Institut für Organischen Landbau

Occasional direct-seeding of grain legumes in Organic Agriculture in Germany and Brazil

fertilisation with P and S

&

weed control with natural herbicides

Dissertation

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Michael Giepen

aus

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Gutachter:

Prof. Dr. Ulrich Köpke

Prof. Dr. Heinrich Scherer

Prof. Dr. Matthias Becker

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Abstract

The aim of this work was to test strategies, which improve nutrient supply and weed control in occasional direct-seeded (DS) grain legumes in Organic Agriculture. The effect of intra-row fertilisation of rock phosphate (RP) and several sulphur fertilisers on crop growth, yield and nutrient uptake was studied in field trials with faba bean (*Vicia faba*) in Germany (two sites in NRW, 2011 and 2012) and with soybean (*Glycine max*) in Brazil (three sites in Paraná, 2012 and 2013). The second part of this work investigated weed control with natural herbicides (NH). Therefore, eighteen screening trials in the field and five field trials with soybean and common bean (*Phaseolus vulgaris*) were conducted in Brazil. Additionally, the effect of different amounts of oats straw residue on faba bean and weed shoot growth were examined as a second trial factor within the 2012 fertiliser trials in Germany.

Soils at all fertiliser trials in Germany exhibited sufficient P-contents (LUFA C) and the intra-row fertilisation of RP (50 kg ha^{-1} P) did not have any effect on faba bean growth and P-uptake, neither alone nor in combination with elemental sulphur, which by way of *in situ* acid formation can enhance RP solubility. On the contrary, the sulphur fertilisers potassium sulphate, gypsum and elemental sulphur did result in a markedly increased S-uptake in both trial years, while faba bean growth and yield were only affected positively and in part significantly in the second trial year. The clear effect was assumed to be due to low soil sulphate contents during initial crop development, caused by low sulphate adsorption and hence high leaching rates during winter and also due to low soil organic matter (SOM) mineralisation rates under DS management. However in Brazil, soybean did not react to S-fertilisation with increased crop growth, yield or S-uptake in any trial. The sufficient S-supply was explained with the high contents of adsorbed sulphate in soil and high SOM mineralisation rates under tropical climate. P-fertiliser application was also ineffective at most sites due to sufficient to high soil P-contents. Only on a field with low P-contents at site *Ponta Grossa* P-fertilisation had a positive effect on P-uptake, crop growth and yield. In both years this positive effect was increased by simultaneous application of elemental sulphur, which presumably increased solubility of RP.

In screening trials with natural herbicides pine oil and acidic acid were found to be the most potent active ingredients with a cell membrane disrupting (CMD) effect. While the sole spray application of high amounts of NaCl resulted in relatively weak plant damage, it was found that the combination of CMD and NaCl resulted in a strongly enhanced weed control. Additionally, a meristem damaging systemic effect of NaCl was identified, which was particularly strong for dicot weed species. In consequence, CMD amounts and hence application costs could be reduced drastically. Furthermore, it was determined repeatedly that emulsifiers influence formulation efficacy strongly at the rates commonly applied in NH and therefore these *inert* ingredients have to be considered as active ones in NH formulations. In the DS soybean field trial in Londrina it was determined that at a fixed total amount of AI (90 L ha^{-1} pine oil or limonene and 90 kg ha^{-1} NaCl) two concentrated applications resulted in a higher weed control than the three diluted ones. Weed control in this trial was not satisfactory, though, and crop growth as well as yield were markedly reduced compared to the clean control treatment. In the DS soybean trial in Ponta Grossa 50 L ha^{-1} pine oil or limonene and 50 kg ha^{-1} NaCl were applied once, twice and three times. Even after three applications weed drymass remained relatively high compared to the weedy control. Nevertheless, soybean shoot drymass was not affected strongly by weed infestation and with three applications grain yield was close to the clean control. Nevertheless, results in this trial were influenced by crop damage due to contact with NH spray, because no working protective screen was available during application. In two field trials with conventionally tilled common bean, weed control was satisfactory in all spray treatments. Crop growth and yield were comparable to those of the weed free control.

Kurzzusammenfassung

Die vorliegende Arbeit erforschte Strategien zur Verbesserung der Nährstoffversorgung und der Unkrautregulierung bei temporärer Direktsaat (DS) von Körnerleguminosen im Ökologischen Landbau. In Feldversuchen wurde die Wirkung der Unterfußdüngung von Rohphosphat und verschiedenen Schwefeldüngern auf Pflanzenwachstum, Ertrag und die Nährstoffaufnahme von Ackerbohne (*Vicia faba*) in Deutschland (zwei Standorte, 2011 und 2012) und von Sojabohne (*Glycine max*) in Brasilien (drei Standorte in Paraná, 2012 und 2013) untersucht. Der zweite Teil der Arbeit behandelte die Unkrautregulierung mit natürlichen Herbiziden (NH). Hierzu wurden achtzehn Screeningversuche im Feld und fünf Feldversuchen mit Soja- und Gartenbohne (*Phaseolus vulgaris*) in Brasilien realisiert. Weiterhin wurde der Einfluss verschiedener Mulchmengen von Haferstroh auf das Ackerbohnen- und das Unkrautwachstum untersucht.

In den Düngungsversuchen in Deutschland waren die P-Bodengehalte ausreichend (LUFA C), und es wurde kein Einfluss der Rohphosphat Unterfußdüngung (50 kg ha^{-1} P) auf das Wachstum und die P-Aufnahme der Ackerbohne gefunden, auch nicht bei gemeinsamer Gabe mit elementarem Schwefel (40 kg ha^{-1} S), der die Löslichkeit des Rohphoshates durch *in situ* Schwefelsäurebildung im Boden erhöhen kann. Die Schwefeldünger Kaliumsulfat, Gips, und elementarer Schwefel (alle 40 kg ha^{-1} S) führten in beiden Versuchsjahren zu signifikant erhöhter S-Aufnahme in Sproß und Korn. Im zweiten Versuchsjahr wurde auch das Ackerbohnenwachstum und die Kornernte positiv und teilweise signifikant beeinflusst. Die deutliche Düngerwirkung wurde darauf zurückgeführt, dass an beiden Versuchsstandorten die Sulfatgehalte zu Vegetationsbeginn aufgrund starker Auswaschung im Winter gering waren, und die Nachlieferung von Sulfat durch Mineralisierung der organischen Bodensubstanz unter Direktsaat nicht ausreichten. Die Sojabohne reagierte hingegen in keinem der Versuche mit erhöhter S-Aufnahme oder gesteigertem Pflanzenwachstum. Die ausreichende S-Versorgung wurde mit den hohen Mengen sorbierten Sulfats im Boden und der schnellen S-Nachlieferung aus der organischen Bodensubstanz erklärt. Die P-Düngung war aufgrund ausreichender bis hoher Bodengehalte von P ebenfalls an fast allen Standorten wirkungslos. Nur auf einem Feld mit niedrigen P-Gehalten (*Ponta Grossa*) wirkte sich die P-Düngung positiv auf P-Gehalte, Pflanzenwachstum und Ertrag aus. Die Wirkung der P-Düngung auf die gleichen Pflanzenparameter wurde durch Mischung mit elementarem Schwefel in beiden Versuchsjahren aufgrund dessen positiven Einflusses auf die Löslichkeit von Rohphosphat verstärkt.

In Versuchen mit natürlichen Herbiziden (NH) wurden in Screeningversuchen Pinienöl und Essigsäure als effektivste Wirkstoffe mit Cuticula und Zellmembran schädigender Wirkung (cell membrane disruptors, CMD) identifiziert. Während die alleinige Applikation hoher Mengen von NaCl nur eine relativ geringe Wirkung zeigte, wurde festgestellt, dass dessen Kombination mit CMD die Wirksamkeit potenzierte und zusätzlich Meristeme durch eine systemische Wirkung stark geschädigt wurden, insbesondere bei dikotylen Unkräutern. Die Menge von CMD und somit die Applikationskosten konnten in der Folge durch NaCl Beimischung deutlich reduziert werden. Es konnte wiederholt nachgewiesen werden, dass die häufig hohen Emulgatormengen in NH eine eigene herbizide Wirkung aufweisen und folglich Emulgatoren nicht als *inerte* Inhaltsstoffe von NH Formulierungen gelten können. Im Feldversuch mit DS Sojabohne in Londrina ergab bei gleicher Gesamtmenge Wirkstoff (90 L ha^{-1} Limonen oder Pinienöl und 90 kg ha^{-1} NaCl) die konzentrierte zweimalige Anwendung eine bessere Unkrautregulierung als drei verdünnte Anwendungen. Die Unkrautregulierung war jedoch nicht ausreichend und Pflanzenwachstum und Ertrag waren deutlich geringer als in der unkrautfreihen Kontrolle. Im DS Sojabohnenversuch in Ponta Grossa wurden 50 L ha^{-1} Limonen oder Pinienöl sowie 50 kg ha^{-1} NaCl ein, zwei und drei Mal appliziert. Obwohl nach drei Applikation die Unkrauttrockenmasse noch relativ hoch im Vergleich zur verunkrauteten Kontrolle war, war die Sproßtrockenmasse der Sojabohne nicht stark negativ beeinflusst, und die Kornernte war mit drei Applikationen sogar vergleichbar mit der unkrautfreien Kontrolle. Die Versuchsergebnisse waren aber insbesondere bei wiederholter Applikation durch Kulturschäden beeinflusst, da bei der Applikation der NH kein zuverlässiger Sprühschutz vorhanden war. In Feldversuchen mit Gartenbohne (konventionelle Bodenbearbeitung) war die Unkrautregulierung in allen Behandlungen ausreichend, und sowohl Pflanzenwachstum als auch Erträge waren vergleichbar mit der unkrautfreien Kontrolle.

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List of abbreviations

AA	acetic acid
AAS	atomic absorption spectroscopy
ADP	adenosine diphosphate
AEC	anion exchange capacity
AI	active ingredient
AMP	adenosine monophosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BBCH	Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie
CA	conservation agriculture
CEC	cation exchange capacity
CMD	cell membrane disruptors
CT	conventional tillage
DAE	days after emergence
DBG	Deutsche Bodenkundliche Gesellschaft
DL	double lactate
DNA	desoxyribonucleic acid
DS	direct seeding
EPA	US Environmental Protection Agency
EU	European Union
FFA	free fatty acids
IAPAR	Instituto Agronômico do Paraná
ICP	Inductively Coupled Plasma
IOL	Institute of Organic Agriculture, Bonn University
LAI	leaf area index
MSO	methylated soybean seed oil
NH	natural herbicide
NPE	nonylphenol ethoxylate
NT	no-tillage
OA	Organic Agriculture
OADS	Organic Agriculture Direct Seeding
oDS	occasional direct seeding
OMRI	Organic Materials Review Institute
P	phosphorus
PA	pelargonic acid
PEG	polyethylene glycol
PET	polyethylene terephthalate
PPG	polypropylene glycol
PRE	pre-emergence
PVC	polyvinylchloride
RBD	randomised block design
RNA	ribonucleic acid
ROS	reactive oxygen species
RP	rock phosphate
S	sulphur

SA	sulphonic acid
SE	standard error of the mean
SiBCS	Sistema Brasileiro de Classificação de Solos
SOM	soil organic matter
SSP	super single phosphate
T80	Tween 80®
TKW	thousand kernel weight
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten
WRB	FAO World Reference Base for soils

1 Introduction

Organic Agriculture (OA) aims to improve soil conditions and to create sustainable production systems. Nevertheless, with the primary aim to combat weeds effectively, the plough is still commonly used as part of seedbed preparation, which can have a negative impact on soil fauna and potentially decrease soil fertility by causing devastating soil erosion, especially on sloping terrain (Carr et al., 2013). Therefore, mechanical soil disturbance should be minimised in order to increase the sustainability of OA production systems. The most extreme form of tillage reduction is direct seeding (DS), a husbandry system in which crops are sown directly into the pre-crop mulch without prior soil tillage. It can be practised occasionally in a crop rotation including tillage, or can be used permanently with zero soil disturbance, then usually referred to as no-tillage (NT) (Derpsch et al., 1988) or conservation agriculture (CA) (FAO, 2007, 2011). In comparison with conventional tillage (CT), DS offers a wide range of economic and ecological advantages, because it reduces labour, labour costs, diesel consumption and CO_2-emissions drastically. One of the most important advantages of DS is the highly effective protection against wind and water erosion. The mulch cover protects the soil physically from the direct impact of raindrops (splash-effect), and thereby prevents superficial soil pore sealing. Moreover, the resulting increase in permeability of the soil (Madari et al., 2005) reduces surface run-off, which otherwise causes the most extreme forms of erosion. Due to frequent torrential rains that severely erode uncovered soils under tillage the use of DS can be considered mandatory in the humid tropics (Derpsch et al., 1988). In the long-term, another advantage of DS is that soil organic matter (SOM) contents and with it soil fertility is increased. Moreover, the longer soil rest in crop rotations with DS benefits soil fauna, for example, the populations of anecic earthworms (Hendrix et al., 1986; Pelosi et al., 2009), which are important for soil structure and nutrient cycling. In conclusion, DS can be regarded as a husbandry system in which soil conditions are closest to a natural state (Blumberg and Crossley, 1983).

DS is widely adopted in large-scale mainstream agriculture in the USA, (sub-) tropical South-America and Australia (Scopel et al., 2013). In Brazil, Argentina and Paraguay DS is even by far the most dominant production system for annual crops, while in temperate climate and small-scale farming the system is far less common (Friedrich et al., 2012). Organic Agriculture Direct Seeding (OADS) means the occasional adoption of the DS system, whenever possible, for suitable crops into OA crop rotations (Köpke and Schulte, 2008). Its implementation is of great interest to OA, especially at erosion prone sites. Brazil, the largest soybean producer in the world (FAOstat, 2013), in theory possesses a great potential for organic soybean production with a growing internal market for organic produce as well as export markets in Europe and Japan which increasingly demand non-GMO (genetically modified organisms) and organic soybean. Equally, the organic production of staple food common bean (*Phaseolus vulgaris*) for the Brazilian domestic market shows great potential. Nevertheless, at present DS is not used in OA on a noteworthy scale worldwide, because it faces severe restrictions. One problem generally associated with DS are soil-borne diseases. Without incorporation of crop residues their decomposition is delayed and pathogens such as *Fusarium sp.* frequently pose problems to subsequent crops (Sturz and Carter, 1995; Bailey, 1996; Sturz et al., 1997; Miller et al., 1998; Dill-Macky and Jones, 2000). However, the current work does not address this issue. A major problem for the realisation of OADS is limited nutrient acquisition by crops. First of all, the N-supply is affected due to lower SOM mineralisation rates in DS, especially in temperate climate (Peigné et al., 2007). Therefore not all crops are suitable for OADS without large additions of organic fertilisers. However, legumes are in general not affected by these N-limitations due to symbiotic nitrogen fixation, and the permanent NT was found to achieve similar N_2-fixation as conventionally tilled faba bean (*Vicia faba*) in Mediterranean conditions (López-Bellido et al., 2006). Faba bean is also well adapted to the wetter and cooler soil conditions of DS in temperate climate (Köpke and Nemecek, 2010) and in Germany similar grain yields between direct-seeded and conventionally tilled soybean were found (Köpke and Schulte, 2008; Massucati et al., 2010). In tropical Brazil, soybean (*Glycine max*) has been planted in the DS mainstream system for nearly four decades, but the system is has not yet been adapted for the use in OA.

In DS of grain legumes, limitations of the nutrients phosphorus (P) and sulphur (S) are likely to occur in Germany and Brazil. On one hand, independently of the tillage system, S-inputs by fertiliser and atmospheric inputs are generally low in both climates. Furthermore, in tropical soils P-contents are frequently low and P-fixation at low pH values can be an issue limiting availability of P to the crop. On the other hand, markedly different soil conditions DS in comparison with tillage systems can aggravate deficiencies of both nutrients. For example, under DS management the soil temperature is lower, soil density higher, aeration decreased and water content increased. All of these factors can lead to more limited root development and growth rates as well as to reduced mineralisation rates under DS management. In order to improve P and S nutrition of DS grain legumes in both tropical and temperate climate, fertiliser field trials with rock phosphate and different S fertiliser were conducted with faba bean in Germany, and soybean in Brazil.

Besides limited nutrient availability, the biggest problem to realise OADS are aggressive weed infestations that can neither be controlled mechanically as in OA conventional tillage systems (CT) nor by synthetic herbicides as in mainstream agriculture. Even though large amounts of mulch residues were found to be able to suppress growth of annual weeds sufficiently in temperate climate, these cannot control perennial weeds (Köpke and Schulte, 2008; Massucati et al., 2010). Also in the humid subtropical climate of Alabama, USA, weed control of mulch of several cover crops was found to be insufficient, making the additional use of conventional herbicides necessary (Price et al., 2006). In order to reconfirm findings of Massucati et al. (2010), trials with different amounts oats straw residue were conducted in direct-seeded faba bean in Germany. As weed control of mulch alone is usually not satisfactory, the second part of this thesis examines non-selective natural total herbicides (NH) as a possibility to control weeds effectively in OADS. Therefore, screenings and field trials with soybean and common bean (*Phaseolus vulgaris*) were conducted, though only in Brazil.

1.1 Nutrient supply in direct-seeding

Soil conditions in DS are distinct from conditions in conventionally tilled soils, which has far reaching consequences for soil temperature, water content, nutrient dynamics and ultimately root development and crop growth (Six et al., 2002). Without loosening, soil bulk density in DS is higher and less air-filled, thus more water-holding pores are present (Fabrizzi et al., 2005). Furthermore, the pre-crop mulch cover physically obstructs moisture exchange with the atmosphere and decreases the heat transfer to soil from solar radiation (Azooz et al., 1997). All of these aspects lead to higher water contents and markedly decreased evaporation rates. Therefore, the soil temperatures in DS are generally lower compared with CT. Another possible alteration of soil conditions in occasional DS is top soil compaction caused by farm machine traffic, which can obstruct crop root development due to higher impedance (Munkholm et al., 2003).

The differences in soil properties influence both soil chemical processes and crop growth. The SOM mineralisation rate decreases with reduced soil temperature and aeration (Six et al., 1999). In tropical or arid environments this and the higher water retention are beneficial for crops, because in tilled soils it is difficult to maintain sufficient levels of SOM and plants are more prone to suffer drought and roots heat stress. Despite lower rates than in tilled soils, SOM mineralisation in the tropics usually does not limit crop N-supply and most field crops could be direct seeded in OA. Also, soil compaction is generally not a problem in Brazilian ferralsol soils, as bulk density can be as low as $1.1\,\mathrm{kg\,cm^{-3}}$. Nevertheless, strong root development is often hindered by low pH values in subsoil and associated Al-toxicity.

In temperate environments elevated soil temperatures are hardly ever a cause of stress to crops, and rather the lower soil temperatures under DS are problematic. The slower soil heating in spring leads to later sowing and crop emergence, slower root growth and therefore delayed crop development in general. The slower mineralisation rates can especially limit the supply of N during early crop development and elevated quantities of N-fertilisers have to be applied for compensation. Legume crops are not affected by N-limitations, because they are self-sufficient for N through symbiotic N_2-fixation and are therefore predestined crops for the use in OADS. But in both tropical and temperate climates a major concern for grain legumes is the nutrient supply with P and S. Deficiencies of both nutrients can limit efficacy of N_2-fixation, overall crop performance as well as nutritive quality of grain.

1.1.1 Phosphorus deficiency

The macro nutrient P is present in plants in the form of phospholipids (e.g. cell membranes), ribonucleic acids (DNA, RNA) and adenosinphosphates (ATP, ADP, AMP), which are involved in energy metabolism (Marschner and Marschner, 2012). Legumes in general are highly demanding for P and a deficiency can have a strong negative impact on N_2-fixation (Leidi and Rodríguez-Navarro, 2000; Vance, 2001; Marschner and Marschner, 2012), which is mainly due to the high ATP requirements for nodule formation and function (Ribet and Drevon, 1996). For *Phaseolus vulgaris* and soybean the shoot and root P-concentrations are decreased with P-deficiency (Olivera et al., 2004) and due to reduced N_2-fixation, also the total N-uptake into shoot is decreased. P-deficiency results in decreased shoot growth, leaf number, leaf area index (LAI) and yield, however, root growth is less affected. Another symptom commonly found is the reduction of the number of flowers and increased pod abortions (Marschner and Marschner, 2012).

Apart from weathering of primary minerals and deposition of dust there are no natural inputs of phosphorus to agricultural fields. As large parts of phosphorus are not cycled back to fields but rather lost to surface waters and ultimately to the oceans, the input of mineral P-fertilisers is mandatory (Paulsen et al., 2016). However, mineral P is a non-renewable resource, and mineable deposits are expected to be depleted in 50–150 years (Cordell et al., 2009). As DS decreases erosion and with it P-losses, this system is an efficient indirect measure to safe on P-inputs. But on the other hand, P-deficiency is a general problem in tropical soils and also more likely to occur in temperate climate under DS management, hence likely to occur in OADS in both Germany and Southern Brazil. The concentration of PO_4^{3-} in soil solution is low over the whole pH range, because di- and trivalent ions in the soil cause the sorption or precipitation of PO_4^{3-}. At low pH values these are different iron oxides and free Al^{3+} ions and at neutral or alcaline pH Ca^{2+} ions (Horn et al., 2010). Thus, P can hardly be transported by mass flow and is therefore relatively immobile in soil. In consequence, P reaches roots mostly by diffusion, which is effectively limited to the direct root proximity. Hence an extensive root and fine root development of crops is necessary to absorb sufficient P for crop development.

Soil aggregation only changes under long-term or permanent no-tillage and more water-stable aggregates as well as more macro-aggregates are formed with increasing duration of soil rest (Beare et al., 1994), which favours rooting and improves crop nutrient supply. However, due to soil disturbance, this does not apply for occasional direct seeding (oDS). In temperate climate, the higher bulk density or even compaction of soils in oDS lead to higher impedance for root penetration and fine root formation. Furthermore, root growth rates decrease with the lower soil temperatures in DS compared with conventional tillage. One limitation for P-nutrition in tropical ferralsols, which predominate the study region in Paraná state, is the usually low total P-content of soil. Furthermore soil acidity, and the ample presence of Fe- and Al-oxides contribute greatly to P-fixation, which further decreases plant availability of P (Horn et al., 2010). On one hand the physical structure of ferralsols favours rooting strongly, also under non permanent no-till (oDS), as they possess a low bulk-density of about $1.1\,\mathrm{g\,cm^{-3}}$ and a high porosity, commonly referred to as 'coffee powder structure'. But on the other hand, Al-toxicity may limit rooting and hence nutrient acquisition of phosphorus.

For both climate regions in study, a limitation of P-supply is therefore likely to occur in the DS system and P has to be supplemented for by fertilisers. OA guidelines prohibit the use of easily soluble super-phosphate or triple-phosphate fertilisers and permit only mechanically treated rock phosphate (RP), which due its low solubility contains only a relatively low proportion of directly plant available phosphorus (Paulsen et al., 2016). Fertilisers in DS can be applied intra-row and the deposition of RP in direct crop root proximity may benefit grain legumes, because the low pH in the rhizosphere which has a positive effect on its availability. Nevertheless, intra-row application of RP in DS faba bean in a previous work at *Wiesengut* (trial site also used in the current thesis) resulted in a deficient P-status of the crop (Seehuber, 2014), which indicates that RP alone is an unreliable source to meet P requirements of faba bean in DS. Several publications of research in temperate and tropical climate showed that mixtures of RP with elemental sulphur are more effective P-fertilisers than RP alone (Rajan, 1982a; Stamford et al., 2005, 2007; Aria et al., 2010). In soil, elemental sulphur becomes oxidised relatively fast to sulphuric acid by soil bacteria (*Thiobazillus species de fact*), which does not only make sulphur available to the crop

(SO_4^{2-}), but also leads to a sharp decline of pH in proximity to elemental sulphur. The acid formed can help to solubilise simultaneously applied RP *in situ* and may thereby increase P availability to crops.

1.1.2 Sulphur deficiency

Sulphur is an essential macro nutrient, which is mostly taken up in the form of sulphate (SO_4^{2-}), yet uptake is also possible as SO_2 gas through leaf stomata. In plants sulphur can be found in the proteinogenic amino-acids cysthein, cysthin and methionin and is therefore part of proteins in general. It is a key element in functional groups of co-enzymes and vitamins and forms part of iron-sulphur proteins involved in oxidation-reduction reactions (ferredoxin), energy metabolism and nitrogen fixation (nitrogenase). Furthermore, sulphate esters are components of sulpholipids and as such structural components of biological membranes. Another large fraction of S-containing substances are formed in plant secondary metabolism, e.g. glycosinolates in *Brassicaceae* or alliins in *Alliaeae* (Scherer, 2001; Marschner and Marschner, 2012).

When S-deficiency occurs the S-concentrations in shoots, roots and nodules can be drastically decreased (Pacyna et al., 2006). The shortage of S-containing amino acids cysteine and methionine inhibits protein synthesis and decreases chlorophyll and protein contents in leafs and reduces shoot growth and leaf area (Marschner and Marschner, 2012). Also, the lower contents of the these amino-acids, which are essential in the nutrition of non-ruminants, influence the protein quality and hence the nutritional value of grain negatively (Kim et al., 1999; Gayler and Sykes, 1985). This change in nutritive quality may occur without necessarily decreasing grain yield or protein content: when S is deficient, the grain content of S-rich storage protein glycinin decreases, and is compensated for by β-conglycinins, which are practically void of S-containing amino-acids (Gayler and Sykes, 1985; Sexton et al., 1998). Furthermore, under conditions of S-deficiency levels of ferredoxin, ATP and leghemoglobin decrease (Scherer et al., 2008), which causes an obstruction of energy metabolism. With sulphur deficiency nodule weight is usually lower (Scherer and Lange, 1996) and even moderate S-deficiency leads to a sharp decline in nitrogenase-activity (Scherer and Lange, 1996; Scherer et al., 2008), which can induce N-deficiency in legume crops (Pacyna et al., 2006).

To be sustainable, OA strongly depends on efficient symbiotic N_2-fixation by legume crops, which in comparison with other crop families have a relatively high demand for sulphur (*Brassicaceae* > *Leguminosae* > *Gramineae*). For both German and Brazilian sites S-deficiency is likely to occur direct-seeded grain legumes. In highly industrialised European countries like Germany, atmospheric sulphur depositions were as high as $33\,\mathrm{kg\,ha^{-1}\,a^{-1}}$ S in the 1980's (Prechtel et al., 2001), which apart from causing negative effects such as acid rains, delivered sulphur to crops in sufficient quantities. However, sulphur gas emission to the atmosphere could be decreased strongly by obligatory flue-gas desulfurisation (Srivastava and Jozewicz, 2001) and the use of low S-containing fuels (Scherer, 2009). Hence, current atmospheric inputs only amount to about $10\,\mathrm{kg\,ha^{-1}\,a^{-1}}$ S (Horn et al., 2010; Umweltbundesamt, 2014). For this reason, the problem of sulphur deficiency in agriculture has gained great importance over the last 20 years. Additionally to low inputs, the leaching potential for sulphate is usually high in Germany, because most soils have a neutral pH, which limits SO_4^{2-} sorption to exchange sites and hence favours leaching strongly (Bolan et al., 1988). Another factor favouring S-deficiency specifically in DS in temperate climate is the limitation of S-supply from SOM mineralisation due to lower soil temperatures and aeration. Also in Brazil sulphur deficiency is suspected to occur. Fertiliser inputs of sulphur are generally low, for OA and mainstream agriculture alike. Widely used in Brazilian mainstream agriculture are high grade P- and N-fertilisers lacking sulphur, for example triple phosphate. Application of gypsum ($CaSO_4 \cdot 2\,H_2O$) is not a wide-spread practice or only realised sporadically. Also, mixed farming is rather the exception in Paraná state and generally no sulphur is applied in the form of farm manure or slurry. As a consequence atmospheric immissions are presumably the only relevant input of sulphur to soils. However, the soybean cultivation regions in Paraná state are generally in rural areas with low industrialisation, and so are the experimental sites. In rural sites in Brazil sulphur wet-deposition was measured ranging from $0.2\,\mathrm{kg\,ha^{-1}\,a^{-1}}$ S (Dias et al., 2012) to $2.1\,\mathrm{kg\,ha^{-1}\,a^{-1}}$ S (Pelicho et al., 2006). Even considering that dry deposition rates are of the same magnitude as wet-deposition (Garland, 1978), the total sulphur immission rate expected for experimental sites in Paraná state is unlikely to exceed

5 kg ha^{-1} a^{-1} S, which cannot balance the harvest exports of most crops, e.g. 10–15 kg ha^{-1} for soybean. Losses of sulphate by leaching may also be considerable. On one hand permeable clayey and sandy ferralsols dominate in Paraná state. The high rainfall incidence and intensity might lead to an elevated leaching potential of SO_4^{2-} ions in these soils. On the other hand, the low pH of soils brings about a high anion exchange capacity (AEC) and SO_4^{2-} can be adsorbed to soil particles (e.g. Fe-, Al-oxides), thereby restricting leaching losses (Horn et al., 2010). Another argument against a high leaching potential is that bare fallows hardly exist in Paraná state and cereals or green manure crops are grown in winter. Also in contrast to temperate climate, SOM mineralisation kinetics are not considered to be a limiting factor for plant availability of S. In the tropics rather low SOM contents caused by rapid mineralisation may be problematic, especially in soils with a sandy texture. Overall, there is evidence for S-deficiency in agricultural soils in Paraná state, and if not acute, deficiency can be expected to occur in the long-term due to a negative nutrient balance. This problem is not assumed to be limited to OADS, but relevant for Brazilian mainstream soybean cultivation as well.

Prior works in Germany found that fertilisation with K_2SO_4 (35 kg ha^{-1} S) increased S-content from 0.25 % to 0.36 % in shoot dry mass compared with control treatment and slightly increase in N-content, indicating a positive effect on symbiotic N_2-fixation (Seehuber, 2014). Also in several regions of Brazil (including Paraná state), sulphur fertilisation has proven efficient to increase soybean grain yields (Sfredo and Lantmann, 2007).

1.1.3 Research approach and hypotheses

From the current state of the art it is assumed that at German and Brazilian trial sites nutrient deficiencies of phosphorus and sulphur are likely to occur in direct-seeded grain legumes in OA. Therefore, the aim of the thesis at hand is to improve the P and S nutrition of grain legumes in DS in both tropical and temperate climate, with intra-row applied, OA permitted rock phosphate and sulphur fertilisers. In field trials, the effects on crop growth and yield as well as nutrient concentrations in shoot and grain are examined. Aiming to improve P-supply of grain legumes, rock-phosphate is applied intra-row in field trials, alone and in combination with elemental sulphur. When elemental sulphur is oxidised by soil bacteria, sulphuric acid is formed and is assumed to solubilise RP *in situ* and increase the crop availability of RP. The mixture, also known as ’biosuper’, has knowingly not been studied extensively as intra-row application in DS. In order to determine whether S-deficiencies are in fact present and if fertiliser application can improve sulphur supply of DS grain legumes, the S-fertilisers gypsum, K_2SO_4, and elemental sulphur, which all possess a differing solubility, were applied intra-row. In North-Rhine-Westfalia (NRW) Germany, a total of six trials at two experimental sites were conducted with faba bean, *Vicia faba* (L.), in *Hennef* (2011) and *Rheinbach* (2012). In Paraná state, southern Brazil, a total of eight fertiliser trials were realised with DS soybean, *Glycine max* (L.), at the three experimental sites *Londrina*, *Ponta Grossa* and *Umuarama*. For fertiliser trials in Germany and Brazil the following hypothesis are postulated:

Box 1.1 Hypotheses: P and S fertiliser trials

1. Rock phosphate (RP) application increases shoot P-uptake and -contents.
2. RP increases crop growth and yield.
3. Joint application of RP and elemental S further increases uptake and contents of P and S.
4. Joint application of RP and elemental S further enhances crop growth and yield.
5. S fertiliser increases S-uptake and contents.
6. S fertiliser increases crop growth and yield.
7. Soluble sulphur forms K_2SO_4 and gypsum are most effective.

1.2 Weed control with mulch and natural herbicides in direct-seeding

1.2.1 Mulch from harvest residues

Compared to conventional tillage the mulch layer in DS can influence weed populations physically, chemically and biologically. Important physical factors of mulch are that less direct sunlight reaches the soil surface and that diurnal soil temperature variation is reduced, which both inhibits weed seed germination. Furthermore, weed emergence is obstructed physically (Christoffoleti et al., 2007). Chemical changes occur in the C:N ratio of topsoil which has an influence on nitrate contents. Depending on residue type, either higher nitrate contents may occur at the soil surface, which enhance weed seed germination and growth (Christoffoleti et al., 2007), or the reverse effect of N-immobilisation (Rice and Smith, 1984), which does not favour weed germination. Nevertheless, the most important chemical weed suppression effect of mulch residue is the release of allelopathic substances, which obstruct weed seed germination and weed growth (Bhowmik et al., 2003; Singh et al., 2003; Weir et al., 2004; Belz et al., 2007). Biological effects of mulch are enhanced parasitism and predation of weed seeds by microorganisms, insects and small mammals (Christoffoleti et al., 2007). Overall, weed control may be improved in DS: life-time of weed seeds, weed number and occurrence of grassy weeds is reduced. In order to take advantage of weed control effect of mulch for the realisation of occasional OADS a large mulch biomass is considered essential as a base to implement the system. In tropical Brazil, Vidal et al. (1998) and Theisen et al. (2000) found that increasing amounts of black oats or wheat decreased weed growth, especially that of *Brachiaria* grasses, and increased soybean yields. Works of Massucati et al. (2010) and Massucati and Köepke (2011) in Germany showed that with an oats straw biomass of at least $4\,\mathrm{t\,ha^{-1}}$ acceptable faba bean grain yields can be accomplished, but with the restriction that weed pressure of perennials such as *Cirsium spp.* and *Rumex spp.* has to be low. In these experiments, perennial weeds were removed manually. In general mulches do not control perennials sufficiently and even organic mulches in combination with a polypropylene cover did not yield satisfactory results (Skroch et al., 1992). But also independent of perennials, mulch cover alone is apparently not always able to grant acceptable weed control. A work by Price et al. (2006) in Alabama state, USA, found that mulch of rye and oats granted efficient weed control, but only in combination with a soybean pre-emergence (PRE) herbicide application. Thus, the authors concluded that mulches allow for herbicide reductions, but that none of the cover crops granted effective weed control without the use of any herbicide.

1.2.2 Natural herbicides

As pre-crop mulch is not able to control weeds effectively, and due to the prohibition of synthetic herbicides in OA by self-imposed guidelines, manual weeding is currently the only applicable weed control measure in DS after crop emergence. However, costs for this measure are prohibitive. Due to the lack of effective weed control strategies OADS is currently not practised in Germany and Brazil. While faba bean is commonly planted using CT in temperate climate OA, organic soybean is rarely cultivated in OA in Brazil due to the low efficiency of erosion control in the CT system. In fact, the low proportion of OA in Brazilian husbandry systems in general may largely be attributed to the fact that DS cannot be realised. For both regions in study the key to enable grain legume OADS is the development of an effective weed control strategy involving the use of natural herbicide (NH). Their temporary use could enable more organic farmers to perform judicious tillage coupled with soil structure improvement of occasional DS at least for grain legumes (Köpke and Nemecek, 2010), as well as offering the broad-scale beneficial environmental effects of OA that have been documented by Hole et al. (2005).

Active ingredients: cell membrane disruptors (CMD) Commercial NH formulations, which are certified for use in OA in certain countries (USA, Oceania), contain as active ingredient (AI) the essential plant oils citronella oil (e.g. Barrier H® [1] or GreenMatchEX®), pine oil (Organic Interceptor®), clove oil (e.g. BurnOut II®, EcoExempt HC®, Matran 2®) or δ-limonene (NaturesAvenger®), as well as on organic acids, such as pelargonic (Finalsan®) or acetic acid (AllDown®). These AI, on which most NH research has been focused on, posses a similar mode of action: they penetrate the leaf cuticle and change

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[1] Barrier H® contains 22.9 % wt/wt citronella oil java type, extracted from *Cymbopogon winterianus.*

its permeability. Within the leafs they damage cell and organelle membranes, which is why they can be referred to as cell membrane disruptors (CMD). Ultimately cell contents leak out and cells die of uncontrolled water loss, resulting in non-selective damage to plants (Tworkoski, 2002; Bakkali et al., 2008). The effect of CMD is not systemic. Thus, non-contacted meristems can resprout and plant recovery may happen quickly. This also means that application volumes need to be large to assure good coverage. As NH are generally not selective, their application can only occur broadcast before crop emergence or after crop emergence as a banded application with the use of a protective screen. In agricultural practice the use of this AI class is therefore limited to row crops such as faba bean, soybean, common bean or maize.

Most essential oils, including citronella oil, clove oil, eucalypt oil and natural pine oil, are obtained by way of steam distillation. This process is energy-intensive, costly and oil yields are generally low, resulting in elevated prices of these AI. For example, the annual oil yields of *Cymbopogon winterianus* in India are in the range of 200–250 $\mathrm{kg\,ha^{-1}}$ are under favourable conditions [2]. For *Cymbopogon nardus* (L.) Rendle, reported annual oil yields are as low as 76 $\mathrm{kg\,ha^{-1}}$ (Mahalwal and Ali, 2003). The situation for clove oil, the most potent CMD of a wide range of essential oils tested by Tworkoski (2002) is similar. Not only is the production and harvest process laborious (cloves are handpicked), but the annual production of buds is only about 250 $\mathrm{kg\,ha^{-1}}$ on average, and up to 850 $\mathrm{kg\,ha^{-1}}$ under good management [2]. With a clove oil content of 14–21 % [3] annual oil yields are in the range of 35–170 $\mathrm{kg\,ha^{-1}}$. Besides low yield, alternative uses for both clove and citronella oil are numerous, for example in cosmetics, food, and pharmaceutical industry. Natural pine oil is used in fragrances, inhalants or disinfectants [4] and the price is also highly elevated: for example, oil from *Pinus ponderosa* costs approximately 250 € per litre (Kelkar et al., 2006). Nevertheless, most pine oil available commercially is a semi-synthetic product based on the oxidation of pine resin distillate containing α-pinene, which is oxidised in presence of an acetic acid catalyst (Budiman et al., 2015). This reaction yields a mixture consisting of 65 % α-terpineol and original α-pinene, which is a pine oil far lower in price (about 4 € per litre) and of a different chemical composition than the steam distilled product. δ-limonene is most abundant monoterpene in nature and is obtained as a byproduct of orange juice production as it is present in juice and citrus peel. Currently, São Paulo state, Brazil is the worlds largest producer of orange juice and δ-limonene. With multiple uses, in cosmetics, food industry, solvents and cleaning products the demand for δ-limonene is high, yet it is still one of the cheapest essential oils, with a price in a range of 2–6 € per litre (oral communication Citrovita, 2013). Several middle chain fatty acids from C_9-C_{11}, especially pelargonic acid (PA) (C_9), cause rapid electrolyte leakage and non-selective damage to plants (Lederer et al., 2004). One AI of this class that is widely used in commercial NH formulations is PA, also called nonoanic acid (e.g. in Finalsan® or Scythe®). Its advantages are its ideal environmental and toxicological profile (Opdyke, 1978). Even though PA is a substance present in nature, for example in *Pelargonium* species, the extraction of sufficient quantities is not feasible and the commercialised products all use synthesised PA in its soluble ammonium salt form. Another common in AI is acetic acid (AA), which is contained in the OMRI [5] certified AllDown® or in WeedPharm®. AA is a naturally occurring chemical with a simple structure, and a very positive environmental profile (Webber et al., 2006), yet AA as a weed control agent possesses several disadvantages considering safety and transportation.

Emulsifiers Practically all pesticidal, including NH formulations contain emulsifiers, which are also commonly referred to surfactants (surface active ingredients). AI, which are not miscible with water require the use of emulsifiers, and spreaders are used in order to improve leaf coverage and thereby guarantee product efficacy. Emulsifiers do not belong to a defined chemical family and can be classified by their action as emulsifiers, penetrants, spreader, stickers and activators or grouped into ionic and non-ionic emulsifiers (Hazen, 2009). However, there is no strict separation between these actions: e.g. an emulsifier allows mixing of oils and water, but also influences the spreading of the mixture on the leaf

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[2] www.horticulture.kar.nic.in, accessed on 29.10.2016.

[3] www.essentialoil.com, accessed on 14.11.2016.

[4] www.fao.org, accessed on 09.10.2016.

[5] OMRI: Organic Materials Review Institute, USA

surface. The preference in mainstream herbicide formulation is mostly given to non-ionic emulsifiers (Young, 2008), because these do not interact with salts present in herbicides or water (e.g. Ca_2^+ in hard water). On product labels of conventional and natural herbicide formulations the identity of emulsifiers is not disclosed and are referred to as *inert ingredients*.

Application rates and costs Typically AI concentrations of commercial products are about 20 % for essential oils, 10–20 % for AA, and about 5 % for PA containing products. For effective weed control the leaf surface needs to be covered thoroughly. To achieve full coverage all commercial products recommend application rates of 1000 L ha^{-1}, which results in AI amounts of 50–200 L ha^{-1}. For the application of NH formulations containing citronella or clove oil this means that the agricultural area needed to produce the amount of AI for a single application would exceed the area that can be treated. Due to high AI rates, the application costs are extremely elevated for most products. For example, the cost of a single citronella oil application would be as high as 9000 € per hectare. Also the application cost of the currently cheapest formulation based on pelargonic acid (Finalsan®) is elevated with about 750 € per hectare and application. OA certified products are at present only available in the USA (e.g. AllDown®) and Oceania (e.g. Organic Weedfree Rapid® in New Zealand), but neither in Europe nor in Brazil. However, no known examples of actual use of the currently available commercial NH formulations in agricultural practice exist, at least not in annual field crops. Their application appears to be limited to urban gardening, which is reflected in the generally small container sizes for areas of only up to 100 m^2.

State of the art of natural herbicide research Most scientific literature on CMD is based on results from laboratory experiments such as germination tests or leaf disk essays (Tworkoski, 2002; Vaid et al., 2010; Bainard et al., 2006; Batish et al., 2004; Singh et al., 2009; Setia et al., 2007). However, these studies do not possess any explanatory power for potential uses in agricultural practice and can at most evaluate substances for their comparative cytotoxicity or ability to disrupt cell membranes. Some papers with more practical relevance deal with application of substances on weed plants in greenhouses, e.g. Abouziena et al. (2009), but scientific literature on efficacy and potentials of NH under field conditions is scarce. In an outdoor pot trial Boyd and Brennan (2006) showed that rates of clove oil for effective control of dicot weeds are about 12–60 L ha^{-1}, while control of the monocot rye was not possible. The costs for one application calculated in this study were prohibitive with about 750 € per hectare. Young (2004) examined the use of pine oil and AA for weed control along roadsides and found that even repeated applications of 50–70 kg ha^{-1} pine oil at an interval of two weeks yielded unsatisfying results. Further work by Barker and Prostak (2014) on weed control on roadsides found that various applications of formulations containing CMD were necessary to control weeds season-long, because resprouting of weeds was an issue. James et al. (2002) found applications of 50–100 kg ha^{-1} of pine oil on newly emerged weeds to be as effective as commercial glyphosate and glufosinate formulations, nevertheless, established weeds needed higher rates for good control. Evans et al. (2009) compared different concentrations of vinegar and clove oil for the control of redroot pigweed (*Amaranthus retroflexus*) and velvetleaf (*Abutilon theophrasti*). In this study it was found that plant morphology has an influence on weed susceptibility, and that good control is only possible at early growth stages. At present, only a limited number of peer-reviewed papers on NH use in annual crops exists, such as works of Evans and Bellinder (2009); Evans et al. (2011) with vinegar and clove oil application in the vegetable crops bell pepper and broccoli in CT. These works found that efficient weed control can be achieved at high application volumes (e.g. 15 % AA at a rate of 640 L ha^{-1}), and that application timing and growth stage of weeds influenced control strongly. Both works advert that crop injury may cause significant yield reductions if not shielded appropriately.

Due to limited weed control efficacy and extremely elevated application costs the use of any of NH formulations which only contain CMD as AI do not need further consideration for the use in OADS. Even though all commercial NH formulations and most recent research is based on CMD, other AI options do exist. Historically, NaCl has been used for weed control and was applied in quantities of several tons per hectare in winter cereals (Smith and Secoy, 1976; Timmons, 2005). Large amounts of NaCl in soils cause osmotic stress to roots, ion toxicity, and nutrient imbalances in plants (Marschner and Marschner, 2012). Similar effects can be assumed for NaCl spray application on aerial plant parts. Recent works on weed control with seawater in a seashore paspalum lawn found that effective broad-leaf control and little damage to lawn can be achieved (Zulkaliph et al., 2011; Uddin et al., 2013). In a work of Lukashyk et al. (2008) spray applications of 250 $\mathrm{kg\,ha^{-1}}$ kainit fertiliser [6] with an NaCl equivalent of 140 $\mathrm{kg\,ha^{-1}}$ were found to have potential for weed control of *Vicia hirsuta* in winter wheat and winter rye. NaCl is cheap, abundant in nature and possesses a predictable behaviour in the environment, and therefore its potential as an AI in NH formulations is examined in this work.

1.2.3 Research approach and hypotheses

To improve weed control in OADS (grain legume) row crops, an integrated weed control strategy is proposed as a solution: the first aspect of the strategy is the use of allelopathic pre-crop mulches such as oats straw or rye, which control weeds physically and through the release of allelopathic substances. The second aspect of the control strategy is the use of NH.

Oats straw residue Mulch is the base for OADS, but in the current work it is only a secondary research topic. One aim was to reconfirm findings of Schulte (2007), Massucati (2013) and Köpke and Schulte (2008) on the effect of different amounts of oats mulch residue on weed growth and faba bean development in Germany. For this purpose the straw mulch biomass treatments (0, 4 and 6 $\mathrm{t\,ha^{-1}}$) were included into the fertiliser trial design in the second trial year at organically managed experimental farm *Wiesengut*, Hennef (`WG`), with the following hypotheses:

Box 1.2 Hypotheses: oats straw residues

With increasing amount of mulch from oats straw residues

8. Weed biomass decreases.
9. Faba bean growth and yield increases.

Natural herbicides For NH to become a relevant weed control tool in OA the efficiency of formulations needs improvement and the costs have to be reduced. Importantly, formulations have to be studied at field level and in crop stands to investigate agronomic aspects under realistic conditions. With scarce scientific literature on trials in crop stands, the NH research project was started with limited experience on behaviour of AI and formulations under field conditions, which is why the character of this research part is exploratory to a large extent. NH research was only conducted in Brazil and originally, the aim was to evaluate efficiency of commercial NH in DS field trials. As these products did not have a registration in Brazil the import even for experimental purposes was not approved by the Brazilian ministry of agriculture. Consequently the research goal in 2011/12 was to identify effective AI and formulations and to study the susceptibility of a range of weed species in screening trials on previously tilled fields. To elaborate formulations, AI used in commercial formulations or described in literature were used in combination with a range of emulsifiers.

Screening trials focused on one hand on different CMD, including several essential oils, acidic acid (AA) and pelargonic acid (PA) and on the other hand on phytotoxic salts, such as NaCl. Mixtures of different AI seemed promising, and a special focus was given to combinations of CMD with NaCl. The research goal of the screenings was to develop efficient, yet - as far as possible - cost-effective NH formulations. In the course of screening trials the formulations containing the AI pine oil, δ-limonene, AA and NaCl proved to be most effective and other AI were discarted either due too inefficiency or high cost. In the second year five field trials were conducted at two Brazilian sites with the purpose to study

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[6] Kainit mineral is a hydrated potassium-magnesium sulphate-chloride ($\mathrm{KMg(SO_4)Cl{\cdot}3\,H_2O}$). However, mined kainit fertiliser (e.g. Magnesia-Kainit®) does not consist only of the pure kainit mineral, but contains about 56 % NaCl. The fertiliser composition of Magnesia-Kainit® is as follows: K: 9.1 %, Mg: 3.0 %, S: 4 %, Cl: 43 %, Na: 20 % (K+S Kali GmbH, 2015).

the effect of effective formulations on weed control and crop growth under field conditions. Parallel to field trials the screening trials were continued in 2012/13.

In summary, the eighteen screening trials examined different aspects of formulation in order to elucidate their effect on weed control. The research objectives can be summarised as follows:

Box 1.3 Research objectives screening trials

1. Determine and compare the efficacy of different essential oils and acidic acid (CMD).
2. Determine the efficacy of NaCl, alone and in combination with CMD.
3. Study the effect of vegetable oils, alone and in combination with essential oils.
4. Study the effect of application rate, and AI concentration.
5. Study the effect of emulsifiers, alone and in formulation with CMD.
6. Determine if combination of different AI can improvement efficacy.

For a total of five field trials the following hypotheses were postulated:

Box 1.4 Hypotheses: natural herbicide field trials

10. Efficient weed control with NH is only possible with multiple applications.
11. Weed density influences weed control efficacy.
12. Weed control with NH is efficient - crop growth and yield are comparable to clean control.
13. NaCl does not influence crop growth negatively by causing salinisation.

2 Materials and Methods

Research was conducted in two climate zones - Germany (temperate) and Brazil (tropical) and on two different topics. In order to avoid redundancies, descriptions of materials and methods are summarised whenever there are similarities between the experiments conducted.

2.1 Trial sites

2.1.1 Germany

The fertiliser trials at research farms of *Bonn University*, Germany were implemented at *Campus Klein-Altendorf* in Rheinbach (`KA`, 2011) and *Wiesengut* in Hennef (`WG`, 2012), certified organic since 1985 (*Bioland* and *Naturland*).

The soil of site `KA` is classified as a *hypereutric, siltic, haplic luvisol* developed from loess (soil analysis of Pätzold, 2012 - DFG Research Group FOR1320) or *Normparabraunerde aus Löss* according to German Soil Classification (Eckelmann et al., 2005). At this site the soil score (*Ackerzahl* [7]) according to Deutsche Bodenkundliche Gesellschaft (DBG) is 70–90 (table 2.1). Site `WG` is situated in the lea of river *Sieg* and soils have developed on river sediments (WRB [8]: *fluvisol*). The soil possesses a heterogeneous texture, ranging from loamy silt to sandy silt. Furthermore, irregularly distributed gravel reaches up to near the soil surface at some locations. At `WG` the soil score for plant production is 20–70 points. A detailed description of `WG` soils can be found in Haas (1995). According to the Köppen (1940) climate classification (Kottek et al., 2006), both `KA` and `WG` are classified as *Cfb* (figure 2.1).

Table 2.1: Site description of German trial sites *Campus Klein-Altendorf*, Rheinbach (`KA`) and *Wiesengut*, Hennef (`WG`).

	Klein-Altendorf 2011 (`KA`)	Wiesengut 2012 (`WG`)
Coordinates	50.63° N, 6.95° E	50.78° N, 7.28° E
Elevation (m.a.s.l.)	173	68
Climate (*Köppen*) (1)	*Cfb*	*Cfb*
Precipitation (mm)	603	850
Mean temperature (°C)	9.4	10.3
Soil texture	Ut3: silty to clayey loam	slU–sU: sandy to loamy silt
Soil classification (WRB) (2)	*Haplic Luvisol*	*Fluvisol*
Ackerzahl	70–93	20–70
Mean groundwater level	20 m	4.5 m

[1] *Cfb*: Marine west coast climate (Kottek et al., 2006)

[2] WRB: World reference base for soils (IUSS Working Group et al., 2006)

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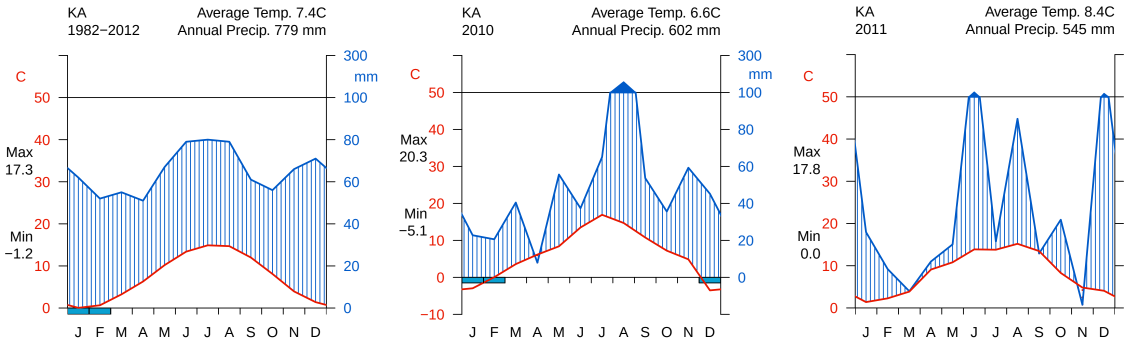
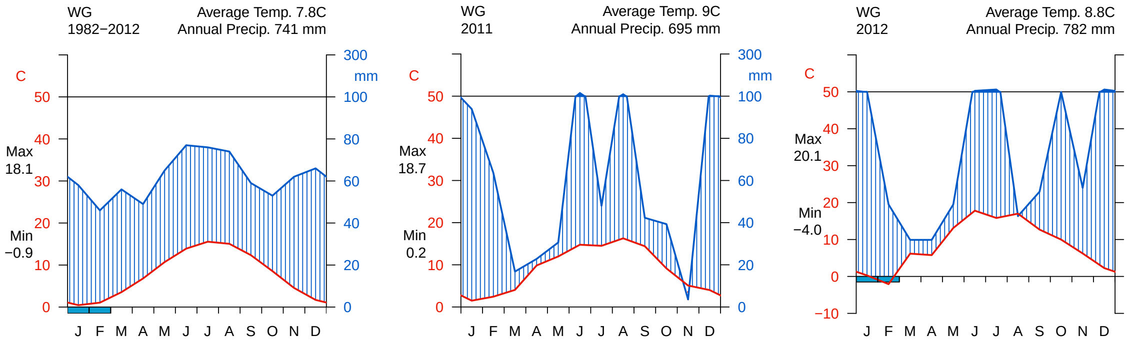
[7] The soil score describes the site's crop production potential and takes into account soil, site and climate conditions on a scale from 1–120 (low to high potential).

[8] WRB: Food and Agricultural Organization (FAO) - World reference base for soil resources - International soil classification system for naming soils and creating legends for soil maps



(a) *Campus Klein-Altendorf*, Rheinbach (173 m).



(b) *Wiesengut*, Hennef (67 m).

Figure 2.1: Long-term and experimental season climate data of *Campus Klein-Altendorf*, Rheinbach (`KA`, 2011) and *Wiesengut*, Hennef (`WG`, 2012). Data representation according to Walter & Lieth (1960).
Sources: Long-term data: `www.climate-data.org`. Experimental year data: on-site meteorological stations.

