize which lead to growth retardations when applied under stressful environmental conditions or in susceptible maize cultivars (FRANK et al., 1983; WEIDENHAMMER et al., 1989). Plant breeders as well as plant protection companies require early information on unwanted side effects of sulfonylurea type herbicides on maize to detect susceptible cultivars in which such herbicides would cause injuries and growth retardations. The common procedure to quantify herbicide injuries on field scale is to rate vitality after herbicide application by visual observations (DONALD, 1998). Visual fatigue and the numbing effect of looking at plants over long periods make rating imprecise (THEUNISSEN and LEGUTOWSKA, 1992). So, visual rating is subjective and may be inaccurate over time (SPOMER and SMITH, 1988). Sensor- and GIS-technologies enable digital measures of crop status. JACOBI and KÜHBAUCH (2005) quantified differences in crop vitality in field plots of winter wheat resulting from deficient nitrogen fertilisation and fungal infection using very high resolution (VHR) satellite images. Remote sensing imagery coupled with geospatial technologies could potentially be used to identify herbicide drift-affected field sites (HENRY et al., 2004). Even with site-specific weed control, where some field areas remain untreated, crop vitality loss resulting from herbicides may clearly appear on images of VHR satellites (DICKE and KÜHBAUCH, 2005). The aim of this study was firstly to detect vitality losses in maize varieties caused by herbicides and secondly to test remote sensing as a tool for quantifying vitality losses caused by herbicides.

## 2. Materials and methods

## 2.1 Field trial in 2005

The influence of two sulfonylurea type herbicides on vitality and yield of the maize cultivars Fuego (insusceptible) and Abraxas (susceptible) was studied in a split plot design with four repetitions within each cultivar at Dikopshof Research Station in 2005. The soil type was a homogeneous sandy loam (loess).

The two maize cultivars were sown on 13.05.2005 with customary sowing rates (9 seeds/m<sup>2</sup>). In all plots, a customary dose of *Click Pro*<sup>®</sup> (225 g/kg bromoxynil and 350 g/kg terbuthylazin) was applied in 200 I water against dicot weeds over all plots, when maize had reached the 5-6 leaf stage using an ordinary field sprayer on 17.06.2005. As trial variants, 50 g Cato<sup>®</sup> (250 g/kg rimsulfuron) (variant 1) and 150 g/ha MaisTer<sup>®</sup> (300 g/kg foramsulfuron + 10 g/kg iodosulfuron + 300g/kg isoxadien-ethylen) (variant 2) were applied on 17.06.2005 at the intended plots. The size of each plot was of 50 m x 21 m. The control plots (variant 3) were not treated with sulfonylurea type herbicides. One week after spraying, multispectral images of the experimental design were taken from a helicopter using a Nir-R-G-Digicam [green (500-600 nm), red (<600-700 nm) and near infrared channel (<700-950 nm); 1 CCD-chip; 1.1 Mio pixel]. The image analysis was processed using ERDAS Imagine 8.4-software. Firstly, the experimental field was excluded out of the geo-referenced air-borne image. Secondly, from the near infrared (NIR) (760-900 nm) and the red spectral bands (630-690 nm), the normalized difference vegetation index (NDVI) [R (NIR) -R (Red)]/[R (NIR) +R (Red)], an indicator for crop vitality (ROUSE et al., 1973) was calculated across all pixels within the parts of interest.

Fresh weight (straw differentiated from corncob) was assessed in each plot four weeks before harvest directly on the field. Dry weight was measured in the lab. During harvest, yield was mapped using CERES 2, a dGPS connected combine-mounted yield monitor system of RDS-Company. Yield and NDVI-data of the plots of interest were merged using ESRI-Software. Yield and NDVI-data were correlated afterwards.