The crop rotation at conventional farm `KA` consists of barley, winter wheat and sugar beat. Mineral fertiliser application to these crops consists of UAN (solution of urea and ammonium nitrate), calcium ammonium nitrate, triple phosphate and Korn-Kali® [9]. After 2006 no more organic fertilisers were applied (before: pig slurry, on average about 10 t ha^{-1} per year). Barley and winter wheat each receive 140 kg ha^{-1} N, 40 kg ha^{-1} P and 10 kg ha^{-1}, while sugar beat receives 150 kg ha^{-1} N, 80 kg ha^{-1} P and 20–25 kg ha^{-1} S. The crop preceding the `KA` trials was barley. After harvest, the straw stubble was left over winter and no winter cover crop was sown. At site `WG`, the crop rotation consists of a clover grass mixture (1.5 years), potato, winter wheat, faba bean, summer wheat, winter rye and oats. The fertilisers used are Patentkali® and rock phosphate. No regular application quantities of these fertilisers can be cited, as both are applied to adjust the nutrient level to LUFA C. For example, Kainit was not applied between 2003 and 2009. Nevertheless in 2010 - two years before the `WG` trials - a large quantity of 1000 kg ha^{-1} Patentkali® was applied. On the mixed farm *Wiesengut* cattle manure of on average 17 t ha^{-1} per year is applied. The crop of 2010 was winter wheat, followed by a mustard green manure crop which remained over winter. Mustard was ploughed in spring and oats was sown as a summer crop. As for site `KA`, the stubble was left over winter without sowing any winter cover crop.

2.1.2 Brazil

Fertiliser and natural herbicide trials in Brazil were set up in *Londrina* (`LO` 2012 & 2013), *Ponta Grossa* (`PG`, 2012 & 2013) and *Umuarama*: (`UM` 2013). In 2012 `LO` fertiliser trials were realised at the experimental farm of *Londrina State University* (UEL). All other trials were conducted research farms of Instituto Agronômico do Paraná (IAPAR). The two major sites `LO` and `PG` represent the most common soil type in Paraná state, Latossolo vermelho (SiBCS) / *ferralsol* (WRB), and its two different climate zones *Cfa* and *Cfb*, respectively (figure 2.3 and table 2.2). The climate at site `UM` is classified as *Cfa* and the soil originated from the cretaceous sediment *arenito Caiuá*, which covers 15 % of the area in Paraná (IBGE) and is classified as a sandy ferralsol. This latter trial site was chosen for fertiliser trials because it was

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[9] Korn Kali® contains: 33 % K, 3.6 % Mg and 5 % S

assumed to be especially prone to sulphur deficiency. A more detailed description of soil physics and chemistry relevant for the interpretation of results will be presented in the soybean fertiliser trial results sections. The cropping system mainly used in Paraná state and at experimental sites `LO` and `PG` is a rotation which consists of about 50–85 % soybean. Usually soybean is planted in rotations with maize in summer, and wheat, oats or green manure plants in winter, e.g. hairy vetch (*Vicia villosa*) or wild radish (*Raphanus raphanistrum*). Site `UM` was under pasture management in the past, however, use was converted to annual cropping of soybean and maize.

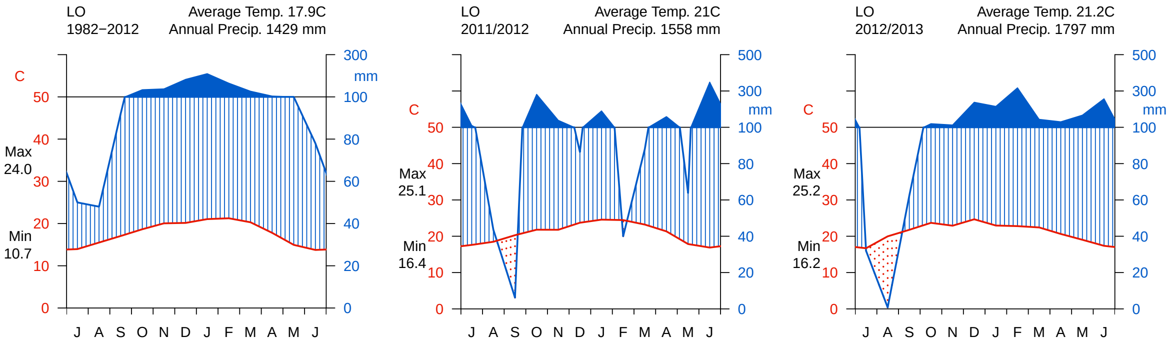
Table 2.2: Site description of Brazilian trial sites *Londrina* (`LO`), *Ponta Grossa* (`PG`) and *Umuarama* (`UM`).

	Londrina (`LO`)	*Ponta Grossa* (`PG`)	*Umuarama* (`UM`)
Year (harvest)	2012, 2013	2012, 2013	2013
Coordinates	23.3° S, 51.2° W	25.1° S, 50.2° W	23.8° S, 53.3° W
Elevation (m.a.s.l.)	610	975	430
Climate (*Köppen*) (1)	*Cfa*	*Cfb*	*Cfa*
Precipitation (mm)	1429	1495	1512
Mean temperature (°C)	20.9	17.5	20.7
Soil texture	Clay	Clay / Loam	Sand
Soil classification (WRB) (2)	Clay ferralsol	Clay ferralsol	Sandy ferralsol
Soil classification (SiBCS) (3)	Latossolo vermelho	Latossolo vermelho	Latossolo vermelho

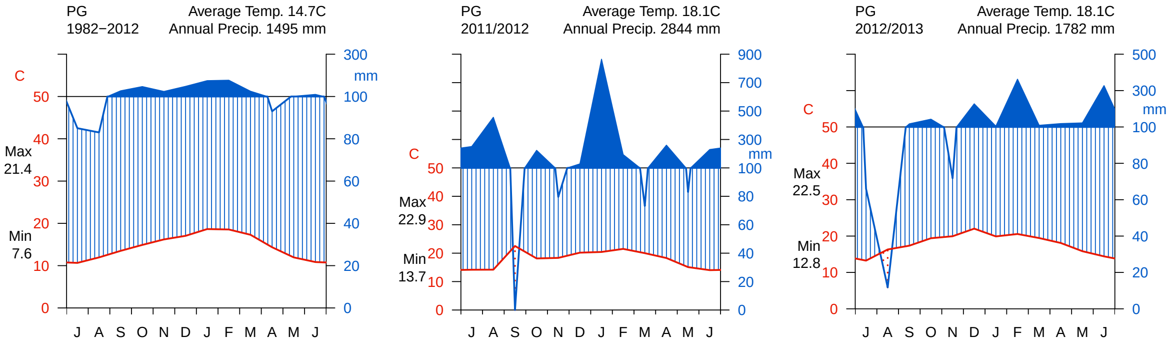
[1] *Cfa*: humid subtropical climate, *Cfb*: marine west coast climate

[2] WRB: World reference base for soils (IUSS Working Group et al., 2006)

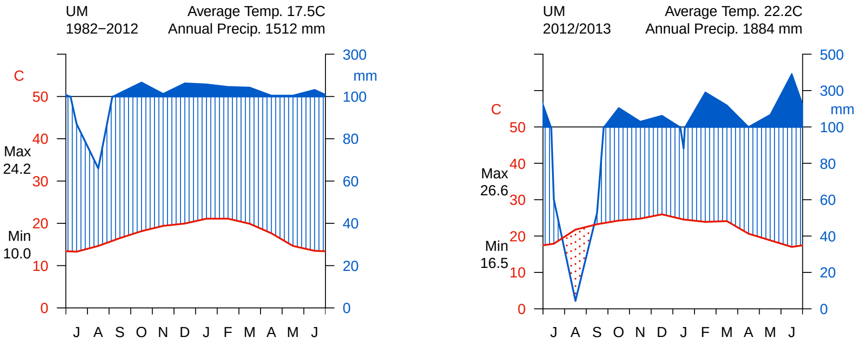
[3] SiBCS: Sistema Brasileiro de Classificação de Solos - Brazilian soil classification (Embrapa Solos, 2006)



(a) Site *Londrina* (603 m).



(b) Site *Ponta Grossa* (966 m).



(c) Site *Umuarama* (452 m).

Figure 2.3: Long-term (1982-2012) and experimental season climate date of sites *Londrina* (`LO`, 2012 and 2013), *Ponta Grossa* (`PG`, 2012 and 2013), and *Umuarama* (`UM`, 2013). Data representation according to Walter & Lieth (1960).
Sources: Long-term data: `www.climate-data.org`. Experimental year data: on-site meteorological stations.

2.2 Fertiliser trials (Germany and Brazil)

2.2.1 Phosphorus and sulphur fertiliser trials (Germany and Brazil)

Phosphorus fertiliser trials (design referred to as `P-trial` [10]) were conducted in order to study the effects of intra-row applied rock phosphate (RP), alone and in combination with potentially RP dissolving elemental sulphur, on nutrition and development of direct seeding (DS) faba bean (Germany) and soybean (Brazil). Accordingly, in the sulphur fertiliser trials (`S-trial`) effects of different intra-row applied sulphur fertilisers on DS faba bean and soybean nutrition and development were studied. The trials were setup in the following design:

Box 2.1 Design `P-trial` and `S-trial` (Germany and Brazil)

One factorial randomised block design (RBD) with the treatment factor *fertiliser*, ($n = 4 - 6$)

Faba bean Germany	**Soybean Brazil**
Plots: 12 m long, 7 rows with 0.34 m row width	Plots: 10 m long, 6–8 rows with 0.45 m row width

Treatments `P-trial`

1.	`Ctl`	Control	
2.	`S0`	Elemental Sulphur (S^0)	$40\,kg\,ha^{-1}$ S
3.	`P1`	*Gafsa* rock-phosphate (1)	$50\,kg\,ha^{-1}$ P
4.	`P1S`	*Gafsa* + S^0	$50\,kg\,ha^{-1}$ P + $40\,kg\,ha^{-1}$ S
5.	`P2`	*Alvorada* rock-phosphate (only Brazil) (2)	$50\,kg\,ha^{-1}$ P
6.	`P2S`	*Alvorada* + S^0 (only Brazil)	$50\,kg\,ha^{-1}$ P + $40\,kg\,ha^{-1}$ S

[1] Tunesian sedimentary rock phosphate: citric acid (2 %) solubility: 40–45 %, P-content: 12.4 %

[2] Brazilian apatite rock phosphate: citric acid (2 %) solubility: 17 %, P-content: 10.5 %

Treatments `S-trial`

1.	`Ctl`	Control	
2.	`KCl`	KCl	$95\,kg\,ha^{-1}$ K
3.	`KS`	K_2SO_4	$40\,kg\,ha^{-1}$ S + $95\,kg\,ha^{-1}$ K
4.	`S0`	Elemental Sulphur (S^0)	$40\,kg\,ha^{-1}$ S
5.	`CaS`	Gypsum ($CaSO_4 \cdot 2\,H_2O$)	$40\,kg\,ha^{-1}$ S

`P-Trial`, `S-Trial` or the combined `P+S-Trial` were conducted at the following sites:

Site		**Harvest 2011**		**Harvest 2012**		**Harvest 2013**	
Klein-Altendorf	`KA`	`KA-P`	`KA-S`				
Londrina	`LO`			`LO1-P`	`LO1-S`	`LO2-P`	`LO2-S`
Ponta Grossa	`PG`			`PG1-PS`	`PG1-Low`	`PG2-PS`	`PG2-Low`
Umuarama	`UM`					`UM-PS`	

The sedimentary, reactive RP *Gafsa* was used as a fertiliser treatment both in Germany and Brazil. In Brazil a locally available, yet less soluble apatite RP named *Alvorada* was additionally included as a second RP treatment into the `P-trial`. As considerable amounts of potassium were applied with the `KS` [11] treatment of `S-trial`, a `KCl` treatment with an equivalent potassium rate $95\,kg\,ha^{-1}$ was introduced in order to detect whether possible effects of potassium fertilisation on crop development exist. Importantly, at site `KA` elemental sulphur was applied as a pellet of ∅ 2–3 mm. However, the pellets were found not to disintegrate well in soil due to the low surface of the pellets. Therefore it was decided to apply the sulphur as a fine ground powder at site `WG` and in all Brazilian trials. The particle size (not granule size) of the sulphur contained within pellets and of the ground sulphur is unknown.

Nearly always both `P-trial` and `S-trial` were setup side-by-side on the same field (`KA`, `LO1` [12]) or

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[10] Trial or site abbreviations are written with bold typewriter font

[11] Treatment abbreviations are written with typewriter font

[12] Sites used only in one year are not numbered, e.g. `KA`. Sites used in two trial years are numbered, e.g. `LO1` = Londrina 2011/12

were combined into one single `P+S-trial` (`PG1-PS`, `PG2-PS` & `UM-PS`). Trial conduction was then in parallel, meaning that variety as well as sowing, evaluation and harvest dates were identical (*ceteris paribus*). For `P+S-trial` the repetitions were increased to six to counteract potentially increased heterogeneity in larger experimental blocks. Of all trials only in the second year in *Londrina* (`LO2`) two distant fields within the experimental farm were used for the two trial types and also the conduction was not totally parallel with an offset of about a week. At site `PG` an additional `P-trial` was set up on a small experimental area with extremely low P-contents (`PG-Low`). Furthermore, this same area was reused in the second year, maintaining plot and treatment locations, which means that the fertiliser effect was cumulative in the second year. The trial conduction of `PG-Low` was in both years parallel with the `P+S-trial` at `PG`. The faba bean variety used at `KA` in 2011 was *Fuego*. In Brazilian fertiliser trials the soybean variety used was *BRS 284*, with exception of `UM` for which *BRS 184* was planted.

2.2.2 Fertiliser and oats straw residue trials Wiesengut (Germany)

On *Wiesengut* in 2012 (`WG`) four faba bean DS trials were conducted in parallel on the same field under organic management, which not only examined fertiliser effects in DS faba bean but also mulch effects on weed and crop development. These trials included one three-factorial RBD trial and three two-factorial split-plot trials. The three-factorial RBD trial consisted of treatment factors *fertiliser*, *straw* and *cut*. The factor *fertiliser* compared `P1S` with a non fertilised `Ctl`, and the trial is therefore referred to as `WG-PS`. For the `PS` treatment the same fertilisers and amounts were applied as in `P-trial`, but in contrast to `KA` the elemental sulphur in treatment PS was applied as finely ground powder. Only in `WG-PS` the factor pre-crop oats cutting height (*cut*), with a high (`High`) and a low cut (`Low`) was included. There are two ideas behind the high cut treatment: on one hand this measure saves the energy used by the chopper of the combine harvester. On the other hand the high cut straw remains secured to the ground and potential clogging of the straw in the direct seeding drill at planting is avoided. For treatment `Low` the pre-crop oats was harvested conventionally, cutting the plants at the base about 10 cm from the ground. For treatment `High`, oats were cut just below the panicle about 50–60 cm from the ground (strip cutting). The standing straw was subsequently rolled with a roller-crimper (Davis, 2010) to ensure soil coverage. The factor *straw* simulated different amounts of pre-crop oats straw residue. After oats harvest the straw residue was determined at around ten spots per trial and the straw amounts present in each plot were interpolated. The straw residue already present in the `Low` cut treatments was found to be around 0.75 t ha^{-1}, and for `High` cut between 1.2–3 t ha^{-1}. For the `0t` treatment no additional straw was added, therefore different straw amounts for `Low` and `High` cut treatments resulted for this straw level. For `4t` and `6t` treatments only the missing straw to complete the respective amounts was added and hence the total straw amounts of `Low` and `High` were equal. As a reference to the DS system ten ploughed plots were inserted into the design of `WG-PS` with the treatment factor *fertiliser*. However, several plots had to be eliminated due to damage by wild boar, which resulted in an unbalanced design. The results of ploughed treatments are not presented in this work, because data is not considered reliable and because `WG-PS` was the only trial with ploughed reference plots.

Box 2.2 Design `WG-PS` trial (Germany)

Three-factorial randomised block design (n = 4), with the treatment factors *fertiliser*, *straw* and *cut*.
Plots: 6 m long, 5 rows with 0.45 m row width

Fertilisation (*fertiliser*)
1. `Ctl` Control
2. `PS` Gafsa + S^0

Oats straw residue (*straw*)
1. `0t` 0 t ha^{-1} oats straw
2. `4t` 4 t ha^{-1} oats straw
3. `6t` 6 t ha^{-1} oats straw

Oats cutting height (*cut*)
1. `Low` High cut
2. `High` Low cut

The three split-plot trials at site `WG` consisted of the treatment factors *fertiliser* (whole-plot) and *straw* (split-plot) and differed only in their fertiliser treatments. Each trial had two different *fertiliser* treatments. In the gypsum trial (`WG-G`, n = 5), `CaS` and in the potassium sulphate trial (`WG-K`, n = 4)

`KS` was compared to a `Ctl` treatment. The third trial was the molybdenum trial (**`WG-M`**, n = 3), which consisted of the *fertiliser* treatments `KS` and a combination of K_2SO_4 (same dosage as `KS`) with 1 kg ha^{-1} Na_2MoO_4, referred to as `Mo`. The dosage of all *fertiliser* treatments was equivalent to the treatments used in **`P-trial`** and **`S-trial`**. In all three split-plot trials strip-cutting of oats was used (*cut* = `High`) and the same three *straw* treatments used as described for **`WG-PS`**. **`WG-M`** was conducted in order to examine effect of molybdenum on N_2-fixation and crop growth of faba bean, but more detailed studies on this nutrient were later resigned and no laboratory analysis of Mo conducted. The results of factor *fertiliser* for this trial are hence omitted. Nevertheless, results of *straw* effect are presented together with other **`WG`** trials (there were no significant interactions between *fertiliser* and *straw* for any of the parameters studied). The faba bean variety used in all **`WG`** trials was *Limbo*.

Box 2.3 Design `WG-G`, `WG-K`, `WG-M` trials - Germany

Two factorial split plot design (n = 3–5), with the treatment factors *fertiliser* and *straw*.
Plots: 12 m long, 7 rows with 0.34 m row width

Fertilisation (*fertiliser*)			**Oats straw residue** (*straw*)		
1.	`Ctl`	Control	1.	`0t`	0 t ha^{-1} oats straw
2.	`CaS`, `KS`, `Mo`	**`WG-G`**, **`WG-K`**, **`WG-M`**	2.	`4t`	4 t ha^{-1} oats straw
			3.	`6t`	6 t ha^{-1} oats straw

2.2.3 Evaluation dates

Both **`KA`** trials were established and conducted in parallel. In **`WG`** trials, sowing, evaluation dates and harvest were nearly equal over all trials - only shoot dry mass sampling dates differed from 1 to 3 days between trials (table 2.3).

Table 2.3: Dates of sowing, shoot dry mass evaluations (with the respective plant age and development stage) and harvest of fertiliser trials in Germany.

Site	Year	Sowing	Shoot dry mass 1		(Stage)	Shoot dry mass 2		(Stage)	Harvest
`KA`	2011	05/03	16/06	73 DAE (1)	BBCH 73 (2)	18/07	102 DAE	BBCH 81	10/08 (125)
`WG`	2012	16/03	31/05	57 DAE	BBCH 65	04/07	89 DAE	BBCH 77	20/08 (136)

(1) DAE: days after emergence
(2) BBCH crop development stage (Meier, 2001)

Apart from trials **`PG1-Low`** and **`PG1-PS`** sowing was realised within the recommended period (Embrapa Soja, 2007). The **`PG1`** trials had to be replanted in mid December due to a failed herbicide application, which caused severest crop injury, and this delayed the trial by about one month. Shoot dry mass evaluations were all carried out at R3–R4 stage (table 2.4).

Table 2.4: Dates of sowing, shoot dry mass evaluation (with the respective plant age and development stage) and harvest of fertiliser trials in Brazil.

Trial		Year	Sowing	Shoot dry mass		(Stage) (1)		Harvest	
`LO1-P`	**`LO1-S`**	2012	07/11	30/01	73 DAE	BBCH 73	R3	12/03	115 DAE (2)
`LO2-S`		2013	05/11	24/01	74 DAE	BBCH 72	R3	22/03	131 DAE
`LO2-P`		2013	31/10	15/01	71 DAE	BBCH 72	R3	19/03	134 DAE
`UM-PS`		2013	29/10	22/01	78 DAE	BBCH 79	R4	05/03	120 DAE
`PG1-PS`	**`PG1-Low`**	2012	15/12	13/03	82 DAE	BBCH 79	R4	24/04	124 DAE
`PG2-PS`	**`PG2-Low`**	2013	19/11	20/02	85 DAE	BBCH 75	R4	08/04	132 DAE

(1) *Left*: BBCH growth stage. *Right*: growth stage adapted from (Fehr et al., 1971; Pedersen and Elbert, 2004)
(2) Soybean crop in trials `LO1-P` and `LO1-S` died off during grain filling stage (infection with *Macrophomina sp.*)

2.3 Natural herbicide trials (Brazil)

Prior to the description of screening and field trials an overview is given on the active and 'inert' ingredients used and the elaboration method of natural herbicide (NH) formulations.

2.3.1 Natural herbicide formulation and application

Active ingredients Due to restrictions by the Brazilian Ministry of Agriculture, commercial NH products could not be imported for trials. Therefore pure ingredients were obtained to elaborate and test formulations in screening trials from 2011–2013. The essential oils used were δ-limonene (99 %), semi-synthetic pine oil (containing 66 % α-terpineol), citronella oil, eucalypt oil (*Eucalyptus citriodora*) and natural turpentine (*Pinus spp.* species unknown). Furthermore the active ingredients acetic acid (AA, 99.9 %) and NaCl were used. δ-limonene was donated by *Citrovita Agro Industrial* company in São Paulo, São Paulo state, Brazil. The other essential oils and acetic acid (AA) were purchased at *Farmanilquima* company in Curitiba, Paraná state, Brazil. NaCl was purchased at local supermarkets.

Inert ingredients Essential oils are not miscible with water and therefore emulsifiers had to be used in NH formulations to obtain stable emulsions. Several emulsifiers were tested, which are also called adjuvants or surfactants (short for surface active ingredients). The emulsifiers were also purchased from *Farmanilquima* company, Curitiba. Most of the emulsifiers used were non-ionic: these were Oxiteno Renex 95®, (nonylphenol-ethoxylate 9.5 EO [13] - abbreviated as NPE), Tween 20® (Polyoxyethylene (20) sorbitan monolaurate - abbreviated as T20), and Tween 80® (Polyoxyethylene (80) sorbitan monooleate - abbreviated as T80). Furthermore, the anionic emulsifiers sodium ricenoleate and SA (specifically sodium dodecylbenzenesulphonate) were employed. The latter is commonly used in formulations of household detergents. Furthermore Rimulgan® (Temmen GmbH, Germany) was used. Another emulsifier studied was methylated soybean seed oil (MSO), not in its pure, but in a formulated form as the commercial product Bayer Aureo® [14]. In order to reduce volatility of essential oils the refined vegetable oils maize and soybean oil were tested as additives to essential oils. These were purchased at local supermarkets. The water used in the elaboration of all NH formulations was soft tap-water at both experimental sides `PG` and `LO` (no analysis made). In all formulations of screening and field trials the spreader-sticker Nu-Film P® (Intrachem Bio, Germany) was added, which is certified for use in Organic Agriculture in Europe.

Elaboration of formulations In order to form a stable emulsion the oil droplet size has to be as small as possible. This size is influenced by the type of emulsifier used, by the order of addition of ingredients and by the mechanical energy applied (agitation intensity or ultra-filtration). Lacking sophisticated equipment for agitation or ultra-filtration it was intended to exploit the effect of phase inversion (Fernandez et al., 2004) on droplet size: at first the essential oil and the emulsifier were mixed and if NaCl was included in the formula, it was added subsequently. The idea behind adding NaCl secondly was that salt crystals add mechanical energy upon agitation and thereby help to mix the components more thoroughly. The large amounts of NaCl were not expected to interfere in emulsion formation as the emulsifiers used were non-ionic. In the next step water was added slowly under constant agitation, which led to the formation of a water-in-oil emulsion. The slow addition of water was continued until the point of phase inversion, where emulsion is transferred to an oil-in-water emulsion. Passing through this point presumably results in extremely small oil droplets in the emulsion (Fernandez et al., 2004) and thereby in enhanced emulsion stability. After passing phase inversion the missing amount of water was added rapidly and in a final step spreader / sticker Nu-Film-P® was added at the recommended dose of 300 mL ha^{-1}.

Application method Application of products was realised with a custom-made CO_2 pressured agricultural sprayer with an adaptor to receive PET bottles as tanks for spraying solutions. The advantage of this PET bottle system was that sprays could be changed quickly, and that very low volume applications

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[13] 9.5 EO: degree of ethoxylation: 9.5 moles of ethylene oxide

[14] 720 g/L methylated soybean seed oil, 188 g/L inert ingredients

were possible in screenings as well as that residual spray solution in tanks was minimal. Application pressure in all cases ranged from 1.4–2.0 bar depending on nozzle and application rate. Before application gauging was carried out twice for 30 s in order to calculate the walking pace for application. In order to ensure precise application rates the necessary pace was practised several times prior to application and a technician assisted during spraying with a stopwatch. The spray tank was shaken manually before application to inhibit separation of emulsions.

Screening trials were all conducted with a 1.0 m wide spraying bar with three 110-02 flat fan nozzles with overlapping spray fans to guarantee even spray distribution. In field trials a single nozzle was used, either a 80-03E band spray or a 110-02 nozzle.

Crops in field trials had to be protected from phytotoxic sprays. However, the protective screen available for field trials caused problems during application as it interfered strongly with the roller-crimper treated straw residue and could therefore not be used successfully. Instead, in the soybean direct-seeding NH trial in Londrina (`LO-NH`) a cut-in-half PVC tubing (∅ 12 cm) was used as a protective cover for the soybean crop during application. However, in the soybean direct-seeding NH trial in Ponta Grossa (`PG-NH`), and in the common bean conventional tillage NH trials in Ponta Grossa (`PG-Lim`, `PG-Pin` and `PG-NaCl`, see description below) no protection was used. In these trials near-surface application using a wide angle 110-02 nozzle largely avoided spray contact with the crop, with spray passing underneath the first and second trifoliar leaf of soybean or common bean. Nevertheless, a problem with this nozzle was the uneven spray distribution, with the centre of the crop inter-row receiving a slightly larger amount of spray. A well working screen was only developed later and used successfully in common bean trials in Londrina in 2013. However, these trials could not be considered in this work, because a drought and high incidence of virus lead to extremely poor crop and weed development.

In broadcast application of screening trials the spray volumes reflect the total spray applied on a hectare basis. However, the spray application volumes described for treatments in field trials have to be interpreted with caution: as band application was employed in field trials, the spray did not cover the complete row width of 45 cm but rather only 40 cm. Within the spray band between crop rows the application rate was equivalent to the value stated (e.g. 600 L ha^{-1}). As the area within the crop row did not receive any spray, the actual rates (and hence costs) applied on a hectare basis were in fact only about 85–90 of the value stated in the treatment description.

Before all spray applications the time, temperature (T), relative humidity (rH), cloudiness (% coverage) and the development stage of weeds were recorded.

2.3.2 Design screening trials

Most screening trials were realised at IAPAR experimental farm in *Ponta Grossa* (18 screening trials) from 2011–2013 and only two screening trials at IAPAR Londrina in season 2012/13. No soil sampling was realised for these trials, however, soil granulometry and chemistry can be assumed to be similar to results obtained for fertiliser trials in `LO` and `PG`. Experimental plots were 5 m long and 1 m wide. The majority of screenings were set up without repetitions in order to allow for higher treatment number, which was often greater than ten. In this case treatment comparisons which were of highest interest were selectively placed side-by-side or near each other to facilitate detection of subtle differences in control efficacy among them. Whenever repetitions were used, randomisation was applied. In a few instances the test plant in screenings was soybean (*Glycine max*), but in nearly all screenings the test plants were spontaneously emerged weed species. The experimental areas were cultivated prior to screenings in order to promote weed emergence and to guarantee that their growth stage at application is as uniform as possible. The formulations employed often contained a large number of ingredients. Box 2.4 summarises all abbreviations for AI, emulsifiers and plant species used in screenings and field trials.

Box 2.4 Abbreviations: natural herbicide ingredients and weed species

Active ingredient	
AA	Acetic acid
Cit	Citronella oil
Euc	Eucalypt oil
Lim	δ-limonene
NaCl	NaCl
Pin	Pine oil
Tur	Turpentine
Soybean	Soybean oil
Rape	Rape oil
Corn	Corn oil

Emulsifiers	
Emustab	Emustab®
MSO	Methylated soybean seed oil
NPE	Nonylphenol-ethoxylate
Ric	Sodium ricenoleate
SA	Sulphonic acid
T20	Tween 20®
T80	Tween 80®
Energic	Energic®
Rimulgan	Rimulgan®

In order to clearly describe these formulations in figures and text a codified description using abbreviations is used. The formulation descriptions are arranged and codified according to the following example in the info box:

Box 2.5 Codified description of natural herbicide formulations

Essential oil	+	**NaCl**	+	**Emulsifier type**	-	**Application rate**
50-8 Pin	+	50 NaCl	+	2 NPE	-	600
50 L ha^{-1}-8.3 % pine oil	+	50 kg ha^{-1} NaCl	+	2 L ha^{-1} NPE	-	600 L ha^{-1}

`50-8 Pine` means that 50 L ha^{-1} pine oil with a concentration of 8.3 % (rounded to 8 % in the abbreviation) is contained in the spraying solution. For essential oils and AA both the total volume applied per area and the concentration (v/v) are presented as these play a role both for cost and for product efficacy. NaCl and emulsifier quantities are only presented based on weight (kg ha^{-1}) or volume (L ha^{-1}) applied per hectare and not their concentration in the spraying solution. One reason is to keep abbreviations as short as possible. Another reason is that the concentration of NaCl and emulsifiers for weed control efficacy is not assumed to be important: during the drying process of NH spray on the leaf surface, the concentrations of salt and emulsifiers increase in any case as both do not evaporate as essential oils and - to a minor extend - AA do. If the last term which describes the application rate is omitted, this means that the application rate is 600 L ha^{-1}. Most applications were realised at this rate.

The info boxes from box 2.6 to box 2.25 contain a brief description of the research objectives of each screening trial and the (codified) spray treatments used. Furthermore, the weed species and their (abbreviated) growth stage are specified as well as the date, hour and environmental conditions at NH application and evaluation date.

Box 2.6 Screening trial Londrina 1 - `S-L1`

Objective:

- Comparison of weed control efficacy of different essential oils
- Determine if addition of vegetable improves weed control effect of essential oils

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	25/03/2013	GLXMA	2L	1	100 Lim
Time:	15:00			2	100 Cit
Temperature:	27 °C			3	100 Euc
Cloud cover:	20 %			4	100 Pin
Humidity:	60 %			5	100 Lim + 50 Corn
Evaluation:	3 DAA			6	100 Cit + 50 Corn
				7	100 Euc + 50 Corn
				8	100 Pin + 50 Corn
				All	**0 Emulsifier - 600 L ha^{-1}**

Box 2.7 Screening trial Londrina 2 - `S-L2`

Objective:

- Determine the weed control effect of formulations containing only emulsifiers: 1–4
- Determine the weed control effect of refined vegetable oils: 5–7

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	24/03/2013	BIDPI	3L	1	20 NPE
Time:	17:00			2	10 NPE
Temperature:	26 °C			3	20 T80
Cloud cover:	10 %			4	10 T80
Humidity:	60 %				
Evaluation:	4 DAA			5	50 Corn oil + 5 Rimulgan
				6	50 Rape oil + 5 Rimulgan
				7	50 Soybean oil + 5 Rimulgan
				All	**600 L ha^{-1}**

Box 2.8 Screening trial Ponta Grossa 1 - `S-PG1`

Objective:

- Comparison of weed control efficacy of different essential oils

Conditions		**Weed**	**Stage**	**Formulations** (4 repetitions)	
Date:	02/12/2011	GLXMA	3L	1	60-20 Lim + 3 Emustab
Time:	14:20			2	86-29 Euc + 3 Emustab
Temperature:	24 °C			3	90-33 Pin + 3 Ricenoleate
Cloud cover:	40 %			4	75-25 Cit + 3 Ricenoleate
Humidity:	55 %			**All**	**300 L ha^{-1}**
Evaluation:	4 DAA				

Box 2.9 Screening trial Ponta Grossa 2 - `S-PG2`

Objectives:

- Comparison of low and high concentration of pine oil and δ-limonene: 1+2, 3+4, 9+10, 11+12
- Comparison of 300 L ha^{-1} and 600 L ha^{-1} application volume: 1+9, 2+10, 3+11, etc.
- Evaluate combined effect of essential oil and acetic acid: 6–8 and 14–16
- Comparison of effect of concentrated vs. diluted application (same amount AI): 2+10, 4+11

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	09/01/2012	BRAPL	1-3L	1	30-10 Lim + 3 Emustab
Time:	16:15	IAQGR	2L	2	60-20 Lim + 3 Emustab
Temperature:	24 °C			3	45-15 Pin + 3 Ricenoleate
Cloud cover:	70 %			4	90-30 Pin + 3 Ricenoleate
Humidity:	62 %			5	30-10 AA + 3 Emustab
Evaluation:	3 DAA			6	60-20 Lim + 30-10 AA + 3 Emustab
				7	45-15 Pin + 30-10 AA + 3 Ricenoleate
				8	90-15 Pin + 30-10 AA + 3 Ricenoleate
				1-8	**600 L ha^{-1}**
				9	60-10 Lim + 3 Emustab
				10	120-20 Lim + 3 Emustab
				11	90-15 Pin + 3 Ricenoleate
				12	180-30 Pin + 3 Ricenoleate
				13	60-10 AA + 3 Emustab
				14	120-20 Lim + 60-10 AA + 3 Emustab
				15	90-15 Pin + 60-10 AA + 3 Ricenoleate
				16	180-30 Pin + 60-10 AA + 3 Ricenoleate
				9-16	**300 L ha^{-1}**

Box 2.10 Screening trial Ponta Grossa 3 - `S-PG3`

Objective:

- Comparison of two concentrations of pine oil and δ-limonene: 2+3, 6+7
- Evaluation of effect of essential oils and acetic acid alone and their combination: 1–9

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	02/03/2012	BRAPL	4,2T	1	30-5 AA
Time:	10:00	IAQGR	4-6L	2	30-5 Lim
Temperature:	25 °C	EPHHL	4-6L	3	60-10 Lim
Cloud cover:	30 %	RAPRA	4-8L	4	30-5 Lim + 30-5 AA
Humidity:	60 %			5	60-10 Lim + 30-5 AA
Evaluation:	3 DAA			6	15-2.5 Pin
				7	30-5 Pin
				8	15-2.5 Pin + 30-5 AA
				9	30-5 Pin + 30-5 AA
				All	**0.3 NPE - 600 L ha^{-1}**

Box 2.11 Screening trial Ponta Grossa 4 - `S-PG4`

Objective:

- Evaluation of NaCl and AA alone and their combination: 1–3
- Comparison of δ limonene formulated with different emulsifiers: 4+5
- Determine if addition of vegetable improves weed control effect of essential oils: 6+7

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	03/04/2012	BRAPL	2-3L	1	120 NaCl
Time:	15:00	IAQGR	2-4L	2	30-5 AA
Temperature:	23 °C	RAPRA	4-5L	3	30-5 AA + 120 NaCl
Cloud cover:	20 %				
Humidity:	56 %			4	120-20 Lim + 30 T20
Evaluation:	7 DAA			5	120-20 Lim + 30 NPE
				6	120-20 Lim + 30 Energic
				7	120-20 Lim + 30 Ricinus + 30 Energic
				All	**600 L ha^{-1}**

Box 2.12 Screening trial Ponta Grossa 5 - `S-PG5`

Objectives:

- Comparison of the essential oils δ-limonene and pine oil
- Evaluation of the combinations of the essential oils with AA, NaCl and AA + NaCl

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	19/04/2012	RAPRA	9-11L	1	30-5 Pin
Time:	15:30	IAQGR	10-14L	2	30-5 Pin + 30-5 AA
Temperature:	21 °C			3	30-5 Pin + 120 NaCl
Cloud cover:	50 %			4	30-5 Pin + 30-5 AA + 120 NaCl
Humidity:	52 %				
Evaluation:	11 DAA			5	30-5 Lim
				6	30-5 Lim + 30-5 AA
				7	30-5 Lim + 120 NaCl
				8	30-5 Lim + 30-5 AA + 120 NaCl
				All	**8 NPE, 600 L ha^{-1}**

Box 2.13 Screening trial Ponta Grossa 6 - `S-PG6`

Objectives:

- Evaluate different combinations of pine oil and δ-limonene with AA

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	25/04/2012	RAPRA	2-4L	1	15-3 Pin + 15-3 AA + 4 NPE
Time:	11:30	BRAPL	3L,2T	2	15-3 Pin + 30-5 AA + 4 NPE
Temperature:	22 °C			3	30-5 Pin + 15-3 AA + 8 NPE
Cloud cover:	60 %			4	30-5 Pin + 30-5 AA + 8 NPE
Humidity:	61 %				
Evaluation:	5 DAA			5	15-3 Lim + 15-3 AA + 4 NPE
				6	15-3 Lim + 30-5 AA + 4 NPE
				7	30-5 Lim + 15-3 AA + 8 NPE
				8	30-5 Lim + 30-5 AA + 8 NPE
				All	**120 NaCl - 600 L ha^{-1}**

Box 2.14 Screening trial Ponta Grossa 7 - `S-PG7`

Objectives:

- Determine lowest effective dose of pine oil and AA in combination with NaCl

Conditions		**Weed**	**Stage**	**Formulations** (4 repetitions)	
Date:	30/04/2012	RCHBR	4L,3T	1	8-3 Pin + 8-3 AA + 2 NPE
Time:	14:00	RAPRA	4-6L	2	15-3 Pin + 15-3 AA + 4 NPE
Temperature:	16 °C	BRAPL	3L,3T	**All**	**120 NaCl - 600 L ha^{-1}**
Cloud cover:	50 %	IAQGR	10-12L		
Humidity:	54 %	EPHHL	6-10L		
Evaluation:	3 DAA				

Box 2.15 Screening trial Ponta Grossa 8 - `S-PG8`

Objectives:

- Determine lowest effective dose of pine oil and AA in combination with NaCl

Conditions		**Weed**	**Stage**	**Formulations** (2 repetitions)	
Date:	02/05/2012	BRAPL	3L,3T	1	8-3 Pin + 8-3 AA + 2 NPE
Time:	14:00	IAQGR	10-12L	2	15-3 Pin + 15-3 AA + 4 NPE
Temperature:	22 °C	RAPRA	4-6L	**All**	**120 NaCl + 8 NPE - 600 L ha^{-1}**
Cloud cover:	20 %	EPHHL	6-10L		
Humidity:	69 %	RCHBR	4L,3T		
Evaluation:	5 DAA				

Box 2.16 Screening trial Ponta Grossa 9 - `S-PG9`

Objectives:

- Comparison of five doses of NaCl (0, 15, 30, 60 and 90 kg ha^{-1}): 1–5
- Comparison of four doses of AA (0, 0.5, 1 and 2 L ha^{-1}): 6–9
- Comparison between Renex 95® (NPE) and Tween 20® emulsifier: 10–13

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	16/10/2012	IAQGR	2-4L	1	30-5 Pin + 6-1 AA + 0 NaCl
Time:	15:30	RAPRA	4-6L	2	30-5 Pin + 6-1 AA + 15 NaCl
Temperature:	24 °C	BRAPL	1-2L	3	30-5 Pin + 6-1 AA + 30 NaCl
Cloud cover:	85 %			4	30-5 Pin + 6-1 AA + 60 NaCl
Humidity:	62 %			5	30-5 Pin + 6-1 AA + 90 NaCl
Evaluation:	3 DAA				
				6	30-5 Pin + 0 AA + 60 NaCl
				7	30-5 Pin + 3-0.5 AA + 60 NaCl
				8	30-5 Pin + 6-1 AA + 60 NaCl
				9	30-5 Pin + 12-2 AA + 60 NaCl
				10	60-10 Pin + 7.5 NPE
				11	60-10 Pin + 7.5 T20
				12	120-20 Lim + 7.5 NPE
				13	120-20 Lim + 7.5 T20
				All	**8 NPE - 600 L ha^{-1}**

Box 2.17 Screening trial Ponta Grossa 10 - `S-PG10`

Objectives:

- Comparison of NaCl application, with and without emulsifier

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	17/10/2012	IAQGR	2-4L	1	30 NaCl + 0 NPE
Time:	14:00	RAPRA	4-6L	2	30 NaCl + 7.5 NPE
Temperature:	27 °C	BRAPL	1-2L	**All**	**600 L ha^{-1}**
Cloud cover:	30 %				
Humidity:	60 %				
Evaluation:	2 DAA				

Box 2.18 Screening trial Ponta Grossa 11 - `S-PG11`

Objectives:

- Comparison of four doses (2.5, 3.3, 5, 10 L ha^{-1}) of NPE emulsifier: 1–4
- Comparison of three types of emulsifiers (NPE, T20, T80): 5–8

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	05/03/2013	1AMAG	4-6L	1	50-8 + 50 NaCl + 10 NPE
Time:	09:30			2	50-8 + 50 NaCl + 5 NPE
Temperature:	24 °C			3	50-8 + 50 NaCl + 3.3 NPE
Cloud cover:	90 %			4	50-8 + 50 NaCl + 2.5 NPE
Humidity:	75 %				
Evaluation:	3 DAA			5	50 NaCl + 10 NPE
				6	50 NaCl + 10 T80
				7	50 NaCl + 10 T20
				8	50 NaCl + 0 Emulsifier
				All	**600 L ha^{-1}**

Box 2.19 Screening trial Ponta Grossa 12 - `S-PG12`

Objectives:

- Comparison of four doses (0, 25, 50, 100 L ha^{-1}) of δ-limonene and pine oil: 1–4 and 5–8
- Comparison of four doses (2.5, 3.3, 5, 10 L ha^{-1}) of NPE emulsifier: 9–12
- Comparison of three types of the emulsifiers NPE, T20, T80 and MSO: 13–16

Conditions		**Weed**	**Stage**	**Formulations** (2 repetitions)	
Date:	18/03/2013	RAPRA	4-5L	1	0-0 Lim 25 NaCl + 10 NPE
Time:	09:50	EPHHL	3L	2	25-4 Lim + 25 NaCl + 10 NPE
Temperature:	17 °C	IAQGR	3L	3	50-8 Lim + 25 NaCl + 10 NPE
Cloud cover:	99 %	BRAPL	4L	4	100-17 Lim + 25 NaCl + 10 NPE
Humidity:	81 %				
Evaluation:	2 DAA			5	0-0 Pin + 25 NaCl + 10 NPE
				6	25-4 Pin + 25 NaCl + 10 NPE
				7	50-8 Pin + 25 NaCl + 10 NPE
				8	100-17 Pin + 25 NaCl + 10 NPE
				9	50-8 Lim + 50 NaCl + 10 NPE
				10	50-8 Lim + 50 NaCl + 5 NPE
				11	50-8 Lim + 50 NaCl + 3.3 NPE
				12	50-8 Lim + 50 NaCl + 2.5 NPE
				13	50-8 Lim + 50 NaCl + 10 NPE
				14	50-8 Lim + 50 NaCl + 10 T20
				15	50-8 Lim + 50 NaCl + 10 T80
				16	50-8 Lim + 50 NaCl + 10 MSO
				All	**600 L ha^{-1}**

Box 2.20 Screening trial Ponta Grossa 13 - `S-PG13`

Objectives:

- Comparison of three doses of NaCl (0, 25, 50 kg ha^{-1})
- Comparison of three doses of emulsifier (0, 5, 10, 15 L ha^{-1})
- Comparison of the emulsifiers NPE, T80 and MSO

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	24/04/2013	EPHHL	6-8L	1	0 NaCl + 0 Emulsifier
Time:	10:30			2	25 NaCl + 0 Emulsifier
Temperature:	17 °C			3	50 NaCl + 0 Emulsifier
Cloud cover:	90 %				
Humidity:	79 %			4	0 NaCl + 5 NPE
Evaluation:	8 DAA			5	0 NaCl + 10 NPE
				6	0 NaCl + 15 NPE
				7	25 NaCl + 5 NPE
				8	25 NaCl + 10 NPE
				9	25 NaCl + 15 NPE
				10	50 NaCl + 5 NPE
				11	50 NaCl + 10 NPE
				12	50 NaCl + 15 NPE
				13	0 NaCl + 5 T80
				14	0 NaCl + 10 T80
				15	0 NaCl + 15 T80
				16	25 NaCl + 5 T80
				17	25 NaCl + 10 T80
				18	25 NaCl + 15 T80
				19	50 NaCl + 5 T80
				20	50 NaCl + 10 T80
				21	50 NaCl + 15 T80
				22	0 NaCl + 5 MSO
				23	0 NaCl + 10 MSO
				24	0 NaCl + 15 MSO
				25	25 NaCl + 5 MSO
				26	25 NaCl + 10 MSO
				27	25 NaCl + 15 MSO
				28	50 NaCl + 5 MSO
				29	50 NaCl + 10 MSO
				30	50 NaCl + 15 MSO
				All	**600 L ha^{-1}**

Box 2.21 Screening trial Ponta Grossa 14 - `S-PG14`

Objectives:

- Determine effect of sulphonic acid applied alone: 1+2
- Comparison of different mixtures of SA with MSO
- Comparison of three doses of NaCl (0, 25, 50 kg ha^{-1})

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	24/04/2013	EPHHL	6-8L	1	2 SA
Time:	10:30			2	4 SA
Temperature:	17 °C				
Cloud cover:	90 %			3	0 NaCl + 2 MSO + 2 SA
Humidity:	79 %			4	0 NaCl + 2 MSO + 4 SA
Evaluation:	8 DAA			5	0 NaCl + 4 MSO + 2 SA
				6	0 NaCl + 4 MSO + 4 SA
				7	25 NaCl + 2 MSO + 2 SA
				8	25 NaCl + 2 MSO + 4 SA
				9	25 NaCl + 4 MSO + 2 SA
				10	25 NaCl + 4 MSO + 4 SA
				11	50 NaCl + 2 MSO + 2 SA
				12	50 NaCl + 2 MSO + 4 SA
				13	50 NaCl + 4 MSO + 2 SA
				14	50 NaCl + 4 MSO + 4 SA
				All	**600 L ha^{-1}**

Box 2.22 Screening trial Ponta Grossa 15 - `S-PG15`

Objectives:

- Determine effect of sulphonic acid factorial combinations of SA and T80 at different rates

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	03/05/2013	RAPRA	2-4 L	1	2.5 SA
Time:	14:30	BRAPL	2-4 L	2	5 SA
Temperature:	27 °C	EPHHL	2-4 L	3	10 SA
Cloud cover:	50 %			4	2.5 SA + 5 T80
Humidity:	50 %			5	5 SA + 5 T80
Evaluation:	8 DAA			6	10 SA + 5 T80
				7	2.5 SA + 10 T80
				8	5 SA + 10 T80
				9	10 SA + 10 T80
				All	**600 L ha^{-1}**

Box 2.23 Screening trial Ponta Grossa 16 - `S-PG16`

Objectives:

- Comparison of three different essential oils in combination with SA

Conditions		**Weed**	**Stage**	**Formulations** (3 repetitions)	
Date:	08/05/2013	IAQGR	2-4L	1	60-10 Lim + 3 SA
Time:	13:40	RAPRA	3-4L	2	60-10 Pin + 3 SA
Temperature:	18 °C			3	60-10 Ter + 3 SA
Cloud cover:	0 %			**All**	**600 L ha^{-1}**
Humidity:	60 %				
Evaluation:	2 DAA				

Box 2.24 Screening trial Ponta Grossa 17 - `S-PG17`

Objectives:

- Comparison of three different emulsifiers in formulation with pine oil

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	14/05/2013	IAQGR	2-4L	1	60-10 Pin + 3 SA
Time:	13:50	RAPRA	3-4L	2	60-10 Pin + 3 T80
Temperature:	20 °C	RCHBR	2-4L	3	60-10 Pin + 3 T20
Cloud cover:	90 %			**All**	**600 L ha^{-1}**
Humidity:	65 %				
Evaluation:	4 DAA				

Box 2.25 Screening trial Ponta Grossa 18 - `S-PG18`

Objectives:

- Comparison of three different emulsifiers in formulation with pine oil

Conditions		**Weed**	**Stage**	**Formulations**	
Date:	20/05/2013	RCHBR	4-6L	1	50-8 Pin - 3 T20
Time:	15:00	RAPRA	6-8L	2	50-8 Pin - 3 SA
Temperature:	22 °C			3	50-8 Pin - 3 T80
Cloud cover:	90 %			**All**	**600 L ha^{-1}**
Humidity:	65 %				
Evaluation:	4 DAA				

2.3.3 Design field trials

The field trials were all realised in season 2012/13. At site `LO` one trial with soybean, and three trials with phaseolus bean were implanted. However, none of the phaseolus bean trials could be evaluated due to strong virus infection and a prolonged draught while the main irrigation pump of the experimental farm was defect. In Ponta Grossa, one trial with soybean, and three trials with phaseolus bean crop were realised. Of the above mentioned active ingredient (AI) and emulsifiers tested in screening trials, only the AI δ-limonene, pine oil and NaCl and the emulsifiers Renex 95®, Tween 80® and Nu-Film P® were

Table 2.5: Soil granulometry, cation exchange capacity (CEC), base saturation (BS), saturation with Al^{3+} and soil pH in natural herbicide trials. All analyses were realised at IAPAR, Londrina. For Ponta Grossa herbicide trials soil analysis data of trial `PG1-PS` is shown, because the same field was used for experiments.

Trial	Clay %	Silt %	Sand %	CEC cmol$_c$ dm^{-3}	BS %	Al^{3+} %		pH
`PG-NH`, `PG-Lim`, `PG-Pin`, `PG-NaCl`	64	16	20	14.8	55.7	0.0	L (1)	5.1
`LO-NH`	77	20	3	13.0	40.0	5.0	M	4.8

[1] L: low level, M: medium level, and H: high level of exchangeable Al^{3+} according to (Embrapa Soja, 2007).

employed in field trials. The two essential oils were selected due to their relatively low price, efficacy (pine oil) and local availability. Only in field trial `LO-NH` soil samples were taken. Nevertheless, trials `PG-Soja`, `PG-Lim`, `PG-Pin` were all installed adjacent to the area of trial `PG1-PS`, hence soil analysis data from this trial was adopted (table 5.1). For more information on soil nutrient contents of Ponta Grossa NH trials, please refer to table 5.2 in the soybean fertiliser trial results section.

Soybean trial Ponta Grossa (`PG-NH`) Results of screening trials of 2012 strongly suggested that a single application does not assure efficient weed control as plant recovery was often observed and it was estimated that at least two or three applications were necessary. Therefore it was decided to investigate the effect of different numbers of NH applications with two NH formulations in a field trial. The soybean natural herbicide trial in *Ponta Grossa* (`PG-NH`) was a two-factorial split-plot trial. The whole-plot factor was the degree of weed infestation. Large plots with different weed densities were available, because in years preceding the experiment a RBD design trial with four repetitions on weed seed-bank reduction had been conducted in the experimental area of `PG-NH`. This trial left four plots per block with different weed densities, each of which had an area of approximately 14 × 14 m. The `High` and `Low` weed density plots of each block were selected as the whole-plot treatment in a split-plot trial design. The split-plot treatments consisted of six NH treatments as well as a `Weedy` and a `Clean` control treatment. Two formulations nearly identical NH formulations were used, which only differed in the essential oil used. They consisted of 50 L ha^{-1} essential oil - δ-limonene or pine oil - 50 kg ha^{-1} NaCl and 10 L ha^{-1} nonylphenol ethoxylate (NPE) (ratio oil:emulsifier of 5:1). The application rate was 600 L ha^{-1} (box 2.26). Both NH formulations were applied once, twice, or three times. For herbicide trials, the same abbreviations used in screening trials are used (refer to box 2.4). The design of `PG-NH` is as follows:

The pre-crop black oats, which left a residue of about 4.5 t ha^{-1} was treated with a roller crimper three weeks before soybean planting. Trial conditions in `PG-NH` cannot be considered fully organic, as a conventional herbicide was applied after roller-crimper treatment of straw residue, in order to create homogeneous conditions for all plots. The major weed species emerging afterwards were *Bidens pilosa*, *Euphorbia heterophylla*, *Brachiaria plantaginea*, *Alternanthera tenella*, *Digitaria sp.*, *Ipomoea largifolia*, *Oxalis sp.*, *Cyperus rotundus* and *Richardia brasiliensis*. As in most fertiliser trials the soybean variety *Embrapa BRS 284* was used. Aiming for an interval between applications of around a week, applications were realised 7 days after emergence (DAE), 22 DAE, and 27 DAE. For data analysis `Clean` and / or `Weedy` were removed depending on the observed parameter. For example, for the analysis of the parameters weed shoot dry mass or weed injury rating the inclusion of `Clean` treatment is not meaningful. Also, for analysis of variance (ANOVA)s with the factors *formulation* and number of spray *applications*, which were contained within the split-plot treatments, both `Clean` and `Weedy` control treatments were left out in order to obtain orthogonality.

Box 2.26 Soybean direct-seeding NH trial Ponta Grossa (`PG-NH`)

Objectives:

- Determine the effect of one, two and three NH applications on weed and crop development.
- Study the effect of high and low weed density on NH efficacy.

Trial setup

Design Two factorial split-plot (2 × 8, $n = 4$) – Plots: 6 m long, 5 rows with 0.45 m row width
Sowing 07/11/2012
Spray application 110-02 spray nozzle - No protective screen was used!
Weed and soybean shoot dry mass 18/02/2013 - 96 DAE, growth stage: BBCH 73 or R3
Harvest 25/03/2013 (131 DAE)

Whole-plot: weed density

1	`Low`	Low density
2	`High`	High density

Split-plot: spray treatments

1	`Clean`	Clean control
2	`Weedy`	Weedy control
3	`1x Lim`	1x: 50-8 Lim + 50 NaCl + 10 NPE - 600
4	`2x Lim`	2x: 50-8 Lim + 50 NaCl + 10 NPE - 600
5	`3x Lim`	3x: 50-8 Lim + 50 NaCl + 10 NPE - 600
6	`1x Pin`	1x: 50-8 Pin + 50 NaCl + 10 NPE - 600
7	`2x Pin`	2x: 50-8 Pin + 50 NaCl + 10 NPE - 600
8	`3x Pin`	3x: 50-8 Pin + 50 NaCl + 10 NPE - 600

Leaving out the two control treatments, the factors *formulation* (`Lim`, `Pin`) and number of *applications* (`1x`, `2x`, `3x`) are nested within the split-plot NH treatments.

Application (1)		Time	T (°C)	rH (%)	Cloud cover (%)	Evaluation (2)
1st	8 DAE	15:30	30	35	20	6 DAA-1
2nd	14 DAE	18:00	29	36	0	8 DAA-2
3rd	22 DAE	14:30	26	76	80	5 DAA-3

[1] Application date in days after emergence (DAE)
[2] Date of visual weed control rating in days after application (DAA)

Soybean trial Londrina (`LO-NH`) In the natural herbicide trial in *Londrina* (`LO-NH`) two different application strategies were compared using NH formulations containing either the essential oil δ-limonene or pine oil and NaCl. Assuming that two or three applications are necessary for efficient weed control it was questioned whether application of given amounts of AI should rather be realised in a concentrated form with two applications (*concentrated*) or in a more diluted form with three applications (*diluted*). The total amounts of AI applied were 90 L ha^{-1} essential oil, 90 kg ha^{-1} NaCl and 18 L ha^{-1} NPE. The trial was set up with the following design:

`LO-NH` was the only herbicide trial conducted under conditions which closely resemble those of Organic Agriculture (OA). The pre-crop oats was sown into superficially harrowed soil before winter, chopped in spring, and soybean was direct-seeded two weeks after. The pre-crop black oats left a straw residue of about 3.5 t ha^{-1}. No noteworthy weed infestation was present at seeding, and only few mature weed plants had to be removed manually to guarantee similar conditions between plots. Before the first application the weed density was determined to be around 100 plants m^{-2}. The major weed species were *Bidens pilosa*, *Brachiaria plantaginea*, *Digitaria*, *Ipomoea largifolia*, *Oxalis sp.*, *Alternanthera tenella*, *Cyperus rotundus*, *Richardia brasiliensis* and *Euphorbia heterophylla*. In trial `LO-NH` weed injury ratings were not recorded and instead weed cover and weed shoot dry mass evaluated. The soybean parameters evaluated were soybean shoot dry mass, soybean height, soybean harvest and soybean grain quality. Shoot dry mass of weed and soybean were evaluated 88 DAE at early grain formation of soybean (BBCH 75, about R2).

Box 2.27 Soybean direct-seeding NH trial Londrina (`LO-NH`)

Objectives:

- Study the effect of NH formulations under field conditions in direct-seeding soybean
- Compare the effect of two concentrated with that of three diluted applications.

Trial setup:

Design Latin square 6 × 6 – Plots: 6 m long, 5 rows with 0.4 m row width
Sowing 15/10/2012
Spray application 80-03E spray nozzle - Plants were protected with PVC tubing (∅ 12 cm)
Soybean and weed shoot dry mass 17/01/2013 - 88 DAE, growth stage: BBCH 72 or R3
Harvest 28/02/2012 - 130 DAE

Treatments

1	`Clean`	Clean control
2	`Weedy`	Weedy control
3	`2x Lim-C`	2x: 45-8 Lim + 45 NaCl + 9 NPE - 600
4	`3x Lim-D`	3x: 30-5 Lim + 30 NaCl + 6 NPE - 600
5	`2x Pin-C`	2x: 45-8 Pin + 45 NaCl + 9 NPE - 600
6	`3x Pin-D`	3x: 30-5 Pin + 30 NaCl + 6 NPE - 600

In all spray treatments the same total quantity of ingredients is applied: 90 L ha^{-1} essential oil + 90 kg ha^{-1} NaCl + 18 L ha^{-1} NPE

Application (1)		Time	T (°C)	rH (%)	Cloud cover (%)	Evaluation (2)
1st	6 DAE	10:30	26	64	30	8 DAA-1
2nd	17 DAE	10:00	24	57	40	7 DAA-2
3rd	24 DAE	11:00	22	63	0	15 DAA-3

[1] Application date in days after emergence (DAE)
[2] Date of visual weed control rating in days after application (DAA)

Common bean trials Ponta Grossa (`PG-Lim`, `PG-Pin` and `PG-NaCl`) In late season 2013 three field trials with phaseolus bean crop (*Phaseolus vulgaris*) were conducted on the same field as `PG1-PS` in the previous year. Lacking a suitable pre-crop in the late season the field was prepared with conventional tillage (CT). Variety IAPAR Tuiuiú was planted with *Kuhn PG 700* planter (7 rows), and NPK 4-30-10 fertiliser applied at 350 kg ha^{-1}. After emergence the trial plots were inserted into the field. All three trials were implemented as a RBD with $n = 4$. Plots were 5 m long, with 4 rows and a row width of 0.45 m (box 2.28). Results from screening trial `S2` suggested that the dilution of a given amount of essential oil has an effect on formulation efficacy, with a concentrated application yielding better weed control. A more concentrated application of AI could therefore possibly enhance formulation efficacy and/or lower application costs. In field trials `PG-Lim` and `PG-Pin` it was intended to examine the effect of concentration and dilution in a crop stand, with a formulation containing δ-limonene in trial `PG-Lim` and pine oil in trial `PG-Pin`. In `PG-Lim`, δ-limonene doses of 25, 50 and 75 L ha^{-1} were applied at an application rate of 300, 600 and 900 L ha^{-1}, however, not all combinations of dose and application rate were tested. Pine oil in trial `PG-Pin` was applied at 25 and 50 L ha^{-1}, each with an application rate of 300 and 300 L ha^{-1}. Spray treatments in both trials contained a fixed dose of 50 kg ha^{-1} NaCl and 2.5 L ha^{-1} of the emulsifier Tween 80$^{®}$ (T80). Each of the spray treatments was applied twice. The first of the two applications was realised 11 DAE (`PG-Lim`) and 13 DAE (`PG-Pin`) at common bean growth stage V2. Compared to the other two NH field trials, the interval between the first and second application was shorter, with three days after the first application for `PG-Pin` and four days for trial `PG-Lim`. Different from other NH field trials, weed shoot dry mass and common bean shoot dry mass were not evaluated simultaneously, and were realised 46 DAE and 73 DAE, respectively. The third CT common bean trial was set up in order to study possibly negative effects of NaCl application on crop development. Therefore different doses of NaCl were spray applied directly to the soil at quantities similar to those used in other NH trials. In this trial, referred to as `PG-NaCl`, 50 kg ha^{-1} NaCl was band sprayed at an application rate of 600 L ha^{-1} between the common bean rows. Treatments were a control treatment with no application (`Ctl`) as well as one, two three applications (`1x NaCl`, `2x NaCl`, `3x NaCl`, respectively), each at an interval of five days and without the use of a protective screen. All plots in this trial were kept weed free manually.

Box 2.28 Common bean tillage NH trials Ponta Grossa (`PG-Lim`, `PG-Pin` & `PG-NaCl`)

Objectives:

- `PG-Lim`, `PG-Pin`: Compare the effect of formulations containing different AI rates and dilutions on weed control and crop growth under field conditions.
- `PG-NaCl`: determine if high doses of NaCl applied to soil affect crop growth negatively.

Trial setup:

Design one-factorial RBD, $n = 4$ – Plots: 6 m long, 5 rows with 0.45 m row width
Sowing 01/02/2013
Spray application 110-02 spray nozzle - In all three trials no protective screen was used!
Common bean shoot dry mass 26/03/2013 - 46 DAE, growth stage: BBCH 65 or R1
Weed shoot dry mass 22/04/2013 - 73 DAE
Harvest 09/05/2013 - 90 DAE

`PG-Lim`		`PG-Pin`		`PG-NaCl`	
1	Clean control	1	Clean control	1	Control
2	Weedy control	2	Weedy control	2	1x 50 NaCl - 50 kg ha^{-1}
3	50-16 Lim - 300	3	50-16 Pin - 300	3	2x 50 NaCl - 100 kg ha^{-1}
4	50-8 Lim - 600	4	50-8 Pin - 600	4	3x 50 NaCl - 150 kg ha^{-1}
5	50-6 Lim - 900	5	25-8 Pin - 300	**2–4**	**600 L ha^{-1}**
6	25-8 Lim - 300	6	25-4 Pin - 600		
7	75-8 Lim - 900	**3–6**	**50 NaCl + 2.5 T80**		
3–7	**50 NaCl + 2.5 T80**				

Trial	Application (1)		Time	T (°C)	rH (%)	Cloud cover (%)	Evaluation (2)	
`PG-Lim`	1st	11 DAE	12:00	28	62	20	4	DAA-1
`PG-Lim`	2nd	15 DAE	11:30	24	79	40	6 & 13	DAA-2
`PG-Pin`	1st	13 DAE	12:00	26	59	20	2	DAA-1
`PG-Pin`	2nd	16 DAE	11:30	25	68	30	5 & 12	DAA-2
`PG-NaCl`	1st	13 DAE	- (3)	-	-	-	-	
`PG-NaCl`	2nd	16 DAE	-	-	-	-	-	
`PG-NaCl`	3rd	25 DAE	-	-	-	-	-	

[1] Application date in days after emergence (DAE)
[2] Date of visual weed control rating in days after application (DAA)
[3] `PG-NaCl` only aimed on determining the effect of soil applied NaCl on the crop.

2.4 Evaluations

Straw residue was determined by sampling (4 × 0.5 m^2 or 8 × 0.25 m^2 per trial) in all DS trials apart from `KA` where estimation occurred visually. Per plot stand density was determined about 10 days after emergence with a standard of three counts ranging from 1–3 m each depending on experiment. For NH trials weed density was determined shortly after crop emergence, differentiating between weed species. Therefore, each `Weedy` control plot was counted three times with the help of a metal frame of 50 × 50 cm (0.25 m^2), or alternatively, five times with a *Göttinger Schätzrahmen* (0.1 m^2).

2.4.1 Shoot dry mass, grain yield and thousand kernel weight

Shoot dry mass harvests of fertiliser trials in both countries and of NH field trials were carried out with the same methodology. Faba bean shoot dry mass was evaluated twice and soybean and common bean once (table 2.3 and table 2.4). For evaluation of shoot dry mass, plants in areas ranging from 0.6–1.2 m^2 (2–4 rows at a length of 1–3 m) were cut close to the ground, usually at one spot per plot, however, for trials at `KA` and `LO1` two samples were taken for each plot. In all cases a total area of at least 1.0 m^2 per plot was cut. As an addition to stand density counts plants were also counted during shoot drymass harvests in the field (referred to as *sample density*). At first the fresh weight for all shoot samples was determined, then the material was immediately shredded. Of the shredded material an aliquot was taken and weighed. For the determination of moisture content the aliquot was dried for at least 24 h at 60 °C in aluminium dishes until no more moisture was apparent in the sample. Afterwards the sample was dried another 24 h at 105 °C and weighed immediately upon removal from the oven. From sample humidity total dry mass was calculated. The dried sample material was stored for nutrient analysis. All analysis of plant material were conducted at IOL institute. In `WG` 2012 trials and NH field trials `LO-NH` and `PG-Soja` in Brazil, weed shoot dry mass was evaluated simultaneously and analogous to crop shoot dry mass. After removal of crop shoots, 0.7–1.2 m^2 of the weeds growing in the same area were cut. Usually, weed species were not differentiated for determination of shoot dry mass. For the two common bean trials `PG-Lim` and `PG-Pin` weed dry mass of a similar area was determined, but evaluation occurred late in the crop cycle and not simultaneously with the crop shoot dry mass evaluation. Furthermore, in these trials weed shoot drymass was determined separately for the different weed species present. In both countries crop grain harvest was realised with experimental harvesters with a working width of 1.5 m (Germany: *Firma Hege*, Brazil *Fankhauser*). After harvest, the grain was cleaned from impurities and the moisture content determined analogous to shoot dry mass. Thousand kernel weight (TKW) was measured from 500 grains per plot (seed counting machine in Germany, counting boards in Brazil) and again, residual humidity determined. For both countries grain harvest and thousand kernel weight (TKW) are reported with a moisture content of 13 %. Harvest components were only determined for trials at `KA`, `WG`, `LO` in 2012, and `PG` in 2012. Before harvest 10 plants were removed from each experimental plot and grains per pod, pods per plant and grains per plant determined. However, in the second trial year in Brazil no resources were available for their determination as trial number was markedly higher than in the first year.

2.4.2 Visual weed injury ratings and weed cover

Visual weed injury ratings were usually realised between two to five days after application (DAA) of NH. Weed control was assessed on a visual rating scale from 0 to 100 (table 2.6) as described by (Burrill et al., 1976), which is a similar method to the one used by Barker and Prostak (2014) with natural herbicides. The score ’0’ means ’no control’ and ’100’ ’complete control’ of vegetation. An injury rating surpassing a score of ’70’ can be considered as an ’acceptable’ weed control. This method is established for conventional herbicide testing where systemic and long lasting effects on weed development are common. However, for NH it has to be taken into account that burn-down NH formulations may cause great damage to leaf tissue, but with no or only slight systemic effect. Hence, meristems often survive and resprouting can occur frequently and rapidly. The ratings in this work are therefore not necessarily comparable with conventional herbicide ratings. Of the weed species emerged only the species abundant in number and with presence in all plots were examined, which included some of the most important weed species in Paraná state. In most screenings and field trials, weed injury was rated separately each species, yet in some trials (e.g. `LO-NH`), only general control over all weed species or weed cover were evaluated. General injury ratings were determined by comparing the plot in question with the adjacent blind control (untreated strip next to plot) or the control plot in the same experimental block. Evaluation of weed cover (% coverage) was realised with a *Göttinger Schätzrahmen.*

2.4.3 Soil sampling

In Germany, soil sampling was realised in the control plots with a Pürckhauer single gouge auger from 0–90 cm soil depth, dividing samples into sub-samples of 0–30, 30–60 and 60–90 cm soil depth. These

Table 2.6: Visual weed injury rating scale used in screening and field trials adapted from (Burrill et al., 1976) and Barker and Prostak (2014).

Injury rating	Effects on weed
0	No effect (all foliage green and alive)
1–10	Very light symptoms (very minor chlorosis and/or leaf curling)
11–30	Light symptoms
31–50	Medium (moderate chlorosis and/or leaf curling)
51–70	Fairly heavy damage
71–90	Heavy damage
91–99	Very heavy damage
100	Complete kill (dead)

samples were stored in cool trays in the field and directly frozen after finishing sampling in order to stop mineralisation processes and allow for later N_{min} determination. At site `KA` soil sampling was realised about a week before crop emergence, while at `WG` soil sampling was only realised after harvest, hence no N_{min} contents were determined. In Brazil only top soil from 0–20 cm was sampled in control plots.

2.5 Laboratory analysis

Soil samples from German sites were all analysed at the laboratory of Institute of Organic Agriculture, Bonn University (IOL). Brazilian soil samples were analysed at IAPAR laboratories in Londrina and Ponta Grossa for pH, cation exchange capacity (CEC), K, P, carbon C and soil granulometry. Methods employed are described in Embrapa (Embrapa Solos, 1997). Additionally, total C, N and S contents of Brazilian soil samples were determined by elementary analysis at IOL laboratory in Germany. Plant shoot and grain samples from Germany and Brazil were analysed at IOL laboratory of Bonn University for N, P, K and S contents. Prior to analysis samples were finely ground with a ball mill (*Retsch MM 200*) or a vibratory disc mill (*Retsch RS 200*). For C/N and C/N/S analysis, 2–4 mg (soybean grain 6–8 mg) of plant and soil material were weight in and measurement was conducted twice. For C/N/S samples, vanadium pentoxide (V_2O_5) was added as a catalyst. For each run a tray of 20 caps, consisting of 7 samples (each twice), two blanks and four standards, were measured. The elemental analyser (E/A) was a *EuroVector EA3000*. Sulphur in samples from trials at `KA` , `LO1` and `PG1` as well as in all soil samples was measured by E/A with a C/N/S column. After the first trial year it was apparent that variations of sulphur between sample repetitions were too high in some runs, frequently making measurement repetitions necessary. Only at the end of laboratory analysis it was realised that these large variations in S-measurement were caused by sporadically too low quantities of V_2O_5, which correctly has to be added in excess (no fixed amount). Due to measurement problems of sulphur it was decided for trials `WG`, `LO-2`, `PG-2` and `UM` to measure only C/N by E/A and to use the Inductively Coupled Plasma (ICP), followed by atomic absorption spectroscopy (AAS) for sulphur measurement (Soil Science Institute of Bonn University). Both measurements between the E/A and ICP method resulted in very similar results, but the results of latter exhibited a lower variance. For analysis of P, K shoot and grain samples a common extraction procedure was carried out. All samples were weight in twice, and each subsample measured twice as well, resulting in a total of four measurements per sample. Ground samples of plant material were weight in at 0.1–0.2 g. Afterwards, 5 ml of 65 % nitric acid and 4 ml H_2O_2 were added. Thereafter, samples were digested in the microwave (*CEM MarsExpress*) in pressure resistant tubes for 10 minutes. For sulphur analysis by ICP the same extracts were used. For soil samples P and K were extracted with double lactate (DL) (Hoffmann, 1991). K samples were measured with Atomic Absorption Spectroscopy (AAS, Model: *Perkin-Elmer AAnalysist200*). P was determined spectro-photometrically with *Molybdenum Blue* (Mo-P) method (*Skalar Continuous Flow Analyser*). 50 g of soil were extracted with 100 ml of water and filtered. N_{min} determination was realised photometrically (*Skalar Continuous Flow Analyser*), but only for `KA` trials (VDLUFA). In Germany soil pH was measured according to VDLUFA (Hoffmann, 1991). Soil texture was not determined for German trial sites as data was already available for `KA` and `WG`.

2.6 Statistical analysis

For statistical analysis and plotting *R statistical package* was used (R Core Team, 2015). Prior to ANOVA, data was tested for normality with the *Shapiro Wilk* test (Shapiro and Wilk, 1965) and for homogeneity of variance (homoescacidicity) with the *Levene* test (Levene, 1960). Transformations were not necessary.

Whenever the prerequesites of normality or homoescacidity were not fulfilled non-parametric Kruskal-Wallis test (Kruskal and Wallis, 1952) of `agricolae` package was used for analysis (de Mendiburu, 2015). In the case that prerequesites were met, mixed model ANOVAs were carried out with *lme* function of `nlme` package (Pinheiro et al., 2015). For latin square design the *lmer* function from `lmer` package (Bates et al., 2015) was employed. If significant effects were found in ANOVA ($p < 0.05$), post-hoc *Tukey Honest Significant Difference* (TukeyHSD) (Tukey, 1949) ($\alpha = 0.05$) were carried out using the `multcomp` package (Graves et al., 2015). All results plots in this work were created with the `ggplot2` package (Wickham, 2009).

3 Results A: faba bean trials (Germany)

3.1 Soil conditions

The *haplic luvisol* [15] in *Campus Klein-Altendorf* (`KA`) and *fluvisol* of *Wiesengut* (`WG`) both had a near neutral pH and a soil organic matter (SOM) content in the topsoil (0–30 cm) of 2.0 % (table 3.1). At both sites the K- and P-contents in topsoil were at recommended levels (VDLUFA C), and P-levels at `KA` were even elevated (VDLUFA D) (Kerschberger et al., 1997; Baumgärtel et al., 1999). At site `KA` soil conditions were homogeneous and the only perceivable difference between trials was a generally increased weed density in the plots of trial `KA-P`. The *Wiesengut* (2012, `WG`) trials were also conducted on the same field, but were not set up side-by-side (distances between trials were about 50–100 m). Especially with respect to soil depth and gravel content the soil conditions were heterogeneous and of different production potential between the trials. According to a site classification of Haas (1995) for `WG`, the soil of trial `WG-G` was classified as *intermediate* quality, because of gravel reaching up close to the soil surface, and all other trials as of *good* quality (`WG-PS`, `WG-M`, `WG-K`).

Table 3.1: Soil texture, nutrient contents and pH in topsoil (0–30 cm) of trial sites `KA` and `WG` in Germany. Due to the direct proximity of trials to each other and the similarity of data at each site only mean site values are presented.

Trial	Texture	N_{min} (1) kg ha^{-1}	N %	C %	S %	P mg 100 g^{-1}		K mg 100 g^{-1}		pH
`KA`	Ut3 (2)	53	0.12	1.01	0.015	15.8	D (4)	31.5 ^{c}	C	6.9
`WG`	slU-Su (3)	-	0.12	1.00	0.013	5.9	C	22.0	C	6.0

[1] N_{min} from 0–90 cm soil depth

[2] Silty loam, *Ackerzahl* 70–90

[3] Clayey-silty to sandy-silty floodplain sediment, *Ackerzahl* 20–70

[4] VDLUFA levels of phosphorus (Kerschberger et al., 1997), and potassium (Baumgärtel et al., 1999). **C**: recommended level, **D**: high level.

Total N_{min} from 0–90 cm soil depth in spring of site `KA` was 53 kg ha^{-1}, for `WG` this data is missing. The S_{min} content in spring was not determined, as it is not suited to reliably predict deficiency for other crops than winter-rape Olfs et al. (2012). Furthermore the method cannot estimate how much sulphur is mineralised during the cropping season (Alt et al., 2000; Olfs et al., 2012). The total S-content gave about equal results for both trial sites, but this value also does not provide useful information on crop availability of sulphur. According to the sulphur estimation framework developed at *Limburgerhof* by BASF (*Schwefelschätzrahmen*) a deficiency is likely to occur at site `WG` (28 points), while for site `KA` (31 points) a S-fertilisation is recommendable.

3.2 Fertiliser effect on crop growth and grain yield

In the faba bean direct seeding (DS) trials in Germany at sites *Klein-Altendorf* (`KA`, 2011) and *Wiesengut* (`WG`, 2012) for none of the parameters interactions were found between the treatment factors *fertiliser*, *straw* and straw cutting height (*cut*, only `WG-PS`). The interaction terms in the statistical models were therefore removed and data analysed with an additive model. The results presentation can therefore generally be separated by the experimental factors. For factor *fertiliser* it was considered convenient to graphically present and describe results of trials `KA` and `WG` together. Even though there was a pronounced early spring drought in 2011 (figure 2.1), water potential recorded at `KA` was never critical for faba bean development. The sowing could be realised on time in both trial years. Stand density of `KA` trials was on trial average 44 and 48 plants m^{-2} for `KA-P` and `KA-S`, respectively. The `WG` trials had lower stand densities with 34 (`WG-G`), 30 (`WG-K`), 31 (`WG-M`) and 31 plants m^{-2} (`WG-PS`). Possible caustic effects of `KCl` (chloride) or S^0 (sulphuric acid formation in root proximity) on seedling roots could not be observed in both trial years, which would have affected either stand density or early crop development. Also, none of the other fertiliser treatments affected stand density. Between the two trial sites, shoot dry mass cannot be compared quantitatively due to different growth stages at evaluation. `KA-P` and `KA-S` did not show any

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[15] 'Normparabraunerde aus Löss' (Eckelmann et al., 2005)

significant differences for shoot dry mass or grain yield at both growth stages BBCH[16] 73 and BBCH 81 (s.d.) or any consistent tendencies (figure 3.1). Also shoot height did not show any differentiation (figure 3.2). The average production level of `KA-P` (3.71 t ha^{-1}) would generally be considered intermediate and that of `KA-S` high (4.62 t ha^{-1}). However, as regional organic faba bean yields in 2011 were far above average, e.g. 6.58 t ha^{-1} at the close-by experimental site *Köln Auweiler* (LWK, NRW), the productivity at `KA` has to be considered relatively low. Due to late manual weeding there was a considerable presence of large *Chenopodium album* plants at `KA`, especially in trial `KA-P` - weed shoot dry mass was not evaluated, though. As no ploughed control plots were present, the effect of the DS system on productivity cannot be deducted.

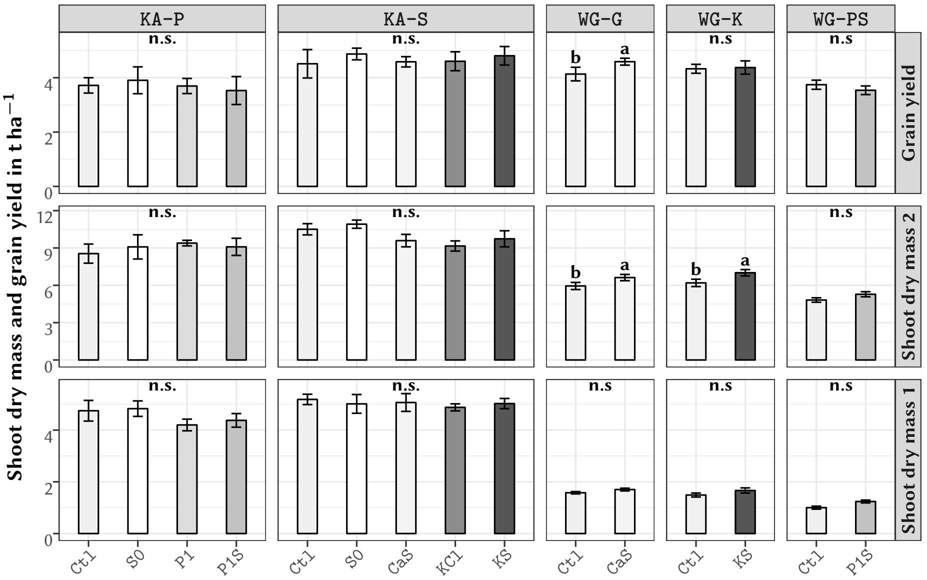

Figure 3.1: Effect of fertiliser treatments on faba bean shoot dry mass and grain yield at sites Klein-Altendorf, *Rheinbach* in 2011 (`KA`) and Wiesengut, *Hennef* in 2012 (`WG`). For `KA` shoot dry mass was evaluated 73 and 102 days after emergence (DAE - growth stages BBCH 73 and BBCH 81, respectively) and grain yield 125 DAE. For `WG` shoot dry mass was evaluated 57 and 89 DAE (BBCH 65 and BBCH 77, respectively), and harvest was realised 136 DAE. Grain yield is reported with 13 % humidity. Treatments were `Ctl`: non-fertilised control, `S0`: elementary sulphur (40 kg ha^{-1} S), `P1`: Gafsa rock phosphate (50 kg ha^{-1} P), `P1S`: joint application of `S0` and `P1`, `CaS`: gypsum ($CaSO_4 \cdot 2\,H_2O$, 40 kg ha^{-1} S) and `KS`: K_2SO_4 (40 kg ha^{-1} S). Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

The two shoot dry mass evaluations of `WG` trials were carried out at an earlier development stage (BBCH 65 and BBCH 77) and average shoot dry mass resulted drastically lower compared with `KA`. Compared with `Ctl`, the shoot dry mass of `CaS` (`WG-G`) was significantly increased at both BBCH 65 (+8 %) and BBCH 77 (+11 %). For the same development stages shoot dry mass for `KS` (`WG-K`) was also increased by 11 % and 13 % (s.d.), respectively. Both `CaS` and `KS` also showed a slightly increased plant height (s.d. for `WG-G`), shoot diameter (s.d. for `WG-K` at 70 DAE) and LAI (figure 3.2, n.s. at both evaluations). For `PS` (`WG-PS`), a slight tendency of increase was visible for height, diameter, and leaf area index (LAI). While `CaS` exhibited a significantly increased yield over `Ctl` (+11 %), there were no yield differences for `KS`. Shoot dry mass for `PS` was higher at both evaluations (+24 % and +9.5 %, both n.s.), however, grain yield was slightly decreased (−5.3 %, n.s.). Also for site `WG` the grain yields in the DS system of mostly above 4 t ha^{-1} (4.9 t ha^{-1} maximum for `WG-M` - not shown) were high compared with yields found in previous years in similar DS trials, such as 3.44 t ha^{-1} for DS compared to 3.8 t ha^{-1} for the mould-board plough (MP) in 2006 and 3.3 t ha^{-1} in 2009 (Köpke and Schulte, 2008; Massucati et al., 2010). Nonetheless, 2012 was also a year with above average faba bean grain yields in the region (6.58 t ha^{-1} at *Köln Auweiler* and 7.28 t ha^{-1} at site `KA`, both conventional tillage). However, even though perennial weeds were removed in `WG` trials, a weed shoot dry mass of 2–3 t ha^{-1} was determined at BBCH 77. Especially in trial `WG-PS`, *Ranunculus sardous* was overgrowing faba bean at the early development in some plots. This is assumed to be one reason why `WG-PS` yield was the lowest of all `WG` trials. In any case lower yields were expected

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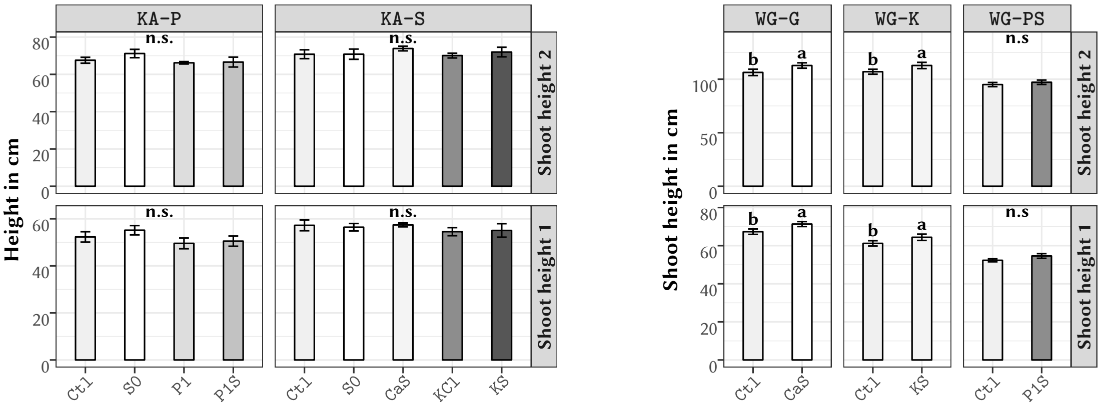
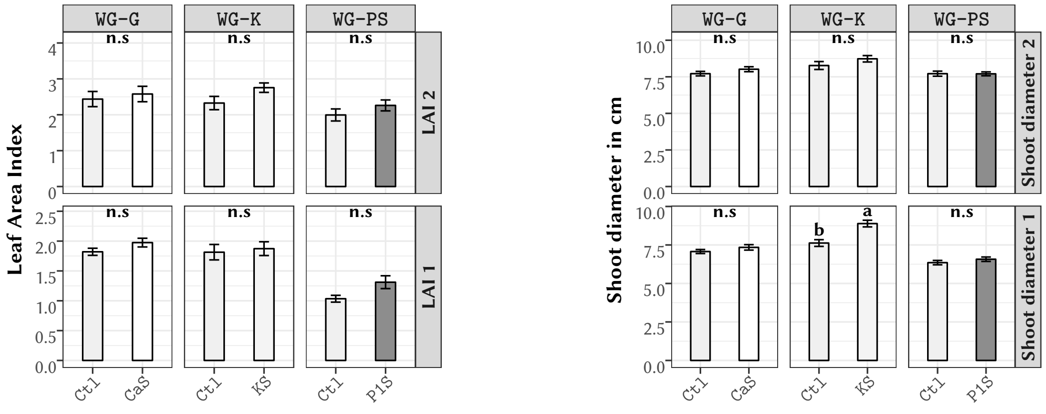
[16] BBCH: Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie weed and crop development scale

for site `WG`. Compared with the profound loam soils at site `KA` with an *Ackerzahl*[17] of 70–80, the soils at `WG` only have an *Ackerzahl* of 20–70.



(a) `KA`: Faba bean shoot height

(b) `WG`: Faba bean shoot height



(c) `WG`: Faba bean LAI

(d) `WG`: Faba bean shoot diameter

Figure 3.2: Effect of fertiliser treatments on shoot height 50 and 70 DAE at site `KA` and on shoot height, shoot diameter and leaf area index 57 and 89 DAE at site `WG`. For treatment abbreviations refer to figure 3.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Similar to all other shoot growth parameters presented, the harvest components thousand kernel weight (TKW), grains per plant, as well as pods per plant were similar within both `KA` trials. The insertion of the first grain containing pod was uniform in all trials with 17–19 cm for `KA` and 33–39 cm for `WG`. Trials `WG-G` and `WG-K` had an increase in the number of grains and the number of pods per plant, while `WG-PS` presented slightly decreased values (none s.d.) for these parameters (figure 3.4).

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[17] *Ackerzahl* means *field score*, which is an index measuring site quality for agricultural production. The base for this index is the *soil score*, which measures soil quality. This number is corrected for with environmental factors favourable or unfavourable for crop production (additions or deductions for climate, terrain inclination, terrain exposition, etc.). The scale has a range from 1 (poor) to 120 (excellent).

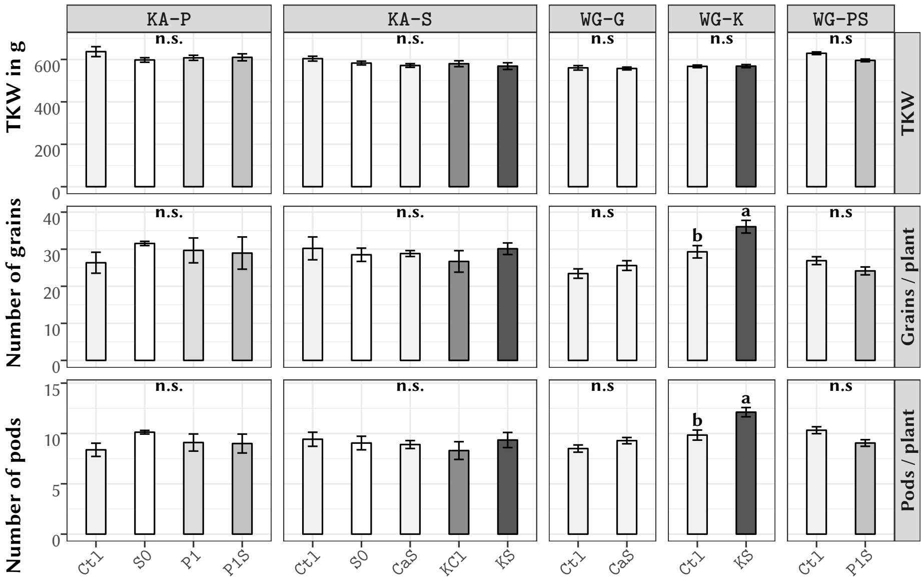


Figure 3.4: Effect of fertiliser treatments on yield components of faba bean at sites `KA` and `WG`. For treatment abbreviations refer to figure 3.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

3.3 Fertiliser effect on nutrient uptake and nutrient concentrations

Both shoot uptake and nutrient concentrations showed similar tendencies for `KA` trials, because both, shoot dry mass and grain yield did not show any differences between treatments. However, as fertilisers did have an effect on crop growth and grain yield in trial `WG` nutrient uptake showed stronger differentiation in nutrient uptake. In order to reference nutrient concentrations observed for shoot and grain in trials, faba bean values found in literature are summarised in table 3.2.

Table 3.2: Literature reference values for faba bean nutrient concentrations in shoot and grain.

Source	Plant part	N (%)	P (%)	K (%)	S (%)
Mayfield et al. 2008	Grain	4.0	0.4	1.0	0.15
Bolland et al. 2000	Grain	4.0	0.3–0.49	1.0	0.16–0.18
Wendland et al. 2012	Grain	4.1	-	1.4	0.2
DLG 1997	Grain	-	0.48	1.3	-
Böhm 2007	Grain	-	0.69	1.4	-
Wahid and Mehana 2000	Grain	-	0.5–0.57	-	-
Griffiths and Thomas 1981	Grain	-	5.9–7.6	-	-
Köhler and Kolbe 2007	Grain	4.2	0.47	1.13	-
Mayfield et al. 2008	Shoot	-	-	-	> 0.2
Hamada and El-Enany 1994	Shoot (40 DAE)	-	-	1.6–2.2	-
Pacyna et al. 2006	Shoot (35 DAE)	-	-	-	0.21
Pacyna et al. 2006	Shoot (50 DAE)	-	-	-	0.14
Pacyna et al. 2006	Shoot (65 DAE)	-	-	-	0.13

3.3.1 Phosphorus

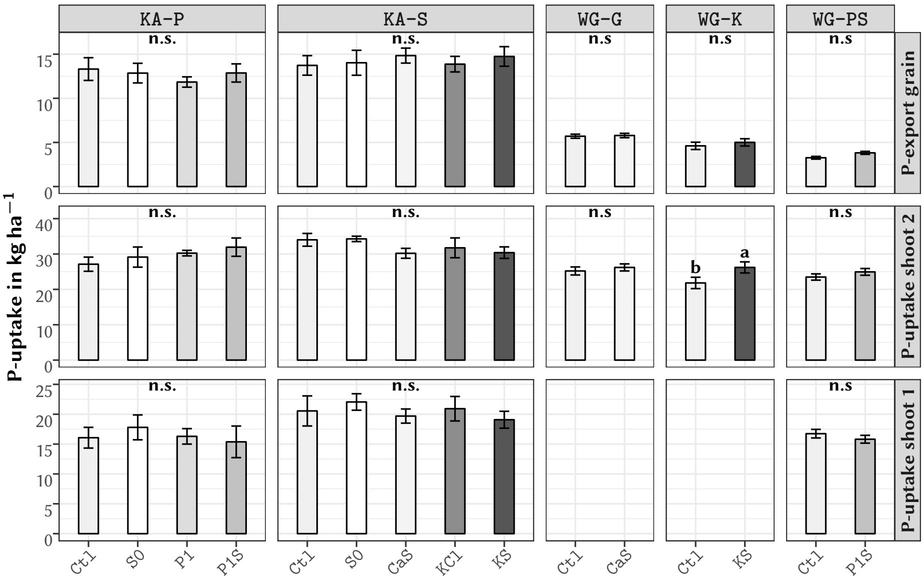
Both `KA` trials showed no clear differentiation with respect to P-concentration and P-uptake in shoot and grain (figure 3.5). The only significant differences were present in `KA-S` and `WG-G`. In `KA-S`, application of both sulphate fertilisers resulted in reduced P-concentrations in grain (`KS` −12.5 %, s.d., `CaS` −5.6 %, s.d.) and reduced P-uptake in grain (n.s.). However, in contrast to that finding shoot P-uptake and -concentrations at BBCH 73 were higher than `Ctl` for treatments `CaS` and `KS` (n.s.), and about equal in all other treatments at BBCH 81. At `WG`, `CaS` had significantly lower shoot P-concentrations (-6.1 %, and -5.7 %), yet shoot uptake was equal between treatments for both evaluations. While shoot P-concentrations of `KS` treatment were equal, P-uptake at BBCH 77 was significantly higher for this treatment. In trial `WG-PS` P-concentration and -uptake did not respond to fertilisation. Reference values in literature for shoot P-concentrations between stages BBCH 65 and BBCH 80 are scarce, however, Nuruzzaman et al. (2005) found shoot P-concentrations of only 1.7–2.0 % about 60 days after emergence (DAE). The values found in both trial years were clearly higher. Grain concentrations of around 0.5 % were in line with concentrations in literature of 0.48 % or 0.50–0.57 % (table 3.2). Also for `Ctl` treatments the values do not appear to be low.

3.3.2 Nitrogen

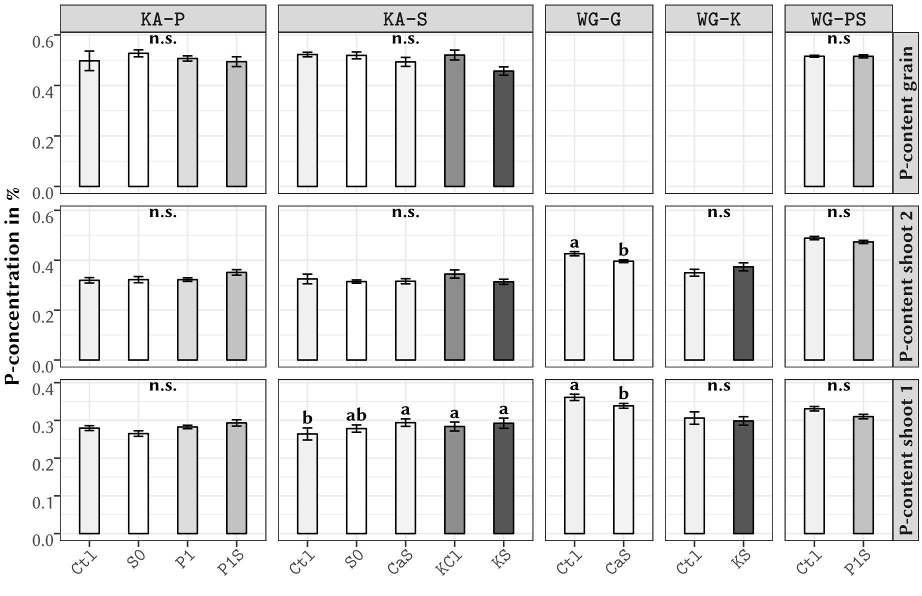
In both trial years N-uptake and -concentration were found neither to differ significantly nor tendentially (figure 3.7). In `WG-G` N-concentration was significantly higher for `CaS` at BBCH 65, but in contrast, was lower at BBCH 77 and in grain (both n.s.) - the significant difference appears to be a random effect in this case. Crude protein concentrations in grain can be calculated by multiplying N-concentration by 6.25 (Kjehldahl nitrogen) which results in concentrations of 25–31 % in grain, values which are commonly found in literature as well (Duranti, 2006).

3.3.3 Sulphur and N:S ratio

In `KA-S` and `KA-P` S-uptake and -concentration showed a clear and mostly significant differentiation for shoot and grain evaluations (figure 3.9). In shoot and grain of `KA-P`, `Ctl` showed the lowest and `P1S` the highest S-uptake (s.d. at stage BBCH 73) and -concentration (s.d.). In both trials, S-uptake and

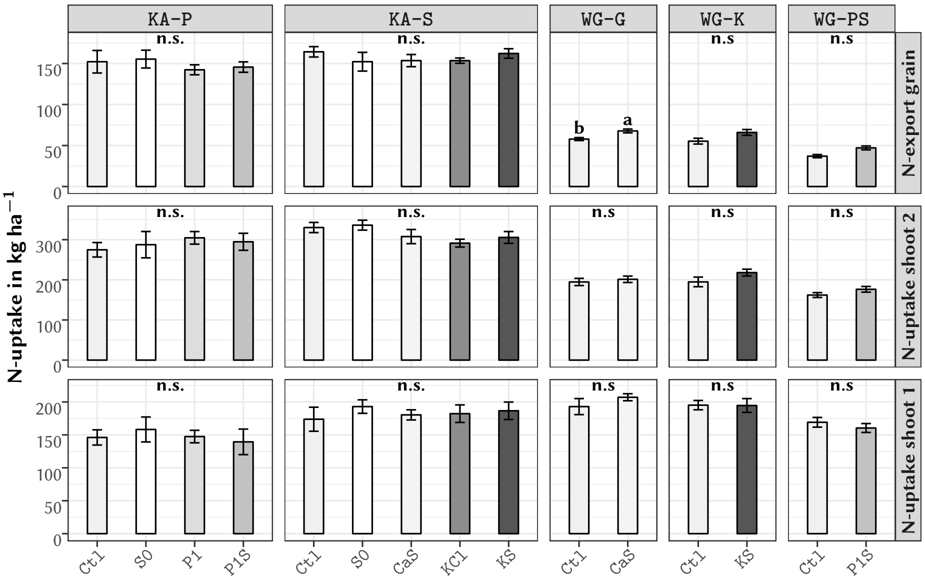


(a) P-uptake into faba bean shoot and grain

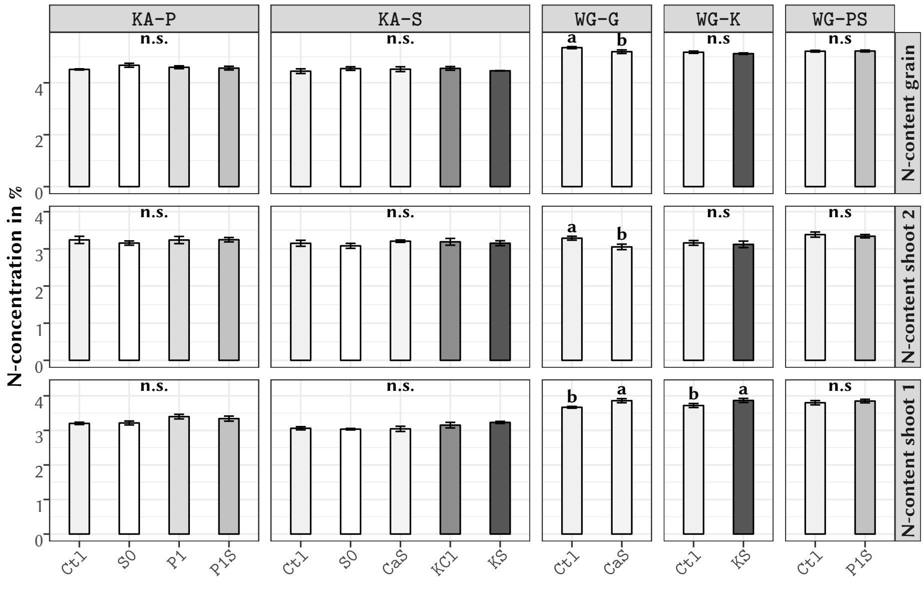


(b) P-concentration in faba bean shoot and grain

Figure 3.5: Effect of fertiliser treatments on P-uptake and P-concentration at both shoot sampling dates and in grain at sites `KA` and `WG`. Grain P-uptake and -concentration data is missing for trials `WG-G` and `WG-K`. For treatment abbreviations refer to figure 3.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).



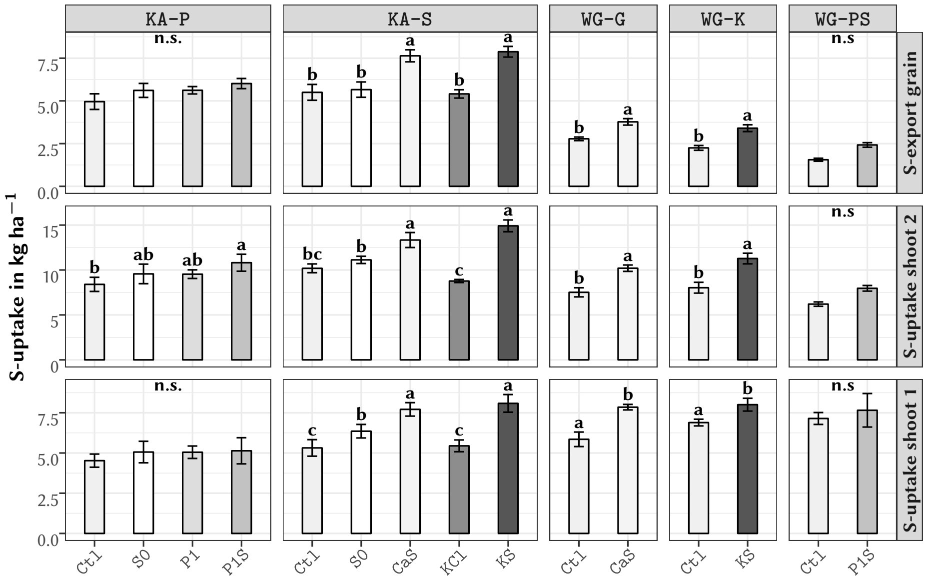
(a) N-uptake into faba bean shoot and grain



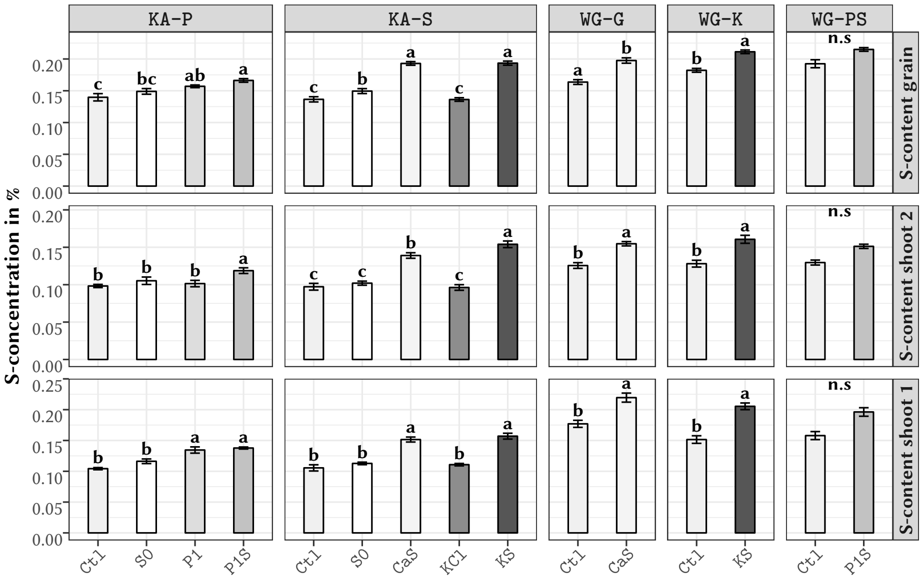
(b) N-concentration in faba bean shoot and grain

Figure 3.7: Effect of fertiliser treatments on N-uptake and N-concentration at both shoot sampling dates and in grain at sites `KA` and `WG`. For treatment abbreviations refer to figure 3.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

-concentration of treatment `S0` were increased (in part significantly) compared with `Ctl`. At growth stage BBCH 73, S-concentration was significantly higher for `P1` than `Ctl`. Nevertheless, values for both these treatments were identical at BBCH 81 and in grain, and S-uptake was also not different for any evaluation. S-concentrations in shoot and grain of **`KA-S`** were observed to form two groups: one of them consisted of treatments `Ctl`, `KCl` and `S0`, which had nearly equal values for S-uptake and -concentration. Only in grain, S-uptake and -concentration were slightly higher (both s.d.) for `S0` compared with `Ctl` and `KCl`. The second group was formed by the sulphate containing treatments `CaS` and `KS`, which both showed strongly increased S-uptake and -concentration for both evaluations (+50–55 % compared with `Ctl`).



(a) S-uptake into faba bean shoot and grain



(b) S-concentration in faba bean shoot and grain

Figure 3.9: Effect of fertiliser treatments on S-uptake and S-concentration at both shoot sampling dates and of grain at sites `KA` and `WG`. For treatment abbreviations refer to figure 3.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Sulphur parameters at `WG` resembled well the results of `KA`. In trials `WG-G` and `WG-K` both shoot and grain S-uptake and -concentration of treatments `CaS` and `KS` were again markedly higher (all s.d.) in comparison to `Ctl`. In two of the experimental blocks of `WG-G` the non-fertilised plots exhibited light green leafs and visibly lower shoot growth, symptoms resembling S-deficiency. During soil sampling it was detected that soil depth was less than 90 cm in some of the trial blocks, which showed particularly high gravel content. Even though not significant, also for `PS` fertilisation a tendency of higher S-uptake and -concentrations was determined. In all `WG` trials, shoot S-concentrations of treatment `Ctl` were similar with 0.15–0.17 % (BBCH 65) and 0.13 % (BBCH 77), while `KS`, `CaS` and `P1S` were markedly higher 0.2–0.23 % and 0.15–0.17 % for the same development stages. The levels of shoot S-concentrations found in both trial years of 0.1–0.23 % are difficult to classify: Olfs et al. (2012) states that for most crops a shoot concentration of 0.3 % is recommended, while the *Grain Legume Handbook* (GRDC: Grains Research and Development Corporation) mentions critical S-levels of 0.2 % (growth stage not specified). Compared with these values shoot S-concentration in trial would have to be considered deficient. However, in a pot experiment by Pacyna et al. (2006) shoot S-concentrations of faba bean were measured at different growth stages, and values continuously decreased from 35 DAE (0.21 %) to 65 DAE (0.13 %) for the S-fertilised treatment. Compared with these results shoot S-concentrations found in `KA` and `WG` trials would all be sufficient. Also grain S-concentrations cannot be classified clearly: for example, reference grain S-concentrations found in literature were 0.15 % (Grain Legume handbook) and 0.16–0.18 % (Bolland et al., 2000). Compared with these values, `Ctl` treatments of `KA` would be considered as deficient, `Ctl` treatments of `WG` as within normal limits, and S-concentrations of the sulphate fertilised treatments of all trials as high. The N:S ratio is helpful to detect S-deficiency and can give information on protein quality (Scherer, 2001). The treatment pattern found was equal to that of S-uptake and -concentration. Nevertheless, in contrast to those parameters all trials apart from `WG-PS` showed significant differences in shoot and grain evaluations (figure 3.11). The N:S ratio was always significantly narrower (s.d.) for both sulphate treatments and `P1S` (only `KA`), as well as for `S0` and `P1` (both in part s.d.).

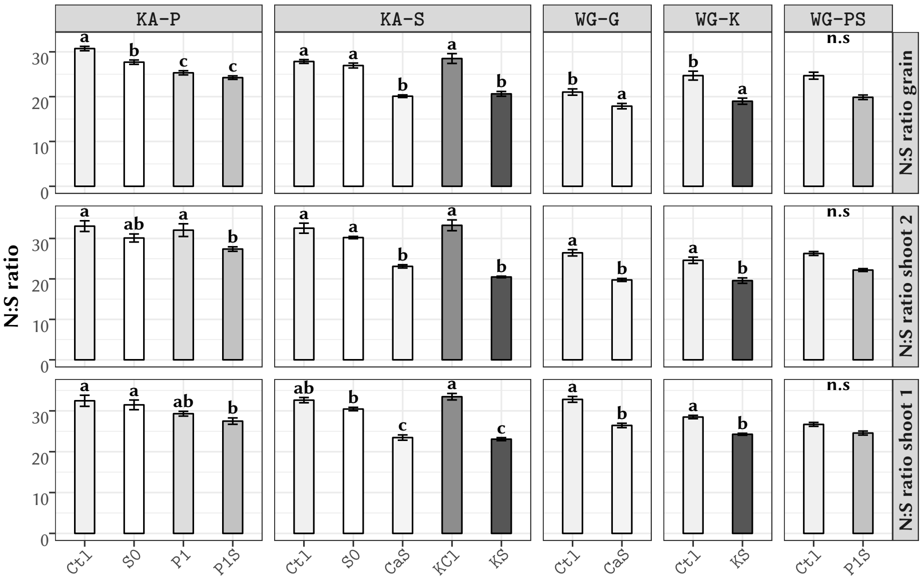


Figure 3.11: Effect of fertiliser treatments on N:S ratio in shoot at both shoot sampling dates and of grain at sites `KA` and `WG`. For treatment abbreviations refer to figure 3.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

3.3.4 Potassium

In all trials, no systematic differentiation for K-concentration in shoot were apparent between fertiliser treatments (figure A.1, appendix on page 127). Elevated K-concentrations in shoot could only be expected for `KS` and `KCl` treatment, but the differences observed were not consistent: at BBCH 73, K-concentration in `KA-S` was highest for `KS` and significantly different from `S0`, while `KCl` and `CaS` were at near identical levels. However, contrasting this result, K-concentrations in shoot at BBCH 81 and in grain were at the same level for all treatments (n.s.). Furthermore, in `WG-K` trial K-concentration of `KS` at BBCH 65 was increased significantly, but at BBCH 77 this difference was only marginal (n.s.). The minimum shoot K-concentration found was 2.0 %, which is at the upper range of values found in literature, e.g. (Hamada and El-Enany, 1994). For interpretation of K-concentrations, Aini and Tang (1998) recommend to apply a critical level of 1.8–2.0 % for the whole shoot-concentration between 48–73 DAE. This critical level was exceeded by far for most treatments. Grain concentrations were also at an acceptable level (DLG, 1997). With soil K-concentrations at both sites at a high level (VDLUFA: D) and in general no response to K-fertiliser application (apart from `KS` of **`WG`** at BBCH 65), the potassium fertilisation with `KS` treatment did not have any effect on crop development. The Ca^{2+} applied with gypsum could also be assumed to not have any effect at both trial sites, which possess a near neutral pH (calcareous application) and hence high levels of Ca^{2+}. In conclusion, the fertiliser effect found for `CaS` and `KS` can be attributed to the application of SO_4^{2-}.