### 2.2 Field trial in 2006

A field trial of BASF Company (Dingbuchenhof, near Erkelenz) was selected, in order to measure vitality losses caused by different dosages of the sulfonylurea type herbicide Motivell $^{(B)}$  (40 g/l nicosulfuron) via remote sensing and to compare the sensor results with the visual ratings from company staff (evaluations were made by one person). The soil type was a very homogeneous sandy loam. Gravel or sand beddings or other interfering differences, which could influence sensor measures were not visible, neither from within the field, nor by bird's eye view onto the whole field via helicopter. However, soil has not been mapped via Em38. In the experiment, 12 maize varieties were sown in strips. Each strip had a width of 4.5 m (6 maize rows). One untreated control, three repetitions of the recommended herbicide dose of 1 l/ha Motivell<sup>®</sup> and two repetitions of the triple amount of the recommended nicosulfuron dose (3 I/ha Motivell®) were established in the field. Before maize germination, the recommended rate of a dimethenamid P + pendimethalin (720 g/l + 400 g/l) mixture was applied against dicot weeds across the field. At 5-6 leaf stage, the herbicide dosages (variants) were applied rectangular to the cultivars in 9 m widths via bicycle sprayer. So, plots sizes were 4.5 m x 9 m. Visual ratings of maize vitality were conducted by company staff 14 days after spraying. The percentage crop vitality losses in each plot were estimated by visual observations (yellowness and crop height). Simultaneously, multispectral images from the experimental design were taken via remote sensing using helicopter in the same way as in 2005. Image analysis was also performed analogical to the field trial in 2005. From the NIR image, a grey value image was derived with a range from 0 (black) to 255 (white). Kernel plots of interest with defined size were extracted out of the experimental plots for further analysis in order to avoid mixed pixels resulting from the neighbor plots. The averaged grey values of each kernel plot were normalized in order to remove the specific reflectance characteristics of the different varieties: The untreated variant (control) of each respective maize cultivar was set to the value 100 for highest crop vitality. Data on yield were not recorded. Visual ratings and sensor measurements were analyzed using a two factorial linear mixed model. Rows of the experimental design were modeled as a random design effect and treatment whereas cultivar and interaction treatment by cultivar were modeled as fixed effects. The estimates and measurements from control plots were not used in the linear mixed model because these plots did not really provide information and would have caused problems related to their zero variance. Nevertheless a statistical test for phytotoxic effects of herbicide application is possible by calculating confidence intervals for the herbicide effect and comparing these intervals with a phytotoxicity of zero. To fulfill the model assumptions of homogeneous variances and Gaussian error distribution, we performed a logit-transformation (eq. 1).

$$y = \log\left(\frac{p}{1-p}\right)$$
, with p = (percent phytox + 0.5) / 100. (eq.1)

The addition of 0.5 was done to avoid problems with a rating of 0 percent. After fitting the linear model we used eq. 2 for back transformation and estimation of means and confidence limits.

$$E_{(percent\_phytox)} = 100 \cdot \left(\frac{1}{e^{-y} + 1}\right) - 0.5$$
 (eq.2)

We calculated the operative heritability as ratio between cultivar and treatment variances divided by the error variance assuming four plots per cultivar and treatment. For example, cultivar heritability is calculated with  $h^2 = var_{cultivar} / (var_{cultivar} + var_{error}/4)$ .

Linear mixed model computations and estimation of variance components were performed with the REML method of procedure MIXED available in the statistical software package SAS 9.2.

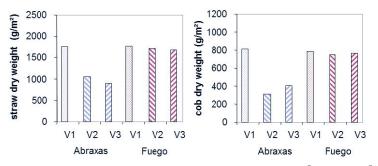
25. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und -bekämpfung, 13.-15. März 2012, Braunschweig

### 3. Results

#### 3.1 Field trial 2005

### Ground truth results:

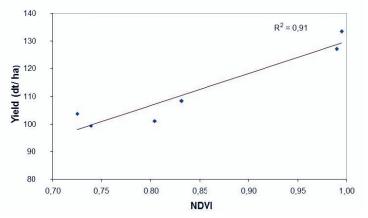
Crop growth of variety Abraxas paused for one month in those plots, where sulfonylurea-type herbicides were applied (data not shown). Corncob and straw biomass was reduced in the treated plots. No herbicide injuries were found in the insusceptible maize cultivar Fuego.

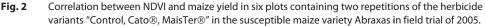


- Fig. 1 Influence of sulfonylurea-type herbicide (V1: Untreated control, V2: Cato<sup>®</sup>, V3: MaisTer<sup>®</sup>) on straw dry weight and corncob dry weight in the varieties Abraxas and Fuego.
- **Abb. 1** Einfluss von Sulfonylharnstoffen (V1: Unbehandelte Kontrolle, V2 Cato<sup>®</sup>, V3: MaisTer<sup>®</sup>) auf Trockenmasse Stroh und Trockenmasse Maiskolben in den Sorten Abraxas und Fuego.

#### Sensor results:

Unfortunately, only data of six plots (two repetitions) of variety Abraxas were of sufficient quality for image analysis, because of vibrations during picture recording which leaded sometimes to camera shake. In the susceptible cultivar Abraxas, the NDVI values were reduced in the plots treated with sulfonylurea type herbicides. In both repetitions the same NDVI-gradation between the treated plots were assessed. There was a high correlation between NDVI and yield, indicating that yield loss resulting from herbicide injury may be predicted from the early measured vitality values.





**Abb. 2** Beziehung zwischen NDVI und Maisertrag in sechs Versuchsparzellen mit zwei Wiederholungen der Herbizidvarianten "Kontrolle, Cato<sup>®</sup>, MaisTer<sup>®</sup>" in der empfindlichen Maissorte Abraxas im Versuch des Jahres 2005.

## 3.2 Field trial 2006

The estimated variance components showed a lower error variance for the sensor data but also lower cultivar and treatment variance for the sensor data compared to the visual data (Tab. 1). The interaction cultivar by treatment was neglectable. The operative heritability for cultivar and treatment effects was higher for data from visual rating compared to the sensor measurement.