3.4 Summary results fertiliser application

Box 3.1 Summary faba bean fertiliser trials

Crop growth

- **`KA`**: no differences between fertiliser treatments were observed.
- **`WG`**: crop growth was significantly increased for `CaS`, `KS` treatments and tendentially for `PS`.

Nutrient concentrations and uptake

- **`KA`**:
  - S-uptake and concentration in shoot and grain were significantly higher for `CaS` and `KS`.
  - S-concentration of `S0` was increased significantly in grain in both **`KA`** trials.
  - S-concentration of `P1` and `P1S` treatments was significantly higher than `Ctl` and `S0`.
  - S-uptake in shoot of `P1S` was significantly higher than that of `Ctl`.
  - N-, K- and P-uptake and -concentration were not affected by fertiliser treatments.
  - *Note*: `S0` was applied in a pelleted form and therefore only had a small contact area with soil.
- **`WG`**:
  - `KS` and `CaS` (in part significantly) increased shoot dry mass and grain yield (only `CaS`)
  - `PS` slightly increased shoot growth (n.s.), but not grain yield
  - S-uptake and concentration in shoot and grain were significantly higher for `CaS` and `KS`.
  - The difference between `Ctl` and `P1S` were more pronounced than at site **`KA`**
  - `KS`: more pods and grains per plant were formed
  - *Note*: `S0` was applied as a fine ground powder and therefore had a larger contact area with soil than in the first year.

Nutrient levels

- S-concentration:
  - `KS`, `CaS`, and `PS` showed sufficient S-concentration in shoot and grain at site **`WG`**
  - `Ctl` and other treatments showed a low level at **`KA`** and a sufficient level at **`WG`**.
- P-concentration in grain was at a sufficient level in all trials, however, the P-nutrient level of shoot was inconclusive.
- K- and N-concentration were at sufficient levels in shoot and grain in all trials

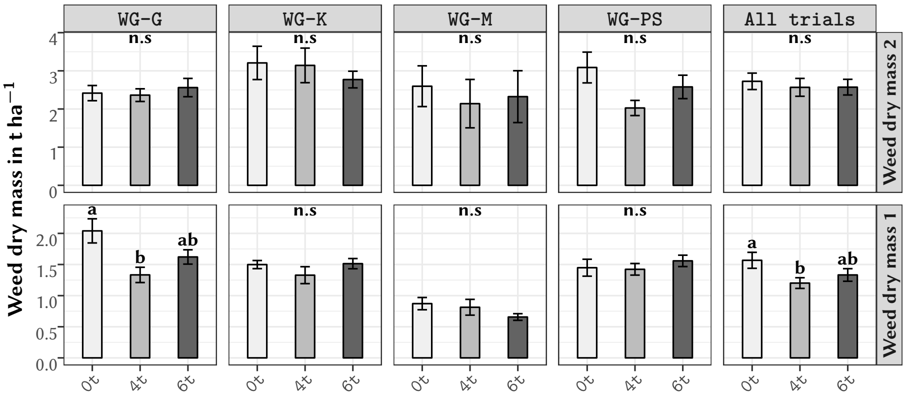
Hypothesis:

1.	Rock phosphate (RP) increases concentrations and uptake of P	-
2.	RP increases crop growth and yield	-
3.	RP+S^0 further increases concentrations and uptake P	-
4.	RP+S^0 further enhances crop growth and yield	-
5.	S-fertiliser increases concentrations and uptake of S	+
6.	S-fertiliser increases crop growth and yield	+ (Only **`WG`**)
7.	Soluble sulphur forms K_2SO_4 and gypsum are most effective	+

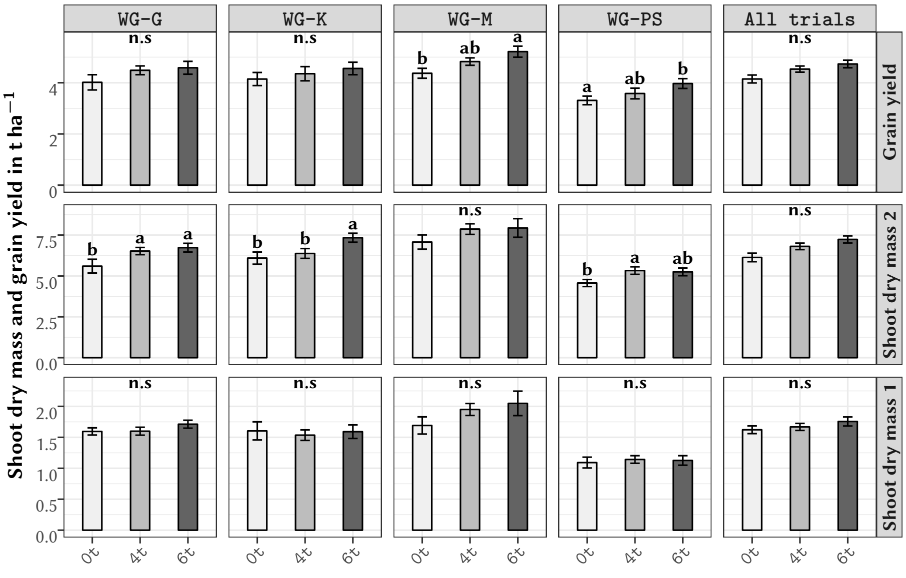
3.5 Oats straw residue

3.5.1 Effects of straw residue mass on crop and weed growth

In continuation, the effects of both experimental factors *straw* and *cut* on crop and weed growth are presented. Additional to single trial results of factor *straw* a joined data set of all `WG` trials was created, named `All trials`. While the block structure of `WG-PS` was maintained for this data set, each whole-plot of the three split-plot trials was coded as a block. This data was analysed as a mixed model randomised block design (RBD), and results are presented in conjunction with the other `WG` trials. In none of the `WG` trials effects of oats straw residue mass and cutting height on crop nutrient concentrations were observed. Therefore, these results are not presented.



(a) `KA`: Weed shoot drymass



(b) `WG`: Faba bean shoot drymass and grain yield

Figure 3.12: Effect of oats straw mulch biomass on weed and faba bean shoot dry mass ass well as faba bean grain yield at site `WG`. Weed and crop shoot dry mass were evaluated 57 and 89 DAE (BBCH 65 and BBCH 77, respectively). Harvest was realised 136 DAE. Results of trial `WG-M` as well as the summary data set of all `WG` trials are included (`All trials`). Treatments: `0t`: no straw addition, `4t`: 4 t ha^{-1} oats straw, `6t`: 6 t ha^{-1} oats straw. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

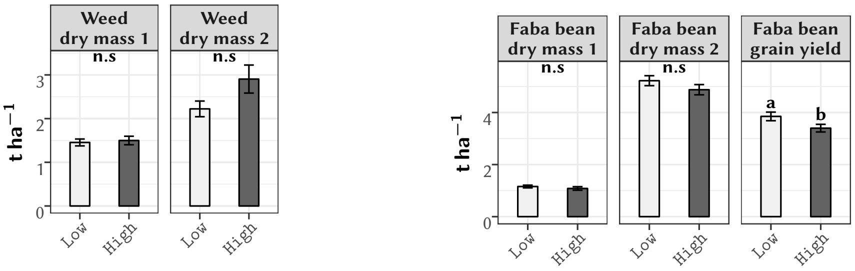
At both shoot dry mass evaluations, weed shoot dry mass did not clearly present the pattern of `0t` > `4t` > `6t`, but in most trials and evaluations both straw additions (`4t`, `6t`) lead to a decrease in weed shoot dry mass compared to the `0t` control (figure 3.12). The weed suppressing effect was more pronounced at BBCH 77, but differences for straw addition were only significant in `WG-G` and `All trials`. However, at the second shoot dry mass evaluation these differences were less pronounced and in no case significant. As for fertiliser treatments, the stand density of all trials was not influenced by the amount of straw mulch cover. In most evaluations shoot dry mass and grain yield increased in the treatment order `0t`

< `4t` < `6t`. While at the first shoot dry mass evaluation this pattern was only apparent for `WG-G`, `WG-M`, and for the joined data set `All trials`, it was universal at the time of the second evaluation, only with exception of `WG-PS` (figure 3.12 b), with often significant differences between control and straw addition treatments. In all trials grain yield exhibited the pattern `0t` < `4t` < `6t`, however, differences were only significant in `WG-M` and `WG-PS`.

Furthermore, crop height and diameter were tendentially increased in nearly all trials with increasing amounts of mulch (figure A.3 on page 128 in the annex), and in many cases the differences were statistically significant between `0t` and `4t` or `6t` treatments. Straw mulch biomass effects on LAI were not consistent at the first evaluation, and only in `WG-M` and `All trials` the common `0t` < `4t` < `6t` pattern was apparent. For the second evaluation only the LAI of treatments `0t` and `6t` was determined: in all trials the LAI was 15–25 % higher for `6t` than `0t`, though differences were not significant. Furthermore, there were no significant effects and no apparent tendencies over all trials between straw mulch treatments on yield components (figure A.5 on page 129 in the annex).

3.5.2 Effects of cutting height on crop and weed growth

The stand density was not influenced by oats straw cutting height. However, `High` cutting of oats straw at harvest affected crop growth negatively: even though there were no differences between crop and weed shoot dry mass at the first shoot dry mass evaluation (BBCH 65), at the second evaluation weed growth of treatment `High` was more elevated (n.s.) while faba bean shoot growth was decreased (n.s., figure 3.14), which resulted in significantly lower grain yield was (about −0.5 t ha^{-1} or −13 %). No differences were observed for shoot height and diameter at both evaluations. Even though not significant, LAI was also decreased for `High` (−17.1 %). Yield components were mostly not affected by the cutting height and no significant differences were present for the number of pods and grains per plant. However, TKW was significantly lower for `High`, even though absolute differences were marginal. Results of shoot height, shoot diameter, LAI and yield components are presented in figure A.6 on page 129 in the annex.



(a) Weed shoot dry mass

(b) Faba bean shoot dry mass

Figure 3.14: Effect of oats cutting height on weed and faba bean shoot growth as well as faba bean grain yield at site `WG`. Both weed and shoot growth were evaluated 57 and 89 DAE (BBCH 65 and BBCH 77 of faba bean, respectively). Treatments were `Low`: low cut at oats harvest at the base of the plant, and `High`: high cut at oats harvest beneath the panicle. Error bars: SE. n.s.: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

3.6 Summary results oats straw residue

Box 3.2 Summary effect oats straw biomass

Straw

- Straw addition increased faba bean shoot dry mass, plant height, plant diameter and leaf area.
- For most trials and evaluations weed shoot dry mass was not reduced by straw addition.

Cut

- `High` cutting of straw at harvest favours weed growth.
- `High` affects crop growth and yield negatively

Hypothesis

9) With increasing mulch biomass, weed shoot dry mass decreases -
10) With increasing mulch biomass, faba bean growth and yield increases +

4 Discussion A: faba bean trials (Germany)

In both trial years sulphur fertilisation resulted in a marked increase in sulphur uptake and contents. Nevertheless only at site `WG` plant growth was affected slightly positive. None of the parameters measuring crop growth of faba beans responded to the applied P- and S-fertiliser treatments. Only in the second year at `WG`, positive fertiliser effects on crop growth were observed. At both experimental sites, crop growth was obviously restricted by weed infestation. Under totally weed free growth conditions nutrient demands for P and S would have been higher and may have allowed for nutrient deficiencies to show up clearer and for a better differentiation between fertiliser treatments.

4.1 Phosphorus

When P-deficiency occurs, shoot P-concentration is decreased (Olivera et al., 2004). The energy metabolism is affected strongly, and nitrogen fixation in grain legumes decreases and with it N-uptake into shoot, even though N-concentrations may be equal between deficient and sufficient conditions (Leidi and Rodríguez-Navarro, 2000; Marschner and Marschner, 2012). When deficient in P, shoot growth and LAI, as well as number of pods and decreased yield are common symptoms (Marschner and Marschner, 2012). Only after many years of permanent direct seeding (no-till) a soil structure which is favourable to rooting can be formed. However, most notable under occasional direct seeding is that the soil has a higher density due to lack of loosening through tillage, which apart from lower soil temperatures hinders root formation and the diffusion of P. Therefore it was hypothesised, that phosphorus acquisition in the DS system is obstructed and that P-deficiencies are likely to occur. However, no signs of P-deficiency were observed in field trials and the shoot and grain P-concentrations were in line with values found in literature for conditions of nutrient sufficiency. Furthermore, the application of RP, alone and in combination with elemental sulphur did not affect shoot growth, LAI, grain yield, yield components or P and N- nutrient uptake or concentration. It is therefore concluded, that independent of fertiliser application phosphate supply was not limited, even under conditions of occasional direct seeding (oDS) in temperate climate. This can be explained with the soil P-levels, which were high (VDLUFA D) at `KA` and at a recommended level (VDLUFA C) at `WG`. In similar DS faba bean trials at `WG`, RP application in a work of Seehuber (2014) also did not affect yield and P-concentrations in leaf.

4.1.1 Rock phosphate and elemental sulphur

Nevertheless, shoot growth, grain yield, and P-uptake of `P1S` treatment in `WG-PS` showed a positive tendency, but it cannot be concluded clearly if this increase was due to the application of RP or S^0 or to the combined effect of the two. The hypothesised effect that combined application of RP and S^0 could improve RP-availability to crops, which has been demonstrated in previous works (Rajan, 1982a,b), could not be eluded with the experimental setup in Germany. One problem was that only at `KA`, all **`P-trial`** treatments were applied, while `WG` trial only included the `P1S` treatment. Secondly, the fertiliser application at `KA` of RP as a granule and S^0 as pellets, both with about 3 mm diameter, was not ideal: the contact area between the two fertilisers and between their mixture and soil was reduced in comparison with the application as a powder, and with it the oxidation rate of elemental sulphur and the dissolution of RP. Especially the oxidation rate of S^0 influences effectiveness of the RP + S^0, which depends heavily on sulphur particle size (Rajan, 1982a,b; Germida and Janzen, 1993; Yang et al., 2010). Nevertheless, Olfs et al. (2012) adverts that not the pellet size, but the particle size of the sulphur contained within is important. For a rapid oxidisation, sulphur should ideally be of colloidal size of about 1 µm (Germida and Janzen, 1993). However, it is unknown, which particle size was used in the elaboration of the pellets used in trials. In any case the large pellets did effectively limit soil and microbe contact, as they did not disintegrate with humidity in soil. The pellets were found largely intact about two months after planting. Therefore acidification and dissolution of RP were presumably not as strong as they could have been, affecting fertiliser performance negatively. In the `P1S` treatment at `WG` fine ground elemental sulphur was used. This mixture did have a positive effect on faba bean growth, P-uptake and S-concentration, but due to missing `S0` and `P1` treatments the solubilisation effect could not be estimated. Nevertheless it is assumed, that the effect was rather brought about by the elemental sulphur. As it was applied as a powder it became oxidised quicker than in the first year, and already at the first shoot dry mass evaluation shoot S-concentration of `S0` was similar to that of treatments `KS` and `CaS`.

4.1.2 Effects on sulphur availability

Interestingly for **`KA-P`**, the RP fertilisation alone (`P1`) did also have a significant effect on shoot and grain sulphur concentrations, which is interpreted as an interaction between P and S in soil. PO_4^{3-} and SO_4^{2-} compete for binding sites in soil, e.g. on goethite surfaces. As the PO_4^{3-} ion has a stronger affinity, the presence of excess phosphate may have increased sulphate concentration in the soil solution as found by Geelhoed et al. (1997a,b). A further study of Bolan et al. (1986, 1988) also affirms this hypothesis, which found that both lime ($CaCO_3$) and P-fertiliser additions strongly increase leaching of sulphate from soils. The displacement effect may therefore explain why `P1` increased shoot S-concentration.

4.2 Sulphur

4.2.1 Effect of sulphur fertiliser application

Sulphur deficiency affects glucose and adenosine triphosphate (ATP)-concentrations in faba bean, reduces nodule weight and affects nitrogen fixation negatively (Pacyna et al., 2006; Scherer et al., 2008). Also, the S-concentrations in shoots, roots and nodules can be drastically decreased (Pacyna et al., 2006). The shortage of S-containing amino acids cysteine and methionine inhibits protein synthesis. Therefore chlorophyll and protein contents in leafs are decreased and shoot growth and leaf area reduced (Marschner and Marschner, 2012). In faba bean field trials S-concentrations and -uptake were the parameters affected strongest by fertilisation at both sites. As literature values are mostly only available for leafs, the ranges observed in shoot cannot be put in relation to values in literature and hence used as an indicator for the presence of S-deficiency. Even though large differences in S-concentration between sulphur fertilised and non-fertilised plots, the S-supply in **`KA`** was not limiting crop growth and N-fixation, which indicates that not even a latent deficiency was present. Another possible interpretation could be that the low plant S-concentration was in fact limiting N-fixation (Scherer et al., 2008), but that this could be compensated for by native soil N, and that therefore N-deficiency symptoms were absent. On the other hand, trials **`WG-G`** and **`WG-K`** were indicating the presence of S-deficiency. While N-concentrations and -uptake were about equal between fertiliser and control treatments, `CaS` and `KS` lead to a strong increase in S-uptake. In some plots of **`WG-G`** even visible symptoms typical for S-deficiency were found. Also a slight, but partially significant increase in crop shoot growth was found, with a maximum increase in faba bean shoot dry mass of +11 %. Under S-deficiency the protein quality and hence the nutritional value of grain is known to change (Gayler and Sykes, 1985; Kim et al., 1999), due to a lower amount of essential amino-acids methionine and cysteine (Sexton et al., 1998). Protein quality was not determined directly, but the strong increase of grain S-concentration, and the more narrow N:S ratio may indicate an improvement (Gayler and Sykes, 1985; Scherer, 2001). Studies with soybean have shown that S-deficiency does not necessarily lead to a decrease in grain protein content or yield, but rather to a change in the type of storage protein formed. About 70 % of the protein in soybean seed is present as glycinin (also termed 11S) and β-conglycinin (7S) (Thanh et al., 1975; Derbyshire et al., 1976). Under S-deficient conditions the amount of 11S protein, which is rich in S-containing amino-acids (3–4 % S), decreases. Instead, more 7S protein is formed, which contains less than 1 % S (Sexton et al., 1998). A similar change in storage protein can be proposed for faba bean, and with about equal grain concentration of crude protein (N-concentration x 6.25) for all treatments, in both years the increase of about 50 % in grain S-content and a more narrow N:S ratio for `KS` and `CaS` compared with `Ctl` may indicate an increase in S-containing amino-acids and hence an improvement of grain nutritive quality. Yet, depending on legume species great amounts can be present as sulphate in grain, which would not improve its nutritional quality. While lupine grain contains 30 % sulphate, common bean hardly contains any, independent of S nutritional status (Tabe and Droux, 2001, 2002). As for faba bean no literature data was found on sulphate contents in grain, no definite conclusion can be drawn if the higher S-contents do indeed indicate a higher nutritional value of the grain.

4.2.2 Experimental sites and susceptibility to S-deficiency

The site specific risk for the occurrence of S-deficiency is affected by a range of factors. On one hand the crop specific demand for S and the yield level are decisive. Site specific, the amount of SOM, soil texture, soil chemistry, atmospheric S-deposition, sulphate leaching rates and potential influence of groundwater play an important role. Furthermore, the farm management with respect to fertiliser application and presence of life stock is important. For both German experimental sites these factors need to be observed.

Influence of groundwater on sulphur supply The experimental site `KA` is not influenced by groundwater (groundwater table approximately 20 m below soil surface), but the experimental fields at site `WG` are located in the lea of river *Sieg*. Therefore it was suspected that groundwater may have an influence on crop sulphur supply, as capillary rise of sulphate rich groundwater can largely meet crop demand, depending on sources up to 50–70 % (Bloem, 1998; Schnug and Haneklaus, 1998). However, the groundwater table at the drinking water protection zone located only about 150 m from the experimental field is on annual average 4–4.5 m below field level[15]. In the data set of 2016 the groundwater table reached its maximum of 2.5 m below field level in January. Capillary rise of groundwater only occurs if the soil above the groundwater table has dried off sufficiently to form a pronounced moisture gradient, which can presumably not occur before late spring. But at this time the groundwater table has already dropped considerably, e.g. in the 2016 data to 4 m in April. In conclusion, any sulphur supply from groundwater can also be ruled out for the `WG` experiments.

Sulphate leaching potential To determine the risk for sulphur deficiency at both sites at first the soil sulphate content at the beginning of the experiments needs to be estimated. Similar to nitrate, sulphate can easily be lost by leaching during winter. Soil chemistry at both experimental sites and in temperate climate soils in general is favourable for high sulphate leaching rates. This is because soils in temperate climate mostly possess a neutral pH value, which is due to low weathering state and common calcareous application. anion exchange capacity (AEC) and hence SO_4^{2-} sorption is low under these conditions compared with more acidic soil conditions (Bolan et al., 1988). Leaching is generally dependent on the exchange rate of soil water, which in turn is conditioned by precipitation and soil texture: a coarse soil texture favours leaching strongly, while loam soils possess an intermediate and clay soils a low leaching potential. Soil skeleton (> 2 mm) also increases leaching potential as the water absorbing soil volume is decreased leading to faster saturation. Soil at site `KA` is a loam with a high clay content (Ut4 according to KA5) and does not posses any skeleton. From the perspective of soil texture the leaching potential is relatively low. In comparison, leaching potential is higher for site `WG`, at which soil is a sandy loam with at places considerable presence of soil skeleton (highly heterogeneous). The symptoms interpreted as sulphur deficiencies in some of the unfertilised plots of trial `WG-G` could be explained with the reduced soil volume, but also with higher sulphate leaching rates at this particular location as soil saturation occurs quicker due to an elevated gravel content.

Studies from England have shown that most of the SO_4^{2-} in soil solution is leached in winter (Kopáček et al., 2014). In Denmark, a work of Eriksen et al. (2002) identified leaching as the quantitatively most important item affecting sulphur balance. In Central Europe fields without soil cover or growing vegetation potentially have leaching rates of 20–120 kg ha^{-1} a^{-1} S, with a mean of 50–60 kg ha^{-1} a^{-1} S (Horn et al., 2010). Even though weeds formed limited biomass at `WG` it is assumed that this did not lower the soil water content notably and therefore the soil was in a saturated state most of the winter. Catch crops can take up NO_3^- and SO_4^{2-} in autumn and winter and may prevent most S-leaching (Eriksen and Thorup-Kristensen, 2002), but after harvest of cereals (barley at `KA` and oats at `WG`) no catch crops were planted afterwards. The sulphur in harvest residues is liberated quickly and is susceptible to leaching in winter. Therefore, only a small proportion of approximately 10 % of sulphur in pre- or cover crop residue is available to the subsequent crop (Schnug, 1988). Hence the sulphur contained in cereal harvest residues at both experimental sites is thought to be of little relevance for the nutrition of faba bean.

For the region (Niederrheinische Bucht) in which both trial sites are located a groundwater recharge of 160–250 mm has been determined (Bogena et al., 2003), which is a good approximation for seepage water on a larger scale. For site `WG` seepage water amounts to 228 mm per year on average[15], which at places with higher gravel content is assumed to be considerably higher. The movement of nitrate and sulphate in soil have been determined to be higher than that of water in pH neutral soils, which is explained by the fact that water can enter soil aggregates while nitrate is repelled by negatively charged clay humus complexes (Körschens and Mahn, 2013). While nitrate is leached 4–4.5 mm per mm of seepage water (Körschens and Mahn, 2013), sulphate moves about 3.5 mm per mm seepage water in soils with a neutral pH (Seeger et al., 2005). Based on sulphate movement and seepage water rates, even the large amount of sulphate applied at `WG` in 2010 (Patentkali application of 1000 kg ha^{-1}, equivalent to 170 kg ha^{-1} sulphur) was presumably transferred to a soil depth greater than 70 cm and was hence not available to the crop early in the season. The mineral sulphate available to plants at the beginning of the season was not determined at both sites, but it can be assumed that these were extremely low due to leaching losses in winter.

Mineral and organic fertiliser management At site `KA` sulphur is applied annually in the form of Korn-Kali® at rates of 10 kg ha^{-1} S for wheat and barley and about 20–25 kg ha^{-1} S for sugar beet, while no organic fertilisers are applied. At the organic mixed farm `WG`, Patentkali® [18] is used as a potassium source, which contains considerable amounts of sulphur. Nevertheless, Patentkali® application is not realised on a regular basis (e.g. no application between 2003 and 2009) as its application has the objective to maintain soil contents of potassium within the boundaries of LUFA class C. With punctual applications of large amounts of Patentkali® no sulphur reserves are build up in soil and most sulphate is lost by leaching. Therefore, S applied in mineral fertilisers can hardly be taken into account for nutrition of faba bean. Cattle manure is applied at an average rate of 17 t ha^{-1} per year, though not to the faba bean crop. It contains approximately 0.05 % (LWK North Rhine-Westphalia), which results in S-inputs of about 9 kg ha^{-1} S per year. Nevertheless, sulphur from organic fertilisers to a large extent is not directly plant available and similar to SOM has to be mineralised first.

Balance of sulphur fluxes As 95 % of S in soil (Scherer, 2009) is bound organically, without mineral fertilisation and presumably low soil sulphate contents in spring, crop S-supply has to be met to a great extent by mineralisation of SOM. In consequence, the biological and biochemical mineralisation kinetics play a key role for S-supply to the crop, which in turn are strongly influenced by soil temperature. Under direct-seeding management soil temperatures rise slower in spring than with conventional seedbed preparation. Hence, also SOM mineralisation rates are lower, which may have caused the interpreted (latent) S-deficiency in `WG` trials and the relatively low sulphur levels in shoot and grain in `KA` trials. However, it cannot be concluded if the DS management in the trials was indeed a cause for the S-deficiency as control tillage plots were missing in the trials. Net annual mineralisation rates are about 1.7–3.1 % of total sulphur in soil (Eriksen et al., 1995), which would correspond to 8–14 kg ha^{-1} and 7–12 kg ha^{-1} according to the total S-contents determined in top soil (0–30 cm) for sites `KA` and `WG`, respectively. In DS, net mineralisation amounts can be assumed to be rather at the lower end of these values. But even if total annual sulphur mineralisation rates were sufficient, slow mineralisation kinetics could cause S-deficiency in spring in the absence of external inputs. Apart from mineralisation, only atmospheric depositions are available to the crop without additional fertilisation, which are about 10 kg ha^{-1} a^{-1} (Horn et al., 2010; Umweltbundesamt, 2014). Nevertheless these depositions are only partially available to the crop (approximately 60–70 % according to annual rainfall distribution and assuming equal dry deposition rates throughout the year). With yields in a range of 3–5 t ha^{-1} for faba bean and a grain concentration of 0.15–0.2 % the total harvest export is in the range of 7–10 kg ha^{-1} S. SOM mineralisation and atmospheric depositions are hardly able to meet the total crop S-demand at both trial sites and due to the slower SOM onset of mineralisation especially in the early season sulphur deficiency is likely to occur.

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[18] Patentkali®: Potassium sulphate with magnesium: 24.9 % K, 6.0 % Mg, 17 % S

4.2.3 Effect of sulphur fertiliser form

Due to the presence of a positive effect of sulphur fertiliser application it can be assumed that nutrient liberation kinetics and atmospheric inputs cannot ensure a sufficient S-nutrient supply to the faba bean crop under DS management. Additional S fertilisation can therefore be recommended. The sulphur pellets applied at `KA` did exhibit an effect on S-concentration, but this effect was low compared with `KS` and `CaS`. Apparently the grain concentrations were affected stronger than the shoot concentrations for `S0`. Likely this is due to the late onset of the fertiliser effect, because S^0 had to be oxidised by soil bacteria (*Thiobacillus sp.*) to sulphate first to become plant available, which - as mentioned above - was restricted by the large pellet size. Therefore the impact of elemental sulphur remained small in the first year. The effect of `CaS` and `KS` on growth (only `WG`) and sulphur nutrient parameters was similar. Even though the `P1S` treatment in `WG-PS` did result in a weaker growth increase than `CaS` and `KS`, the S-concentrations in shoot and grain were also comparable to those treatments. This indicates that the powder application of elemental sulphur in fact had a better effect than the pellets in the first year. Additionally, the RP in this treatment also have had a positive effect on sulphate concentration in soil solution, as described above for `P1` treatment. Again, due to missing `P1` and `S0` treatments, the single effect of each of the two fertilisers cannot be separated. Even though plant availability of the sulphur form applied plays a role, the different solubility of gypsum and K_2SO_4 did not matter, and both supplied plants equally well with S. Possibly in practice, elemental sulphur is able to supply S just as effective as the more soluble sulphate forms, if applied as fine ground powder, preferentially with particles of colloidal size. Micronised elemental sulphur was found to be oxidised to 50 % within 6–10 days at soil temperatures of 20 °C, 23–26 days at 7 °C, and 36–42 days at 2 °C (Chapman, 1989). Therefore, even the rates at initially low soil temperatures in spring in the trials were apparently sufficient to meet crop demand and increase sulphur uptake drastically.

4.3 Oats straw residue

The experimental data on oats straw cutting height was limited to trial `WG-PS`. Nevertheless, from the results of this trial it can be assumed that the `High` cut, which was used for all split-plot trials, had a negative impact on weed control of oats straw mulch, and was therefore unfavourable for crop development. The decreased weed control compared with `Low` cut is assumed to be due the reduced soil cover of rolled straw compared with chipped straw. Moreover, while chipped straw is evenly distributed, the rolled straw is only combed into one single direction, often points up slightly and is therefore not in close contact with soil. Soil shading is reduced and more solar radiation can reach the soil surface, which reduces the physical obstruction of weed germination and emergence considerably. Furthermore, the standing straw dries faster after rainfall, which may retard or obstruct their decomposition and hence the release of allelopathic substances and thereby decrease their associated weed control effect. The results from the factor oats straw residue (*straw*) confirm the finding of works at *Wiesengut* in previous years (Schulte, 2007; Köpke and Schulte, 2008; Massucati et al., 2010, 2011; Massucati and Köepke, 2011; Massucati, 2013). Over all trials a similar pattern could be observed, even though results were not always significant. Oats mulch residue suppressed weeds at the initial development of faba bean, which favoured crop development. While at the first evaluation clear differences in weed dry mass were found between straw residue treatments, this effect was only weak at the second evaluation. Apparently, at later stages of faba bean development weed growth did not impede crop growth as strongly anymore: weed dry mass was similar over all straw treatments, but faba bean growth showed the same treatment differentiation as at the first evaluation. This confirms findings of Massucati and Köepke (2011) that the early suppression of weeds is decisive for the positive effect of mulch. Rather than season-long weed control, the positive effect lays in the improvement of initial crop development.

Compared with trials of (Massucati et al., 2010) weed shoot dry mass was higher for all `WG` trials (evaluated at about equal growth stages). However, in Massucatis works even for the `0t` treatment a weed shoot dry mass of only 0.75 t ha^{-1} was determined (88 DAE) and strong reductions were achieved, down to 0.51 t ha^{-1} for `4t` and 0.27 t ha^{-1} for `6t` oats straw treatment (Massucati et al., 2010). The `0t` plots in the cited work even had a lower weed shoot dry mass than the weed shoot dry mass found for the `6t` treatments of `WG` trials (BBCH 65 / 89 DAE). Weed pressure in `WG` trials therefore can be considered distinctly higher. Despite the higher weed infestation yields were also higher at `WG`. Both increases in weed shoot dry mass and faba bean yield can be explained by the excellent growing conditions in 2012, with reference trials of *Landwirtschaftskammer* (LWK, NRW) achieving yields exceeding 6 t ha^{-1}. Another reason for higher weed growth and smaller differentiation between treatments at `WG` trials was the `High` cutting height used in all split-plot trials (see above). Furthermore, lower weed control was presumably due to the amount of oats straw biomass added in trials: in order to make `Low` and `High` cut treatments comparable both the stubble of `Low` and `High` treatments were determined and the missing straw was added up to 4 t ha^{-1} and 6 t ha^{-1}. The difference between `0t` and `4t` treatment in the split-plot trials was therefore not as large as the treatment nomination alleges, because `High` cut straw alone already left 2–3 t ha^{-1} of straw residue. Straw additions were therefore only in the range of 1–3 (`4t`) and to 3–5 t ha^{-1} (`6t`). The total mulch biomass used by Massucati (2013) was higher: in these trials stubble was not quantified (assumed to be 0 t ha^{-1}) and hence 4 t ha^{-1} and 6 t ha^{-1} of straw were added. Therefore, including the stubble weight of presumably 1–2 t ha^{-1} total amounts of 5–6 t ha^{-1} and 7–8 t ha^{-1} were actually present. Combined with a `Low` cutting of oats straw in these trials the soil coverage was better, which is the most likely explanation for better weed control in comparison to the trials conducted in the work of Massucati.

5 Results B: soybean fertiliser trials (Brazil)

5.1 Soil conditions

Soils at `LO` and `PG` were classified as clayey *ferralsol*[19] (clay content of 64–79 %) with a base saturation of less than or only slightly above 50 % (distrophic). For the sandy ferralsol soil at site `UM` a base saturation of around 29 % and exchangeable Al in the topsoil of 18 % were determined (table 5.1). With missing irrigation facilities the latter trial site was extremely drought prone due to its low water holding capacity.

At sites `LO1` and `UM` soil was only sampled from 0–20 cm soil depth. Of all other sites samplings were realised from 0–10 cm and 10–20 cm, which is the typical procedure for soils under DS management in Brazil. Among other sources, Embrapa Soja (2007) was used for referencing nutrient levels found in soil and plant parts. Only soils in trials `LO1` and `PG2-PS` had a pH above 5 and hence no exchangeable Al^{3+}, while most other sites exhibited medium levels of exchangeable Al^{3+} (table 5.2). The highest levels of topsoil exchangeable aluminium were found at `LO2-S` and `PG-Low`, with the latter exhibiting elevated and potentially toxic levels already at 20 cm soil depth. Most experimental sites exhibited medium to high plant available P-levels in soil according to Embrapa Soja (2007), and only `PG-Low` showed a strikingly low content of available P. Also the measured Ca^{2+}-, Mg^{2+}- and K^{+}-contents were at a medium to high level for all trial sites and K-fertilisation above the amount of crop export was not indicated. From the soil carbon contents in table 5.2 SOM can be deduced by applying the conversion factor 2 according to Pribyl (2010). The SOM and soil S-contents differed greatly between experimental sites: for trials at `LO` 3.5–4.0 % SOM in 0–10 cm soil depth were determined, while `PG` has up to 6 %, with S contents of 0.25–0.33 %. Exceptional was `PG-Low` with nearly 8 % SOM and 0.4 % S, which can be attributed to the long fallow period of several years before trials. The lowest SOM content was found for site `UM`, with only 1.7 %.

5.2 Fertiliser effect on crop growth and grain yield

The graphical presentation of results is realised according to trials in Germany, combining trials and related parameters into faceted diagrams. This allows for visual comparisons between trial sites and years as well as for easier detection of systematic tendencies in response to fertilisers between treatments. However, due to the higher amount of trials in Brazil, results need to be presented in two separate plots in order to allow for better legibility.

5.2.1 Crop density and weed infestation

There were clear differences in stand density between trials: the sowing machine used in season 2011/12 distributed seeds unevenly, causing an irregular stand density. Nonetheless, `LO-1` trials were accidentally planted with an increased sowing density, nevertheless, a subsequent manual resulted in an even stand

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[19] Ferralsol according to FAO World Reference Base for soils (WRB). According to Sistema Brasileiro de Classificação de Solos (SiBCS) all soils can be classified as *Latossolo vermelho*.

Table 5.1: Soil granulometry, cation exchange capacity (CEC), base saturation (BS), saturation with Al^{3+} and pH in 0–10 cm soil depth of the soils used for fertiliser trials at sites *Londrina* (`LO`), *Ponta Grossa* (`PG`) and *Umuarama* (`UM`).

Trial	Clay %	Silt %	Sand %	CEC $cmol_c\,dm^{-3}$	BS %	Al^{3+} %		pH
`LO1`	66	22	12	14.0	56.8	0.0	L (1)	5.3
`LO2-P`	74	21	5	13.5	46.6	2.0	M	4.9
`LO2-S`	79	18	3	14.1	38.4	10.8	M	4.5
`PG-Low`	79	14	7	16.3	33.3	17.2	M	4.4
`PG1-PS`	64	16	20	14.8	55.7	0.0	L	5.1
`PG2-PS`	64	16	20	12.5	42.8	6.5	M	4.7
`UM`	16	2	82	7.4	28.9	17.9	M	4.5

[1] L: low nutrient content, M: medium content, H: high content according to (Embrapa Soja, 2007).

Table 5.2: Soil nutrient contents in 0–10 cm soil depth of fertiliser trials at sites *Londrina* (`LO`), *Ponta Grossa* (`PG`) and *Umuarama* (`UM`).

Trial	Ca^{2+} $^{(1)}$ cmol$_c$ dm^{-3}		Mg^{2+} $^{(1)}$ cmol$_c$ dm^{-3}		K^{+} $^{(1)}$ cmol$_c$ dm^{-3}		P $^{(2)}$ ppm		C $^{(3)}$ %		C $^{(4)}$ %	N $^{(4)}$ %	S $^{(4)}$ %
`LO2-P`	4.11	H $^{(5)}$	1.80	H $^{(5)}$	0.40	H $^{(5)}$	13.20	H $^{(5)}$	1.58	H $^{(5)}$	1.72	0.16	0.028
`LO2-S`	3.59	M	1.40	H	0.43	H	12.82	H	1.56	H	1.71	0.16	0.026
`PG-Low`	3.00	M	2.16	H	0.28	M–H	1.43	L	2.81	H	3.89	0.25	0.041
`PG1-PS`	5.31	H	2.72	H	0.22	M–H	6.54	M	2.27	H	2.96	0.22	0.038
`PG2-PS`	3.40	M	1.62	H	0.37	H	3.46	L	2.85	H	2.44	0.17	0.033
`LO1`	5.62	H	1.72	H	0.64	H	13.01	H	1.75	H	1.97	0.19	0.033
`UM`	1.45	M	0.58	M	0.15	M	33.82	H	0.84	L–M	0.38	0.04	0.007

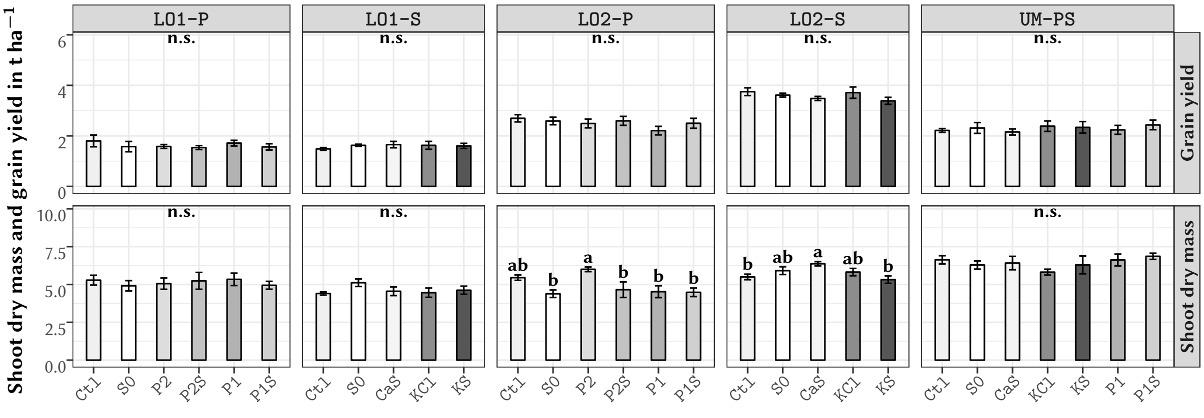
[1] Ca^{2+}, Mg^{2+}, K^{+}: extraction with ion exchange resin (Van Raij et al., 1986), IAPAR Londrina soil laboratory
[2] P: Mehlich I method (Mehlich, 1953). IAPAR Londrina soil laboratory
[3] C: Walkley-Black method (Walkley and Black, 1934), IAPAR Londrina soil laboratory
[4] C, N, S: elementary analysis (E/A), IOL laboratory, Bonn University, without nutrient levels
[5] Nutrient levels according to Embrapa Soja (2007): L: low, M: medium, H: high nutrient level

with a density of 25 plants m^{-2}. Both trials `PG1-PS` and `PG1-Low` exhibited failures despite high stand densities (`PG1-PS`: 32 plants m^{-2}, `PG1-Low`: 37 plants m^{-2}). Therefore, shoot dry mass sampling in these trials was only carried out in failure free areas of the plots. In order to correct for gaps in the crop stand, the total failure area was determined for each plot and excluded for grain yield calculation. In any case, the amount of failures between plots was fairly similar and therefore equal conditions were assumed for all plots. The sowing machine used in 2012/13 distributed seed more evenly and the counted stand densities were in a range of 22–27 plants m^{-2} on trial average. Despite even seed distribution, `LO2-P` presented a strong variation in stand density (s.d.) between plots and treatments, with treatment averages from 17–26 plants m^{-2}. Visual symptoms of seedling damage or depressed growth were not observed. One reason for the generally irregular stand density was the shallow sowing depth. On one hand this deprived seeds of water for germination and on the other hand it facilitated seed predation by pigeons. The significant treatment differences between treatments of `LO2-P` were also attributed to a slight change in sowing depth between treatments and not to fertiliser effects on crop emergence (treatments were planted subsequently). Not only because of problems during seeding results of `LO-P2` have to be assessed critically: due to a delay in herbicide application this trial exhibited a noteworthy weed infestation, while all other fertiliser trials in Brazil could be considered weed-free. In comparison with other trials, high standard errors of results were evident for many parameters in `LO-P2`. With treatment `KCl` there was a risk of damage to seedling roots due to high chloride concentration. To avoid root damage, the maximum recommended fertiliser dose of intra-row applied KCl is around 65 kg ha^{-1} (Embrapa Soja, 2007), which was exceeded with the `KCl` treatment (95 kg ha^{-1}). Also elevated acidity caused by oxidation of elemental sulphur in `S0`, `P1S` and `P2S` treatments could have possibly damaged roots, which apparently also was not the case. As in all treatments signs of growth depression at early crop development, decreased stand density or visual seedling damage were absent it was concluded that the fertilisers `KCl` and `S0` had no negative effects on crop emergence and early development.

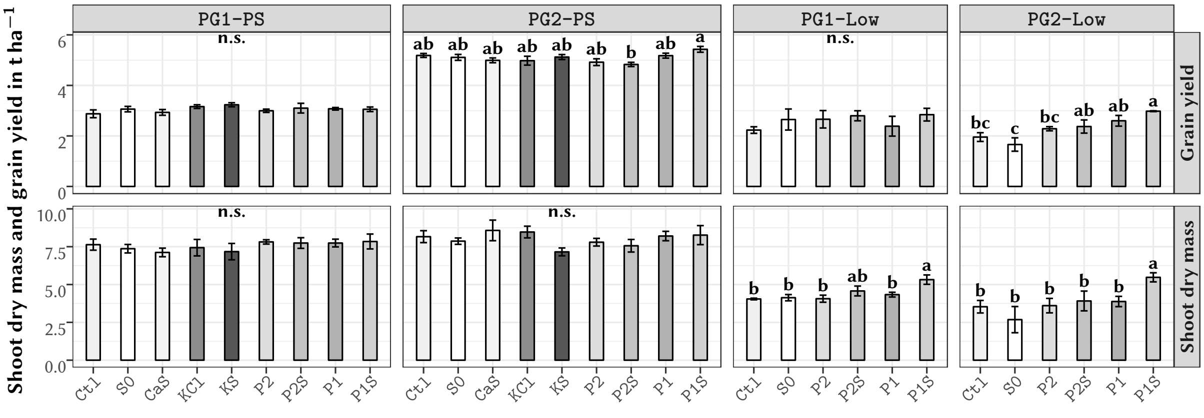
5.2.2 Site Londrina

Shoot dry mass, grain yield and shoot height were not affected by any of the fertiliser treatments in both `LO1` trials (figure 5.1 - the shoot height data is presented in figure B.1 in the appendix A on page 130). In contrast to the other Brazilian fertiliser trials two shoot dry mass evaluations were realised in these trials. At the first evaluation (53 DAE) no differences were found in both trials for shoot dry mass, shoot height and shoot diameter B.7 in appendix A on page 132). Yield data and TKW could not be taken into account in both `LO1` trials: symptoms of a *Macrophomina phaseolina* infection became visible during grain filling stage (85 DAE) and the trials had died off completely by 100 DAE (TKW results in figure B.3 on page 130 in the appendix A). At this stage no differences in grain yield or TKW were present. In order to compensate for lost grain yield data, harvest components were examined (figure B.9

in appendix on page 133): none of the `P-trial` treatments affected the formation of grains and pods per plant or the number of grains per pod. Nonetheless, the `CaS` treatment (`S-trial`) formed more grains per plant (+56.9 %, s.d. Wilcox) and more pods per plant. This is assumed to be a random effect, as there was no effect on plant nutrient uptake by sulphate fertilisers (figure 5.8) and because a soil ameliorating effect of gypsum can be ruled out: no exchangeable aluminium was present in topsoil and the limited amounts applied would not greatly mitigate potentially existing Al-toxicity in deeper subsoil layers. The most reasonable explanation for the effect is the high variability of measurements in this trial: for example `Ctl` measurements were in a range of 40–100 grains per plant, while treatment `CaS` presented relatively high values (not the highest, though) with less variability.



(a) Londrina & Umuarama



(b) Ponta Grossa

Figure 5.1: Effect of fertiliser treatments on soybean shoot dry mass and grain yield at trial sites in *Londrina* (`LO`), *Ponta Grossa* (`PG`) and *Umuarama* (`UM`). Shoot dry mass was determined 73 days after emergence (`LO1`), 71 DAE (`LO2-P`), 74 DAE (`LO2-S`), 78 DAE (`UM-PS`), 82 DAE (`PG1-PS`) and 85 DAE (`PG2-PS`). Grain yield is presented with 13 % humidity. `LO1-P` harvest data is not representative due to stand dying off at early grain filling stage 101 DAE. Treatments were `Ctl`: non-fertilised control, `S0`: elementary sulphur (40 kg ha^{-1} S), `P1`: *Gafsa* rock phosphate (50 kg ha^{-1} P), `P2`: *Alvorada* rock phosphate (50 kg ha^{-1} P), `P1S`: joint application of `S0` and `P1`, `P2S`: joint application of `S0` and `P2`, `CaS`: gypsum ($CaSO_4 \cdot 2\,H_2O$, 40 kg ha^{-1} S) and `KS`: K_2SO_4 (40 kg ha^{-1} S). Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

In season 2012/13 P2 treatment of `LO2-P` formed significantly higher shoot dry mass than all other treatments except `Ctl`. Yet these differences were apparently not related to fertiliser effect, but rather to the irregular stand density in this trial, which on one hand was reflected in the strong and highly significant correlation between shoot dry mass and sampling density ($r = 0.74^{***}$, $R^2 = 0.54$), and on the other hand in the absence of differences in grain yield. The average level of grain production in this trial was low (2.3 t ha^{-1}) compared with other trials. Nevertheless the TKW was similar to other trials (152 g). In `LO2-S` shoot dry mass of `CaS` was significantly higher than `Ctl` (+15.0 %) and `KS` (+19.7 %). The same held true for shoot height (figure B.1 in appendix on page 130), however, grain yield and TKW did not show any differentiation. The grain yield was at an intermediate level [20] with 3.8 t ha^{-1}.

5.2.3 Site Ponta Grossa

Shoot dry mass was similar for most sites, and only both `PG-High` trials stood out with far higher shoot dry mass production. This can be explained by the sites cooler temperate climate, which prolongs the crop cycle and the vegetative growth period (Embrapa Soja, 2007). Shoot dry mass, shoot height and grain yield of `PG1-PS`, were similar for all treatments. In season 2011/12 plant diameter, LAI, and weight of the plant parts stem, leaf and pods were determined during the evaluation of shoot dry mass. Most of this component data showed a strong and highly significant correlation to the sample density, which means that the irregular stand interfered with the data quality. Therefore treatment comparisons are not meaningful for these parameters (figure B.10 in the appendix on page 134).

Curious about the results was that the yield level remained extremely low, only slightly higher than `PG1-Low`, despite distinctly higher shoot dry mass weight in trial comparison. The reason for this can be found in the ratio of pods (wt/wt) per plant, which was only 0.2 for `PG1-PS` and 0.31 in trial `PG1-Low`. The low yield of `PG1-PS` could therefore be attributed to the low number of pods formed. This was assumed to be a consequence of the late reseeding (consequence of herbicide damage) of both `PG1` trials in mid-December 2011, which lead to a reduced period for flowering and pod-set, and ultimately to lower yields. Due to the generally lower production level `PG1-Low` was not affected as strongly by the late sowing date. The `PG2-PS` trial had the highest production level of all trials with an average yield of 5.1 t ha^{-1}, and significant treatment differences were apparent between `P1S` and `P2S`. Compared with `Ctl`, the yield was decreased for `P2S` (−6.9 %) and marginally increased for `P1S` (+4.7 %). As no plausible reason exists as to why `P2S` application would impact yield negatively a random effect was assumed. Compared with treatment `Ctl`, shoot height and shoot dry mass of `PG1-Low` were found to be increased for treatments `P1S` (+32 %, s.d.) and `P2S` (+13 %, n.s.), and both treatments also yielded most (n.s.). On average, production was relatively low with only 2.6 t ha^{-1}, but grain TKW was in a normal range with an trial average of 141 g. The treatment differentiation in 2012/13 of `PG-Low` was far stronger: shoot growth of `P1S` was highest (+55 %, compared with `Ctl`, s.d.) while `S0` exhibited a depression in shoot dry mass with the lowest value of all treatments (−24 %), even though differences of `S0` in comparison with `Ctl` were not significant. The treatment pattern found for both shoot dry mass and grain yield was `P1S` > `P1` > `P2S` > `P2` > `Ctl` > `S0`. Grain yield of `P1S` was also together in a group with `P1` and `P2S` treatment (all three s.d.). Even though TKW was notably higher in season 2012/2013 (162 g), production level of `PG2-Low` remained low, with an average of only 2.3 t ha^{-1}.

5.2.4 Site Umuarama

All fertilisation treatments in `UM-PS` resulted in about equal shoot dry mass (n.s.), shoot height, grain yield and TKW. Even though shoot dry mass was at similar levels as in most other trials, the mean production level remained low with only 2.3 t ha^{-1}. The explanation for this is that a drought occurred during the grain filling stage (experimental site did not have irrigation facilities) which is the growth stage of soybean that has the highest demand for water (Embrapa Soja, 2007).

5.3 Fertiliser effect on nutrient uptake and nutrient concentrations

Nutrient concentrations in soybean shoot were all determined at development stages R3 and R4 (BBCH 73–74) and can therefore be compared within and between trials. No literature values for shoot nutrient concentrations of the exact same growth stage (R2–R4) were available, therefore shoot concentration values for soybean growth stage R4.5, found by Sexton et al. (1998), were used as a reference in Brazilian trials. Leaf concentrations, which were only determined in the second trial season at stage R3 were compared with literature values, which are usually obtained at the earlier development stage R2. The literature sources used to reference grain and leaf concentrations determined in this work, and to qualify them as *deficient*, *sufficient*, or *high* were Nogueira and Melo (2003), Hitsuda et al. (2004), Urano et al. (2006), Sfredo and Lantmann (2007), Embrapa Soja (2007) and Marschner and Marschner (2012). Furthermore, in the first of the two shoot dry mass evaluations of `LO1` trials (53 DAE) nutrient concentrations

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[20] Source: Instituto Brasileiro de Geografia e Estatística (IBGE)

and uptake were determined. The nutrient uptake of both trials, which presented no differences in P, S N and K uptake into shoot can be observed in figure B.8 on page 132 in the appendix A.

5.3.1 Phosphorus

None of `P-` nor `S-trial` treatments had any effect on shoot and grain P-concentrations (figure 5.3) and absolute values were similar for all trials. Differences in P-uptake were therefore only a function of shoot growth and grain production. The only significant, differences found for P-concentration were those in grain of trial `PG2-Low`, which were negligible in absolute and relative terms. As *Alvorada* RP (P2) is less soluble than *Gafsa* RP (P1), differences would be expected to be the opposite between the two treatments, therefore this is concluded to be a random effect.

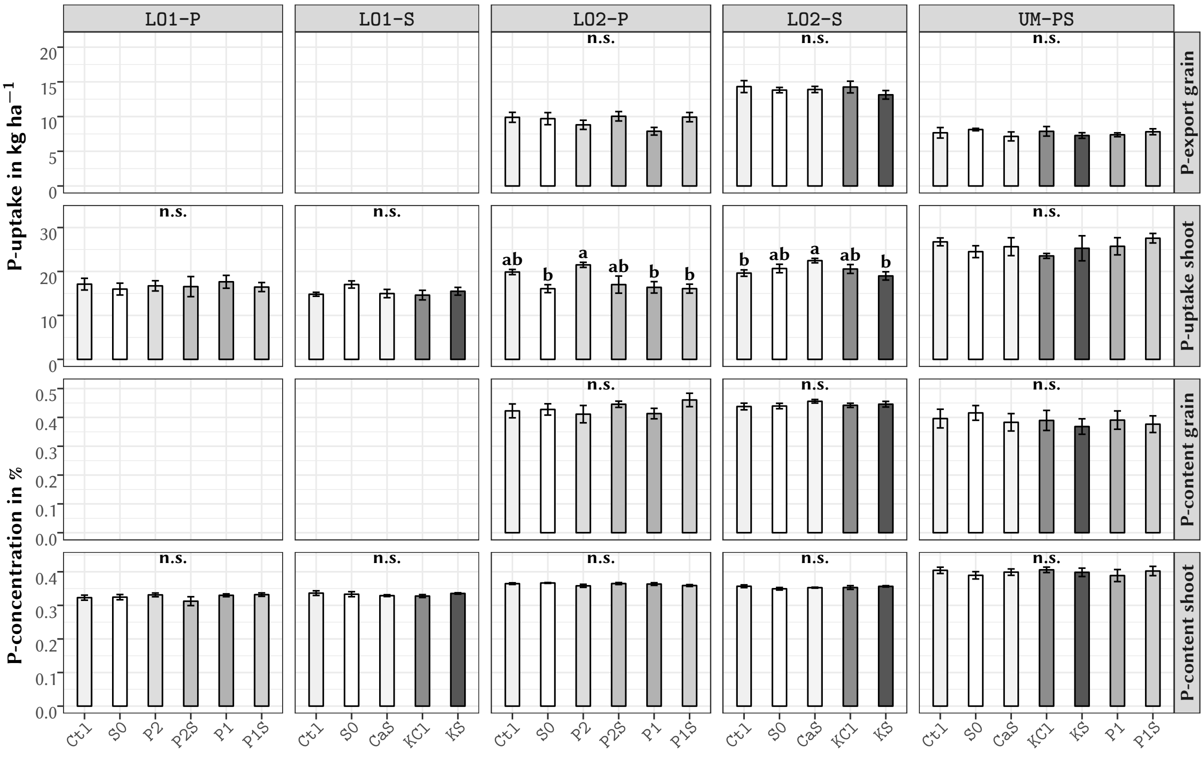
The significant differences for leaf P-concentration observed in trial `PG2-PS` were also concluded to be random (figure 5.5), because it is not plausible that `P2` treatment showed lower leaf P-concentrations than all other non P-fertilised treatments. While all other trials in 2012/2013 showed adequate P-concentrations in leafs, values of all `PG2-Low` treatments were deficient for P. The same was also true for grain P-concentration in both `PG-Low` trials, which also presented slightly deficient levels (Embrapa Soja, 2007; Marschner and Marschner, 2012). The significant increase in grain P-concentration of only 4.9 % in treatment P2 was again assumed to be a random effect: P1 (*Gafsa*) was the more soluble phosphate, and hence would have to be expected to possess a greater effect on P-concentration. For shoot concentrations no reference values were found for the respective growth stage, but average values between trials were mostly similar. Notably lower were concentrations in both `PG-Low` trials. Nonetheless, the exceptionally low concentrations found in `PG1-Low` may also be due to a measurement error during laboratory analysis.

5.3.2 Nitrogen

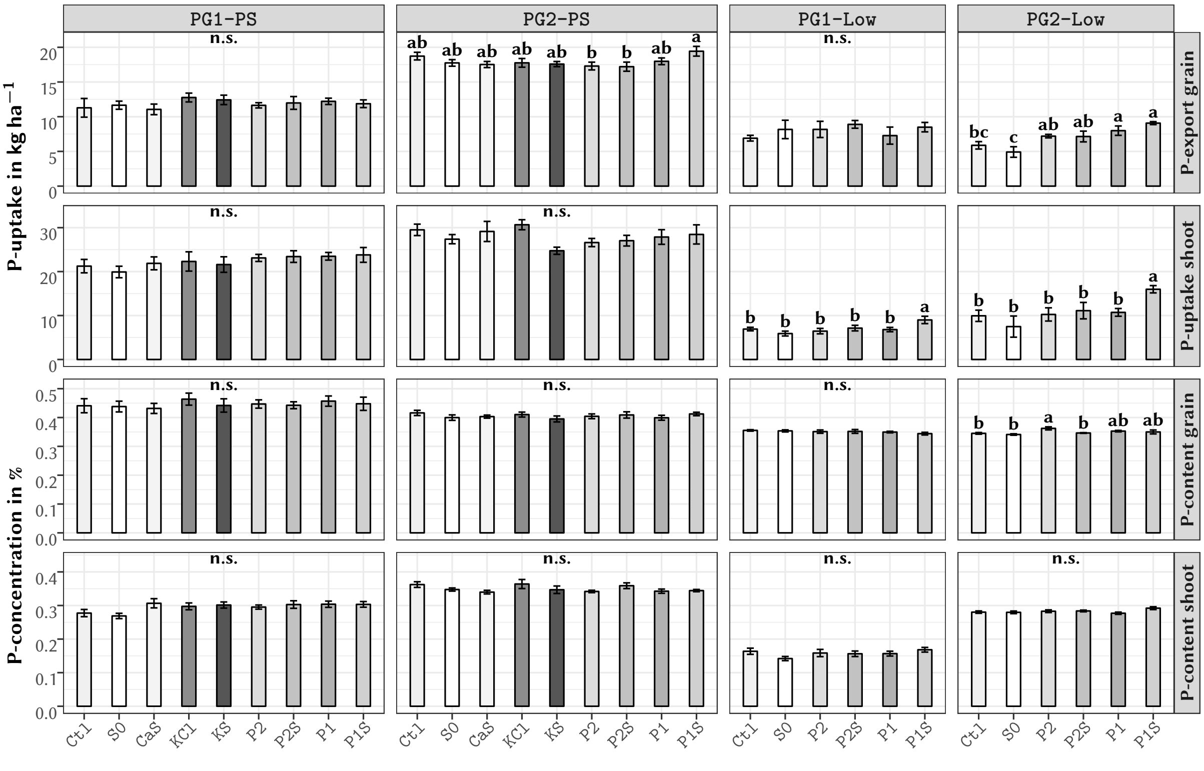
Both `LO1` trials and `UM-PS` did not show differences in N-uptake and -concentration in shoot and grain (figure 5.6). Shoot concentrations of `LO2-P` were also similar. Analogous to shoot dry mass, the significant differences observed for shoot N-uptake (s.d.) were presumably connected to the heterogeneous crop stand. Compared with literature reference values the leaf N-concentration of this trial was relatively low for all treatments except P1 (n.s.). At the same time grain N-uptake and N-concentration were similar for all treatments and the N-concentrations at a rather elevated level. Shoot N-concentrations were also equal over all treatments in trial `LO2-S`. Therefore differentiation of uptake was analogous to shoot dry mass, with a significantly higher N-uptake for CaS compared with Ctl and KS treatments. The leaf N-concentrations were also nearly equal for all treatments and at a relatively low level. Even though significant differences were present in grain N-concentrations, the values were all in a narrow range from 6.77–6.98 %, and the relative differences of fertilised treatments compared with Ctl were negligible (CaS: +4.34 %, KS: +3.1 %). The grain N-uptake was about equal for all treatments (n.s.).

All nitrogen parameters of `PG1-PS` were found not to differ. For season 2012/2013 results were also similar: there were no differences in N-concentrations of `PG2-PS`, however, analogous to grain yield grain, N-uptake did present significant differences. The leaf N-concentration was found to be at sufficient levels for all treatments and the grain concentration at a high level.

In the `PG-Low` trials differences were present in N-parameters in both trial years. Shoot N-concentration of `PG1-Low` was similar between treatments despite the presence of significant differences. Shoot N-uptake was distinguished more clearly: P1S and P2S showed higher values than all other treatments (s.d.). Grain concentration was practically equal for all treatments and moreover the lowest on average of all trials, even though still above the critical concentration according to Embrapa Soja (2007). There was a slight tendency of higher grain yields for the treatments P1S and P2S, but the differences of these compared with P2, S0 and P1 were only small. Grain N-concentrations of `PG2-Low` were equal over all treatments, but for shoot and grain uptake a clear differentiation was apparent. In shoot only P1S was significantly higher than other treatments. It was also visible that S0 treatment showed decreased growth, with all other treatments being nearly identical. Grain yield showed a similar, yet more differentiated pattern, in the order S0 < Ctl = P2 < P2S < P1 < P1S. The leaf N-concentration in the second trial season was low for all treatments. For the N-parameters in `UM-PS` the results were similar for all



(a) Londrina & Umuarama



(b) Ponta Grossa

Figure 5.3: Effect of fertiliser treatments on P-uptake and -concentration in shoot and grain. For treatment abbreviations refer to figure 5.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

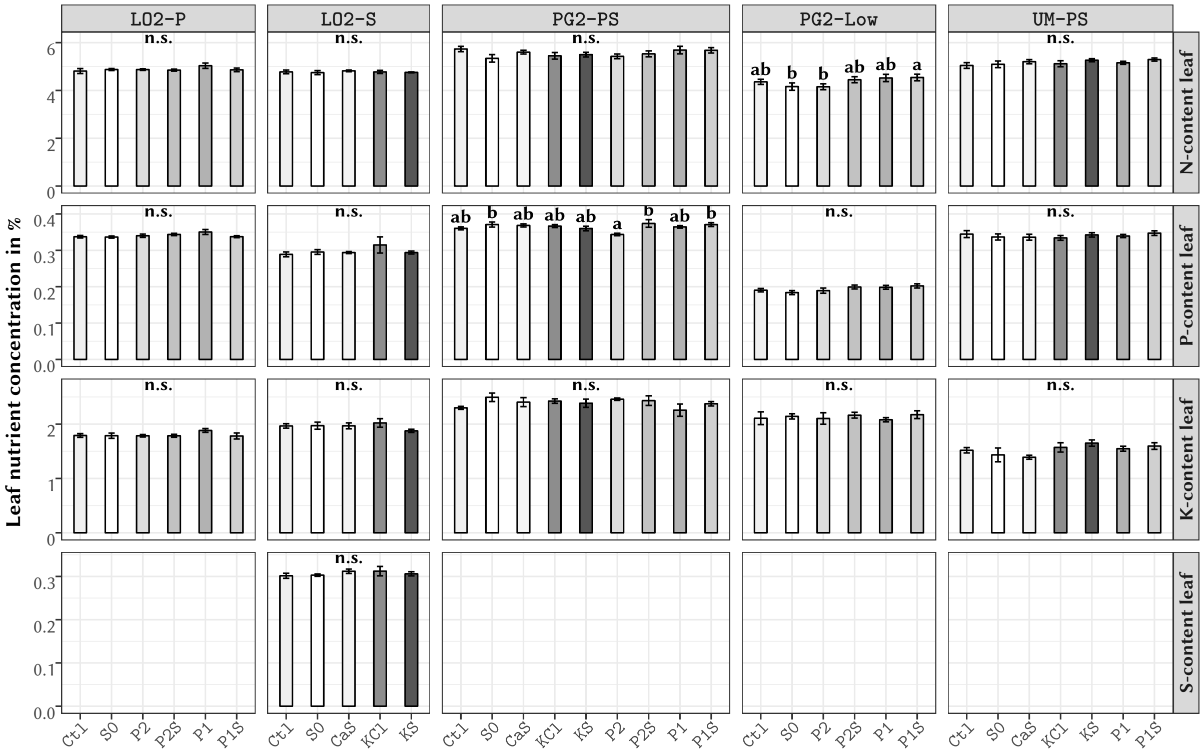


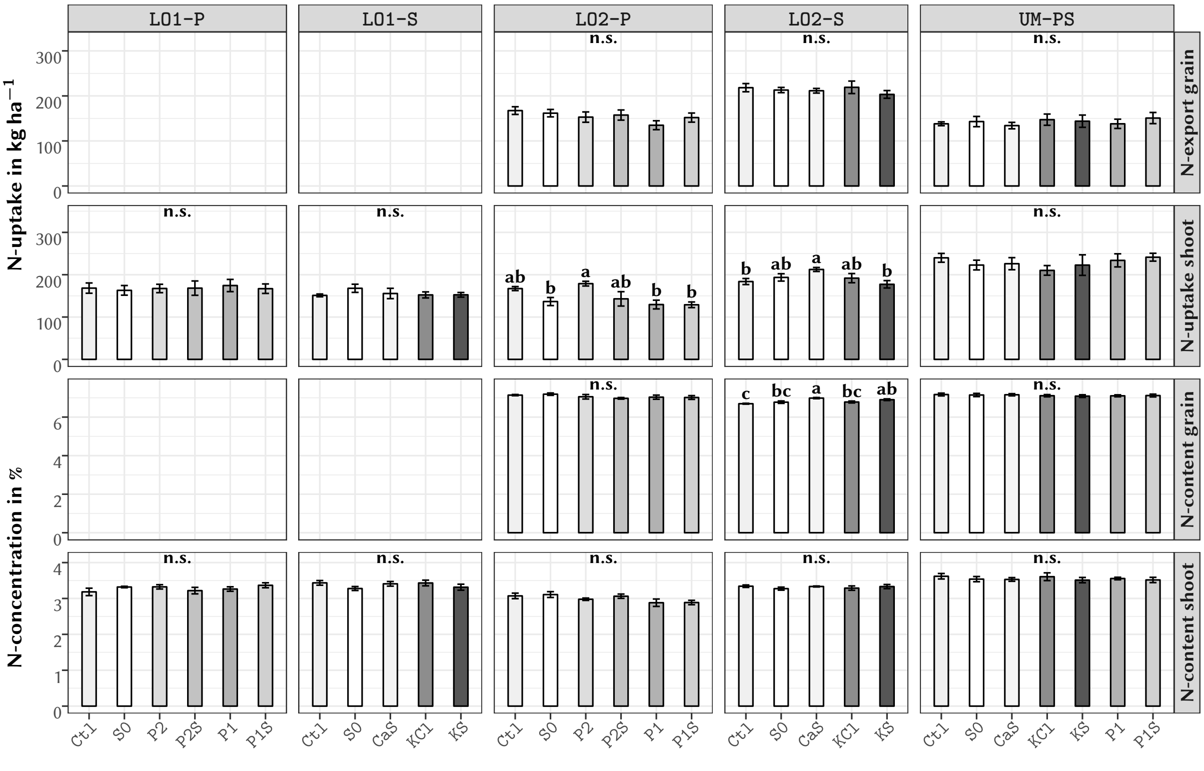
Figure 5.5: Effect of fertiliser treatments on soybean leaf nutrient concentrations contents at R3 growth stage at sites `LO`, `PG` and `UM` in 2013. For treatment abbreviations refer to figure 5.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

treatments. The leaf N-concentration was considered sufficient and the concentration in grain (and hence crude protein content) was the highest of all trials.

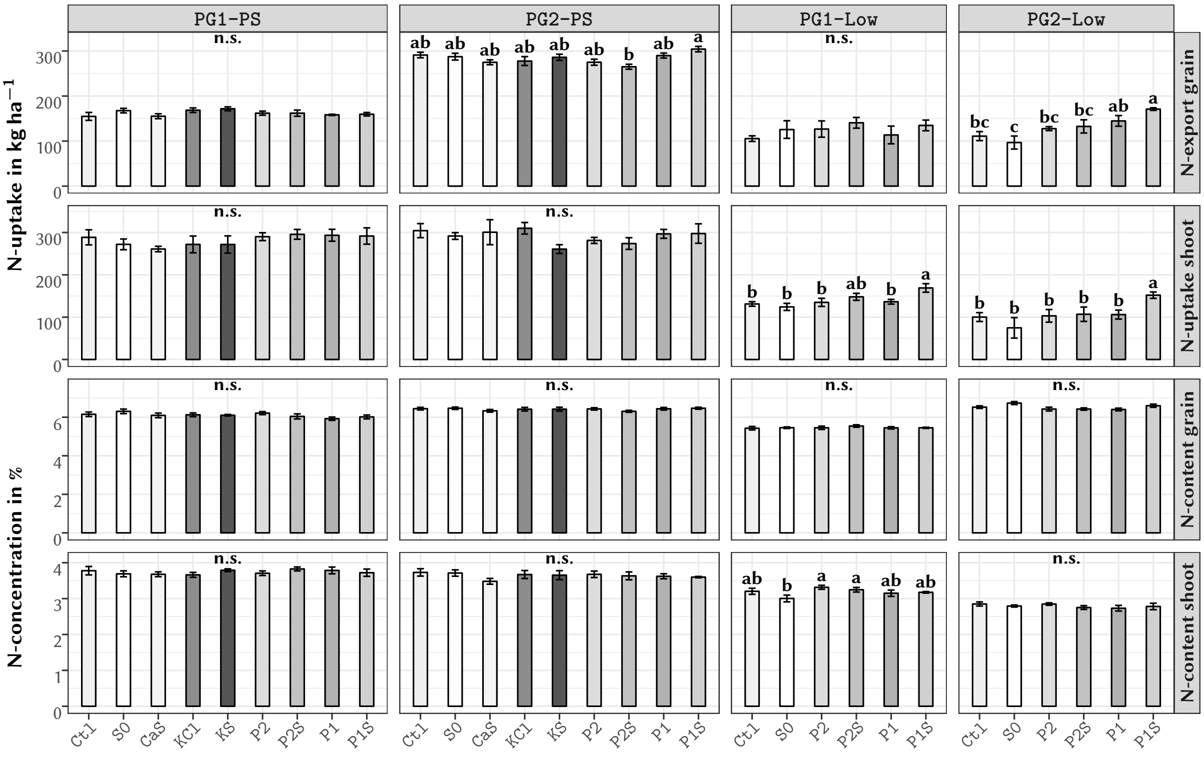
For all trials shoot and leaf N-concentrations of nitrogen were far above values found for growth stage R4.5 by Sexton et al. (1998). Leaf N-concentrations were at sufficient levels for **`PG2-PS`** and **`UM-PS`**, while **`PG2-Low`** exhibited low concentrations for all treatments, with the lowest concentrations for treatments `S0` and `P2`. Nevertheless, these differences were rather minor. Even though considered low, the leaf N-concentrations in trials **`LO2-S`** and **`LO2-P`** were near a sufficient level for all treatments (Embrapa Soja, 2007).

5.3.3 Sulphur and N:S ratio

In both **`LO1`** trials the fertiliser treatments resulted in almost equal S-uptake and -concentration. Shoot S-concentration in trial **`LO2-P`** was significantly higher for `S0` and `P2S` (maximum difference of +8.9 %) compared with treatments `Ctl`, `P2` and `P1`, while grain S-contents did not present any differentiation. In trial **`LO2-S`** shoot S-concentration was about equal for all treatments. Grain S-content of treatment `CaS` was slightly higher than `Ctl` (s.d., +5.5 %). Nevertheless, the other sulphate fertiliser treatment `KS` did not cause an increase in grain S-concentration, while S-free `KCl` treatment presented significantly higher values than `Ctl`. This indicates that the significant differences, which were relatively small in absolute terms, were probably random effects. In trials **`PG1-PS`** and **`PG2-PS`** no differences in shoot or grain concentration were apparent. Due to low differences between treatments in shoot and grain S-concentrations of trials **`LO2-P`**, **`LO2-S`**, **`PG1-PS`** and **`PG2-PS`**, the S-uptake was merely a function of shoot growth and grain yield and hence the same treatment pattern was observed (figure 5.1 on page 70). In trial **`PG1-Low`** significant differences were only present for shoot S-concentration, with highest values for `S0` and `P2`, and lowest for `P1S`, while grain S-content was equal for all treatments. Even though shoot and grain S-uptake did not show significant differences, in both evaluations `Ctl` treatment showed the lowest, and `P1S` and `P2S` the highest values. The `S0` treatment of **`PG2-Low`** showed by far the highest sulphur concentrations in shoot of all trials, but at the same time the lowest total uptake into shoot, which indicates a concentration effect. On one hand, the lowest shoot and grain concentrations (s.d.)



(a) Londrina & Umuarama



(b) Ponta Grossa

Figure 5.6: Effect of fertiliser treatments on N-uptake and -concentration in shoot and grain. For treatment abbreviations refer to figure 5.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

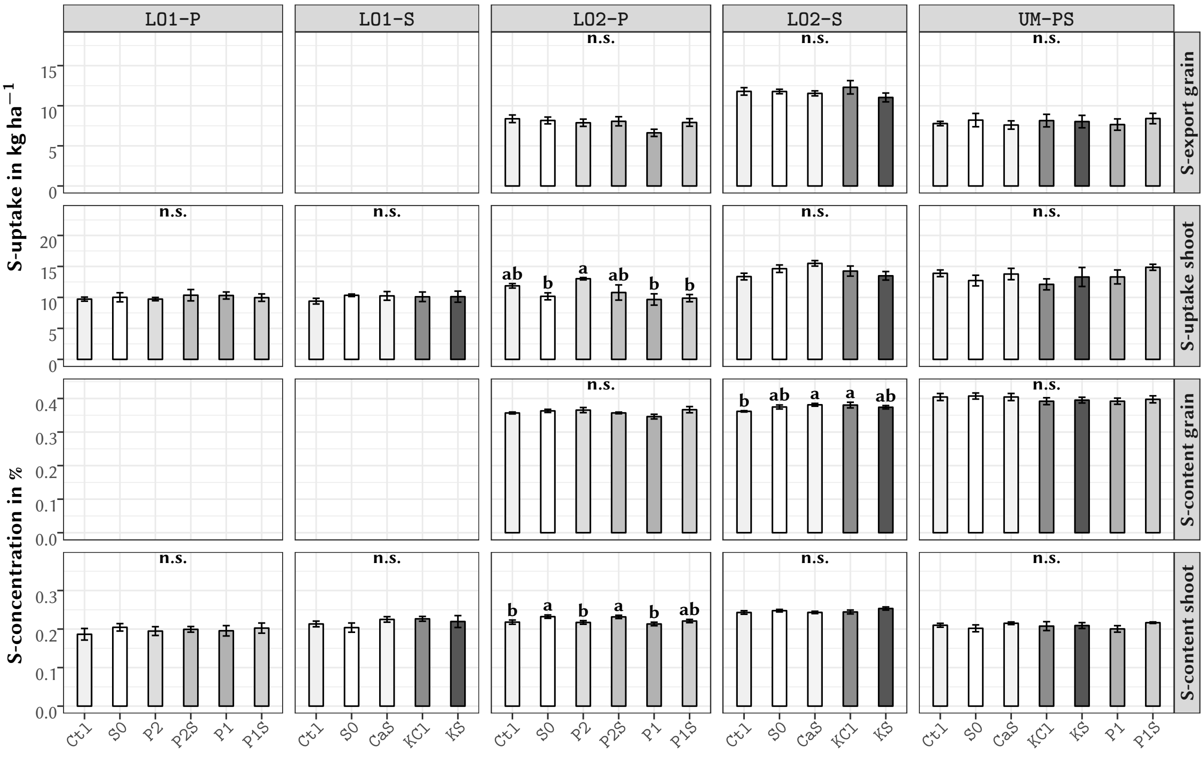
in this trial were found for treatments `P1` and `P1S`. On the other hand shoot and grain S-uptake of treatment `P1S` was highest, hence indicating a dilution effect. Similar results were repeated for grain S-parameters: while the concentration of `S0` was highest (s.d.) and that of `P1S` lowest (s.d.), the contrary was the case for grain S-uptake. In **`UM-PS`** trial identical values for shoot and grain S-concentrations and no differentiation for S-uptake were observed.

The shoot S-concentrations, which were over trials in the range of 0.19–0.25 % were generally double of the values determined by Sexton et al. (1998) for sulphur sufficient plants at stage R4.5. The leaf S-concentration was only measured for **`LO2-S`** which resulted in contents of about 0.31 %, which is a value at the lower end of the level considered sufficient according to Embrapa Soja (2007). Soybean grain S-concentration is a reliable index for detecting S-deficiency as it is highly correlated with yield, and using values of Hitsuda et al. (2004) as a reference, the grain S-concentration was without exception in a normal range (> 0.23 %), with minimum values just below 0.3 % and maximum values of 0.4 %, which points to sufficient S-supply. Nevertheless, grain sulphur reference contents mentioned by Embrapa Soja (2007) are far higher than those in trials with 0.54 %.

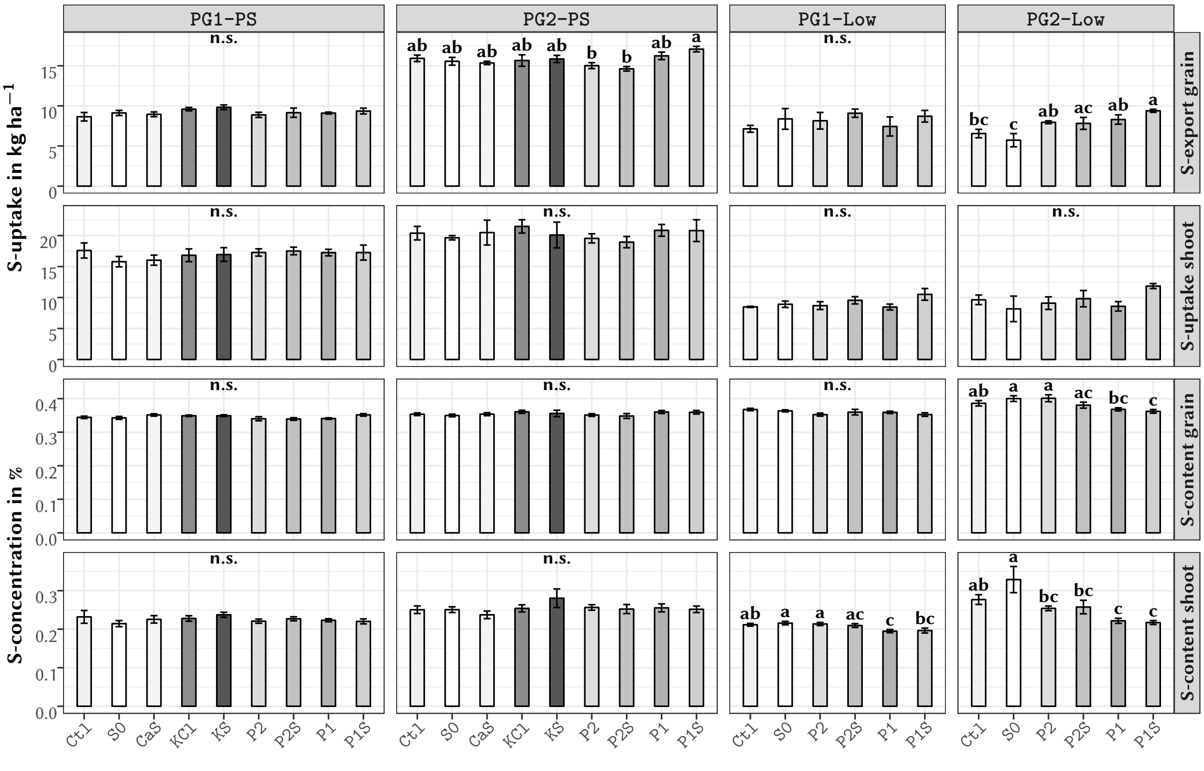
The N:S ratio was only affected by fertiliser treatments in **`PG-Low`** trials (figure 5.10). In season 2011/12 clear differences were only observed in shoot N:S ratios, with `S0` exhibiting the narrowest and both `P1` and `P1S` the widest ratio. In **`PG2-Low`** the differences in shoot were even more pronounced with N:S ratio of `P1S > P1 = P2S > P2 > Ctl > S0`. Significant differences in N:S ratio were also determined in grain: N:S ratio of `P1S` was significantly wider than that of `P2S`, `S` and `Ctl`. Furthermore, the N:S ratio of `Ctl` and `P2S` was significantly wider than that of `P2`.

5.3.4 Potassium

K-uptake and K-concentration in shoot, leaf and grain did not show an increase for K-containing fertiliser treatments `KS` and `KCl` compared with `Ctl` (figure B.5 in appendix on page 131) in all fertiliser trials. The few significant differences found in K-concentrations were only marginal in absolute terms (e.g. grain of **`LO2-S`** and **`PG2-PS`**) and apparently random effects at times. For example, in trial **`LO2-S`** the `Ctl` treatment exhibited slightly higher K-concentration (s.d.) compared with K-fertiliser treatment `KCl`, which is obviously not plausible. In **`LO2-P`**, a minor statistically significant difference was present for the shoot K-concentration, but the maximum difference between treatments was only 7.4 %. Leaf concentrations were sufficient (> 1.76 %) or high (> 0.24 %, **`PG2-PS`**) for all trials apart from **`UM-PS`**. In the latter trial the leaf K-concentration was in general slightly deficient and K-fertiliser treatments were able to raise concentrations to near sufficient levels. With no reaction to fertilisation and generally sufficient plant concentration levels and soil levels even at site **`UM-PS`** it was concluded that K was not a limiting factor for soybean development in any of the trials.

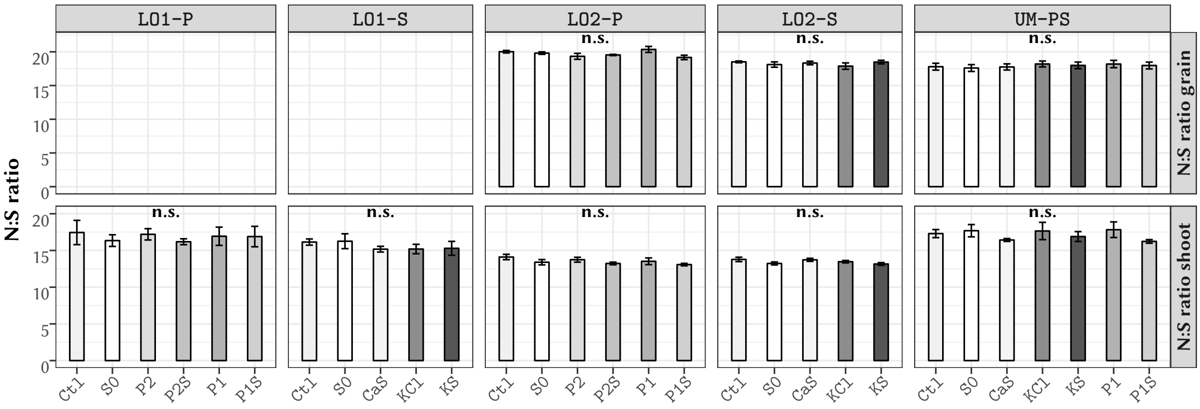


(a) Londrina & Umuarama

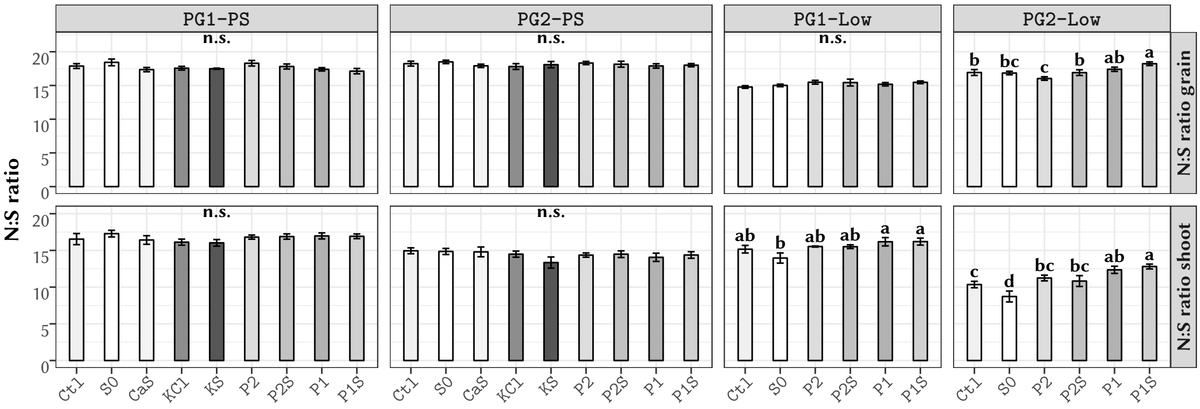


(b) Ponta Grossa

Figure 5.8: Effect of fertiliser treatments on S-uptake and -concentration in shoot and grain. For treatment abbreviations refer to figure 5.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).



(a) Londrina & Umuarama



(b) Ponta Grossa

Figure 5.10: Effect of fertiliser treatments on N:S-ratio in shoot and grain. For treatment abbreviations refer to figure 5.1. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

5.4 Summary results soybean fertiliser trials

Box 5.1 Summary soybean fertiliser trials

Soil

- P- and K- contents were generally at sufficient to high levels (except for `PG-Low`).
- Exchangeable Al^{3+} was present in top soil at most sites, however, (near) toxic levels were only found in trials `PG-Low` and `LO2-S`.
- `UM-PS` was the only site with low SOM contents.

Crop growth and yield response to fertiliser application

- `P1S` treatment increased crop growth in `PG1-Low` trial, and additionally yield in trial `PG2-Low`.
- In trial `LO2-S`, crop growth was significantly higher for treatment `CaS` (+15.8 %).
- In all other trials no differences in crop growth and grain yield were present.

Nutrient parameters

- `PG1-Low`:
  - The N:S ratio in shoot was slightly more narrow for treatments `P1` and `P1S`.
  - The N-concentration in shoot was decreased for treatment `S0`.
  - The S-concentration in shoot decreased with `P1` and `P1S` application, however, no differences in shoot uptake were present.
- `PG2-Low`:
  - The N-concentration in leaf was increased for treatments `P2S`, `P1S` and `P1`.
  - The S-concentration in shoot and grain decreased with `P1` and `P1S` application, but shoot S-uptake did not show any response.
  - The N:S ratio in shoot differentiated strongly: `P1S` > `P1` = `P2S` > `P2` > `Ctl` > `S0`.
  - The N:S ratio in grain showed a slight differentiation: treatment `P1` exhibited the widest and `P2` the narrowest ratio.
- `LO2-P`: minor differences were observed for S-concentration in shoot.
- `LO2-S`: minor differences were observed for N- and S-concentration in grain.
- No response to fertiliser treatments was found in trials `LO1-P`, `LO1-S`, `UM-PS` and `PG1-PS`.

Nutrient levels according to reference values in literature

- K-concentration in shoot, leaf and grain were sufficient in all trials except `UM-PS`.
- N- and S-concentration was sufficient in shoot and grain in all trials.
- N-concentration in leaf was slightly deficient for `LO2-P`, `LO2-S`, and `PG2-Low`.
- S-concentration in leaf was at a sufficient level for trial `LO2-S` (only trial tested).
- For P-concentration in shoot no reference data was available.
- The P-concentration in leaf and grain was slightly deficient in both `PG-Low` trials and at sufficient levels in all other trials.

Conclusion Except for site `PG-Low` no deficiencies of P, K or S were limiting crop development.

	Hypothesis	`PG-Low`	Others
1.	RP increases concentrations and uptake of P	+	-
2.	RP increases crop growth and yield	+	-
3.	RP+S^0 further increases concentrations and uptake P	+	-
4.	RP+S^0 further enhances crop growth and yield	+	-
5.	S-fertiliser increases concentrations and uptake of S	-	-
6.	S-fertiliser increases crop growth and yield	-	-
7.	Soluble sulphur forms K_2SO_4 and gypsum are most effective	-	-

6 Discussion B: soybean trials (Brazil)

In several occasions relatively small, but statistically significant differences between fertiliser treatments were found for crop growth and nutrient parameters. However, these differences often did not seem to be meaningful and were also not repeated over several trials. Furthermore, the relative differences determined were mostly below 10 %, which for many parameters is only a subtle difference in the context of field trial. Realising statistical testing with a total of nine trials which more than ten independent parameters each (i.e. not including the parameters nutrient uptake or export, which are calculated from shoot dry mass, grain yield and their respective nutrient contents) a considerable number of false positives naturally arises by chance. Therefore statistical differences, which were not observed to be consecutive over trials, which were relatively small or which showed flawed logic, were interpreted as random effects. In the results section these random effects have been identified and the corresponding treatment differences were assumed not to be directly related to fertiliser effects. They will hence be ignored in the following discussion, which will merely focuses on the results that are directly related to the effects of phosphorus and sulphur fertilisation.

6.1 Phosphorus

6.1.1 Londrina (`LO`), Ponta Grossa (`PG-High`) and Umuarama (`UM`)

At the clay rich ferralsol sites `LO` and `PG-High` and at sandy ferralsol site `UM` soil P-contents were medium to high according to (Embrapa Soja, 2007), and no additional P fertiliser application was recommended. P-deficiency symptoms (described in faba bean discussion on page 62) absent in the treatments without P-fertiliser application. Nutrient concentrations of P, and N in shoot, leaf and grain were generally at medium to high levels Embrapa Soja (2007) and P-fertiliser application did not increase shoot dry mass, grain yield, and P- or N-uptake. No control treatments with phosphorus fertilisers more soluble than RP, like super single phosphate (SSP) or triple phosphate (TP), were installed in the trials. Therefore it is not possible to fully determine if the solubility of rock phosphates was in fact limiting crop productivity. Nevertheless growth limitation caused by limited phosphate solubility seems unlikely: not even in trial `PG2-High`, which showed the highest shoot growth and grain yields and hence demand for P of all trials and at the same time one of the lowest soil P-contents (still considered medium), rock P-fertiliser application did not have any effect and it is assumed that more soluble P-fertilisers would not have brought about an effect either. Apart from trials on field `PG-Low`, trial results firmly indicate P-sufficiency. For most sites it can be assumed that there were no adverse effect on soybean crop rooting and hence for phosphorus acquisition. Different from direct-seeding in temperate climate, the lower soil temperatures compared to soils under conventional tillage do not limit root growth but rather maintain soil temperatures at more optimal levels. Furthermore, ferralsol soils possess a low density and a good porosity, also frequently referred to as *coffee powder* structure, which facilitates diffusion processes and root penetration. The sandy *ferralsol* at site `UM` also possesses a great medium soil pore volume which favours rooting. Apart from trial `LO2-S`, Al-toxicity was not a serious constraint to crop development at these sites despite the low pH values, according to reference values in Embrapa Soja (2007),

6.1.2 Ponta Grossa: Low P content site (`PG-Low`)

The soil conditions of `PG-Low` were most distinct from the other ferralsol trial sites: the soil had by far the lowest P-contents and Al-toxicity was strongest. It was the only site for which fertiliser application was repeated in two consecutive trial years and also the only site at which fertiliser application had an effect on crop parameters.

Al-toxicity The soil P-contents of `PG-Low` were extremely low and limiting to crop development. However, soil acidity, which was the severest of all trials, probably even had a more negative impact on crop growth, with exchangeable Al^{3+} reaching near-toxic concentrations already in the topsoil 0–20 cm. With presumably even more increased subsoil acidity and Al-toxicity, the rhizosphere was effectively limited to the topsoil and the acquisition of water and nutrients was compromised. Despite extremely low soil contents, phosphorus was probably less of a limiting factor than the soil volume effectively available for rooting. A possible explanation for the finding that with treatment `S0` shoot dry mass and grain yield decreased strongly in season 2012/2013 could be the acidifying effect of elemental sulphur: upon oxidation by *Thiobacillus* bacteria in soil forms sulphuric acid (Yang et al., 2010). Over the two years of S^0 application, the already present Al-toxicity was thereby intensified in direct root proximity, which further deteriorated crop growth conditions. Nevertheless, the negative effect that `S0` had applied alone was not found when applied in combination with rock phosphates. This could be explained with RP buffering the acidity caused by S^0 oxidation. Furthermore, calcium, which is liberated from RP on dissolution, plays a key role in protecting roots against low pH and Al-toxicity stress (Caires et al., 2002).

Phosphorus fertiliser effect The shoot P-concentration was lower in both years than in the other Brazilian fertiliser trials. However, the drastic difference in shoot P-concentrations between seasons 2011/12 and 2012/13 cannot be explained: the P-concentrations in the first trial season resulted extremely low, and were possibly the result of a laboratory measurement error. The cumulative effect of repeated fertiliser application in season 2012/13 lead to a more pronounced treatment differentiation. The effect of RP was apparently dependent on its solubility, which is assumed to be the reason for *Gafsa* RP giving better results than *Alvorada* (citric acid (2 %) solubility: 40–45 % vs. 17 %, respectively). Changes in tissue P-concentrations still remained relatively low between treatments, but crop growth and P-uptake were clearly increased with rock-P fertiliser treatments. P-concentrations in leaf were found to be deficient in the second trial season, making this the only Brazilian site where presence of P-deficiency could be confirmed. The only significant increase in leaf-P was found for `P1S`, but concentrations remained in a deficient range. The buffering effect of both RP indicated that the acid formed by oxidation of elemental S was indeed buffered by RP and helped in its solubilisation (Rajan, 1982a,b) and therefore liberation of PO_4^{3-}. As a result, a positive effect of the joint application of RP and elemental sulphur in treatments `P1S` and `P2S` can be concluded in trial **`PG-Low`**, which was not related to the fertiliser effect of elemental sulphur.

6.2 Sulphur

6.2.1 Effect of sulphur fertiliser application

In the field trials conducted, sulphur was not a limiting factor for crop production and for none of the trials a sulphur induced effect on soybean shoot dry mass, grain yield was found. Furthermore, neither S-concentrations of shoot, leaf (only limited data) nor grain did react to fertilisation and also N-uptake remained unchanged. Nutrient levels of S in shoot and grain were nearly always sufficient. Evidence for a beneficial effect on the grain protein quality could not be found either, as an increased content of cysteine and methionin would have been indicated by a more narrow N:S ratio (Gayler and Sykes, 1985). This confirms results of Caires et al. (1998) and Caires et al. (2011), who in soybean direct-seeding trials on a ferralsol in Ponta Grossa also did not find any effect for gypsum application on grain yield. However, in works of Sfredo and Lantmann (2007) in *Londrina* and *Ponta Grossa* gypsum and elemental sulphur application both resulted in yield increases for soybean crop.

The reaction to S-fertiliser application observed in trial **`PG2-Low`** with a significantly higher S-uptake into grain in `P1S` treatment was attributed to the positive impact of elemental sulphur on P-availability, which in turn increased crop growth and yield and hence S-uptake. A further effect of application of S-fertiliser `CaS` was observed in trial **`LO2-S`** where crop growth was increased significantly. Nonetheless, also in this trial S-concentrations of leaf, shoot and grain remained equal for all treatments. The application of sulphur fertiliser cannot explain this difference as the more soluble fertiliser `KS` did not have any effect on sulphur uptake. The cause for the effect of gypsum was rather thought to be due to the presence of Al-toxicity. In an adjacent trial with *Phaseolus vulgaris* on the same field tap root deformations starting at about 15 soil depth were observed. Al-toxicity was the most likely cause for these deformations as soil pH in the field of trial **`LO2-S`** was one of the lowest of all trials (only **`PG-Low`** lower) and the level of exchangeable aluminium one of the highest determined in soil samples. Both factors presumably hindered the root development of the soybean crop (Marschner and Marschner, 2012). With no response to `KS` treatment it can be concluded that the increased crop growth of `CaS` was not caused by an improved supply of SO_4^{2-}, but can rather be attributed to the positive effect of gypsum in the mitigation of Al-toxicity. Gypsum application has an direct effect on soil pH, as sulphate is exchanged with OH^- ligands and increases the adsorption of cations (Curtin and Syers, 1990). Furthermore, it enhances root growth by providing Ca^{2+}, which is limited in acid soils and competes with Al^{3+} for exchange sites on the root surface (Marschner and Marschner, 2012) and can alleviate deleterious Al effects (Rengel, 1992). Furthermore, the SO_4^{2-} provided with gypsum application is capable of decreasing Al-toxicity by the formation of non-toxic $AlSO_4^+$ ions (Caires et al., 2002; Marschner and Marschner, 2012). The total absence of fertiliser response in all trials clearly indicates that soybean S-demand could fully be met by SOM mineralisation and with the sulphate that is adsorbed or free in the soil solution, even for the sites with average to high yields and accordingly relatively high S-demands (e.g. `PG-High`).

6.2.2 Experimental sites and susceptibility to S-deficiency

Sulphur in organic matter The largest S-pool at all ferralsol sites is the organically bound sulphur in SOM, which only in the topsoil (0–20 cm) with S-contents of 0.026–0.041 % (to the largest extent present in SOM) at `LO` and `PG` amount to 570–902 kg ha^{-1} S. For the sandy ferralsol site `UM` these values were far lower, with topsoil contents of 1.7 % SOM topsoil and an S-content of only about 150 kg ha^{-1} S and further yet presumably minor amounts (no data present) in subsoil. No data from field trials on annual sulphur mineralisation rates or quantities were found for tropical soils under direct seeding management. Taking the mineralisation rate of total organic sulphur found by Eriksen et al. (1995) for Danish soils of 3 % of amounts mineralised in top soil would amount to 18–28 kg ha^{-1} S for the clayey and about 5 kg ha^{-1} for the sandy ferralsol soils. In a laboratory pot experiment on sulphur mineralisation with Brazilian ferralsol soils with similar texture and SOM contents rates of about 11 kg ha^{-1} were found both for sandy and clayey textures (Silva et al., 1999). The higher SOM mineralisation rate (with respect to SOM content) for the soil with the sandy texture is plausible due to the high soil temperatures and the excellent aeration of sandy soils. Additionally to SOM mineralisation, S contained in oats straw residue of 4 t ha^{-1}, with a S-content of about 0.23 % S (Plant Nutrition Institute, 2014), amounting to about 9 kg ha^{-1} S can largely become available to the crop during the soybean growing season.

Sulphate adsorption In general about 95 % of S in soils is contained in SOM, while mineral sulphate, which is adsorbed or free in the soil solution forms only a minor fraction of the total S pool (Scherer, 2009). However, this only holds true for moderately weathered soils with about neutral pH values. In calcareous soils and in intensely weathered acid tropical soils adsorbed sulphate can form an important pool for crop S-supply. With an ample presence of the variable charge sesquioxides hematite and goethite and a low soil pH in the range of 4.5–5.5 the AEC and sulphate sorption are high in ferralsol soils (Alves and Lavorenti, 2004). For example, Neptune et al. (1975) found a ratio of 8:11 soluble to adsorbed sulphur for a Brazilian ferralsol, meaning that the amount of adsorbed sulphate may exceed that of free sulphate ions in the soil solution.

According to data of Churka Blum et al. (2013), the maximum adsorption of SO_4^{2-}-S in the topsoil (0–20 cm) of a loamy ferralsol in Ponta Grossa was 56.8 mg kg^{-1} and 217.4 mg kg^{-1} in the subsoil (180–200 cm) and in a clayey ferralsol in Guarapuava, Paraná 133.3 mg kg^{-1} and 384.6 mg kg^{-1}, respectively. The reason for the lower values in topsoil is that sulphate adsorption is influenced negatively by high pH (Bolan et al., 1986), soil organic matter and phosphate ions (Couto et al., 1979; Bolan et al., 1988; Liu and Hue, 2001; Scherer, 2001). In another study of Alves and Lavorenti (2004) on representative soils in São Paulo state, Brazil, a maximum SO_4^{2-}-S adsorption capacity of clay in the subsoil of ferralsols (80–170 cm soil depth) was determined to be between 163–473 mg kg^{-1} of clay, which is equivalent to a range of 118–411 kg ha^{-1} maximum sulphate adsorption for a subsoil layer of 10 cm thickness. Given the clay content of the clayey ferralsols at experimental soils at `LO` and `PG` of 64–79 % maximum sulphate-S adsorption of 118–411 kg ha^{-1} would be possible and 29–83 kg ha^{-1} for the sandy ferralsol at site `UM` (16 % clay).

Actual values of extractable sulphate found by Churka Blum et al. (2013) could be taken as a reference for the magnitude of the amount of adsorbed sulphate potentially present in the clayey ferralsol soils studied in the work at hand: between 0–100 cm soil depth a total of 72 kg ha^{-1} adsorbed S was found in

Ponta Grossa, 13 years after gypsum application of 12 t ha^{-1}, compared to 39 kg ha^{-1} for the non-fertilised plots, and in Guarapuava, 3.5 years after application 297 kg ha^{-1} compared to 48 kg ha^{-1}, respectively. Reference values for extractable sulphate in sandy ferralsols were not found in literature and it can only be assumed that the magnitude would be about 4–5 times lower according to the lower clay contents, hence about 10 kg ha^{-1}, assuming no gypsum application. Therefore even without gypsum application, adsorbed mineral sulphur can be assumed to be an important S-pool in ferralsol soils, which should largely be available to the crop. The total soybean S-demand for yields of 3–4 t ha^{-1} is in the range of 45–60 kg ha^{-1} (Embrapa Soja, 2007). According to the values cited above, this demand can be met by sulphur mineralisation and soil mineral sulphur, which explains why S-fertiliser treatments did not have any effect.

Sulphate leaching potential Both clayey and sandy ferralsols exhibit fast infiltration rates, but only the clayey ones have a high water holding capacity. Therefore, the leaching potential of sandy ferralsols is certainly higher. Up to a certain point, the high potential for sulphate adsorption can inhibit leaching. For example, in the experiment of Churka Blum et al. (2013), 13 years after application of 12 t ha^{-1} of gypsum in Ponta Grossa, only 52 % had been leached below 200 cm soil depth and concentrations up to 100 cm were still higher than those for the non-fertilised treatment, while 3.5 years after gypsum application in the clayey ferralsol at Guarapuava, 100 % of the gypsum was still present up to same soil depth, with highest concentration in the soil layer from 20–60 cm. For sandy ferralsols no data on leaching rates was found, but rates can be expected to be far higher due to the lower sulphate adsorption potential. An important fact to consider when discussing leaching potential is that bare fallows hardly exist in crop rotations in Paraná state and for most of the year a growing vegetation cover can actively remove SO_4^{2-} from soil solution, which recycles sulphur back to topsoil (Eriksen and Thorup-Kristensen, 2002) and eventually to SOM. While at site `LO` and `PG` annual cropping is practised, at site `UM` conversion has only occurred recently. During the long-term pasture use, S-exports were minimal and leaching was inhibited more effectively than with annual crops by the relatively extensive root system of pastures. In conclusion, the combination of SOM preservation in DS, near-permanent vegetation cover and high sulphate adsorption potential in acid soil conditions limit sulphate leaching and at least for the clayey ferralsol soils sulphate leaching rates can be assumed to be much lower than expected initially.

6.2.3 Balance of sulphur fluxes

Cropping system exports A typical cropping system used in mainstream agriculture in Paraná state is a rotation consisting of about 50–85 % soybean, which is planted in rotations with maize in summer, and wheat, oats or green manure plants in winter, e.g. hairy vetch (*Vicia villosa*), or wild radish (*Raphanus raphanistrum*). Soybean is the major sulphur exporting crop in Brazilian agriculture (Yamada and Lopes, 1998), and in comparison with maize or wheat, the S-demand of soybean is highest (Sfredo and Lantmann, 2007). With average soybean grain yields of 3.4 t ha^{-1} in Paraná state (IBGE 2014 [21]) and a grain concentration of 0.3–0.54 % (Embrapa Soja, 2007), the total export of S is in the range of 9–15 kg ha^{-1}, which is similar to values found in this work (7–15 kg ha^{-1}). With a grain concentrations of maize and wheat of about 0.1 % and 0.15 % S typical yields result in S-exports of 7–9 kg ha^{-1} and 3–6 kg ha^{-1} S, respectively. Annual S-exports are therefore in the range from about 8 kg ha^{-1}, e.g. for maize cropping in summer plus a green manure winter crop, to 12–21 kg ha^{-1} S for a rotation of soybean followed by wheat in winter. On average a minimum annual export of sulphur between 10–15 kg ha^{-1} can be assumed.

Sulphur fertilisation and atmospheric inputs Atmospheric immissions are the most relevant input of sulphur to agricultural soils. High resolution data on regional variations in S-immission in Brazil are not available but several studies on rain water chemistry have been conducted in metropolitan, near-industrial and rural areas. For the metropolitan areas of São Paulo and Rio de Janeiro, wet-depositions of 8.2 kg ha^{-1} a^{-1} S (Rocha et al., 2003) and 7.2 kg ha^{-1} a^{-1} S (De Mello, 2001) have been determined,

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[21] IBGE: Instituto brasileiro de geografia e estatística - Brazilian institute for geography and statistics

respectively. Near coal power plants, which are one of the strongest S-emitters, wet depositions were 13.3 kg ha^{-1} a^{-1} and 15 kg ha^{-1} S (Migliavacca et al., 2004; Flues et al., 2002). Nonetheless, the soybean cultivation sites in Paraná state are generally in rural areas with low industrialisation, and so are the experimental sites. For Cuiaba, Mato Grosso state, a non-industrialised city in the interior of Brazil, sulphur wet deposition is as low as 0.2 kg ha^{-1} a^{-1} S (Dias et al., 2012). The trial site and second largest city in Paraná state Londrina, has a low degree of industrialisation and the wet-deposition at this site is only about 2.1 kg ha^{-1} a^{-1} S (Pelicho et al., 2006). Similar immissions are assumed for *Ponta Grossa* and even lower values for the trial site outside of the small town of *Umuarama*. Considering that dry deposition rates can be assumed to be of the same magnitude as wet-deposition (Garland, 1978), the total sulphur immission rate to be expected at experimental sites in Paraná state are unlikely to exceed 5 kg ha^{-1} a^{-1} S.

Therefore an imbalance between harvest exports and atmospheric inputs is apparent in Paraná state, with a negative balance of at least 10 kg ha^{-1} a^{-1} S, because further amounts are removed by leaching, with presumably elevated rates for soils with a sandy texture. Hence, fertiliser inputs of sulphur become necessary to avoid S-deficiency in the long term.

Mixed farming systems are not wide-spread and therefore no on-farm sulphur recycling with organic fertiliser application is practised in general. Cattle livestock farming is realised as grazing systems, and sheds that would enable manure collection are only used for pig and poultry production. Typically, it can be assumed that harvested sulphur is entirely exported off-farm. In most fertilisers sulphur is absent or contents are low, as more concentrated fertilisers are used preferentially (Schnug, 1988; Scherer, 2001). For example, the more concentrated P-fertiliser triple phosphate is preferred over the sulphur containing super-single-phosphate (SSP).

Nonetheless, amelioration of soils with a combination of limestone and gypsum is common practice in Brazil. The purpose of limestone application is to correct for acidity in the topsoil, but applied in this form Ca^{2+} is not mobile enough to move to and mitigate low Ca^{2+} contents in the subsoil. The purpose of gypsum application is to effectively bring Ca^{2+} into the subsoil as it is far more mobile in soil. The supply of S as a plant nutrient is - if at all - only a secondary objective of soil amelioration. Apart from forming less toxic Al-SO_4^{2-} salts, the sulphate contained in gypsum also exhibits a *self-liming* effect, by exchange with OH^- ligands upon adsorption. This combined effect of gypsum in the subsoil effectively ameliorates Al-toxicity (Caires et al., 2011). Gypsum application is recommended when subsoil Ca^{2+} contents are below 0.5 cmol$_c$ dm^{-3} and saturation of Al^{3+} above 20 %.

No soil analysis of subsoil layers is available for the experimental sites, yet the high saturation of Al^{3+} in the topsoil of `PG-Low`, `UM` and `LO2-S` makes the presence of Al-toxicity likely. In fact, for the latter site symptoms resembling Al-toxicity were found. Nevertheless, at none of the experimental sites gypsum application has been realised in the past. Rather than a intra-row application of sulphur fertilisers, broadcast gypsum application has to be recommended. Due to the relatively fast infiltration into the soil the broadcast application of gypsum is a much more effective way to supply sulphur to the crop, which furthermore helps to ameliorate a much larger soil volume than a intra-row application. With typical recommended rates of 700 kg ha^{-1} gypsum for sandy soils and up to 3200 kg ha^{-1} for clay soils (rule of thumb: 50 x % clay kg ha^{-1}), large sulphur amounts of 110 kg ha^{-1} and 500 kg ha^{-1} S are applied, respectively, and due to the adsorption capacity of tropical variable charge soils, there is a residual effect of 5–15 years (de Sousa et al., 2005). Gypsum broadcast application can therefore avoid occurrence of S-deficiency in the long-term effectively. Nonetheless, excessive gypsum applications have to be avoided as SO_4^{2-} is known to move through the soil profile together with cations, which can result in leaching losses of potassium and magnesium (Liu and Hue, 2001).

7 Results C: natural herbicide trials (Brazil)

7.1 Screening trials

As there was little knowledge on natural herbicide (NH) formulation at the beginning, screening trials were of exploratory nature and intended to answer a wide range of questions. Research evolved strongly during the two trial seasons in Brazil and especially screening trials have to be understood in this context. Beforehand, no analytic statistics of the weed injury ratings were intended and conclusions were only drawn from descriptive statistics. In plots, the ratings for the individual weed species are represented by different shapes and the mean value averaged over all species by horizontal bars. Thereby observations on overall treatment efficacy and susceptibility of different weed species can be made. A rating above 70 is considered *acceptable weed control*. Individual screening trials mostly contained a large number of treatments and sub-trials with different experimental questions. For graphical presentation of results, trials were often subdivided into orthogonal sub-trials, which sometimes results in data of a single treatment being presented in several sub-trial plots. In a number of occasions results of sub-trials of different screenings on the same research question were summarised into a single plot arrangement in order to visualise common tendencies. This is most evident for a compilation of the effect on formulation efficacy of the *emulsifier type*, in which the results of eight trials are compiled. As NH formulations often contain a variety of ingredients a codified form is used for treatment descriptions in plots and occasionally in text.