		5		
Variance component	logit_sensor_pct	logit_visual_pct	sensor_pct	visual_pct
Cultivar	0.174	0.312	3.85	13.31
Treatment	0.049	0.461	0.96	9.31
Cultivar*treatment	0.000	0.030	0.00	0.00
Row(treatment)	0.054	0.045	2.30	0.55
Error	0.098	0.152	2.34	2.49
Heritability for cultivar differences	0.88	0.89	0.87	0.96
Heritability for treatment differences	0.67	0.92	0.62	0.94

 Tab. 1
 Variance components and heritability (ratings from control plots not used).

 Tab. 1
 Varianzkomponenten und Heritabilität (Bonituren der Kontrollvarianten sind unberücksichtigt).

The significance test with logit-transformed data showed the same tendency (Tab. 2). With visual rating, the differences between cultivars and the dose effect was clearer.

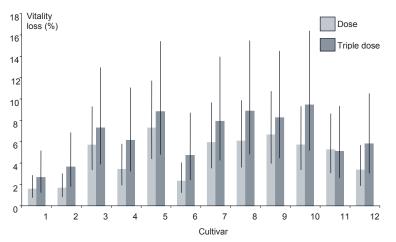
- Tab. 2
   F-test for cultivar and treatment effects on logit-transformed phytox ratings (ratings from control plots not used).
- Tab. 2
   F-test f

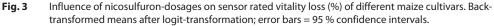
   F-test f

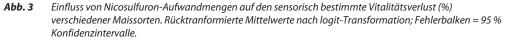
   Sorten- und Behandlungseffekten auf die logit-transformierten Phytotoxwerte (Werte der Kontrollvarianten sind unber

	Logit visual pct				Logit sensor pct			
Effect	Num DF	Den DF	F value	$\Pr > F$	Num DF	Den DF	F value	Pr > F
Cultivar	11	33	10.31	<.0001	11	33	7.78	<.0001
Treatment	1	3	20.30	0.0204	1	3	2.89	0.1879
Cultivar* Treatment	11	33	1.35	0.2442	11	33	0.46	0.9154

Nevertheless, treatment effects and cultivar differences were detectable with sensor measurement (Fig. 3). Confidence intervals for all cultivar\*treatment combinations were significantly different from zero. The highest vitality losses with more than 6 % from the recommended nicosulfuron dosage were achieved in the cultivars 5, 7, 8, 9 and 10, whereas in cultivar 1 and 2 only marginal vitality losses of 1-2 % could be achieved. The triple dosage of the recommended rate of nicosulfuron resulted in vitality losses of more than 8 % in the maize cultivars 5, 7, 8, 9 and 10.







#### 4. Discussion

Herbicide applications resulted in high vitality and yield losses when applied in susceptible maize cultivars. The specific susceptibility of maize cultivars against sulfonylurea type herbicides could be detected and guantified in a numerically manner by use of remote sensing. In sulfonylurea susceptible plants, an herbicide attaching or binding to an enzyme (acetlactate synthase or ALS) is responsible for disrupting amino acid biosynthesis. Sulfonylurea herbicide resistant plants have a modified ALS enzyme that prevents herbicide binding (HOCK et al., 1995). Different amounts of such enzymes within the different cultivars may influence their individual response to herbicides. Due to the possibility to normalize the grey values, it was possible to exclude the effects of cultivar specific reflectance patterns, which also may fudge visual ratings. However, parameter estimates and standard errors resulting from statistical treatment must be interpreted with care since the randomization of cultivars and treatments was restricted by technical reasons. Due to this lack of randomization, only one strip per cultivar was sown and divided into six plots by cross application of the herbicide and the control treatment. Hence cultivar effects and cultivar by treatment interaction might have been confounded with soil effects. For the control, only one row with twelve plots was available, for the single dose three rows and for the high dose two rows. Whereas cultivar and treatment effects might have been biased due to the lack of randomization, the method comparison of sensor measurements against visual ratings should still be valid. One question that generally comes up against sensor based approaches to quantify crop vitality is how to distinguish other factors affecting crop vitality like drought stress originating from soil heterogeneities, plant diseases or within field nutrition deficiencies etc. from the influences of herbicides. Most authors agree that all measurable information relating to the field of interest should be analyzed (LUSCHEI et al., 2001). In the last decades, geographical information systems and GPS guided sensors for measuring spatially referenced field information like within-field soil variation (CORVIN and LESCH, 2005), weed distribution (GERHARDS and CHRISTENSEN, 2003), yield variation (NOACK et al., 2003), etc. were developed and continually improved. DICKE and GEBHARDT (2007) showed how different thematic information can be merged to tables in order to distinguish the effects of each input factor and to study their specific interactions. However, in further experiments those additional influencing factors should be measured and analyzed. As a suggestion for future experiments, the herbicides of interest could be

applied in certain spatial patterns at different locations within the field. If the spatial patterns correspond to the spatial vitality patterns resulting from remote sensing, influences of herbicides on vitality would be evident. The influences of additional measured field information on crop vitality could be observed within the patterns and taken into account for analysis afterwards.

The current approach is (1) suitable for detecting herbicide injury in maize at early growth stages in a numerically manner, (2) all experimental plots can be analyzed in one run and thus (3) may cheapens company costs in field trial operations.

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