The abbreviations used for active ingredient (AI) and emulsifiers as well as the code to describe NH formulations are all presented in boxes 2.4 and 2.5 on page 32. Weed species in plot legends and screening trial treatment descriptions are abbreviated to their 5-letter Bayer code. The full plant species name can be found in the following box:

Box 7.1 Abbreviations: 5-letter Bayer code of weed species

Bayer code	Species	Bayer code	Species
1AMAG	*Amaranthus sp.*	GLXMA	*Glycine max*
BIDPI	*Bidens pilosa*	IAQGR	*Ipomoea grandifolia*
BRAPL	*Brachiaria plantaginea*	RAPRA	*Raphanus raphanistrum*
EPHHL	*Euphorbia heterophylla*	RCHBR	*Richardia brasiliensis*
GASPA	*Galinsoga parviflora*	SONOL	*Sonchus oleraceus*

In order to further shorten treatment descriptions in plots and to direct the readers attention only to the part of the formulation which changes between treatments, ingredients or spray volumes used in all treatments of a screening trial are described in the title box of each (sub-) plot. In case it is not specified in treatments or title box the application rate is 600 L ha^{-1}.

7.1.1 Essential oils and acetic acid

`S-L1`, `S-PG1`, `S-PG2`, `S-PG3`, `S-PG16`: cell membrane disruptors applied alone In these screening trials weed control of NH containing different cell membrane disruptors (CMD) was compared at application rates of 300 L ha^{-1} and 600 L ha^{-1}. The CMD tested were acetic acid (AA) and the essential oils δ-limonene, pine oil, citronella oil, eucalypt oil (of *Eucalyptus citriodora*), and turpentine (not used in commercial formulations). Emulsifiers were employed in all trials apart from `S-L1` and treatment `30 AA + 0 Emulsifier` of `S-PG2`. Due to unlimited solubility of AA in water there was no implicit need for an emulsifier in this treatment, nevertheless it was used in `S-PG3` and all AA treatments in later screenings. Emulsifiers used in `S-PG1` and `S-PG2` were *Emustab*® (for δ-limonene) and sodium ricenoleate (for eucalypt, citronella and pine oil) as well as nonylphenol ethoxylate (NPE) in `S-PG3`. In `S-PG16` the anionic emulsifier sulphonic acid sulphonic acid (SA), specifically sodium dodecylbenzenesulphonate, was tested as an emulsifier, which is commonly used in laundry detergents. Results are presented in figure 7.1. In `S-PG1` and `S-PG2` the AI rates of eucalypt, citronella and pine oil were not identical with that of δ-limonene. The reason for this was a misperception: for example, the pine oil used had a α

terpineol content of 67 %. The AI application rate was at first erroneously based on α terpineol, and therefore 90 L ha^{-1} pine oil were applied instead of 60 L ha^{-1}. The same error also applies to eucalypt (70 % 1,8-cineole) and citronella oil. Nevertheless, the differences in effect between treatments were that marked that the concentrations did not play a major role for interpretation. Starting from `S-PG3` this error was corrected.

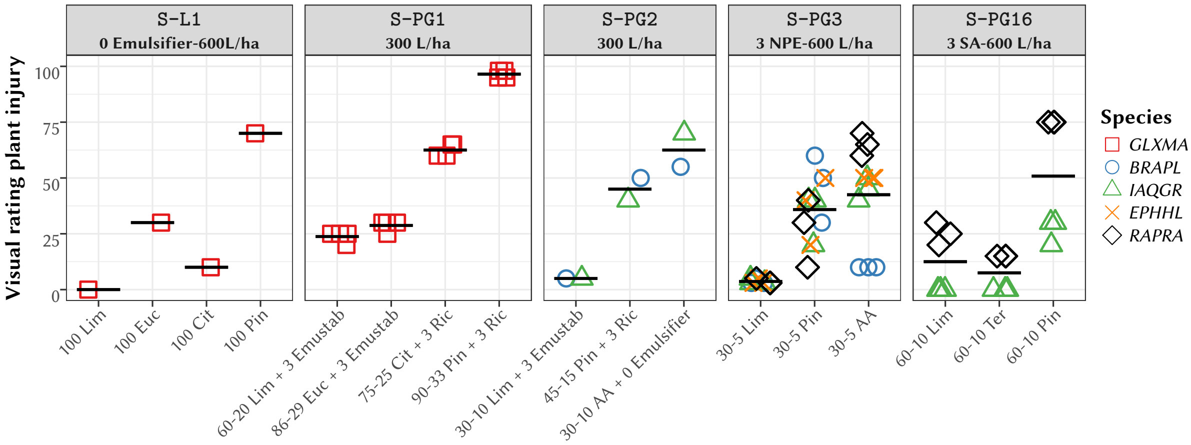
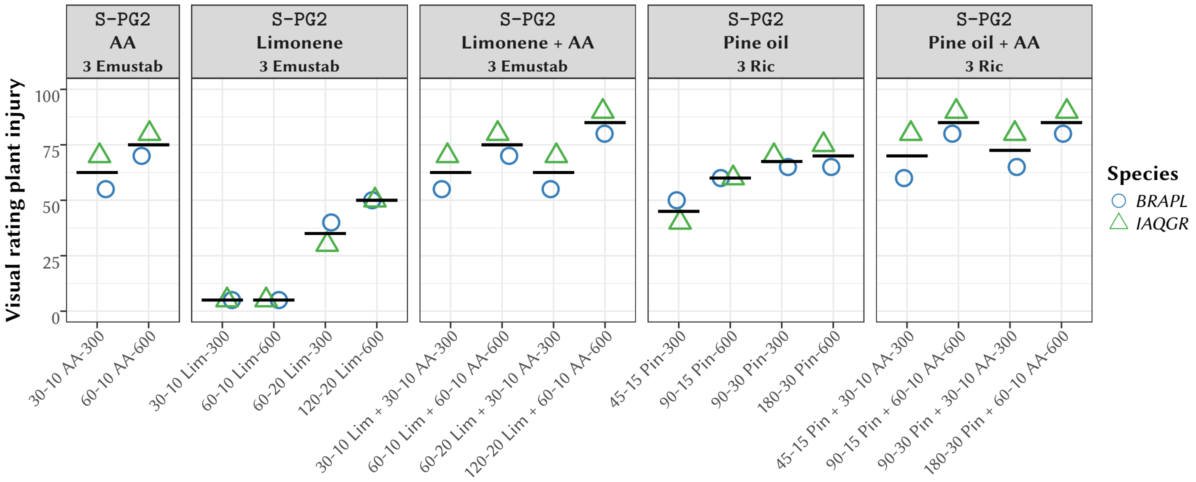


Figure 7.1: Screening trials `S-L1`, `S-PG1`, `S-PG2`, `S-PG3`, `S-PG16`: effect on weed control of NH formulations containing a single CMD. The active ingredients used were δ-limonene (Lim), eucalypt oil (Euc), citronella oil (Cit), turpentine (Tur) and acetic acid (AA). The essential oils were formulated with the emulsifiers sodium ricenoleate (Ric - for Lim and Euc) and Tween 20® (T20 - Pin). No emulsifiers were used in `S-L1` and AA treatment of `S-PG2`. Emulsifiers and application rates common to all treatments are specified in each sub-trial title box. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all plant species. A rating above 70 is considered as *acceptable weed control*.

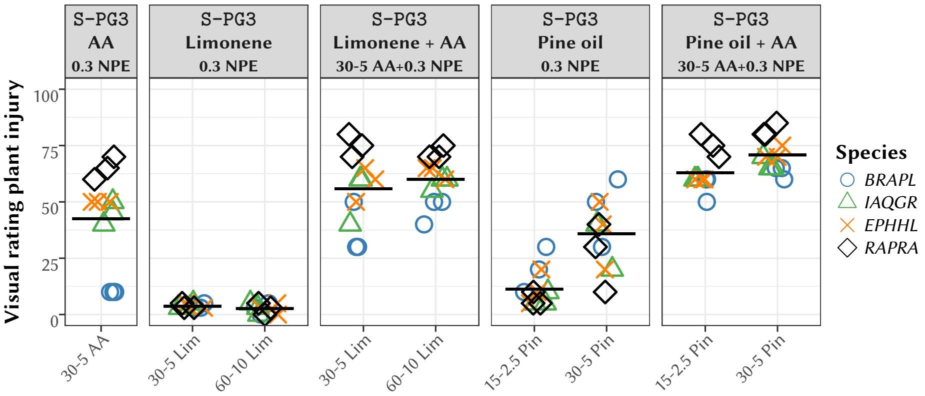
The Londrina screening `S-L1` was the only screening that tested essential oils without emulsifiers. Despite the high AI rate of 100 L ha^{-1} for the essential oils only the effect of pine oil was strong, followed by a notable effect of eucalypt oil, a weak effect of citronella oil and no effect of δ-limonene. At AI rates of 60–90 L ha^{-1} in `S-PG1`, only pine oil showed excellent, and citronella oil moderate weed control. The effect of eucalypt oil and δ-limonene was hardly perceivable. In `S-PG2` and `S-PG3` lower AI rates of 30 L ha^{-1} were used, with application rates of 300 L ha^{-1} and 600 L ha^{-1}, respectively. Weed control was not acceptable in any of the treatments. The effect of δ-limonene was absent, that of pine oil notable and that of AA strongest, but weed control was also not acceptable in both screenings. In `S-PG16` only pine oil showed an intermediate weed control, however, the control of *R. raphanistrum* was even acceptable (>70). The effect of eucalypt oil and δ-limonene was weak in all four screenings. Weed control of citronella oil and pine oil was noticeable but not satisfactory. AA in all three screenings obtained the highest score, however, only in `S-PG2` a nearly acceptable rating (below 70) was obtained. Eucalypt oil and citronella were only tested in `S-L1` and `S-PG1`. Both AI were discarded, eucalypt oil due to its low effect and citronella oil because of its highly elevated cost and the poorer performance compared with the cheaper pine oil. Despite miserable weed control tests with δ-limonene were continued in screenings and later in field trials, because of its relatively low price and the fact that it is used successfully in commercial NH products like NaturesAvenger®. At this stage the low performance δ-limonene was thought to be a problem of emulsion stability, hence improper formulation.

`S-PG2` and `S-PG3`: essential oil in combination with acetic acid Both screenings examined applications of AI pine oil, δ-limonene and AA alone, and combinations of these essential oils with AA. A further objective of `S-PG2` was to test different concentrations of δ-limonene (10 & 20 %) and pine oil (15 & 30 %) as well as different application rates (300 L ha^{-1} & 600 L ha^{-1}, figure 7.2). The single AI results were already shown partially in figure 7.1, but are plotted again as a reference for the treatments with added AA. From `S-PG3` onward, the application rate was raised to 600 L ha^{-1} in all screenings in order to ensure higher spray coverage. It was also intended to decrease AI amounts for pine oil (down to 15 L ha^{-1}) in order to lower spray costs. Therefore, concentrations in `S-PG3` were generally lower than in S-PG2. The NPE emulsifier *Renex 95*®, and *Tween 20*® (T20) were used for the first time in S-PG3. However, the total emulsifier amounts in these formulations were low, each at a rate of 0.2 L ha^{-1}.

The effect of δ-limonene was weak in both screening trials and only rates of 60 and 120 L ha^{-1} had a notable effect. The 90 and 120 L ha^{-1} rate of pine oil showed acceptable weed control in S-PG2, while weed control of 30 L ha^{-1} in S-PG3 was moderate, and that of 15 L ha^{-1} weak. In S-PG2 weed control of 30 L ha^{-1} AA was moderate and 60 L ha^{-1} AA acceptable. Weed control of essential oils combined with AA is in all cases stronger than the effect of each of the AI by themselves. Nevertheless, the increase in effect was rather small in comparison with AA alone. With each pair in S-PG2 the effect of the increase of application rate (at same AI concentration) can be observed. Nevertheless, doubling the application rate and hence cost, the observed increase in effect was rather small. The application cost also doubles when doubling the AI concentration, which can be observed in the following treatments: 30-10 Lim-300 vs 60-20 Lim-300 and 60-10 Lim-600 vs 120-20 Lim-600, as well as 45-15 Pin-300 vs. 90-30 Pin-300 and 90-15 Pin-600 vs. 180-30 Pin-600. This effect was notably stronger than that of doubling the application rate. Different dilutions, meaning that the same amount of AI is applied at different application rates, can only be observed between treatments 60-10 Lim-600 and 60-20 Lim-300, as well as between 90-15 Pin-600 vs. 90-30 Pin-300: when applied in a more concentrated form the effect is increased slightly for pine oil and considerably for δ-limonene, at the same application cost.



(a) Screening trial S-PG2



(b) Screening trial S-PG3

Figure 7.2: Screening trials S-PG2 and S-PG3: effect on weed control of NH formulations containing the CMD δ-limonene (Lim), pine oil (Pin) and AA alone, and the combinations of both essential oils with AA. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all plant species. A rating above 70 is considered as *acceptable weed control*.

The effect of NH containing essential oils and acetic acid can be summarised as follows:

Box 7.2 Summary: effect of essential oils and acetic acid

- Comparison of AI effect: AA > pine oil > citronella oil > eucalypt oil > δ-limonene > turpentine.
- Without addition of an emulsifier only pine oil and acetic acid have any effect.
- The absolute effect of δ-limonene was minimal, even at a high concentration of 20 %.
- Weed control of the mixture of essential oils and AA was not largely improved compared to AA alone.
- At a fixed AI rate, the concentrated application resulted in better control than the diluted one.

7.1.2 Vegetable oil applied alone and in combination with essential oils

Only in screening `S-L2` the refined vegetable oils of corn, soybean and rape oil were applied at rates of 50 L ha^{-1} in combination with the Organic Agriculture (OA) certified emulsifier Rimulgan®, but no visible phytotoxic effects on weeds were observed (figure 7.4). Nevertheless, it was hypothesised that the volatilisation of the essential oils could be reduced when applied in mixture with vegetable oils, thereby prolonging and possibly increasing the phytotoxic effect on weeds. In screening `S-L1` essential oils were applied alone at a rate of 100 L ha^{-1} without the addition of an emulsifier and in combination with 50 L ha^{-1} corn oil. In `S-PG4`, refined ricinus oil (30 L ha^{-1}) was added to a formulation of 120 L ha^{-1} δ-limonene and 30 L ha^{-1} NPE emulsifier. In both screenings the herbicidal effect of the essential oil formulations decreased clearly.

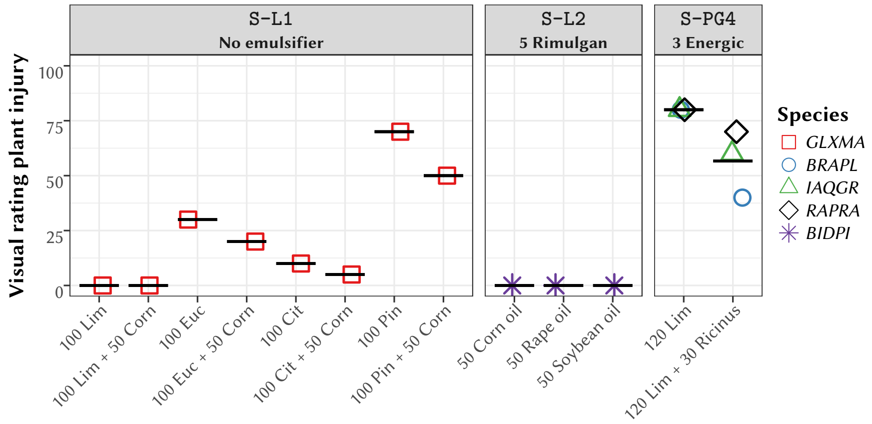


Figure 7.4: Screening trials `S-L2` and `S-PG4`: effect on weed control of NH formulations containing vegetable oil applied alone (`S-L2`) and in combination with the essential oils pine oil (Pin), δ-limonene (Lim) and eucalypt oil (`S-L1` and `S-PG4`). Emulsifiers used were Rimulgan (`S-L2`), Energic (`S-PG4`) and no emulsifier (`S-L1`). The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

7.1.3 NaCl applied alone and in combination with CMD

`S-PG4`, `S-PG5`, `S-PG12` and `S-PG13`: NaCl alone and in combination with AA and essential oils

Works of Lukashyk (2005); Lukashyk et al. (2008) had examined spray application of NaCl containing kainit fertiliser (50 % NaCl) as a weed control strategy. In field trials 350 kg ha^{-1} NaCl applied as powder and 150 kg ha^{-1} NaCl in spraying solution lead to acceptable control of *Vicia hirsuta*, which is a problem weed in temperate climate OA. Inspired by this work, the addition of a large amount of NaCl (120 kg ha^{-1}) to formulations containing CMD was hypothesised to increase the herbicidal effect: as CMD damage and permeabilise leaf cuticle and cell membranes, NaCl could possibly be taken up into the leaf in greater quantities and unfold an phytotoxic effect within the leaf tissue. Screenings `S-PG12`, `S-PG13` and `S-PG4` contained treatments in which NaCl was applied alone without emulsifiers (figure 7.5). The effect varied strongly between screenings and weed species. *Ipomoea largifolia* was controlled effectively with 50 kg ha^{-1} NaCl in `S-PG12`, while the control effect for *Euphorbia heterophylla* was weak to moderate in `S-PG13` with 25 and 50 kg ha^{-1} NaCl. The 120 kg ha^{-1} NaCl rate in `S-PG4` even did not show any herbicidal effect on *B. plantaginea*, *I. grandifolia* or *R. raphanistrum*. The pure AA treatment in this screening (`30-5 AA-600`) only had a weak effect. However, compared with both AI applied alone, the effect of the combination of AA and NaCl in `S-PG4` proved to be extremely potent. In a next step 120 kg ha^{-1} NaCl was added to formulations containing pine oil or δ-limonene, with and without AA (each at a rate of 30 L ha^{-1}). In this screening a NPE emulsifier was used at a ratio of 4:1 (oil:emulsifier). The addition of NaCl to CMD formulations containing the essential oils δ-limonene or pine oil, with and without AA was examined in `S-PG5`. Both the addition of NaCl and AA improved the effect strongly, with even a stronger increase for NaCl addition compared with AA addition, especially for the pine oil formulation.

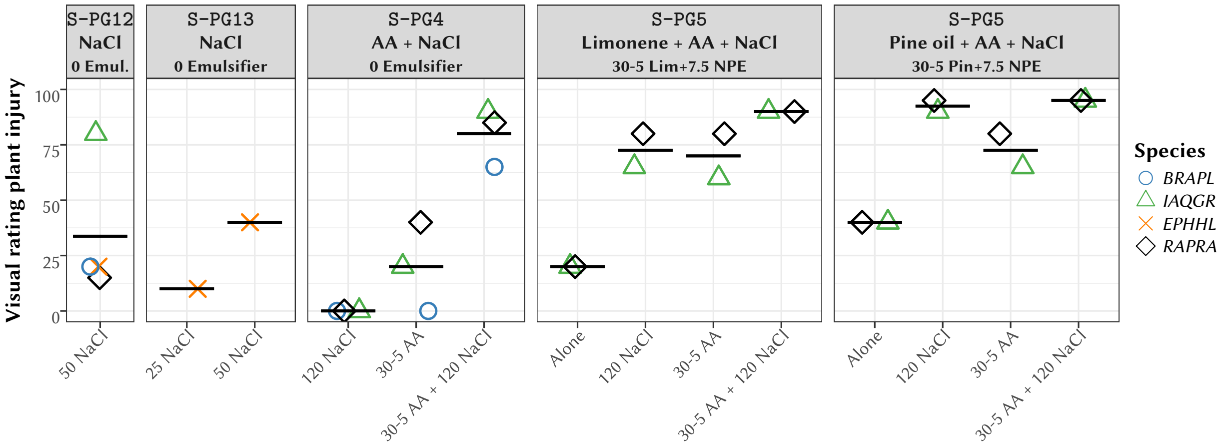


Figure 7.5: Screening trials `S-PG4`, `S-PG5`, `S-PG12` and `S-PG13`: effect on weed control of NH formulations containing the AI AA and NaCl alone as well as the combination of both. No emulsifiers were added in `S-PG4`, `S-PG12` and `S-PG13`. `S-PG5` shows the application of δ-limonene and pine oil alone with NPE emulsifier and the effect of addition of AA and/or NaCl. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

In many dicot species even a systemic effect could be observed for NaCl containing formulations (figure 7.6 b). Even with large dicot weeds, meristems that did not come in contact with spraying solution later died off, despite the fact that leaf area was not damaged severely. This could especially be observed in *R. brasiliensis*, *Amaranthus sp.*, *B. pilosa* and *E. heterophylla*.

(a) Effect on *Brachiaria plantaginea*

(b) Effect on *Bidens pilosa*

Figure 7.6: Effects of CMD-NaCl formulation on monocot and dicot species. In monocot *Brachiaria sp.* plant recovery is evident, while meristems of dicot *Bidens pilosa* have died off.

`S-PG6`, `S-PG7` and `S-PG8`: dose reduction of essential oils and AA With the strong efficiency boost obtained by the addition of NaCl, the next research question was how far the concentrations of terpenes and AA can be reduced, as these AI are elevating NH application costs strongly. Therefore subsequent dose reductions of essential oil and AA were examined in `S-PG6`, `S-PG7` and `S-PG8` (figure 7.8). All three trials consisted of formulations containing varying amounts of terpene and AA with a fixed amount of 120 kg ha^{-1} NaCl. Important to note is that varying amounts of NPE were applied, in accordance to the amount of terpene (ratio 4:1). In `S-PG6` and `S-PG7` good results were obtained with all combinations of essential oil and AA rates from 15–30 L ha^{-1}. With the lowering of the pine oil and AA rate down to 7.5 L ha^{-1} in `S-PG7`, weed control was reduced strongly. In screening `S-PG8` the dose was lowered from 15 to 7.5 L ha^{-1} for either of the two AI. As in pure AI screenings the effect of AA was stronger and a rate of 7.5 L ha^{-1} pine oil and 15 L ha^{-1} AA in combination with 120 kg ha^{-1} was the lowest possible CMD rate to still obtain an acceptable result.

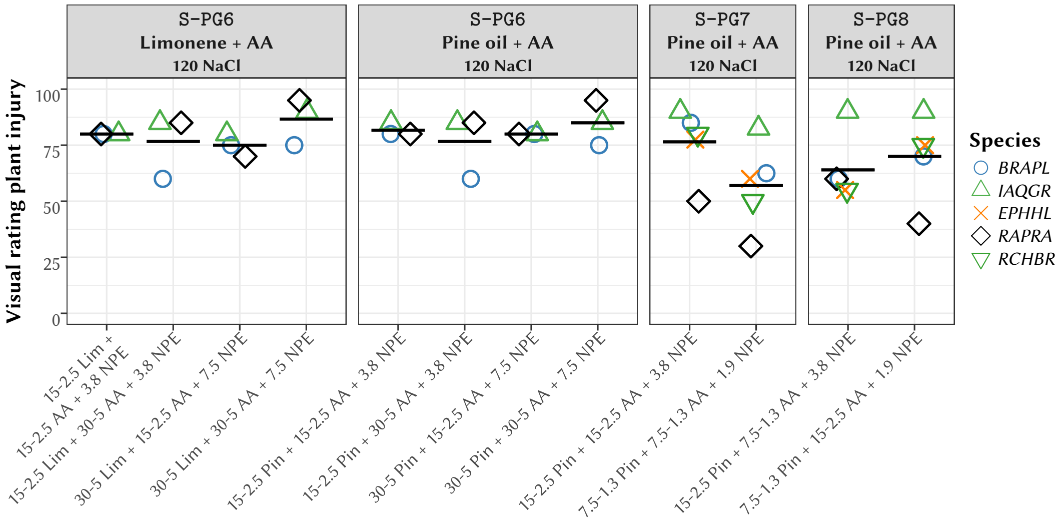


Figure 7.8: Screening trials `S-PG6`, `S-PG7` and `S-PG8`: effect on weed control of NH formulations containing relatively low volumes of δ-limonene and pine oil combination with. In all formulations a high dose of 120 kg ha^{-1} NaCl was used and the emulsifier NPE at a rate of 4:1 (*essential oil* : *emulsifier*). The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

`S-PG9`: AA and NaCl dose One problem with the use of AA in formulations is that natural vinegar only contains AA at concentrations of up to 8 %. Natural vinegar is expensive, and the alternative synthetic, concentrated ('glacial') AA is a dangerous good for transport and handling. Also synthetic AA would be difficult to certify for the use in OA. Therefore, screenings were directed towards the reduction of AA in formulations at least to levels present in vinegar. Also, at this stage of screening it was already evident that repeated spray applications would be necessary to guarantee persistent weed control. With this in mind the NaCl amounts in single applications would have to be reduced as well. Therefore in `S-PG9` the effect of relatively small doses of AA and different amounts of NaCl were tested, in formulations with pine oil. The **`AA-Dose`** sub-trial used 30 L ha^{-1} pine oil (5 %) and 60 kg ha^{-1} NaCl formulation, with 7.5 L ha^{-1} NPE as a base and testing volumes from 0–12 L ha^{-1} (0–2 %). The base formulation in `NaCl-Dose` sub-trial consisted of 30 L ha^{-1} pine oil (5 %), 6 L ha^{-1} AA (1 %), and again, 7.5 L ha^{-1} NPE, with NaCl amounts from 0–90 kg ha^{-1} (figure 7.9). In the AA dose sub-trial of `S-PG9` already the addition of 0.5 L ha^{-1} AA to the formulation increased effect on weeds considerably, while both 6 and 12 L ha^{-1} AA rates achieved high weed control ratings. The addition of 15 kg ha^{-1} NaCl already improved weed control up to acceptable levels, and with 60 kg ha^{-1} and 90 kg ha^{-1} excellent results were achieved.

Results from trials with NaCl and NaCl in combination with a variety of emulsifiers the results can be summarised as follows:

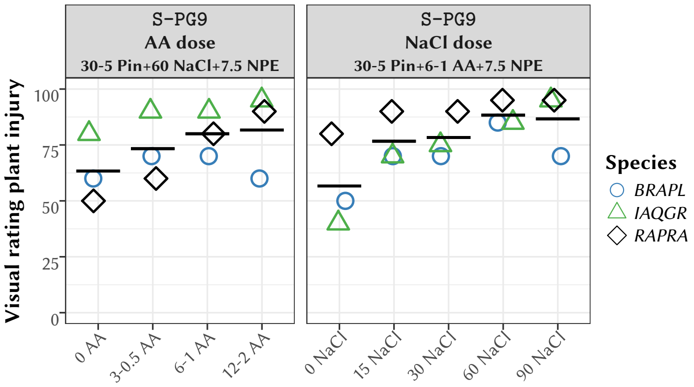


Figure 7.9: Screening trial `S-PG9`: effect on weed control of NH formulations containing different doses of AA and NaCl. *Left*: effect of addition of different amounts of AA to a formulation containing pine, NaCl and the NPE emulsifier. *Right*: addition of different amounts of NaCl to a formulation containing pine oil, AA and emulsifier NPE. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

Box 7.3 Summary: NaCl applied alone and in combination with CMD

`S-PG4`, `S-PG5`, `S-PG12` and `S-PG13`: NaCl alone and in combination with AA and essential oils

- Weed control of NaCl alone without emulsifier was variable, depending on screening and weed species.
- The addition of NaCl showed an extreme boost in efficacy of AA and essential oil formulations.
- Effect: Terpene + NaCl + AA > Terpene + NaCl > Terpene + AA > Terpene (`S-PG5`)

`S-PG6`, `S-PG7` and `S-PG8`: dose reduction of essential oils and AA

- Both pine oil and δ-limonene obtained similar results for all combinations with AA in `S-PG6`.
- Efficacy diminished when reducing pine oil and AA from 15 L ha^{-1} to 7.5 L ha^{-1} in `S-PG7`.
- Based on AI rate, AA had a greater impact on formulation efficacy than pine oil in `S-PG8`

`S-PG9`: AA and NaCl dose

- The addition of 3, 6 and 12 L ha^{-1} AA improved weed control considerably.
- NaCl addition of 15 kg ha^{-1} enhanced weed control strongly.

7.1.4 Effect of emulsifiers

Initially it was assumed that emulsifiers are inert ingredients, which is also suggested on labels of OA certified or conventional agricultural herbicides. Nevertheless, screening results attested a proper herbicidal effect to emulsifiers, which is not only based on improved emulsion stability. Therefore, the effect of emulsifier effect examined in more detail with different types of emulsifiers applied on their own and at different dosages.

`S-L2`, `S-PG14`, `S-PG15`: emulsifiers applied alone The screenings `S-L2`, `S-PG13`, `S-PG14` and `S-PG15` contained treatments in which emulsifiers were applied alone with water (rate: 600 L ha^{-1}). The effect was weak at emulsifier rates of 10 and 20 L ha^{-1} of NPE and T80 (`S-L2`) as well as 2 and 4 L ha^{-1} SA (figure 7.10). In `S-PG15` the emulsifier SA was applied alone and in combination with T80. The mixture of 10 L ha^{-1} SA with 10 L ha^{-1} T80 even granted acceptable weed control. In `S-PG13` MSO (Bayer Aureo®), T80 and NPE were tested alone (5, 10 and 15 L ha^{-1}) and in combination with NaCl (see figure 7.14). No effect was found for any of the MSO rates, a very weak effect for all T80 rates and a moderate (5 and 10 L ha^{-1}) to strong effect (15 L ha^{-1}) for the NPE emulsifier. Results from these screenings clearly suggest that some emulsifiers do possess a herbicidal burn-down effect on weeds. Typical symptoms of emulsifiers applied alone can be observed in figure 7.11.

In mainstream herbicidal formulations the rate of emulsifiers, e.g. NPE in the commercial product Energic®, is only applied at about 0.5–1.0 L ha^{-1}). At this dose no visible damage would occur if applied alone, and in this case emulsifiers could in fact be considered *inert* with respect to their herbicidal action. However, applied at rates used in screenings and commercial products (10–50 L ha^{-1}) emulsifiers would have to be considered as AI themselves. Based on AI rate, the effect of the emulsifier can even be stronger than that of essential oils, e.g. δ-limonene.

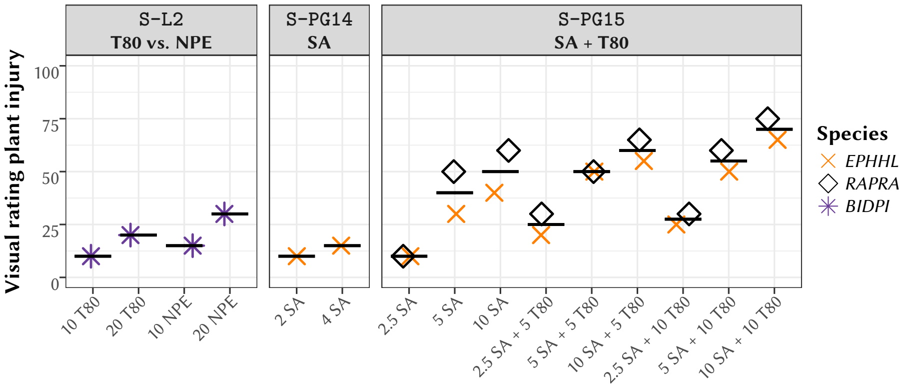


Figure 7.10: Screening trials `S-L2`, `S-PG14` and `S-PG15`: effect on weed control of NH formulations containing only emulsifiers. MSO: methylated soybean oil, T80: Tween 80® (Polyoxyethylen(20)-sorbitan-monooleate), NPE: Nonyl-phenol-ethoxylate. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

(a) 20 L ha^{-1} NPE on *E. heterophylla*

(b) 20 L ha^{-1} Aureo® (MSO based) on *R. raphanistrum*

Figure 7.11: Effects of emulsifiers applied alone at rates of 20 L ha^{-1} on *E. heterophylla* and *R. raphanistrum*.

`S-PG11` & `S-PG12`: emulsifier dose in limonene and NaCl formulation In both `S-PG11` and `S-PG12`, the dose effect of NPE emulsifier was examined, applied at amounts from 2.5–10 L ha^{-1}, in a fixed formulation containing 50 L ha^{-1} δ-limonene and 50 kg ha^{-1} NaCl (figure 7.13).

In `S-PG11` weed control of *Amaranthus sp.* increased with raising NPE doses of 2.5 to 10 L ha^{-1}. Nevertheless, about equal ratings were obtained for the same treatments in `S-PG12`.

`S-PG13` and `S-PG14`: emulsifier dose and type - addition of NaCl One objective in `S-PG13` and `S-PG14` was to examine if high-cost ingredients such as essential oils or AA could be replaced by emulsifiers. Knowing that the effective emulsifier NPE has severe environmental concerns (see *Discussion C*), other emulsifiers were tested as replacements. However, natural emulsifier which would not precipitate in combination with high amounts of NaCl (non-ionic emulsifiers) were unknown. Therefore, Tween 20® and Tween 80® were tested, which - even though synthetic - are emulsifiers that are widely used in the food industry, and which are not toxic to humans or of environmental concern. In `S-PG13` factorial combinations of NaCl dose (0–50 kg ha^{-1}), emulsifier dose (5–15 L ha^{-1}) and three types of emulsifier were examined on *Euphorbia heterophylla*. Furthermore, a methylated soybean seed oil based emulsifier (MSO, Aureo®) was tested, an adjuvant used in conventional herbicide sprays, which is low in cost and

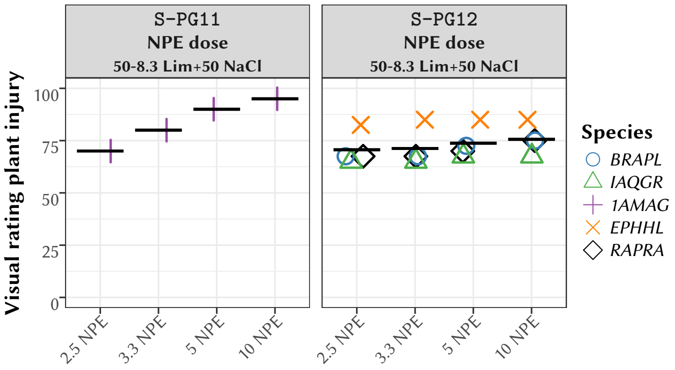


Figure 7.13: Screening trials `S-PG11` and `S-PG12`: effect on weed control of NH formulations containing 50 L ha^{-1} δ-limonene and 50 kg ha^{-1} NaCl with different doses of the emulsifier NPE (2.5, 3.3, 5 and 10 L ha^{-1}). The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

presumably is of little environmental concern[22]. Figure 7.14 presents all combinations of the non-ionic emulsifiers MSO, Tween 80® (T80) and NPE, each at three rates (5, 10 and 15 L ha^{-1}), in combination with three NaCl rates (0, 25, 50 kg ha^{-1}).

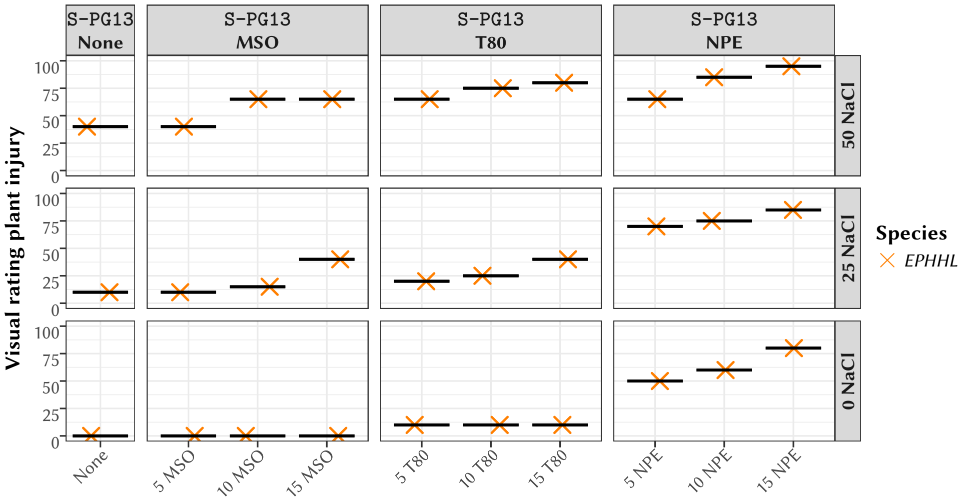


Figure 7.14: Screening trial `S-PG13`: effect on weed control of NH formulations containing only emulsifiers and NaCl. Three emulsifiers were tested at doses of 5, 10 and 15 L ha^{-1}, each in combination with three doses of NaCl (0, 25 and 50 kg ha^{-1}), plus a treatment without any emulsifier. MSO: methylated soybean oil, Tween 80: Polyoxyethylen(20)-sorbitan-monooleate, NPE: Nonyl-phenol-ethoxylate. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

The effect of emulsifiers applied have already been described above (see subsection *Emulsifier alone*). Addition of 25 kg ha^{-1} only lead to weak to moderate weed control with the 15 L ha^{-1} rate of MSO and T80 emulsifier, while all three rates of NPE gave acceptable weed control results. The 50 kg ha^{-1} NaCl rate of NaCl nearly resulted in acceptable weed control for the 10 and 15 L ha^{-1} rates of MSO emulsifier, while the T80 emulsifier achieved acceptable weed control, and NPE acceptable to excellent weed control at these rates. In screening trial `S-PG14` a combination of Aureo® (MSO) and SA emulsifiers was used, both at a relatively low rate of 2–4 L ha^{-1}. As in `S-PG13`, three doses of NaCl were applied (0, 25, 50 kg ha^{-1}).

The addition of 25 kg ha^{-1} NaCl only had a moderate effect on weeds in combination with 4 L ha^{-1} of both emulsifiers (figure 7.15). The 50 kg ha^{-1} NaCl rate showed acceptable weed control with the 4 L ha^{-1} rate of both emulsifiers. The strong effect of both emulsifiers at the 2 L ha^{-1} rate is interpreted as random.

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[22] The emulsifier contains 720 g L^{-1} methylated soybean seed oil, which is of little environmental concern. However, it also contains 188 g L^{-1} *inert* ingredients of unknown nature and environmental profile.

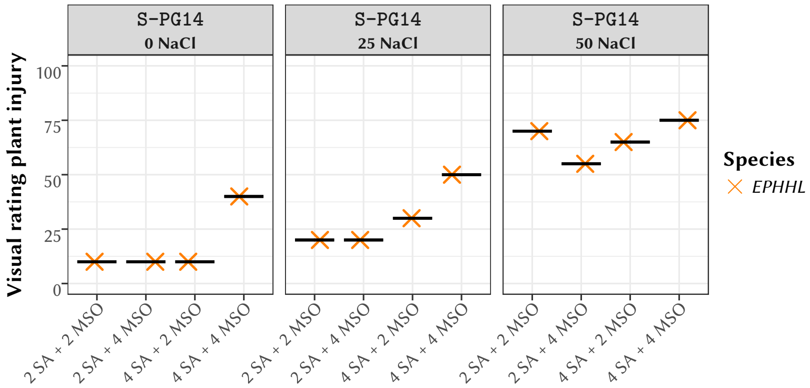


Figure 7.15: Screening trial `S-PG14`: effect on weed control of NH formulations containing a combination of the emulsifiers sulphonic acid (SA) and methylated soybean seed oil (MSO, Aureo®) and NaCl. SA and MSO were combined at rates of 2 and 4 L ha^{-1} and NaCl was added at rates of 0, 25 and 50 kg ha^{-1} NaCl. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all species. A rating above 70 is considered as *acceptable weed control*.

In conclusion `S-PG13` and `S-PG14` showed that combinations of relatively low rates of emulsifier (in comparison with essential oil rates) and 50 kg ha^{-1} can achieve satisfactory weed control.

`Summary emulsifier`: emulsifier type and NH formulation efficacy Resuming all subsets of screenings, in which the emulsifier type was the only difference between treatments it could also be demonstrated clearly, that the emulsifier type in NH formulations has a strong effect on weed control efficacy (figure 7.16). The most extreme example for the strong impact of emulsifier type on weed control can be found in `S-PG4`, where high amounts of δ-limonene with emulsifier Tween 20® did hardly cause any damage, while in combination with NPE complete weed control was achieved.

Box 7.4 Summary: emulsifiers effect

`S-L2`, `S-PG14` and `S-PG15`: emulsifiers applied alone

- 10–20 L ha^{-1} of NPE and T80 or 2–4 L ha^{-1} of SA had a herbicidal effect on weeds.
- The combination of SA and MSO, each at 10 L ha^{-1} achieved acceptable weed control (`S-PG15`).
- Emulsifiers can be considered as AI at the amounts applied in NH formulations.

`S-PG11` and `S-PG12`: emulsifier dose in limonene and NaCl formulation

- Increasing the NPE rate from 2.5 to 10 L ha^{-1} increased weed control strongly in `S-PG11`.

`S-PG13` and `S-PG14`: emulsifier dose and type - addition of NaCl

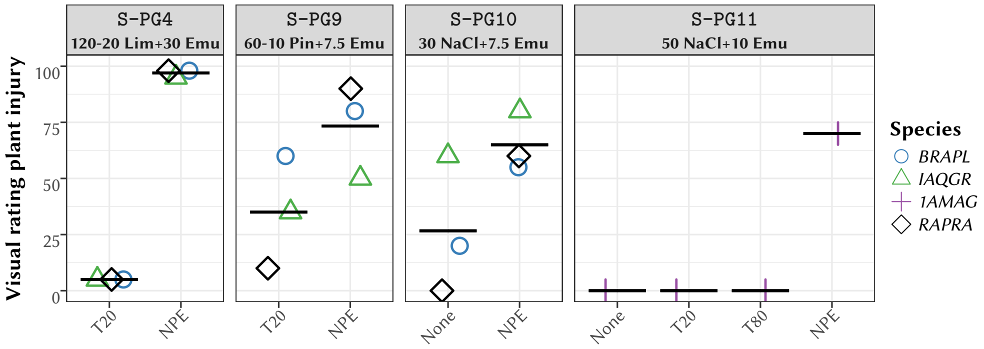
- Efficacy emulsifier: NPE > T80 > MSO.
- Control of *E. heterophylla* with NPE was notable at 5 L ha^{-1} and acceptable at 15 L ha^{-1}.
- Tween 80® only had a weak effect on *E. heterophylla* at doses from 5–15 L ha^{-1} (`S-PG13`)
- Based on volume, weed control of NPE is stronger than that of most essential oils.
- NaCl and emulsifier dose influence effect strongly. The most important factor is the NaCl dose.
- MSO and SA, each at a rate of 4 L ha^{-1}, combined with 50 kg ha^{-1} NaCl achieve acceptable weed control.

`Summary emulsifier`: emulsifier type and NH formulation efficacy

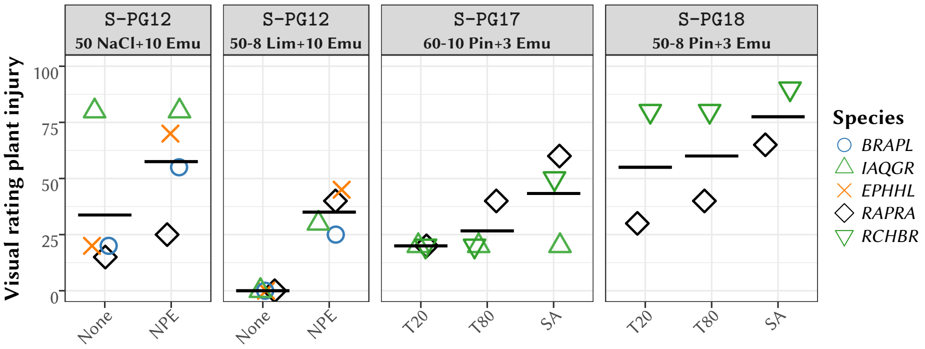
- The emulsifier type influences the formulation efficacy strongly

7.1.5 Weed species susceptibility

The determination of the susceptibility of the different weed species cannot be deducted in an exact way from screening trial data, because the development stages of weeds varied widely (about 1–12 leaf stage) and so did the environmental conditions at application and the formulations applied. The



(a) `S-PG4`, `S-PG9`, `S-PG10` and `S-PG11`



(b) `S-PG12`, `S-PG17` and `S-PG18`

Figure 7.16: `Summary emulsifier` : effect of the emulsifier type on weed control in a variety of NH formulations. NPE: Nonyl-phenol-ethoxylate, T80: Tween 80®, T20: Tween 20®. SA: Sulphonic acid. MSO: Methylated soybean oil. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all plant species. A rating above 70 is considered as *acceptable weed control*.

experimental data on weed susceptibility is summarised in box 7.5. One of the observations made in the field was that *R. raphanistrum* was sensitive to NH applications up to about 3 leaf stage. However, this changed drastically from about 4–5 leaf stage, where it became very resistant to applications and often only showed leaf tip burns after application. *B. plantaginea* was difficult to control and only up to 2-leaf stage control was possible. When it started forming tillers growth could be reduced, but killing this weed was nearly impossible.

Box 7.5 Summary: weed susceptibility

Comparitive Weed susceptibility (L: leafs, T: tillers):

- `S-PG2`: *I. grandifolia* (2L) > *B. plantaginea* (1–3L)
- `S-PG3`: *R. raphanistrum* (4–8L) > *E. heterophylla* (4–6L) = *I. grandifolia* (4–6L) > *B. plantaginea* (4L,2T)
- `S-PG4`: *R. raphanistrum* (4–5L) = *I. grandifolia* (2–4L) > *B. plantaginea* (2–3L)
- `S-PG5`: *R. raphanistrum* (9–11L) = *I. grandifolia* (10–14L)
- `S-PG6`: *R. raphanistrum* (2–4L) > *B. plantaginea* (3L,2T)
- `S-PG7` & `S-PG8`: *I. grandifolia* (10–12L) > *E. heterophylla* (6–10L) = *B. plantaginea* (3L,3T) = *R. brasiliensis* (4L,3T) > *R. raphanistrum* (4–6L)
- `S-PG9`: *I. grandifolia* (2–4L) = *B. plantaginea* (1–2L) = *R. raphanistrum* (4–6L)
- `S-PG12`: *E. heterophylla* (3L) > *I. grandifolia* (3L) = *B. plantaginea* (4L) = *R. raphanistrum* (4–5L)

7.2 Summary results screening trials

The research questions for screening trials can be answered as follows:

Box 7.6 Research questions screening trials

Which AI and formulations are most phytotoxic to weeds?

- Cell membrane disruptors (CMD)
  - AA > pine oil > δ-limonene
  - No systemic effect, only acute plant injury
- NaCl
  - NaCl is crucial for formulation efficacy.
  - NaCl has a systemic effect and causes chronic plant injury.

What are effective AI concentrations and application rates of NH?

- CMD: essential oils at $50\,\mathrm{L\,ha^{-1}}$, and AA at about $25\,\mathrm{L\,ha^{-1}}$
- NaCl: $50\,\mathrm{kg\,ha^{-1}}$ recommended, and at a rate of $120\,\mathrm{kg\,ha^{-1}}$ nearly no additives are needed
- An application rate of $300\,\mathrm{L\,ha^{-1}}$ is sufficient for weed coverage
- At a constant AI rate the concentrated formulation is stronger effect than the diluted one

Do AI mixtures potentiate phytotoxicity?

- No synergistic effects found for CMD mixtures.
- A strong increase in effect was found for mixtures of CMD and NaCl.

How do emulsifiers affect NH formulation efficacy?

- Emulsifiers are in fact AI themselves.
- Emulsifiers have a crucial role for formulation efficacy.
- Phytotoxicity ranking emulsifiers: NPE > SA > T80 > MSO > T20
- Based on volume NPE was more effective than all other emulsifiers and terpenes studied.

Which weeds are susceptible to NH application and up to which stage?

- Comparative susceptibility between weed species is independent of treatment applied.
- Dicot species were more susceptible than monocots.
- Weed susceptibility differed over development stages, and probably also environmentally conditions.

7.3 Field trials

NH field trials were carried out in season 2012/13 at IAPAR research stations in *Londrina* and *Ponta Grossa*. Scientific articles on the use of NH in annual field crops in direct seeding (DS) do not exist to the authors best knowledge. The preconditions for field trials were that neither information on the moment of the first application was present, nor on how many applications and in what interval were necessary to control weeds effectively. Furthermore, the behaviour of the products in DS crop stands was unknown. The experiences with NH from screenings were limited to relatively uniform weed stands without crops in tilled soil. However, under conditions of DS, weed emergence and development stages are more heterogeneous. Additionally there is an interaction between crop and weed growth in field trials. Lacking experience, the timing and application intervals could not be predetermined, and applications were realised depending on weed abundance and development stage. In screenings it was observed that non-killed plants recovered and regrew, especially monocot species. Therefore it was understood prior to the conduction of field trials that at least two applications would be necessary to persistently eliminate weeds or to at least limit their growth effectively. Two or three applications were hypothesised to grant sufficient weed control and give crops a head start until becoming competitive against weeds. Despite positive findings in screening trials it was decided not to use AA in field trial formulations. Its purely synthetic nature was thought to lead to great problems for certification, and in combination with NaCl pine oil and δ-limonene proved to be sufficiently efficient. Despite knowing about its negative environmental effects a NPE emulsifier was used for both soybean field trials. No effective emulsifiers had been identified at this point, yet, and the improved effect of NPE emulsifier was thought to be rather connected to emulsion stability, than to the strong proper effect of this emulsifier, which was only clearly understood after beginning with the field trials.

7.3.1 Soybean trial Londrina (`LO-NH`)

The objective in trial `LO-NH` was to examine the effect of natural herbicides on weed control and crop growth in a direct seeded soybean field. Multiple NH applications were assumed to be necessary to control weeds effectively. Taking into account results from screening trial `S-PG2` it was questioned whether an improved weed control is achieved either by applying a given amount of AI in two concentrated, or in three more diluted applications. The total amount of AI applied were 90 L ha^{-1} of essential oil (δ-limonene or pine oil), 90 kg ha^{-1} of NaCl and 18 L ha^{-1} of the emulsifier NPE.

Weed cover In `LO-NH` no weed injury ratings were recorded and instead weed cover was determined at three evaluation dates (each evaluation consisted of twelve estimations per plot). All spray treatments clearly reduced weed cover and by 39 DAE all treatments reduced weed cover to under 20 % (figure 7.18). Tendentially, weed cover was more reduced in concentrated treatments (`2x Lim-C`, `2x Pin-C`) than in the diluted ones (`3x Lim-D`, `3x Pin-D`).

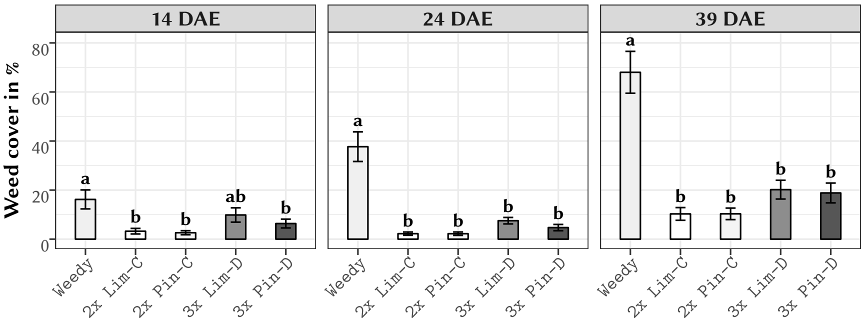


Figure 7.18: Effect of NH treatments on weed cover 14, 24 and 39 days after soybean emergence (DAE). `Weedy`: weedy control, `2x Lim-C`: two applications of concentrated δ-limonene formulation, `2x Pin-C`: two applications of concentrated pine oil formulation, `3x Lim-D`: 3 applications of diluted δ-limonene formulation, `3x Pin-D`: 3 applications of diluted pine oilformulation. The total amount of AI applied in all treatments was: 90 L ha^{-1} essential oil, 90 kg ha^{-1} NaCl and 22.5 L ha^{-1} NPE. Error bars: standard error of the mean (SE). *n.s.*: not significant. Different letters denote significant differences (Kruskal-Wallis, $\alpha < 0.05$).

Weed and soybean growth Weed biomass was reduced by 31–60 % (`2x Lim-C`, `3x Lim-D`) compared with `Weedy` and with more success for the concentrated treatments in comparison with the diluted treatments (figure 7.19). Between both terpene formulations no clear trend was apparent: pine oil treatments once resulted in higher and once in lower weed dry mass. The overall reduction of weed dry mass was not satisfactory: even for the best treatment (`2x Lim-C`) 1.7 $\mathrm{t\,ha^{-1}}$ weed dry mass remained, and for the worst treatment (`3x Lim-D`) 2.3 $\mathrm{t\,ha^{-1}}$.

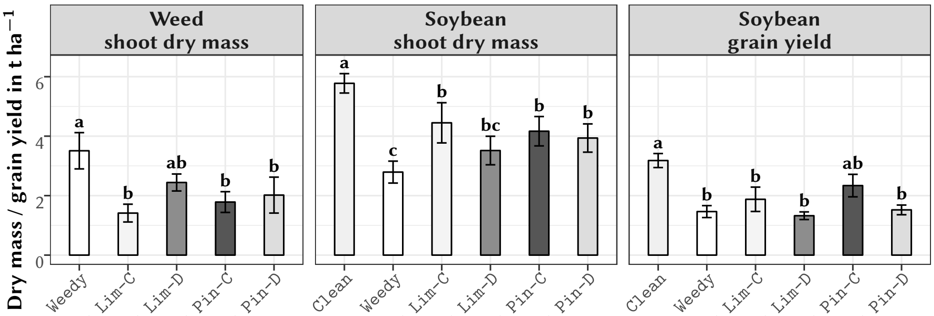
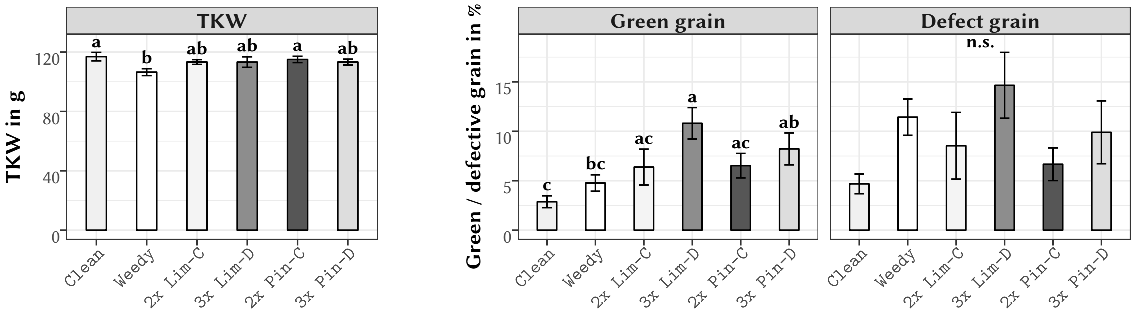


Figure 7.19: Effect of NH treatments on weed and soybean shoot dry mass as well as soybean grain yield. For treatment descriptions refer to figure 7.18. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

The reduction in weed growth led to increased soybean shoot growth, which is reflected in the strong negative correlation between the two parameters ($r = -0.72$, $R^2 = 0.52$). Nevertheless, soybean shoot growth reductions were considerable compared with `Clean`. While `Weedy` showed a reduction of 51 %, the sprayed treatments still exhibited a reduction of between 39 % (`3x Lim-D`) and 23 % (`2x Lim-C`, figure 7.19). Compared with `Clean`, yield reductions were extreme for all treatments: for `Weedy` 54 %, `3x Lim-D` 58 %, `3x Pin-D` 52 %, and least for the two concentrated spray treatments with `2x Lim-C` 41 % and 27 % for `2x Pin-C`. The thousand kernel weight (TKW) was also negatively influenced by weed infestation with the greatest reduction for `Weedy`, which was 8.6 % lower than `Clean` (s.d.). All treatments spray treatments were tendentially lower than `Clean` (n.s. figure 7.20). Grain quality parameters were only determined in **`LO-NH`** trial and quality decreased notably with increasing weed infestation: significantly more green grains were present (s.d.), and the number of defect grains was tendentially increased (n.s.). The green grain is indicative for heterogeneous maturing and can be induced by pronounced weed infestation. The deformed or defect grain can be caused by sucking insects, which find a habitat in weed plants and may therefore be linked to weed infestation.



(a) Thousand kernel weight

(b) Grain quality: occurence of green and damaged grain

Figure 7.20: Effect of NH treatments on thousand kernel weight (TKW), and on occurence of green or damaged grains. For treatment descriptions refer to figure 7.18. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$)

Box 7.7 Summary results `LO-NH`

- Weed cover was reduced strongly in all spray treatments.
- With identical total amounts of AI the 2x concentrated application was clearly more effective than the 3x diluted application.
- Overall the weed growth reduction was not satisfactory.
- Phytotoxicity: all spray treatments reduced soybean shoot growth (23–29 %) and grain yield (27–58 %).
- Grain TKW and quality were also reduced compared with `Clean` (more green and damaged grains).

7.3.2 Soybean trial Ponta Grossa (`PG-NH`)

The research objective in trial `PG-NH` was to determine how many applications are necessary for effective weed control (one, two and three applications), in the context of `Low` and `High` weed densities. Two NH formulations were tested, containing $50\,\mathrm{L\,ha^{-1}}$ essential oil (δ-limonene or pine oil), $50\,\mathrm{kg\,ha^{-1}}$ NaCl and $10\,\mathrm{L\,ha^{-1}}$ of the emulsifier NPE. As no interactions between spray treatments and weed density were found for any parameter the results are presented separated by the two experimental factors *spray* and *weed infestation*.

Weed injury rating The visual weed control ratings for the spray treatments are presented in figure 7.22. With average ratings around 60 for both formulations weed control was only near acceptable at the first evaluation 6 DAA-1 (13 DAE). The second application, realised in the late afternoon, only had a poor effect on weeds and only increased the rating marginally up to 65, compared with 55 for the treatments with one application. By the third evaluation (5 DAA-3, 27 DAE) there was a clear differentiation between treatments and the weed injury rating of `1x Lim` had decreased considerably (40, s.d.). The `1x Pin` and `2x Lim` and `2x Pin` treatments were about equal, while both three-application treatments were highest (both s.d.). Nevertheless, the injury rating remained at score below 70, which is still considered unsatisfactory weed control.

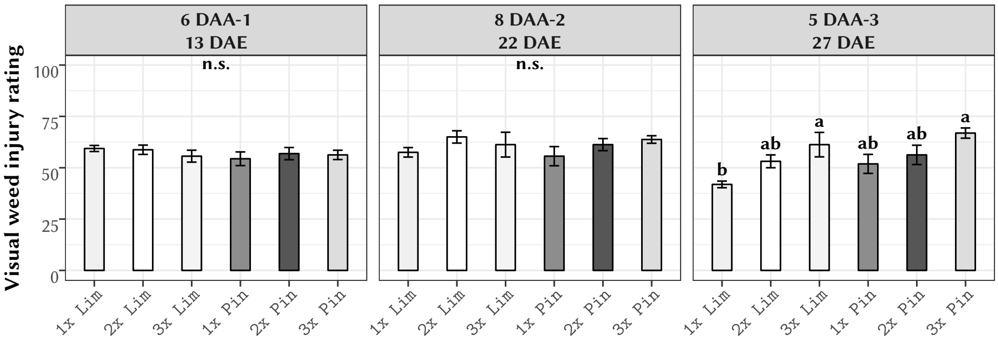


Figure 7.22: Effect of NH treatments on visual weed injury rating 13, 22 and 27 DAE (averaged over species). Estimation occurred 6 days after first application (DAA-1), 8 DAA-2 and 5 DAA-3, respectively. `1x Lim`, `2x Lim`, `3x Lim`: one, two and three applications of δ-limonene formulation (`50 Lim + 50 NaCl + 10 NPE-600`). `1x Pin`, `2x Pin`, `3x Pin`: one, two and three applications of pine oil formulation (`50 Pin + 50 NaCl + 10 NPE-600`). Error bars: standard error of the mean (SE). *n.s.*: not significant. Different letters denote significant differences (Kruskal-Wallis, $\alpha < 0.05$).

Comparing average ratings over all spray treatments by the factor *weed infestation* the weed injury ratings in the `High` plots were consistently lower than in the `Low` plots (s.d. for second evaluation, figure 7.23). However, in absolute terms, differences in weed injury between the two weed densities remained relatively small.

Weed growth and soybean growth The weed dry mass of `Weedy` plots was on average $4.2\,\mathrm{t\,ha^{-1}}$ 96 DAE (figure 7.24). Analogous to results from the third weed injury rating of spray treatments (figure 7.22) weed shoot dry mass decreasing from `1x` > `2x` > `3x`. The difference between one and two applications is apparently smaller than the one between `2x` and `3x`. But compared with `Weedy`, even three applications

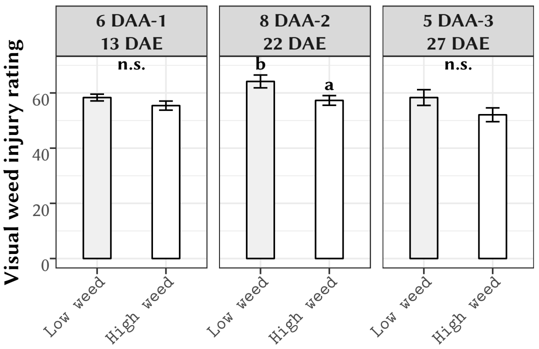


Figure 7.23: Effect of NH weed density on visual weed injury rating at 13, 22 and 27 DAE. The estimation of weed injury occurred 6 days after first application (DAA-1), 8 DAA-2 and 5 DAA-3, respectively. `Low`: low weed density, `High`: high weed density. Error bars: standard error of the mean (SE). *n.s.*: not significant. Different letters denote significant differences (Kruskal-Wallis, $\alpha < 0.05$).

only reduced weed dry mass by 55 % and 59 % for δ-limonene and pine oil, respectively. Soybean was not protected properly against herbicidal sprays, and considerable crop injury occurred, which increased with application repetitions and was highest for pine oil. On the rating scale used for phytotoxicity, a score exceeding about 10 is capable of reducing yields. Of the δ-limonene treatments only the `3x` treatment suffered severe crop injury. However, of the pine oil treatments already the single application caused phytotoxicity (score 14), and the `3x Pine` treatment rated highest of all treatments for crop injury. Due to phytotoxicity it was hard to separate the effect of weed competition from that of phytotoxicity on soybean growth. As expected, the `Clean` treatment did attain the highest shoot biomass and yield, and the `Weedy` treatment the lowest (-21 %). All spray treatments only had a shoot dry mass similar to `Weedy`. Despite reduced weed dry mass a tendency of decreasing crop shoot dry mass with increasing application repetitions was apparent, as a consequence of phytotoxicity. Also, grain yield of `Clean` was highest, and that of `Weedy` lowest with a reduction of 36 %. For δ-limonene, grain yield was higher with increasing number of applications, while yield of pine oil was nearly identical independent of application repetitions. The spray treatment that performed best was `3x Lim`, which had a 10 % lower yield than the `Clean` control treatment.

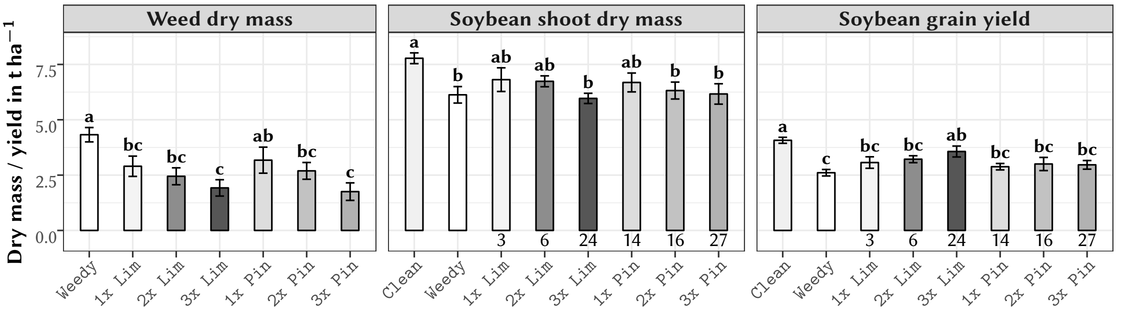


Figure 7.24: Effect of NH treatments on weed and soybean shoot dry mass, evaluated 96 DAE, and soybean grain yield. Numbers below bars of spray treatments indicate crop injury caused by spray contact: scale: 0–100, analogue to weed injury rating scale. `Clean`: weed free control treatment, `Weedy`: weedy control treatment, `1x Lim`, `2x Lim`, `3x Lim`: one, two and three applications of δ-limonene formulation (`50 Lim + 50 NaCl + 10 NPE-600`). `1x Pin`, `2x Pin`, `3x Pin`: one, two and three applications of pine oil formulation (`50 Pin + 50 NaCl + 10 NPE-600`). Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

The whole-plots with high weed infestation (`High`) showed a clearly higher weed dry mass, however, differences were not significant (figure 7.25). At the same time `High` weed infestation lead to decreased shoot dry mass and grain yield.

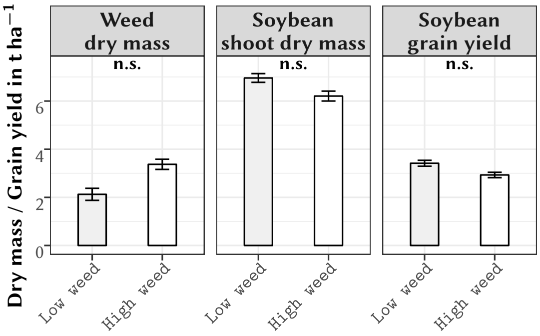


Figure 7.25: Effect of weed infestation on weed and soybean shoot dry mass (evaluated 96 DAE) as well as soybean grain yield. `Low`: Low weed density, `High`: high weed density. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Box 7.8 Summary results `PG-NH`

NH treatments

- Three applications nearly achieved acceptable weed control.
- Weed injury rating: pine oil higher than δ-limonene formulation.
- Weed dry mass, weed injury rating, shoot dry mass and grain yield similar for `1x` and `2x` .
- Phytotoxicity influenced soybean growth strongly.

Weed infestation

- `Low` slightly lower weed shoot dry mass and higher soybean shoot dry mass and grain yield (all n.s.).
- Weed injury ratings similar for both infestations.

7.3.3 Common bean trials Ponta Grossa (`PG-Lim`, `PG-Pin`, `PG-NaCl`)

In the conventional tillage common bean trials in Ponta Grossa formulations with different AI rates and application rates of formulations containing NaCl and δ-limonene (`PG-Lim`) or pine oil `PG-Pin` were studied. In both trials the more environmentally friendly emulsifier Tween 80® was used in formulation.

Visual weed injury rating and weed growth In the weed injury rating of both trials *Amaranthus sp.* reacted more susceptible to herbicide sprays. *Raphanus raphanistrum* mostly had the lowest ratings, and weed injury was intermediate for *Brachiaria plantaginea.* In `PG-Lim` trial the treatment order was tendentially `75-8 Lim-900` > `50-8 Lim-600` > `50-6 Lim-900` > `50-17 Lim-300` > `25-8 Lim-300`. After the first application weed injury ratings of treatments `25-8 Lim-300`, `50-17 Lim-300` and `50-6 Lim-900` were not acceptable, while `50-8 Lim-600` and `75-8 Lim-900` already showed acceptable control (figure 7.26). From the second application onward all treatments showed acceptable (`25 Lim-300`, `50 Lim-300`) and all others even a highly efficient control with weed injury rating up to 90. Of the 50 L ha^{-1} δ-limonene rate the 600 L ha^{-1} application rate achieved the best ratings in all evaluations. In `PG-Pin` all ratings achieved acceptable weed control apart from `25 Pin-600`. At an AI rate of 25 L ha^{-1} pine oil, the concentrated 300 L ha^{-1} application caused higher damage to weeds the 600 L ha^{-1} application rate. Overall, control was acceptable for all trials, with scores around 70 for both evaluations.

In both trials all spray treatments reduced weed growth considerably (figure 7.27). In `PG-Lim` larger amounts of weeds remained for treatment `50-8 Lim-600` (1 t ha^{-1}) despite the high rating score for weed injury, as well as for `25-8 Lim-300` and `50-6 Lim 900`. All weeds were virtually eliminated by treatments `50-17 Lim-300` and `75-8 Lim-900`. In `PG-Pin` weed dry mass was reduced to 0.1 t ha^{-1} on average (2 % of `Weedy`) and differences between treatments were minor. *B. plantaginea* was the most dominant weed by dry mass, followed by *Amaranthus sp.* and to a very minor extent *R. raphanistrum.* In `PG-Pin` only *B. plantaginea* remained after spray treatment. Dry mass of *Amaranthus sp* was notable

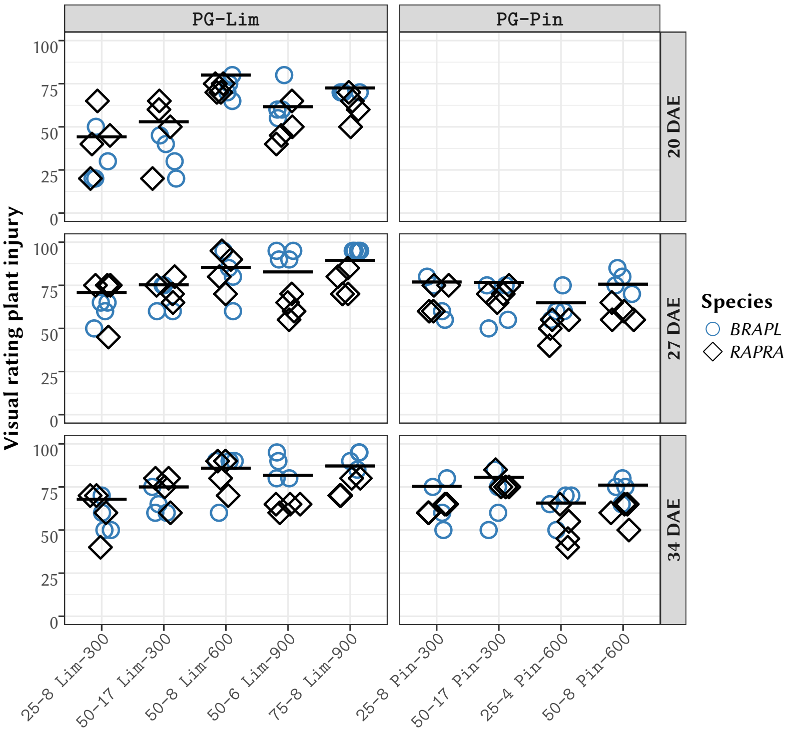


Figure 7.26: `PG-Lim`, `PG-Pin`: effect of NH treatments on visual weed injury ratings after the first, second and third application. All treatments contained 50 kg ha^{-1} NaCl and 2.5 L ha^{-1} of the emulsifier T80. The different shapes represent the visual rating of plant injury for each plant species and horizontal bars the mean rating score averaged over all plant species. A rating above 70 is considered as *acceptable weed control*.

in `PG-Lim`, while *R. raphanistrum* did not form considerable dry mass.

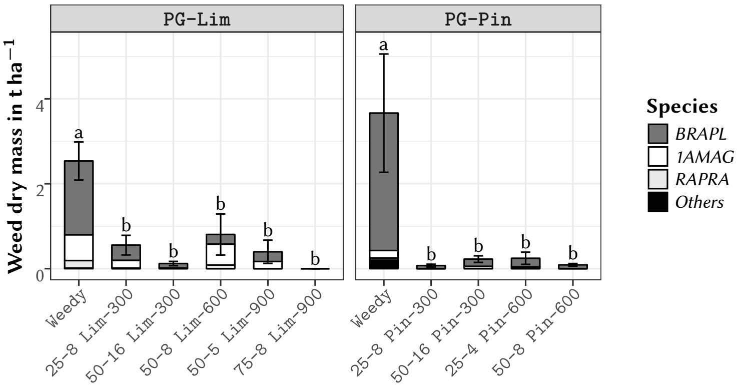


Figure 7.27: `PG-Lim`, `PG-Pin`: effect of NH treatments on weed shoot dry mass at 73 DAE. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences in total weed shoot dry mass (Kruskal-Wallis, $\alpha < 0.05$)

Common bean growth All three trials were established in direct proximity on the same field and evaluated at the same growth stage. Nevertheless, shoot growth of `Clean` control was slightly higher than that in trial `PG-Pin` (figure 7.28). In `PG-Pin` shoot dry mass was nearly equal for all treatments. The differences in `PG-Lim` were stronger: even though not significant, treatments `Weedy`, `50-8 Lim-600` and `50-16 Lim-300` showed growth reductions of 28 %, 23 % and 18 %, respectively. Weeds apparently affected shoot growth stronger in `PG-Lim` than in `PG-Pin`, as the latter did not exhibit growth reductions in `Weedy` treatment. The slight growth reductions in spray treatments of `PG-Lim` compared with `Clean` were related to phytotoxicity as in `PG-NH`, while shoot growth in `PG-Pin` was not effected by the only subtle spray damage to the crop. Clear differences were found for grain yield: `Weedy` treatments of both trials had significantly lower yields with 1.54 t ha^{-1} in `PG-Lim` (-42 % compared with `Clean`) and

1.75 t ha^{-1} for **`PG-Pin`** (-38 %). In both NH trials grain yield was not significantly different for spray treatments, though tendentially lower. In trials **`PG-Lim`** and **`PG-Pin`** the grain yields of the best spray treatments were only 1 % (`75-8 Lim-900`) and 5 % (`25-4 Pin-600`) lower than `Clean`. The lowest yields of spray treatments were 14 % (`50-16 Lim-300`) and 13 % (`50-8 Pin-600`) lower than `Clean`. In trial **`PG-NaCl`** shoot dry mass and grain yield remained unaffected by inter-row spray application of different amounts of NaCl.

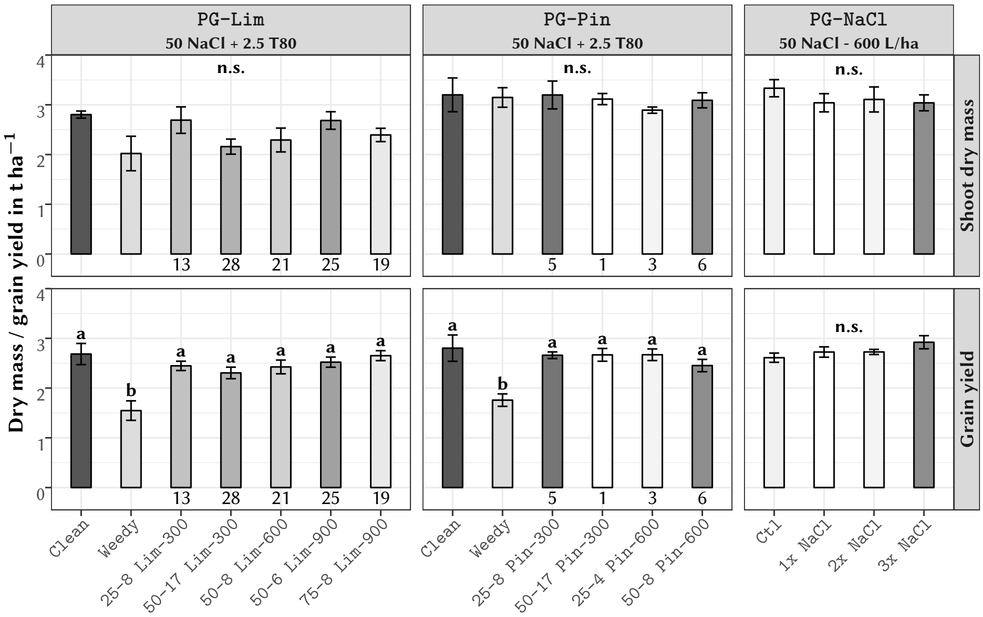


Figure 7.28: Effect of spray applications on shoot dry mass and grain yield in trials **`PG-Lim`**, **`PG-Pin`** and **`PG-NaCl`**. Numbers below bars: phytotoxicity rating. Numbers below bars of spray treatments indicate crop injury caused by spray contact: scale: 0–100, analogue to weed injury rating scale. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$)

Box 7.9 Summary results `PG-Lim`, `PG-Pin`, and `PG-NaCl`

`PG-Lim` & `PG-Pin`

- Weed dry mass was strongly reduced in all treatments, which allowed yields comparable to `Clean`.
- Weed injury ratings were acceptable for nearly all treatments of both trials.
- Phytotoxicity was apparent in **`PG-Lim`** and presumably responsible for growth reduction of spray treatments.
- Concentrated and diluted application did not differentiate in both trials.

`PG-NaCl`

- Soil applied NaCl did not have any negative effect on common bean growth even at the highest dose.

7.4 Summary results field trials

Box 7.10 Summary of results NH field trials

- **AI**: Pine oil formulations were generally more efficient than δ-limonene formulations.
- **Application repetitions**: 2–3 NH applications necessary in summer, 1–2 in late season (March).
- **Phytotoxicity**: strong in `PG-NH`. Effective shielding indispensable.
- **Weed control**: in soybean `PG-NH` and `LO-NH` not sufficient.
- **Grain quality**: Weed infestation in trial `LO-NH` lead to unacceptable grain quality.
- **NaCl in soil**: No negative effects on crop growth found for soil applied NaCl up to 150 kg ha^{-1}.

Hypothesis	`LO-NH`	`PG-NH`	`PG-Lim`	`PG-Pin`
1. NH grant sufficient weed control	-	-	+	+
2. Crop growth and yield comparable to `Clean`	-	-	+	+

8 Discussion C: natural herbicide trials (Brazil)

8.1 Active ingredients and their formulations

8.1.1 Cell membrane disruptors

Essential oils, AA, emulsifiers and MSO caused very similar damage symptoms to weed plants in screenings. Many literature sources support this finding: within the class of essential oils the mode of action is the same and only the magnitude of their effect differs (Bakkali et al., 2008). All cell membrane disruptors (CMD) penetrate the leaf cuticle and change its permeability. Inside the leaf they interact with and damage cell and organelle membranes which causes rapid electrolyte leakage and leads to universal interference in most of cells metabolism (Tworkoski, 2002; Bakkali et al., 2008). Findings of Baker (1970) for mineral oil are similar: penetration of hydrocarbons causes cell membrane damage, a decrease of membrane potentials and consecutive formation of reactive oxygen species (ROS), which in turn leads to further membrane damage. Another consequence of the membrane damage is described by Lederer et al. (2004): chlorophyll displaced from the thylakoids is sensitised in the light and generates radicals (ROS), which cause peroxidation and increase membrane damage further. Ultimately cell contents leak out and cells die of uncontrolled water loss (Tworkoski, 2002; Bakkali et al., 2008). Despite the same mode of action, Evans et al. (2009) found slight differences in damage symptoms between clove oil and AA. While damage symptoms of AA were found widespread throughout the leaf tissue, including upper and lower epidermal cells, the damage for clove oil treatment was mostly located in the mesophyll cells. Epidermal cells appeared less affected by clove oil than by AA in vinegar. Even though both destroy cell membranes they are distinct in their ability to penetrate cuticle and leaf tissue. This is assumed to be due to their difference in polarity and hence solubility in water. Due to their common general effect on cell membranes, essential oils, mineral oils, AA, pelargonic acid (PA) and also emulsifiers can all be grouped into the same AI class, denominated CMD. The effect of all CMD is not systemic as no translocation within plants occurs. Remaining, non-contacted meristems may resprout and plant recovery can happen quickly. Common to all CMD are the large concentrations and total amounts of AI needed to effectively kill or severely damage plants. Following, some of the presented ingredients are discussed in more detail. Due to the large quantities of water insoluble (essential) oils in most NH formulations, also large quantities of emulsifiers are needed to elaborate stable emulsions. This deserves special attention as it has far reaching consequences for the environmental profile of NH and their certification and is therefore discussed separately in more detail.

Essential oils Essential oils usually consist of a wide range of chemical constituents, but mainly of terpenes (mostly monoterpenes and sesquiterpenes) and volatile aromatics. The type and quantity of constituents vary between species of a genus, a variety within a species and the environmental conditions of their respective production sites (Ioannou et al., 2014). Thus, essential oils are by no means defined substances, which may limit reproducibility of experimental findings due to different oil qualities.

δ-limonene was found to be an extremely inefficient AI and cannot be considered for NH formulations. Its effect is apparently more based on the accompanying emulsifier than the δ-limonene itself. Pine oil proved to be effective, and even caused damage to weeds without the use of any emulsifier. Nevertheless, also this ingredient performed better when combined with an effective emulsifier, such as NPE. The other essential oils tested were discarted in screenings due to insufficient effect or high cost. One problem in the use of essential oils as AI for weed control is their volatility. The cuticle and membrane dissolving effect is therefore limited in time and a reason why they only cause acute and no chronic plant injury. The volatility of substances (vapour pressure high) is affected by its polarity. Pine oil (α-Terpineol) as an alcohol is more polar than δ-limonene and hence less volatile. Elaborating natural herbicide formulations it was observed that without any emulsifier δ-limonene separated quickly when mixed with water whereas synthetic pine oil (65 % α-terpineol) even showed a milky appearance for a very short time after agitation (not stable though). Weidenhamer et al. (1993) confirmed that solubility of alcohol monoterpenes are one to two magnitudes of order higher than that of pure hydrocarbons, and found a solubility of $13\,\mathrm{mg\,L^{-1}}$ for δ-limonene and $1360\,\mathrm{mg\,L^{-1}}$ for 4-Terpineol (similar to α-terpineol contained in pine oil). The natural turpentine (α-pinene) tested in screenings did not show any notable effect, which may be due to the low solubility (22 mg L^{-1}) and high volatility as is the case for δ-limonene. The lower volatility and higher solubility in water associated with higher polarity may explain the higher toxicity of pine oil to plants compared to δ-limonene (monoterpene-hydrocarbon): due to a lower volatility the time to exert an effect on plant tissue is prolonged and due to a higher solubility also higher concentrations come in contact with target cells. In the case of heavy mineral oils Crafts and Reiber (1948) and Baker (1970) found that the acute injury to plants was caused by volatile unsaturates and volatile acidic compounds, while chronic injuries were caused by high boiling unsaturates. Gauvrit and Cabanne (2006) confirms these findings: inside the different classes of constituents of mineral oils the order of phytotoxicity is *aromatics* > *olefins* > *naphthenes* > *paraffins*. The author concludes that unsaturation and aromaticity generally appear to be the major factor for phytotoxicity of (mineral) oils, and attributes this finding to their higher reactivity. These results may also explain why cinnamon and thyme oil, which respectively contain the aromatic essential oils eugenol and thymol, are among the most phytotoxic essential oils (Tworkoski, 2002).

There are reports of systemic action of constituents of some essential oils, e.g. eucalypt oil (Singh et al., 2005). However, these results were obtained in laboratory bioessays with seeds of *Parthenium hysterophorus* weed. It is assumed that due to their volatility, essential oils cannot come into contact with seeds or seedlings in concentrations to cause any physiological response, but will evaporate on the soil surface. This is even more true for DS management, as the mulch impedes spray contact with the soil. In the few screenings with eucalypt oil (from *Eucalyptus citriodora*) symptoms indicating a systemic effect, e.g. leaf yellowing, tissue necrosis (not acute damage) or growth reduction could not be observed at all, even at rates of 90 L ha^{-1}. Based on the limited experimental data no definite conclusion can be drawn, but a systemic effect under field conditions is assumed to be unlikely. Nevertheless, additions of small volumes of essential oils with supposedly systemic action to NH formulations should be studied further.

Acetic acid Acetic acid was the most effective CMD in screening trials based on concentration. Despite being a very simple chemical with a very positive environmental profile (Webber et al., 2006), AA as a weed control agent possesses several disadvantages. First to mention is the high cost of natural vinegar. AA concentrations in vinegar range from about 5–10 %, but concentrations used for an effective burn-down effect in weeds is 15–20 %, concentrations which are not present in natural vinegar (Evans and Bellinder, 2009; Evans et al., 2009). Typical spray application volumes are 500–1000 L ha^{-1}, or 50–200 L ha^{-1} of AA. Only the use of cheaper synthetic glacial AA can therefore be an option, which difficults or impedes OA certification. The second problem is that concentrations starting around 10 % are caustic, causing skin irritation and severe eye damage upon contact, as well as serious lung injury upon inhalation. Another downside is that volumes needed for application are very large and hence only transport of concentrates would be feasible. But handling concentrates would aggravate the hazards for users even more. Likewise, concentrations exceeding 10 % are considered a dangerous good and special rules for transportation with consequently increased costs apply. Resuming, the use of AA as a standalone AI in natural herbicide formulations can be ruled out at this point. Nevertheless, AA can be used as an ingredient for pH regulation or as a formulation enhancer with concentrations from about 1–3 %. In screening tests an improved weed control was observed with AA added to NaCl formulations.

Combinations of cell membrane disruptors From the results of mixtures of different essential oils or essential oils with AA no interaction or potentiating effects could be observed. However, the screening trials were not suitable for a fine detection of differences. In the case of essential oil - AA mixtures the effect of AA was dominant, which reflects the efficiency of AA as a single ingredient.

Vegetable oils Applied purely, no damage to plants was found in screenings, which is in line with results from leaf disk essays of Tworkoski (2002) in which no electrolyte leakage was found for the refined vegetable oils (rapeseed, canola, soybean). Addition of refined vegetable oils decreased the effect of essential oil formulations strongly, which is assumed to be due to the lower concentration of essential oil that leaf tissue come in contact with. Even though refined vegetable oils are generally not phytotoxic, application of crude ricinus (3 %) and cotton oils (5 %) to control insect pests was reported to result in devastating leaf burns on crops (oral communication Androcioli, IAPAR, Londrina 2013). This observed effect may have been due to the fact that crude vegetable oils contain up to 10 % free fatty acids (FFA), which are in fact phytotoxic. Furthermore, non-saturated vegetable oils oxidise in presence of daylight and form additional FFA in the process (Carlsson et al., 1976). Applying non-refined vegetable oils with a high content of FFA and non-saturated fatty acids could therefore be examined for possible phytotoxic effects on weeds.

Methylated soybean seed oil - Aureo® The product Aureo® tested in screenings in part showed promising results. This product at large contains methylated soybean seed oil (MSO), which in essence is soybean biodiesel. However, apart from 72 % MSO the product also contains about 19 % inert ingredients, in which unknown ingredients are contained. The importance of these *inert* ingredients for the products effect remains unknown, and they may have been influencing the effect strongly. Nevertheless, Vaughn and Holser (2007) studied the phytotoxic effects of 1–2 % aqueous emulsions of different biodiesel. Their phytotoxic effect was found to be higher than that of commercial NH products based on essential oils or PA, at lower concentrations and also at a lower cost. Biodiesel may therefore be an AI in environmentally friendly contact herbicides and their potentials of biodiesel should be examined further.

Emulsifiers With the results found in screenings there is no doubt that emulsifiers are in fact AI themselves at the high concentrations applied in NH formulations. Damage symptoms are similar to those of other CMD, as emulsifier equally interact with the cuticle waxes and cell membranes lipids. Anionic emulsifiers additionally interact with proteins and are considered more cytotoxic than non-ionic emulsifiers (Bartnik, 1992), which can explain the relatively strong weed control effect of sulphonic acid in mixtures with other CMD and/or NaCl, as well as on its own. Nevertheless, toxicity of ionic emulsifiers is formulations containing the anionic emulsifier sodium ricenoleate formed were relatively weak in action compared with those containing NPE. At the rates used in mainstream herbicidal formulations of about 0.5–1.0 L ha^{-1}, both NPE or Tween 80® (T80) emulsifiers do not present any visible effect and can in fact be considered *inert* with respect to their herbicidal action. However, in screening trials weed damage started to occur at rates of 5–10 L ha^{-1} for NPE and 10–20 L ha^{-1} for Tween 80®. Using NPE emulsifier for trials certainly alterated results. For example, a large proportion of damage caused by δ-limonene containing formulations could be attributed to the use of this emulsifier. There is apparently no awareness of the herbicidal effect of emulsifiers in natural herbicide formulations and no scientific literature on the topic was found. The reason for this is assumed to be that commercial NH formulations are the only known case in which such high quantities of emulsifiers are used in agriculture. Furthermore, these substances by law can be declared as 'inert' ingredients without further definition. They are not tested in the certification process and without disclosure there is also no possibility to revise them in independent studies. Emulsifiers are indispensable ingredients in NH formulations, which strongly influence product efficacy, and are one of the most complex issues for certification and practical application of NH. Therefore the implications of emulsifiers for the environmental profile and certification of formulations is discussed separately in section 8.5 *Emulsifiers: 'inert' ingredients in NH formulations* on page 115.

8.1.2 NaCl formulations

The effect of spray applied NaCl alone varied strongly from moderate to no weed control. However the addition of NaCl to CMD formulations potentiated weed control strongly. Subsequently essential oil rate could be lowered considerably from about 200 L ha^{-1} to 25–50 L ha^{-1}, maintaining the burn-down effect at a comparable level. In Germany *Urtica sp.* and *Rumex sp.* showed clear reaction of NaCl application without emulsifiers though, but no representative trials were conducted. The observed susceptibility may be due to the relatively thin cuticle which permits penetration of NaCl into plants (Hull et al., 1975). NaCl application of up to 120 kg ha^{-1} NaCl without the use of any emulsifier mostly did not cause serious, if any leaf damage on screened weed plants. A thick waxy cuticle and trichomes on leafs inhibit the contact of NaCl solution with the permeable cell wall, which was found for mature *Vicia hirsuta* by

(Lukashyk, 2005). However, NaCl in mixture with any CMD potentiated the herbicidal effect strongly. The main obstacle for NaCl penetration into leafs is the cuticle, and the dissolving and penetrating effect of CMD apparently opens an entry door for NaCl absorption into plant tissue. Plants can react to the acute osmotic stress by stomata closure, accumulation of NaCl in the apoplast or formation of organic solutes to balance osmotic potential inside cells (Marschner and Marschner, 2012). However, leaf cuticle and cell membrane permeabilised by CMD render stomata closure ineffective, while the high osmotic potential of NaCl speeds up desiccation. In addition to acute damage some systemic effect was observed and NaCl is apparently transported to meristems, which was especially observed in dicot species. Even lateral meristems that have not gotten into contact with spraying solution died off, retarding or inhibiting resprouting (figure 7.6 b). Furthermore, also advanced growth stages of the weeds *Bidens pilosa* and *Euphorbia heterophylla* could be controlled effectively with NaCl, despite the fact that full spray coverage was not reached. Due to its strong acute and longer lasting effect NaCl is one of the most promising ingredients for NH formulations. Furthermore, NaCl is a cost neutral ingredient compared with CMD. Weed control can be improved further by increasing the application rate of NaCl, but it is not known up to what dose a use is sensible, considering salinisation and impacts on crop (by root absorption). In most experiments the per-application-rate was limited to 50 kg ha^{-1}, and the maximum total amount applied in a cropping period was 150 kg ha^{-1}. In trial `PG-NaCl` this high dose was applied directly to the soil in the inter-row space, which did not result in any negative effect to crops, indicating that at least with occasional applications, no concentrations toxic to plants are reached in the soil. Nevertheless, it has to be pointed out that application must be limited to permeable soils in climates with vertical downward water movement, which would allow for NaCl to be leached from topsoil. In Brazil, apart from the Caatinga biome, these conditions are generally met and salinisation problems are not expected. In screenings the combination of AA and NaCl appeared to be highly effective, which may be related to AA penetrating leaf tissue more profoundly than essential oils (Evans et al., 2009). Furthermore, it may be possible that H$^+$ ions in AA saturate negative binding sites, which are predominant in plant tissue. This could lead to higher presence of 'free' Na$^+$ ions in plant tissue.

8.1.3 Effect of concentration, dilution and volume

Concerning concentrations of ingredients it is difficult to determine exact values for an effective NH formulation, because emulsifiers played a crucial role in the effect of practically all formulations tested. Independent of this fact, formulations that can achieve efficient control, given benign environmental conditions (see below) and applied at an early weed development stage could be proposed to contain the AI amounts presented in continuation. For cost reduction and improvement of herbicidal effect all formulations need to contain NaCl, or another phytotoxic mineral salt. Several times it was shown in screenings that NaCl amounts of 50 kg ha^{-1} per application were necessary for an acceptable effect, while 120 kg ha^{-1} gave excellent results. In mixed formulations of CMD and NaCl the damage caused by NaCl appears to be much larger than that of the CMD component. However, cuticle penetration has to be facilitated by CMD, which is supported by the finding that pure applied NaCl gave extremely varying results, ranging from none to moderate weed control. δ-limonene can be discarted from the list of possible CMD ingredients, and of the tested essential oils only pine oil remains as a candidate for a successful NH formulation. In some cases 25 L ha^{-1} of this ingredient were sufficient for a strong effect. However, to guarantee acceptable weed control, pine oil needs to be formulated with at least 50 L ha^{-1} in combination with NaCl. For formulations containing only AA as AI the dosage can be lower than that of essential oils, with concentrations of about 10 %. Nevertheless, AA has been discarted from the list of standalone ingredients for NH formulations due to the disadvantages discussed above, and only makes sense in combination with NaCl. In this case concentrations of 5 % AA with 50 kg ha^{-1} NaCl may already grant efficient weed control, but data obtained is not sufficient to make a reasonable judgement. In oil-containing formulations emulsifiers are necessary, and a minimum ratio of *oil*:/emulsifier/ of 10:1 can be proposed from screening and formulation testing. As emulsifiers possess a proper effect they may also be considered as the CMD component of a formulation containing NaCl. The formulations containing T80, SA or NPE in combination with NaCl achieved acceptable weed control in screenings `S-PG13` and `S-PG14`. The combination of NaCl and an effective emulsifier could lead to tremendous reductions in application costs. However, an environmentally friendly, or even natural emulsifier with CMD action first has to be found. Estimating from findings with NPE and Tween 80® the necessary dose of a emulsifier to penetrate or permeabilise the leaf cuticle would start from about 10 L ha^{-1}. Commercial CMD formulations are recommended at application rates of 1000 L ha^{-1} presumably because complete coverage is necessary and because evaporation of essential oil has to be counteracted to prolong effect. However, the commercial products are also indicated for grown weeds, for which complete coverage can only be achieved by such large volumes. Nevertheless, the elimination of grown weeds has repeatedly been shown not to be effective (Young, 2004; Barker and Prostak, 2014). As NH formulations based only on CMD will always fail to control large weeds the application at earlier growth stages is considered mandatory. Under these circumstances a rate of 1000 L ha^{-1} is not necessary and would elevate costs unnecessarily. 600 L ha^{-1} gave sufficient coverage in all screenings and field trials conducted and even at a rate of 300 L ha^{-1} insufficient spray coverage did not appear to be a problem. Testing different volumes and concentrations of CMD formulations it was found in `S-PG2` that the same amount of AI, applied in a more concentrated form with 300 L ha^{-1} resulted in higher weed control than its more diluted application with 600 L ha^{-1}. In this screening, the difference between the concentrated and diluted application was more pronounced than the effect of doubling the application rate (AI concentration fixed). Due to the systemic effect of NaCl the improvement of effect by concentrated application may even be more pronounced in NaCl containing formulations. However, concentrated application of NaCl containing formulations was only tested in the field trials `PG-Lim` and `PG-Pin`. In these trials weed control was too efficient in all treatments to allow for a clear differentiation between the different formulations tested. The concentrated 25 L ha^{-1} rate of pine oil (300 L ha^{-1}) scored a higher weed injury rating than diluted one (600 L ha^{-1}), while in `PG-Lim` trial the results for the 50 L ha^{-1} rate are inconclusive: the 300 L ha^{-1} application scored worse than 600 L ha^{-1}, but slightly higher than 900 L ha^{-1}. Therefore, no definite conclusion can be drawn, and further studies would have to be conducted to confirm if concentrated application can be recommended.

8.2 Weed control and susceptibility to natural herbicide application

For interpretation of trial results it needs consideration that none of the trials were conducted on fields under permanent organic management. The flora present was therefore typical for areas treated with conventional herbicides and densities were presumably lower than those found in fields under permanent organic management. Also, apart from *Oxalis sp.* and rare occurrence of *Cyperacea sp.*, no perennial weed species were present, which could also be different in OA. Resuming the results of screening trials with different CMD formulations, alone or combined with NaCl, the susceptibility of weed species was observed to be relatively constant independent of the formulations applied. Mostly the development stage of weeds influenced susceptibility.

Monocots compared with dicots Especially dicot species were found to be susceptible. Within this group are some of the most common and problematic weeds in soybean DS, for example *E. heterophylla*, *Amaranthus sp.*, *Alternanthera tenella*, *Parthenium hysteropherus* and *B. pilosa* (figure 7.6 a). The susceptible species *Alternanthera tenella* and *Amaranthus sp.* can even be controlled with NaCl solutions only with addition of small amounts of emulsifier and without the need of addition of large amounts of essential oil. Therefore at least in theory, control of these species can be achieved at a low cost. An intermediate susceptibility was observed for *Ipomoea grandifolia*, *Richardia brasiliensis* and *Commelina benghalensis*, recently emerged *B. plantaginea* (< 2 leaf) and *R. raphanistrum* (< 4 leaf). Relatively insensitive to application were monocots with more than 2 leafs or with presence of first tillers such as *Brachiaria plantaginea* (figure 7.6 b), *Digitaria sp.* or dicot *Raphanus. raphanistrum* (> 4 leafs). This is reflected in the weed dry mass found in `PG-Lim` and `PG-Pin` which is made up almost exclusively of *B. plantaginea*. *Cyperacea* species rarely occurred in experiments, however, field observations indicated that *Cyperus rotundus* is not susceptible to application at all: not even light leaf burns were observed. One explication for the low susceptibility of monocot species is the higher amount of wax formed on the cuticle, which is for several cereal species 2–4 times that of dicot weeds (Stender, 1902). An exception in screenings was *Commelina benghalensis*, a monocot which was fairly easy to control with NH sprays. With the addition of observations made in the field, including species which were not included in screenings, or which were only present in few plots, results are vaguely summarised in table 8.1.

Table 8.1: Summary of weed species susceptibility found in screenings and field trials. Due to varying NH formulations, weed growth stages and environmental conditions at application this list is only a rough interpretation for weed species susceptibility.

Sensitive	Intermediate	Insensitive
Ipomoea grandifolia	*Richardia brasiliensis*	*Raphanus raphanistrum* (>4 leaf)
Amaranthus sp.	*Raphanus raphanistrum* (<4 leaf)	*Brachiaria plantaginea* (>2–4 leaf)
Euphorbia heterophylla	*Brachiaria plantaginea* (1–2 leaf)	*Digitaria sp.*
Alternanthera tenella	*Ipomoea grandifolia* (> 4–6 leaf)	*Cyperus sp.*
Commelina benghalensis		
Parthenium hysteropherus		
Bidens pilosa		

Weed habitus Plant habitus is another factor affecting the spray cover: upright grassy weeds intercept less spraying solution than some dicot species which present a low leaf angle, which is another reason why grasses are especially difficult to combat with CMD. At least in this respect monocot *Commelina benghalensis* is different: leafs are broad and the leaf angle is low, offering a large surface for the absorption of spraying solution. Another reason for the low susceptibility of monocot species is that meristems in grasses are located in the interior of shoots, and tillers or the plant base cannot come in contact with spraying solution. While the number of lateral meristems is generally limited in dicot species (typically two per node), the number of lateral meristems formed at the base of grasses is near infinite, which increases the possibility for plant recovery. In fact, when grasses already formed tillers, the control with NH formulations was found to be nearly impossible.

Weed growth stage and susceptibility Generally weed control should occur until 4-leaf stage of weeds, and shortly after emergence for the relatively insensitive species, especially *B. plantaginea*, *Digitaria sp.* and *R. raphanistrum* (2-leaf stage). The time margin for effective control with the tested NH formulations was therefore rather narrow. To control resprouting and also to account for uneven emergence of weeds, repeated applications were found to be necessary in the DS system. Up to 4-leaf stage control of *R. raphanistrum* is usually effective, but a marked difference in control is observed at later stages when phytotoxicity symptoms become nearly absent and weed control ratings fall extremely low. This is probably reasoned by a change in leaf cuticular wax, which changes as a function of plant age (Baker, 1974). Lukashyk (2005) explains the reasons for the lower susceptibility of larger weeds to kainit application (essentially NaCl) with the more resistant and less permeable cuticula, the relative to total leaf area low area of tissue necrosis and with the larger reserves of assimilates which aid in plant recovery. Roughly, it can be summarised that monocots can be controlled up to two-leaf stage, and dicots up to four-leaf stage.

Weed susceptibility and season In trials `PG-Lim` and `PG-Pin` it was striking that weed control was far better than in the other two field trials, despite a similar NH formulation. On one hand this was certainly due to the more homogeneous weed development stage and the improved application timing in comparison to direct-seeded trials `PG-NH` and `LO-NH`. Another possible explication is that late in the season, lower temperatures and light intensities may have lead to a minor formation of the leafs waxy cuticle, favouring the leaf penetration of applied products. Light intensity not only triggers the formation of cuticle but also the synthesis of cellulosic and pectic materials of the outer epidermal wall of leafs (Baker, 1974; Hull et al., 1975). Therefore the resistance to penetration, leaving out the stomatal path, can be assumed to be higher in mid-summer.

Cell membrane disruptors, NaCl and weed susceptibility The universal problem with formulations only containing CMD is that control can only occur up to a relatively young development stage (Bhowmik et al., 2003; Young, 2004; Batish et al., 2008). The effect of pure CMD formulations is firmly linked to a complete product coverage of the leaf area. With growing plant age the amount of overlapping leafs and lateral meristems, as well as the energy reserves for recovery increase (Evans et al., 2009). Therefore, weed control with CMD becomes unfeasible at advanced development stages. Weed susceptible in the case of pure CMD is a function of habitus, cuticle thickness, protection of meristems, and storage reserves for recovery. However, information on effects of NaCl applied to leafs is hardly found in literature (Lukashyk et al., 2008; Uddin et al., 2013) as research on weed species salt tolerance usually studies the effect of soil salinity. In this case weed tolerance is a function of the osmotic potential at which plants are still able to absorb water, or a function of the NaCl concentration that plants can tolerate their tissue (Marschner and Marschner, 2012). The former mechanism for salt tolerance of plants cannot be expected to play a role for leaf applied products. Differences in weed susceptibilities to spray application of NaCl can rather be explained by the second argument: weed susceptibility is probably a function of resistance to acute osmotic stress, and tolerance of meristems to high NaCl concentrations. However, the experimental data is too limited to derive exact conclusions on how plants susceptibility is working in this case. Monocot species appear to be more salt tolerant than dicots. Dying off of tillers was not observed as commonly as the dying off of lateral buds in dicots. This is supported results of Uddin et al. (2013), who found that seawater application for weed control in turfgrass controlled dicot species, while leaving the grass undamaged.

8.3 Environmental conditions and natural herbicide efficacy

In order to evaluate the optimal time of day for application one screening trial was conducted, in which spray treatments were applied either in the morning, midday or afternoon. Unfortunately, the screening gave no results, which was due to the low dose of AI chosen. Nevertheless, observations made in screenings and field trials indicate that NH efficacy does depend on environmental conditions and the time of the day of application, at least for formulations containing NaCl. Applications in the late afternoon were generally found not to be as effective as applications in the morning or midday. This could for example be observed for the second application of trial `PG-NH`: the visual weed injury ratings resulted in low scores for this application. Furthermore, the difference in weed dry mass compared with the single application treatments was only small. Another example are the results from screenings `S-PG11` and `S-PG12`: with the same NH formulation the midday-application in `S-PG11` resulted in better weed control than the late afternoon application in `S-PG12`. Labels of commercial NH formulation state that application timing is irrelevant. However, compared with formulations only containing CMD, NaCl adds osmotic stress to the acute toxicity of CMD. At midday with highest temperatures the osmotic shock caused to plant tissue is highest. In fact, wilting at midday was observed to start within seconds after application. Stomata closure cannot protect plants as it may be possible in the early morning or late afternoon and leaf tissue dies off immediately. At lower temperatures in the afternoon the leaf do not dry out as quickly and may give more time to plants to adjust to the stress. Apart from stomatal closure plants may be able to form osmotic solutes during nighttime to adjust to the NaCl absorbed (Marschner and Marschner, 2012). Brainard et al. (2013) found that cloud cover, hence reduced solar radiation, did not affect weed control of vinegar and clove oil formulations. Nevertheless, high relative humidity was found to improve weed control. On one hand, this may be due to the slower evaporation rate of the spraying solution at high relative humidity, and hence a prolonged time for AI to act upon plant tissue. On the other hand more stomata are open in these conditions, which may increase the AI entry into the leaf. Supporting this argument, it was also found for mineral oils that in the light, when stomata are open, plants were killed more easily, while when closed at night, their effect was low. Nevertheless, for mineral oils that penetrate plant tissue easily, the environmental conditions were less important. Resuming, phytotoxicity of mineral oils is greatest on sunny and very hot weather (Baker, 1970). Another argument that can explain more effective weed control for midday-application is that the membrane leakage displaces chlorophyll from the thylakoids. In the light this chlorophyll is sensitised and forms radicals, which then cause peroxidation and further damage to membranes (Lederer et al., 2004). Deducing from screenings and field trials the moment most unfavourable for weed control is the late afternoon, and with low temperatures or/and cloudy conditions. In conclusion, NH applications should be realised when temperatures and radiation are highest. Noon is usually avoided for herbicide application in mainstream practice, due to faster drying of spray and the drift caused by stronger winds due to thermal activity. However, drift off is not a problem in banded application close to the ground with a protective screen and midday application would therefore not a problem for NH application in OA.

8.4 Agronomic aspects of natural herbicides

8.4.1 Straw residue cover

One precondition for using the DS system in OA is a dense pre-crop mulch cover to reduce weed infestation efficiently. The rolling of pre-crop straw brings about certain problems: on one hand the soil coverage is worse, on the other hand the problem was encountered that the protective screen interfered with the upstanding straw residue. Furthermore, standing straw absorbs spraying solution and acts as a protection to weeds. For NH application chipping the pre-crop or pre-crop residues can therefore be recommended: the weeds emerge directly through the mulch cover and are not shielded by any straw. At the same time the distribution of chipped straw is more homogeneous and less soil is exposed to solar radiation, which would favour germination of weed seeds (Finch-Savage and Leubner-Metzger, 2006). For soybean, an important consideration is an early seeding date, before weeds begin to emerge. Weeds that emerge prior to soybean could be controlled with NH, but due to high costs applications should be concentrated after crop emergence to limit the number of applications. Of both soybean field trials, only `LO-NH` was managed without prior herbicide application. Seeding occurred early, and most weeds only started emerging after crop emergence.

8.4.2 Application intervals and repetitions

In field trials one single application did not give satisfactory results. Other studies also confirm that multiple applications are necessary for sustainable weed control (Young, 2004; Barker and Prostak, 2014). For the summer crop soybean two to three applications were necessary, and for late season common bean one or two applications. The interval length between applications was observed to be crucial to obtain positive results. With the choice of an application interval in mid-summer of about seven days, weeds had resprouted and had nearly regrown to their original size by the subsequent application, which lead to relatively low weed injury ratings and high weed biomass in `PG-NH`, `LO-NH`. It seems reasonable to assume that the second application has to be timed shortly after the beginning of resprouting of surviving weeds. In dicot weeds the apical dominance is usually broken due to damage to or destruction of the apical meristem. The resprouting of the remaining lateral meristems therefore occurs simultaneously. During resprouting weeds are not able accumulate photosynthates but are forced to consume their energy reserves and to expose sensitive meristems, which is why this is thought to be the ideal moment for the subsequent NH application. Depending on temperature an estimate for the interval between applications ranges from about four days in mid-summer (November / December) to six to eight days for late season crops (March / April).

8.4.3 Requirements for spraying equipment

The tested NH formulations all possess a non-selective burn-down effect on plants, hence crops need to be protected from NH spray. The protective screens available at both sides had the disadvantage of interfering with the rolled straw, which was pointing up frequently. In consequence in none of the `PG` trials a protective screen was used. In common bean conventional tillage (CT) trials the unshielded application did not cause greater damage to crop, but in `PG-NH` damage was considerable, which strongly altered trial results. In contrast, in Londrina large PVC tubes were used to cover the small plantlets from spray, which allowed for sufficient crop protection. Even with a protective screen, post-emergence application can be difficult to realise immediately after crop emergence, and only the centre of the row can be band-sprayed without risking injury to sensitive crop seedlings. This requires specialised spraying equipment, and restricts working widths and performance of mechanised sprayers. Also at later crop development stages the protective screen impedes application in direct crop proximity (approx. 3 cm) and weeds within the crop row are usually not controlled by NH application. One possibility to increase the percentage of sprayed area may be to sow crops with the widest spacing possible while at the same time increasing the plant density within the row. Intra-row weed control with vinegar has been tested by Evans et al. (2011) in broccoli and pepper, and at later crop development stages in cabbage, using a specialised banded directed sprayer. The shields lifted up bottom leafs up and over the spray nozzles and applied spray solution directly into the crop row. However, despite only 5 % foliar injury measurable yield reduction was observed in pepper. A working protective screen was also developed in this work in early 2013 and was used successfully in common bean trials in *Londrina* (see figure 8.1 a). However, due to draught and high incidence of virus these trials had to be abandoned. This protective screen was able to push the first trifoliate leafs of common bean aside and lift them up slightly, allowing for application as close a 1 cm to the stem. But application this close to the crop is risky. Occasionally, leafs can pass below the protective screen and also the partial contact with NaCl containing formulations may reduce growth of crops at early development stages severely, as absorption and translocation of this AI occurs.


(a) Banded application with protection

(b) Left: Application - Right: Control

Figure 8.1: Application and effect of NH in a crop stand of *Phaseolus vulgaris*

During experiments, rubber sealings and tubes of spraying equipment were strongly affected by terpene formulations and leakages were observed in several occasions. Furthermore, the NaCl and AA containing formulations caused strong corrosion of metal parts. In any practical application of NH formulations containing CMD or phytotoxic salts this detrimental effect on spraying equipment has to be considered and materials need to be changed for less susceptible ones. For example, rubber sealings could be replaced by sealings made of silicon. The metal parts would have to be changed to stainless steel and spraying nozzles should preferably be made of porcelain. Common backpack sprayers used by small scale farmers are not apt for some of the tested formulations and will corrode and deteriorate rapidly which could discourage farmers from using these products.

8.4.4 Application costs

Table 8.2 shows examples of the prices of commercial products, single AI and their respective application cost estimates. Typically AI concentrations applied in commercial products are about 20 % (PA only about 5 %) with recommended application rates of 1000 L ha^{-1}. The products are rather aimed for to be used by home gardeners which is reflected in the generally small container sizes on sale, for areas of only up to 100 m^2. Prices even for the cheapest formulation of PA (Finalsan®) are elevated. In fact, the current commercial products need no further consideration for application in Organic Agriculture Direct Seeding (OADS).

In comparison, the prices for single ingredients are far lower. Prices cited are based on small volumes of 1–10 L, which were bought at local chemical distributors in *Curitiba*, Paraná state, and should be

Table 8.2: Example of commercial products based on pelargonic acid, acetic acid and citronella oil. Finalsan® and Barrier H® are only marketed in small volume containers while larger volumes while larger volumes with reduced costs of Weed Pharm® and Avenger Weed Killer® are on sale - application costs are based on the cost of the largest volume containers available. Prices in parenthesis are prices for low volume containers (estimated for Finalsan® and Barrier H®). The concentration of the active ingredient (AI) is generally around 20 %, except for pelargonic acid with only 5 %.

Product (AI)	Product cost (1) € per L	Cost per application (2) € per ha
Finalsan® (pelargonic acid)	0.75 (1.5)	750
Barrier H® (citronella)	9 (18)	9.000
Weed Pharm® (AA)	2 (6)	2.000
Avenger® Weed Killer (δ-limonene)	3 (5)	3.000

[1] Ready-to-use formulated product

[2] Application cost at the recommended application rate of 1000 L ha^{-1}

Table 8.3: Non-formulated AI, which are contained in commercial formulations, their approximate application rates and costs.

Active ingredient	Pure AI € per L	Cost per application (1) € per ha
Citronella oil	20	1000
Eucalyptus oil	10	500
Pine Oil	3.5	175
δ-Limonene	2–7	200
Synthetic AA (99 %)	4	200 (2)
Vinegar (10 % AA)	1	1000
NaCl	0.14	30
Emulsifier (3)	1–4	10-80

[1] Application rate: 1000 L ha^{-1}, concentration: 20 %

[2] Concentration of only 10 %

[3] Estimated costs when applied at 10–20 L ha^{-1}

even lower when bought in bulk (table 8.3). As all the CMD tested possess a similar mode of action, the ingredient choice can be based on cost-effectiveness. With the highly elevated prices of thyme, clove and lemongrass oil, these oils are assumed not to possess any economic potential as natural herbicides, and if than only as additives to formulations based on other ingredients. Generally byproducts are more cost effective, for example pine oil from wood industry and orange oil (δ-limonene) from orange juice production. Nevertheless, despite far lower prices AI costs per application are still highly elevated. A replacement by natural or environmentally emulsifiers could further reduce costs. NaCl - not actually used in commercial formulations - is by far the cheapest AI. With a price of about 50–140 €/t (unrefined / refined) the AI costs are about 5–20 € ha^{-1} when applied three times at 50 kg ha^{-1}. Due to its strong effect on weeds and the low price it is thought to be a key ingredient to make NH application viable. Based on the prices in table 8.3 the typical formulation using 50 L ha^{-1} of pine oil and 50 kg ha^{-1} of NaCl would still cost about 200 € per application, leading to total application costs with two or three applications of 400 to 600 €. In comparison, a formulation only using a emulsifier as CMD ingredient in combination with NaCl would only cost 40–110 € per application.

8.4.5 Summary: ideal conditions for NH application

At this point the ideal preconditions, formulation, application timing and agronomic application for a successful use of NH in OADS can be summarised as follows:

Box 8.1 Summary: ideal conditions for NH application

Preconditions

- High pre-crop straw residue biomass.
- Early seeding to give crop a head start over weeds.

Application

- NH application at early weed development stage.
- 2–3 applications.
- Timing of subsequent applications: during early phase of resprouting, about 3-7 days after previous application.
- Effective protective screen.

Formulation

- Maximise phytotoxicity.
- Cheap mixture of CMD and NaCl a possibility.
- Concentrated instead of diluted application.
- 300 L ha^{-1} results in sufficient coverage.

8.5 Emulsifiers - 'inert' ingredients in natural herbicides

In labelling and registration of agro-chemicals, ingredients are classified as 'active' and 'inert'. The 'inert' substances include emulsifiers and other additives, besides water or solvents. Common labelling policy requires formulations to disclose AI type, chemical form and the amounts present in formulation. However, only the total amount, but not the exact nature of 'inert' ingredients needs to be stated. (Cox and Surgan, 2006) reviews the extend of problematic implications associated with this practice. Starting with the registration of agro-chemicals, 'inert' ingredients do not need to pass through the same registration process as AI: in the U.S., only short term acute toxicity studies are conducted with the whole formulation. Negligently, important long term toxicity studies (e.g. carcinogenicity, genetic damage) are not mandatory with the 'inerts' or the whole formulation and are only conducted with the AI alone. This practice has been criticised extensively, and several publications alert of the toxicity of emulsifiers in herbicidal formulations, which - in some cases - resulted to be more toxic than the AI itself, and hence not passive at all as the term 'inert' would suggest. One recently reported example is that the most human toxic principle in several glyphosate formulations is a frequently used emulsifier (POE-15) and not the AI glyphosate (Mesnage et al., 2013). In another study 'inerts' were found to contribute to about half of the overall toxicity of 2,4-D formulations (Oakes and Pollak, 2000). Apparently the increased environmental or human toxicity of emulsifiers or whole formulations compared to pure AI is not limited to classes of pesticides, types of formulations or the mode of action of AI, but is a common occurrence Cox and Surgan (2006).

As for mainstream herbicides, also emulsifiers used in NH formulations need to be observed carefully, as both type and amounts of emulsifiers applied can be problematic. As currently no products are certified in Europe and Brazil, reference is made to US OMRI and EPA guidelines, in order to elucidate difficulties with NH registration and certification. OMRI certifiable formulations of NH may contain synthetic emulsifiers mentioned on the EPA *List 4* (U.S. Environmental Protection Agency, 2004b,a; U.S. Environmental Protection Agency and Environmental Protection Agency, 2014), among which several are problematic. For example, in the δ-limonene herbicide patent held by the company that produces AvengerAG® (Messerschmidt and Jankauskas, 2012) one *preferred* embodiment includes Tween 80® as a emulsifier, which has a positive environmental profile. Nevertheless, in another *preferred* embodiment a mixture is mentioned which contains two types of NPE, one polypropylene glycol (PPG) emulsifier as well as an organosilicone adjuvant as a wetting agent (example Silwet®) [23], which can all be found

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[23] From the patent claim held by the company that produces NaturesAvenger® it is impossible to derive which ingredients are exactly used in the actual commercialised product. The criticism here is that some of the ingredients mentioned in the

on EPA *List 4* (table 8.4). However, organosilicones have an effect on non-target organisms and possess insecticidal properties, for example on mites (Cowles et al., 2000). NPE make up a highly effective emulsifier class, but are problematic as an ingredient in NH formulations: emulsifiers of this class are known to be endocrine disruptors and toxic xenobiotics (Ying et al., 2002; Soares et al., 2008). For that reason NPE use has been restricted for mainstream pesticide applications in the EU (Directive 2003/53/EC) to concentrations of 0.1 %. Even US EPA states (NP and NPE action plan – RIN 2070-ZA09) that '*NPE are highly toxic to aquatic organisms, and in the environment degrade to more environmentally persistent nonylphenol (NP)*'. Furthermore the text states that '*NP is associated with reproductive and developmental effects in rodents*'. Despite these claims NPE are on *EPA List 4B* and permitted in OMRI certified formulations for application in OA.

Table 8.4: δ-limonene formulation `MM-01` used in field trials described in δ-limonene product patent (Messerschmidt and Jankauskas, 2012), owned by the company *Cerrone Bio Innovations*. NPE: nonylphenol ethoxylate, PPG: Polypropylene glycol. The trial formula was applied at a ratio of 3:1 (*water* : *product*). The table shows the concentration in concentrate and in the applied spray as well as the total amounts of each ingredient at the application rates of 500 L ha^{-1} and 1000 L ha^{-1}. Assuming two subsequent applications, this would result in a sum of total emulsifiers applied of about 7.7–16 L ha^{-1}. According to the label of the δ-limonene containing product of the same company, AvengerAG®, this rate would even be higher.

Ingredient	Concentrate %	Spray (3:1) %	Rate: 500 L ha^{-1} kg ha^{-1}	Rate:1000 L ha^{-1} kg ha^{-1}
δ-limonene	96.0	24.0	120.0	240
Tergitol^{TM} NP-8 (NPE)	1.3	0.4	2.1	4.3
Tergitol^{TM} NP-9 (NPE)	2.0	0.6	2.9	5.8
Pluracol® P-425 (PPG)	2.0	0.5	2.5	5.0
Silwet (organosilicone)	0.2	0.05	0.25	0.5

Nevertheless, not only the permitted types of emulsifiers have to be criticised, but also the total amounts applied. Table 8.5 compares the amounts of 'inerts' applied in commercial natural and mainstream herbicide AI formulations. As high concentrations of essential oil are necessary to achieve efficient weed control, also high total rates of emulsifiers are typically applied in commercial NH formulations.

Table 8.5: Based on the concentration of 'inerts' on product labels and highest recommended application rates, approximate amounts of 'inerts' applied per ha are calculated for commercial limonene, glyphosate, glufosinate, atrazine and 2,4 D formulations. 'Inerts' may include water or other solvents and exact amounts of each are unknown for the mainstream formulations. Nevertheless, for the δ-limonene formulation (AvengerAG®) these 'inerts' are in fact emulsifiers according to the technical data sheet of the product. Additional emulsifiers, which are often recommended for mainstream herbicidal formulations are not included in the calculation, yet would typically amount to 0.5–1 L ha^{-1}.

Product	AI	Rate	Concentration and rate 'Inerts'	
		L ha^{-1}	%	L ha^{-1}
AvengerAG®	δ-limonene	100	25–30	25–30
Barrier H®	Citronella	500–1000	10	50–100
Roundup® Custom	Glyphosate	3	46	1.4
Liberty® 280 SL	Glufosinate	2.0	76	1.5
Atrazine 4L	Atrazine	5.6	57	3.2
2,4 D LV4	2,4 D	1.1	34	0.35

The mainstream herbicides may contain more toxic emulsifiers, as these are not limited to *EPA List 4*, but the overall toxicity of 'inerts' in NH may well be higher, because of the large quantities applied. The addition of NPE as a spreader is common in mainstream herbicide sprays (e.g. in Agral®, Energic®) at rates of about 0.5–1 L ha^{-1}. If NPE were actually used in NH formulations this rate would be exceeded by far. In the δ-limonene patent (Messerschmidt and Jankauskas, 2012) a δ-limonene:emulsifier ratio

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patented formulation are questionable, but yet can be certified by OMRI for use in OA.

of up to 4:1 is described, which at a recommended rate of 1000 L ha^{-1} and a concentration of 20 % δ-limonene would amount to about 50 L ha^{-1} emulsifier per application. Independent of environmental effect, from the screenings conducted in this work it can be concluded that a wide range of emulsifiers has a phytotoxic effect at doses near that magnitude, e.g. T80, MSO, SA or NPE. Furthermore, as δ-limonene effect was found to be extremely dependent on emulsifier choice it can be assumed that the commercial product AvenverAG® also derives its herbicidal effect from the emulsifiers contained, and not from the ingredient δ-limonene, which is declared as the AI on the product label. Thus, the existing OMRI guidelines most certainly have to be reviewed critically with respect to the emulsifiers permitted, especially for NH. Considering the synthetic nature, potential toxicity and the herbicidal effect of emulsifiers, certifications of some of the commercial NH products on the market may have to be revoked.

9 Summary

Direct seeding (DS) is a cropping system, which compared with conventional tillage (CT) reduces labour, diesel consumption, and CO_2-emissions drastically, and is highly effective in protecting soils against erosion. In tropical climates torrential rains occur frequently, and the use of the direct-seeding system has to be considered mandatory for annual cropping, in order to avoid soil degradation by erosion and to thereby preserve soil fertility in the long-term. The same holds true for erosion prone soils on hillsides in temperate climate. Organic farming, which accepts soil fertility losses by strong erosion due to intense tillage management, cannot be considered sustainable. The implementation of occasional DS is of great interest to Organic Agriculture (OA) but the system faces severe restrictions.

Especially plant nutrition with sulphur and phosphorus is assumed to be limited under DS management. In temperate climate, the lower soil temperatures in DS and the higher top-soil density reduce soil organic matter (SOM) mineralisation rates, and crop fine root formation in comparison to tillage systems. In consequence, availability of phosphorus (P) and sulphur (S) is decreased. S-inputs by fertilisation and atmospheric deposition are also generally low, which holds true for rural sites in tropical climate as well. In tropical soils P deficiency is frequently caused by subsoil Al-toxicity, low P-contents and P-fixation. The biggest constraint for the realisation of DS in OA are high weed pressures. Even though straw residues of cereal crops are able to suppress weeds up to a certain extent, weed control is inefficient without further measures. The research goal of the work at hand was to address both weed control and plant nutrition restrictions in DS for grain legumes in OA of Germany and Brazil. In order to improve nutrient supply with P and S, rock phosphate (RP) and different sulphur fertilisers were applied intra-row. Field trials were conducted in seasons 2011/12 with DS faba bean in Germany and in 2012/13 with DS soybean in Brazil.

The second research approach primarily studied weed control with natural herbicides. These were thoroughly examined in Brazil in screening trials with spontaneously emerged weeds, in field trials with direct-seeded soybean, and with common bean under conventional tillage. As a second weed control strategy, results of previous works which studied weed control with straw residues in temperate climate DS were validated. Therefore, treatments with different amounts of oats straw residue were included into the fertiliser trials in Germany in the second trial year.

9.1 P and S fertilisation in direct-seeding in temperate climate (Germany)

Fertiliser trials with direct-seeded faba bean were conducted on a *luvisol* soil formed on loess at *Campus Klein Altendorf* in 2011 (`KA`), and on a *fluvisol* at *Wiesengut* in 2012 (`WG`). In the `S-Trial` at `KA`, sulphur was applied intra-row at a rate of 40 kg ha^{-1} in the forms of gypsum ($CaSO_4 \cdot 2\,H_2O$), potassium sulphate (K_2SO_4), and elemental sulphur (S^0). An additional KCl treatment examined the effect of K-fertilisation at a rate of 97 kg ha^{-1} K, which is equivalent to the amount of K applied in K_2SO_4 treatment. In the `P-Trial`, the effects of intra-row applied *Gafsa* rock phosphate (RP) were studied. As the low solubility of RP limits P-supply, it was intended to increase its dissolution by simultaneous application of elemental sulphur, which upon oxidation by *Thiobacillus de fact* bacteria liberates sulphuric acid *in situ.* The treatments were RP, applied at 50 kg ha^{-1} P, elemental sulphur at 40 kg ha^{-1} S, and the combination of the two. Both `P-` and `S-Trial` had a non-fertilised control.

At site `WG` in 2012, fertiliser treatments were examined in combination with mulch straw biomass treatments under OA management. In the three-factorial trial `WG-PS`, the fertiliser treatment consisted of RP in combination with elemental sulphur, and a control treatment. The second treatment factor was oats straw biomass, which consisted of the treatments 0, 4, and 6 t ha^{-1} oats straw. The third treatment factor was the oats cutting height at harvest, consisting of a conventional low cut at the plant base, and a high cut just below the panicle followed by rolling the straw with a roller-crimper. On the same field three split-plot trials were conducted, in which oats was harvested with a high cut. The gypsum trial (`WG-G`) and the potassium sulphate trial (`WG-K`) each consisted of two whole-plot fertiliser treatments: a control, and a gypsum treatment for `WG-G`, and a control and a K_2SO_4 treatment for `WG-K`. Furthermore, each trial had the same oats straw biomass treatments as the `WG-PS` trial within the split-plots. Of a third split-plot trial with molybdenum application (`WG-M`), only the oats straw treatments were evaluated.

Phosphorus The soil at the trial sites in Germany contained phosphorus at a recommended level (LUFA C). RP fertiliser applications, alone and in combination with elemental sulphur, in both years did not result in a positive effect on crop growth, P-uptake and -concentration. The lower soil temperatures in the DS system, the higher top-soil density, and the reduced aeration compared to tilled soils, all did not limit root development to the extent that P-uptake was compromised. Due to P-sufficiency, for German trial sites no conclusions can be drawn whether the combined application of RP and elemental sulphur is able to improve P-supply: in 2011 elemental sulphur was applied as pellets, which did not disintegrate well in soil, and in 2012 only the combined application of both treatments was examined. Trials would have to be repeated on sites with lower P-content.

Sulphur In both years the sulphate fertiliser treatments had a strong and almost identical effect on S-uptake into shoot and grain, with an increase of up to 40 % compared with the control. The more narrow N:S ratio in grain indicated that grain contained higher amounts of the S-containing amino-acids cysteine and methionin, which would mean an improvement in grain nutritional quality. Elemental sulphur showed a weaker effect, only apparent in grain. This could in part be attributed to the application of S^0 as pellets. These only possessed a small surface in contact with the soil, resulting in delayed sulphate liberation. While in 2011 no impact on crop growth was found, in 2012 shoot growth and grain yield were in part significantly increased.

The sulphur balance determined for the experimental sites in Germany was found to be negative, as atmospheric inputs did not balance harvest exports over the crop rotation. Also, lixiviation losses in the neutral and relatively permeable soils are high. Nevertheless, the main reason for the presence of S-deficiency was attributed to the kinetic limitation of SOM mineralisation caused by lower soil temperatures under DS management. Furthermore, the low SOM-mineralisation rates limit crop S-supply independent of nutrient balance. Therefore, the application of sulphur fertilisers to grain legumes is considered mandatory for occasional DS in temperate Germany. Generally, the S-fertilisers gypsum, K_2SO_4, and S^0 are all able to guarantee sufficient S-supply to the faba bean crop. S^0 has the advantage that the oxidation speed can be roughly controlled by the choice of sulphur particle size. While a long-term effect can be achieved with larger particle sizes, colloidal elemental sulphur has to be chosen to obtain a short-term fertiliser effect (Chapman, 1989; Germida and Janzen, 1993).

The work at hand cannot conclude if lower SOM mineralisation rates in direct-seeding were the cause for the lower S-uptake in non-fertilised plots. To better understand the implications of nutrient supply of P and S in the DS system in comparison to CT, the trials should be repeated including fertiliser treatments in both the DS and the CT system.

Oats straw residue In `WG` trials, increasing the amount of oats straw showed a significant positive effect, or at least a trend to increase crop growth and grain yield, which confirms results of Massucati et al. (2010). In trial `WG-PS`, the high cut at oats harvest had a negative impact on weed control, because soil coverage by mulch and shading was reduced, which lead to the formation of a higher weed shoot drymass and to lower crop growth.

9.2 P and S fertilisation in direct-seeding in tropical climate (Brazil)

The same `P-Trial` and `S-Trial` as in Germany were conducted in Brazil with DS soybean, in 2012 and 2013 (year of harvest) on clayey *ferralsol* soils in *Londrina* (`LO`) and *Ponta Grossa* (`PG`), and in 2013 on a sandy *ferralsol* soil in *Umuarama* (`UM`). Additionally to *Gafsa* RP a less soluble Brazilian *Alvorada* RP was applied, alone and combined with S^0.

Phosphorus For *Gafsa* and *Alvorada* RP, applied alone and in combination with elemental sulphur, no crop response was determined at trial sites with medium to high soil P-contents. On a highly acid and P-poor ferralsol in `PG`, the `P-Trial` was repeated for two consecutive years with the exact same plot treatments. In both trial years positive effects of P-fertilisation were apparent, with increased shoot growth, grain yield, and P-uptake, especially in the second year. Furthermore, in these trials the addition of elemental sulphur further increased P-uptake and elevated shoot growth as well as yield for both

P-fertilisers, with a stronger effect for *Gafsa* treatments. The S^0 application alone caused a distinct growth depression in 2013, presumably because sulphuric acid formation increased the already present Al-toxicity, and thereby restricted crop root development. In combined applications of RP and S^0, this acidity was buffered by the dissolution of RP. On P-poor soils it should be examined if the effect of the combinations of RP and S^0 can further be increased by inoculation of the fertiliser mixture with *Thiobacillus sp.* bacteria, weeks prior to application (Aria et al., 2010).

Sulphur In a total of five trials and over two years no response in crop growth, grain yield, and nutrient uptake was found for sulphur fertilisation, not even at the S-deficiency prone sandy ferralsol site `UM`. Only the gypsum application in *Londrina* 2013 had a positive effect on crop growth, but the effect was interpreted as a result of amelioration of Al-toxicity, and not attributed directly to sulphate application, because the even more soluble K_2SO_4 exhibited no response. In tropical soils mineralisation rates are relatively high. Furthermore, large amounts of plant available SO_4^{2-} are adsorbed to sesquioxides and kaolinite in acid tropical variable charge soils, which also restricts leaching losses despite high amounts of rainfall. In all trials both the combination of high SOM mineralisation rates and large pools of adsorbed sulphate were concluded to be able to fully meet crop S-supply. Sulphur flows at experimental sites were not balanced, and even without considering additional leaching losses, yield exports alone exceeded atmospheric inputs. Therefore, sulphur deficiency may become a problem in the long term. Nevertheless, intra-row application of S-fertilisers is not considered necessary. With low soil pH, Al-toxicity is one of the major limitations for crop productivity in ferralsol soils, and in some of the trials high saturation levels of Al_3^+ were present. Therefore, emphasis should rather be put on amelioration of Al-toxicity. Al-saturation and calcium contents in subsoil should be monitored and gypsum applied periodically whenever critical values are reached. Thereby sufficient quantities of S are supplied to soils and no S-deficiency has to be expected. In conclusion, further research on intra-row application of sulphur fertilisers for Brazilian sites is not considered necessary.

9.3 Natural herbicides

The screening trials were realised in previously cultivated fields with recently emerged spontaneous vegetation. The active ingredient (AI) tested in screenings were mainly the essential oils δ-limonene and pine oil, acetic acid (AA), and NaCl, apart from a variety of emulsifiers. Other essential oils such as citronella or eucalypt oil were discarded due to insufficient effect or prohibitive costs. In all screenings, per-species visual weed injury ratings were estimated, with 0 indicating no weed damage, a rating above 70 acceptable, and 100 complete weed control. Furthermore, a total of five natural herbicide (NH) field trials were conducted, two with DS soybean, and three trials with late season CT common bean, all including a weedy and a clean control plot. In a two factorial split-plot trial in Ponta Grossa with DS soybean (`PG-NH`), the whole-plot factor consisted of a high and a low weed density treatment (different weed densities were the result of seed bank reduction trials in previous years). The two NH-formulations tested contained 50 L ha^{-1} δ-limonene or pine oil, 50 kg ha^{-1} NaCl, and 10 L ha^{-1} emulsifier (Renex 95®). These formulations were band-sprayed between crop rows at a rate of 600 L ha^{-1}. The split-plot treatments consisted of one, two and three applications of both formulations, with a week interval between subsequent applications. In the Latin square soybean DS trial in Londrina (`LO-NH`), a total amount of 90 L ha^{-1} essential oil (δ-limonene or pine oil), 90 kg ha^{-1} NaCl, and 20 L ha^{-1} emulsifier Renex 95® was applied, either concentrated in two applications (45 L ha^{-1} terpene, 45 kg ha^{-1} NaCl) or diluted in three applications (30 L ha^{-1} terpene, 30 kg ha^{-1} NaCl), again with an interval of a week between subsequent applications. In the one factorial common bean trial `PG-Lim`, the spray treatments were `25 Lim-300` (25 L ha^{-1} δ-limonene at a rate of 300 L ha^{-1}), `50 Lim-300`, `50 Lim-600`, `50 Lim-900` and `75 Lim-900`. In the second one factorial common bean trial `PG-Pine`, the treatments were `25 Pin-300`, `25 Pin-600`, `50 Pin-300` and `50 Pin-600`. In both trials all spray treatments additionally contained 50 kg ha^{-1} NaCl and 10 L ha^{-1} emulsifier Tween 80®. Applications were realised twice at an interval of four days. In all NH field trials crop and weed shoot dry mass as well as grain yield were determined. Weed control was estimated with visual weed injury ratings per species (*Ponta Grossa* trials) or weed cover (*Londrina*). In a third CT common bean trial the effect of soil applied NaCl was studied to determine if the total NaCl amounts applied have a negative impact on crop growth by root absorption (`PG-NaCl`). Treatments consisted of a control plot and three NaCl treatments in which 50 kg ha^{-1} NaCl were spray applied to the inter-row space once, twice and three times, at an interval from four to five days between applications. Plots were kept weed free.

Results screening trials The screening trials confirmed the findings of prior works, that application of formulations of cell membrane disruptors (CMD) alone, like AA, essential oils, or pelargonic acid (PA), are not viable for application in agricultural practice. Weeds readily resprout, and already the cost of a single application is highly elevated. In most trials both the essential oils pine oil and δ-limonene were used. However, δ-limonene proved to be extremely inefficient, and most of the effect was related to the emulsifiers contained within the formulation. In screenings it could be shown that at least for AA and essential oil formulations the concentration and dilution of ingredients plays a crucial role, more than the application rate. Applying a given amount of product in a concentrated form at a lower application rate, resulted in better weed control than the diluted application. Spray applied NaCl at rates of 120 kg ha^{-1} caused weak or no weed damage only. However, in combination with CMD, NaCl rates of 50–120 kg ha^{-1} strongly increased phytotoxicity. NaCl on one hand damages plants through strong osmotic stress, but also introduces a systemic effect to formulations as it is translocated to young meristems, which frequently die off. Resprouting of weeds occurs less frequently and is both reduced and delayed. In combination with NaCl the concentrations of CMD ingredients used initially could be decreased from rates of 150–200 L ha^{-1} to 50 L ha^{-1} for pine oil. Emulsifiers were found to affect NH efficacy strongly, not only because of their effect on emulsion stability, but because of a proper herbicidal effect occurring at the large quantities applied in NH formulations, which is similar to that of CMD ingredients.

Results field trials In trial `PG-NH`, weed control with one and two application repetitions was unsatisfactory, while three repetitions of both formulations nearly achieved sufficient weed control. It was concluded that the application intervals were too long, as weeds had resprouted and regrown nearly to their original size at the subsequent applications. Weed shoot dry mass was reduced for all spray treatments compared with control (4.7 t ha^{-1}), but even in the three application treatments about 2 t ha^{-1} weed shoot dry mass remained. As spray application was realised without the use of a protective screen, strong phytotoxicity and reduced crop growth and yield resulted. Despite of crop damage, three applications of the δ-limonene formulation gave comparable yields to the control treatment. Weed density also influenced weed and crop growth: even though not significant (n.s.), the average weed dry mass was 2.1 t ha^{-1} for the low and 3.4 t ha^{-1} for the high weed density. Also, reductions for shoot dry mass (7.0 t ha^{-1}, 6.2 t ha^{-1}) and grain yield (3.4 t ha^{-1}, 2.9 t ha^{-1}) were found for low and high weed density, respectively (both n.s.). In trial `LO-NH` weed cover was reduced strongly by all spray treatments, with concentrated applications achieving improved results. At the last evaluation 39 days after emergence (DAE), weed cover was 68 % for the weedy control, 10 % for both concentrated and 20 % for both diluted application treatments. Weed dry mass (88 DAE) was 3.5 t ha^{-1} for weedy control, 1.4 t ha^{-1} for `2xLim-C`, 1.8 t ha^{-1} for `2xPin-C`, 2.4 t ha^{-1} for `3xLim-D` and 2.0 t ha^{-1} for `3xPin-D`. Shoot dry mass (also evaluated 88 DAE) was 4.4 t ha^{-1} for clean control, 2.8 t ha^{-1} in weedy control, 4.4 t ha^{-1} in `2xLim-C`, 4.1 t ha^{-1} in `2xPin-C`, 3.5 t ha^{-1} `3xLim-D`, 3.9 t ha^{-1} `3xPin-D`. Grain yield was 3.2 t ha^{-1} for clean control, 1.5 t ha^{-1} in weedy control, 1.9 t ha^{-1} in `2xLim-C`, 2.3 t ha^{-1} in `2xPin-C`, 1.3 t ha^{-1} `3xLim-D`, 1.5 t ha^{-1} `3xPin-D`. The concentrated applications clearly showed improved weed control and crop growth. However, crop growth and yield were strongly reduced in all spray treatments compared to control. Also grain quality resulted low for all spray treatments and the weedy control, with a large proportion of green and defect grain. In `PG-Lim` spray treatments achieved sufficient weed control with weed injury ratings frequently surpassing 75. Weed dry mass (73 DAE) was 2.5 t ha^{-1} in the weedy control, 0.6 t ha^{-1} in `25-8.3 Lim-300`, 0.1 t ha^{-1} in `50-16.7 Lim-300`, 0.8 t ha^{-1} in `50-8.3 Lim-600`, 0.4 t ha^{-1} `50-5.6 Lim-900`, and 0.0 t ha^{-1} in `75-8.3 Lim-900`. Common bean shoot dry mass was only reduced in the weedy control treatment with 2.0 t ha^{-1} compared with 2.8 t ha^{-1} in the clean control. Shoot dry mass in the spray treatments was in the range of 2.2–2.7 t ha^{-1}, however, this parameter was in part affected by phytotoxicity. Grain yield was 2.7 t ha^{-1} for `Clean` and 1.5 t ha^{-1} for `Weedy` control, and spray treatments were in the range of 2.3–2.7 t ha^{-1}. In **`PG-Pin`** weed injury ratings of spray treatments also often surpassed a 75. While weed dry mass (73 DAE) in **`PG-Pin`** weedy control was 3.7 t ha^{-1}, the best performing spray treatment achieved near perfect weed control with only 0.2 t ha^{-1} remaining weed shoot dry mass. All treatments exhibited a similar common bean shoot dry mass, with 3.2 t ha^{-1} for the clean control and 3.1 t ha^{-1} for the weedy control, which indicated a relatively low initial weed pressure in this trial. However, a strong yield reduction was present for `Weedy` control with 1.8 t ha^{-1} compared with 2.8 t ha^{-1} for the `Clean` control. Spray treatments had very similar yields compared with `Clean` control in a range of 2.5–2.7 t ha^{-1}. The intra-row application of NaCl in **`PG-NaCl`** did not have any negative effect on crop growth or yield even with three applications, totalling 150 kg ha^{-1} NaCl.

General conclusions NH trials In screening and field trials it was observed that most dicots could be controlled well until about the 4-leaf stage. Monocots were less sensitive. When grasses started to form tillers they were almost impossible to control, therefore application should occur up to 2-leaf stage. With CT common bean it was found that at least two applications were necessary to control weeds. In DS soybean rather three applications were necessary, due to higher weed growth rates in the summer months. It was concluded that subsequent applications should occur when the weeds have started to resprout, and lateral bud meristems form enough area to intercept spray. Approximate recommended intervals for soybean in tropical summer conditions are 3–5 days, and in late summer season common bean crop about 7 days. Furthermore, the results suggest that in order to maximise the herbicidal effect the application of NH formulations containing cell membrane disruptors (CMD) and NaCl should preferably occur midday, when temperatures and irradiation are highest.

10 Outlook: natural herbicides in OA direct-seeding

In order to realise (occasional) direct-seeding successfully in OA, a dense mulch cover is a prerequisite in order to suppress weeds before crop emergence and during early crop development. High cutting of straw at harvest followed by a treatment with a roller-crimper, tested in the work at hand, is not a feasible strategy as it decreases mulch soil coverage, and because it presumably reduces the release of allelopathic substance by the straw. In order to guarantee an equal distribution and maximum soil coverage, the cereal pre-crop should be cut low and chipped at harvest. The biomass of allelopathic oats or rye straw should exceed 4 t ha^{-1}. As weed control by mulches of annual weeds in tropical climate and of perennial weeds in temperate climate is insufficient, the use of NH is thought to be indispensable for the implementation of successful Organic Agriculture Direct Seeding (OADS). NH containing cell membrane disruptors and NaCl could be the key to enable DS in OA. However, weed control is only sufficient if applied at early weed growth stages and if subsequent applications are timed correctly. Currently, the greatest problem are the highly elevated costs of NH formulations and their often unsatisfactory weed control, especially with respect to monocot species.

10.1 Improvement of formulations

NH formulations need to be improved and development should focus on non-selective total herbicides. These need be the most phytotoxic possible, while maintaining costs, human toxicity, and environmental risks at minimum. The primary scenario for the application NH is assumed to be in DS row crops, such as grain legumes or maize, but products could also be applied in all other DS field crops prior to crop emergence. To decrease costs and improve NH efficiency, expensive ingredients need to be substituted by cheaper ones. In order to further improve efficacy of NH, other phytotoxic mineral salts or AI with systemic effects could be introduced to formulations.

10.1.1 Substitution of cell membrane disruptors

For the penetration of leaf cuticle and cell membranes CMD are assumed to be indispensable in NH formulations. Emulsifiers by their effect are also CMD ingredients and could be another AI option for NH formulations: in screenings formulations containing only T80, SA, or NPE, in combination with NaCl gave moderate to acceptable results at a competitive cost. However, these are not of natural origin. Emulsifiers of natural origin could be soaps made from vegetable oils. Nevertheless, a common problem with these ionic emulsifiers is that flocculation may occur when adding salts to the formulation. Allowing synthetic emulsifiers made of natural primary materials could be a reasonable exception for synthetic substances in NH certification. For example, polyethylene glycol (PEG) emulsifiers are a reaction product of sugar (or starch) and vegetable oil. They are proven to possess a low toxicity (Messinger et al., 2007), which is why they are widely used in cosmetics and food industry. Another toxicological safe synthetic substitute for the criticised nonylphenol ethoxylate (NPE) emulsifier is Tween 80®, a synthetic emulsifier used in food industry, which gave acceptable results in screenings.

It may also be possible to substitute expensive CMD by cheaper crude vegetable oils, which have can have a weed control effect, presumably due their content of phytotoxic free fatty acids. Furthermore, biodiesel could be an AI in environmentally friendly contact herbicides, even though strictly speaking, biodiesel cannot fully be considered natural, as a simple transesterification reaction is involved in production (saponification of vegetable oil followed by esterification of the resulting fatty acids with methanol). Another potential candidate for replacement of CMD is fusel oil, a byproduct of ethanol fuel production from sugarcane, which has a similar mode of action. It is available in large quantities and at a low cost in Brazil. Doses of 375 L ha^{-1} and 500 L ha^{-1} fusel oil resulted in fast wilting followed by yellowing and drying of weed plants. The species *Ipomoea hederifolia*, *Ipomoea quamoclit*, *Euphorbia heterophylla*, *Digitaria spp.*, *Cenchrus echinatus*, and *Panicum maximum* were controlled effectively by fusel oil, when treated early after emergence (Azania et al., 2010, 2011). In another work *efficient* (grade B) control of monocots *Digitaria insularis* and *Commelina benghalensis* was achieved with rates of 600–800 L ha^{-1} (Osipe et al., 2009). Promising about fusel oil is its low cost and that in contrast to CMD and NaCl formulations, an efficient control of some monocot species is apparently possible.

10.1.2 Phytotoxic salts

Combining CMD with NaCl was the most successful measure to improve formulation efficacy, and to decrease NH application costs in this work. Further mineral salts with a predictive behaviour in the environment should be examined for their weed control potential in combination with CMD. Chloride is responsible for most of NaCl toxicity, and at an equivalent chloride rate KCl application resulted in similar weed damage as NaCl Lukashyk (2005). Therefore, further chlorides such as $MgCl_2 \cdot 6\,H_{20}$ or $CaCl_2$ could also possess potential as AI in combination with CMD. Another phytotoxic mineral salt for use in NH formulations might be iron-vitriol ($FeSO_4$). It has already been used as a herbicide in the mid 19th century (Smith and Secoy, 1976; Timmons, 2005), and is still applied today as a moss herbicide or in lawn fertilisers. With a relatively high solubility of $256\,g\,L^{-1}$, anhydrous $FeSO_4$ could be spray applied at rates up to $100\,kg\,ha^{-1}$ in NH formulations with relatively low costs: the price of $FeSO_4$ is currently in a range from 55–140 € per tonne. The substance exhibits acute toxicity to humans upon skin contact (skin resorption, caustic action), contact with mucus tissues, or upon swallowing. Nevertheless, the environmental profile is ideal and with careful handling it could be another AI option for NH formulations.

10.1.3 Active ingredients with systemic effect

As CMD enhance the permeability of the leaf cuticle, this port of entry should also be studied for allelopathic extracts or systemic AI. One option is manuka oil, the essential oil from the manuka tree *Leptospermum scoparium*, which shows pre- and post- herbicidal activity. It was successfully tested in a mixture with a commercial lemongrass oil herbicide (Dayan et al., 2011). In trials, monocotyledonous weeds exhibited lower chlorophyll and carotenoid contents in regrown leaf tissue as well as stunted growth, apart from burn-down symptoms (Dayan et al., 2011). The β-triketones present in manuka oil have the same molecular target site as the mainstream synthetic herbicides AI *sulcotrione* and *mesotrione*, namely the enzyme *p-hydroxyphenylpyruvate dioxygenase* (Dayan et al., 2007). Compared with all other essential oils, the herbicidal activity of manuka oil is highest, and application volumes are relatively small with $0.5\text{–}3\,L\,ha^{-1}$. With a systemic action and a pronounced effect on monocot weeds, manuka oil appears to be one of the most promising options to improve NH formulations or to base them upon this oil.

A further possibility could be combining CMD with bialaphos [24], a tripeptide produced by *Streptomyces viridichromogenes & hygroscopicus* bacteria in soil. The substance is a pre-herbicide, which is hydrolysed to 2-Alanin and L-phosphinotricin inside plant cells. Glufosinate, a racemate of L- and D-phosphinothricin, is its synthetic analogue, and a mainstream herbicide commercialised under the name Basta® or Liberty® (Copping and Duke, 2007; Duke and Dayan, 2011). The effects of bialaphos and glufosinate are identical, but the formulated product exhibits a weaker effect on weeds than glufosinate based products. The substance is the only natural systemic AI that has ever been employed in a commercial product (Dayan et al., 2009). It was marketed as Herbiace® in Japan (*Meiji Seika*), yet the registration expired around 2010 and was not renewed. The market price per application was around 180 € (Information *Meiji Seika*), which, compared to current NH prices, can be considered low-priced. Bialaphos fulfils most criteria for an AI in NH: it is of natural origin and can be produced in biofermenters (Satoh et al., 1993). Furthermore, its toxicological profile is acceptable, and the overall environmental profile appears to be positive. No long term effects such as carcinogenicity, teratogenicity, and mutagenicity were found in studies with its synthetic analogue glufosinate ammonium (Ebert et al., 1990), and suspected reproductive toxicity of glufosinate has recently be refuted (Schulte-Hermann et al., 2006). Even though there is a notable toxicity of glufosinate on aquatic organisms (PAN Pesticides Database - Chemicals), glufosinate is rarely found in the environment (Scribner et al., 2007), due to its fast decomposition. Therefore, glufosinate possesses a limited potential to pollute surface waters, which is assumed to be similar for bialaphos. Apparently, bialaphos use in OA has never been considered. It should be tested and reviewed for certification, alone and in formulation with other AI mentioned in this work.

Further substances with a systemic herbicidal action, that could be tested in CMD based formulations,

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[24] Bialaphos or bilanafos: tripeptide, which within the plant is hydrolysed to 2-Alanin and L-phosphinotricin

are allelopathic extracts such as *artemisinin* (from *Artemisia annua*), or *parthenin* (from *Parthenium hysterophorus*) (Chen et al., 1991; Belz et al., 2007).

10.2 Potentials of natural herbicide use in temperate climate

The effects of NH were not studied under temperate conditions, but in principle it should be possible to use NH successfully in temperate climate row crops such as faba bean. Results of this work suggest that weather conditions have a strong influence on weed control efficacy: CMD were found to be more effective with high temperatures and irradiation, as the osmotic stress of NaCl containing formulations is particularly high under these conditions. Considering the lower temperatures in temperate climate, efficacy may be weaker. On the other hand, late season common bean trials higher weed injury ratings were determined than soybean trials in mid summer, despite lower temperatures, which was thought to be related to the thinner cuticles formed in the late summer season. For temperate climate, it can also be assumed that relatively thin cuticles are formed compared with tropical conditions, which might allow efficient weed control with NH products. Another argument to consider is that rainfall events in tropical southern Brazil are mostly concentrated in late afternoon (thunder-) storms. Longer rain periods, typical for the maritime temperate climate of Western Germany, are a rare occurrence. Due to the favourable weather conditions, application in Brazil can usually be timed precisely. Nevertheless, high weed growth rates result in relatively short intervals in which application can be successful. Hence, careful attention is required in order to avoid that weeds surpass the growth stage, which is ideal for application. Prolonged rain periods would difficult an exact application timing in temperate climate, nonetheless, weed growth rates are also lower in these conditions and the intervals ideal for NH application are hence longer.

10.3 Spraying equipment and precision farming

The use of natural total herbicides is projected to be limited to row crops. To become relevant in practice, a working protective screen and specialised spraying equipment needs to be developed for mechanised application. Most CMD and NaCl are capable of damaging conventional spraying equipment. The aggressive AI commonly used in NH formulations (NaCl, essential oils, emulsifiers) deteriorate rubber sealings and corrode metal parts. In consequence, the equipment needs to be resistant to these AI, which requires a modification of the material of sealings and metal parts. If economically viable and certifiable NH formulations can be found, in a next step application volumes could be reduced by the use of camera guided precision sprayers (Oebel and Gerhards, 2006). However, the author of the current work does not believe in a potential for this technology under Brazilian DS conditions. Even at low weed densities the distribution of weeds observed in trials was fairly even, and estimated spray reduction (visual estimation realised in trial `LO-NH`) was not surpassing 20 %. Additionally, a risk arising from selective NH application is suspected: when emerging from the straw layer, weeds are highly susceptible to NH, and could be controlled with relative ease. At this stage, weeds may be partially covered by straw and therefore overlooked by sensors, while a non selective application in this situation may result in successful control. If not detected and sprayed at an early stage, the weed may already have grown too large by the next NH application to be combated effectively.

The potential of camera guided precision sprayers is rather seen for temperate climate OA. If a dense mulch layer (e.g. oats) is present, annual weeds can be controlled effectively, but perennial weeds are limiting for the realisation of OADS (Köpke and Schulte, 2008; Massucati, 2013). Perennial Weeds like *Rumex sp.* and *Cirsium arvense* are distributed unevenly and form patches in fields. In this case the potential of sensors, which detect different weed species and which are able to direct NH spray to problematic perennial species, may have a great potential to reduce NH amounts.

10.4 Certification

Apart from practical aspects of application, the certification of products for OA is considered a major hurdle. For OA certification as well as for acceptance by the public and OA practitioners, ingredients in NH formulations should ideally be of natural origin, possess a positive environmental and toxicological profile, and provide effective weed control at a competitive cost. AI such as citronella, thyme or clove oil are of natural origin, but are too expensive. Despite their presence in nature, pine oil is an AI that is usually semi-synthetic (derived from oxidation of α-pinene), and PA is always, and AA usually synthesised, and therefore these effective ingredients do not fulfil the above mentioned criteria. To avoid acceptance and certification problems, research has to direct towards screening of effective CMD of natural origin.

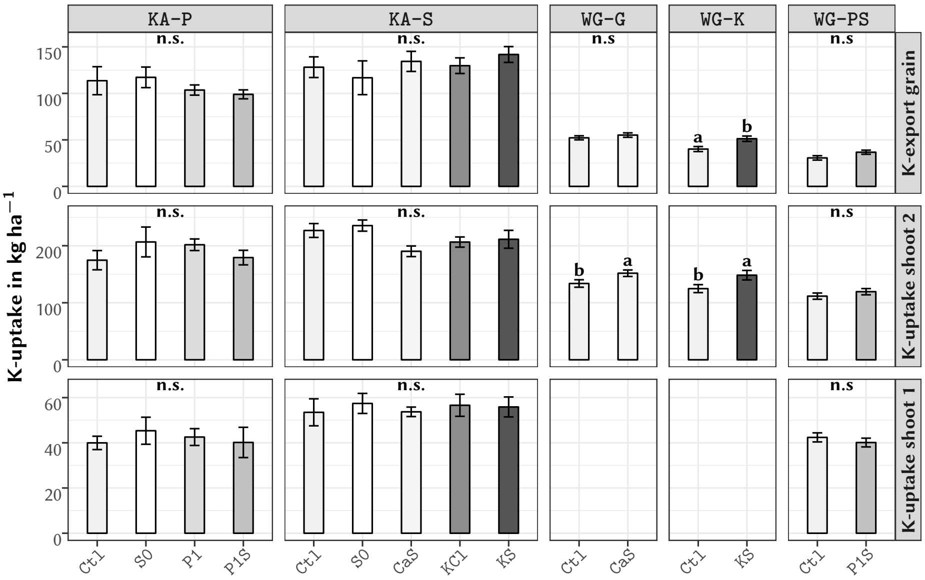
Currently, certified natural herbicides contain some of the above mentioned (semi-) synthetic active ingredients, but even a greater problem is that they contain large quantities of synthetic emulsifiers, which do not need disclosure. Some of the emulsifiers permitted by Organic Materials Review Institute (OMRI) for OA certification are harmful to the environment or toxic. Hence, these certified products have to be revised, if they really are more environmentally friendly than some of the mainstream herbicides. It appears likely, that in some cases product certifications have to be revoked.

An important measure to increase acceptance and transparency would be to make disclosure of the type of 'inert' ingredients in NH formulations mandatory for OA certification. This would allow for independent toxicity studies with all ingredients used in the formulated product, and not only with the ingredients considered active. Research has to be directed towards the screening of CMD and emulsifiers of natural origin. If effective natural emulsifiers cannot be found, sensible exceptions in certification guidelines may have to be made, but only after careful revision of the synthetic emulsifiers for their predictable behaviour in the environment and low toxicity. Certainly, for the assessment of NH for OA certification an overall balance of benefits of the DS system and the necessity of its realisation at erosion prone sites has to be considered.

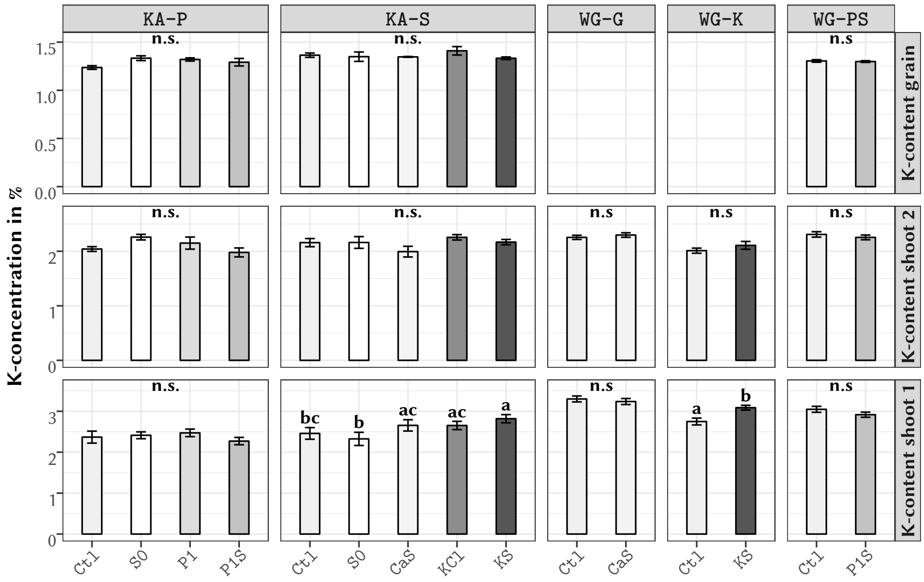
Appendix

A Faba bean trials (Germany)

Fertiliser effect on K-uptake and K-concentration



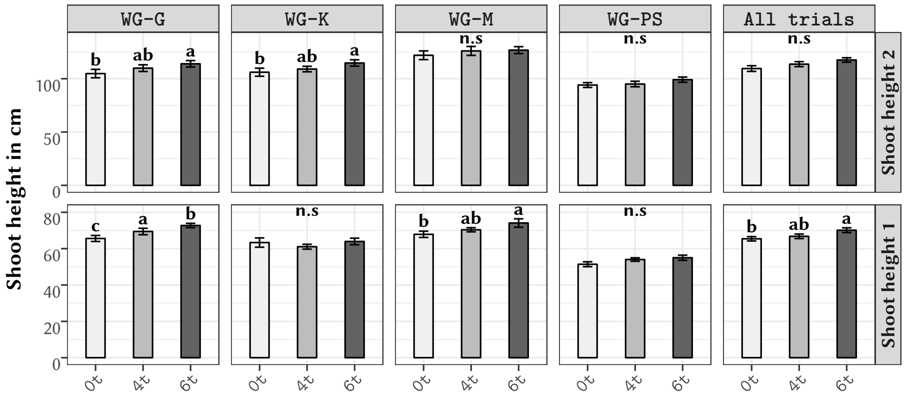
(a) K-uptake into faba bean shoot and grain



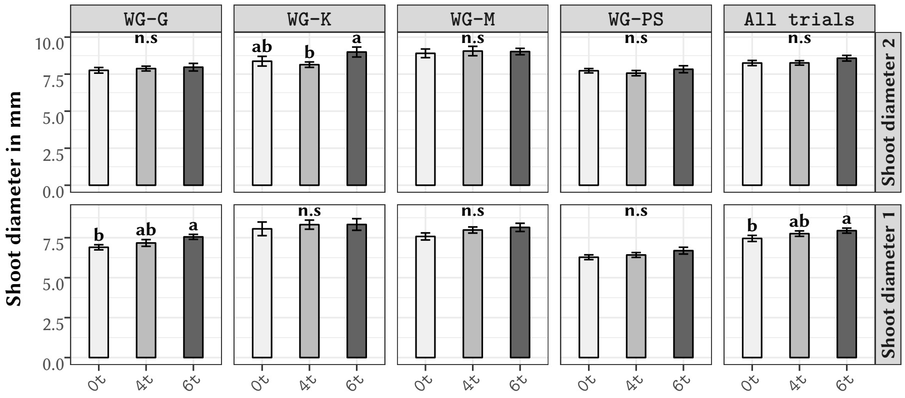
(b) K-concentration in faba bean shoot and grain

Figure A.1: Potassium concentrations and -uptake in shoot (BBCH 65 and BBCH 77) and grain. Treatments were `Ctl`: non-fertilised control, `S0`: elementary sulphur (40 kg ha^{-1} S), `P1`: Gafsa rock phosphate (50 kg ha^{-1} P), `P1S`: joint application of `S0` and `P1`, `CaS`: gypsum ($CaSO_4 \cdot 2\,H_2O$, 40 kg ha^{-1} S) and `KS`: K_2SO_4 (40 kg ha^{-1} S). Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

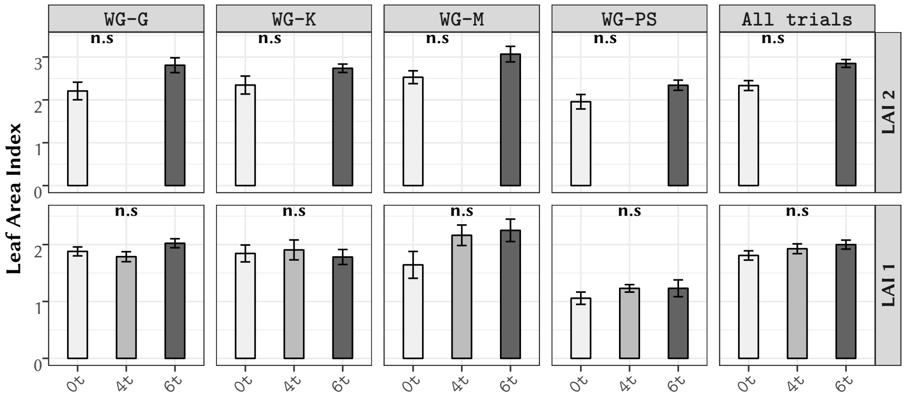
Oats straw effect on shoot height, diameter and LAI



(a) `WG`: Faba bean shoot height



(b) `WG`: Faba bean shoot diameter



(c) `WG`: Faba bean LAI

Figure A.3: Effect of oats straw mulch biomass on faba bean shoot height and diameter as well as leaf area index at site `WG`, evaluated 57 and 89 DAE (BBCH 65 and BBCH 77, respectively). Treatments: `0t`: no straw addition, `4t`: 4 t ha^{-1} oats straw, `6t`: 6 t ha^{-1} oats straw. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Oats straw effect on harvest components

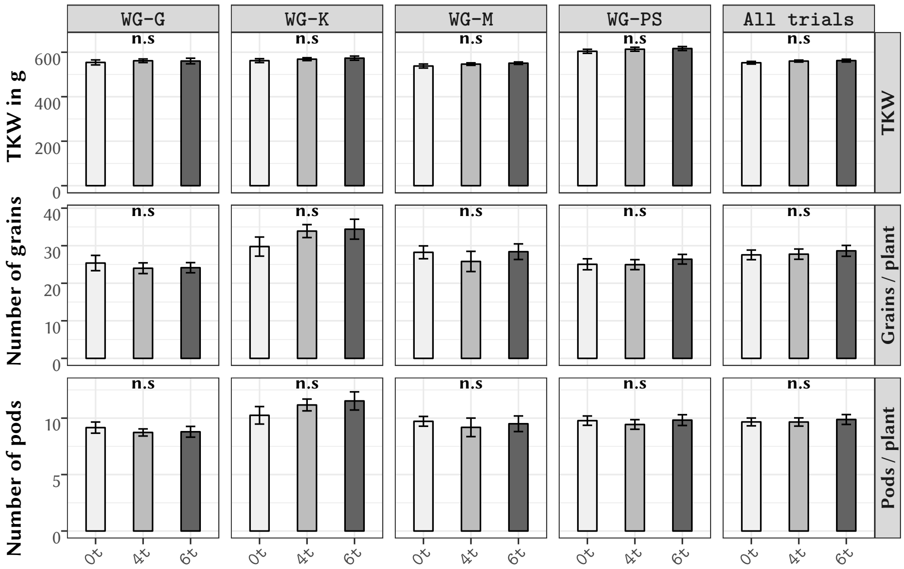
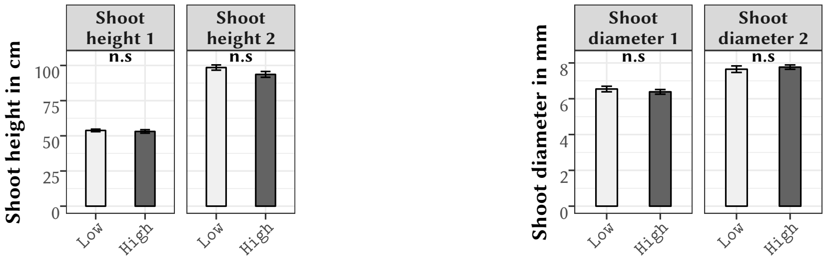


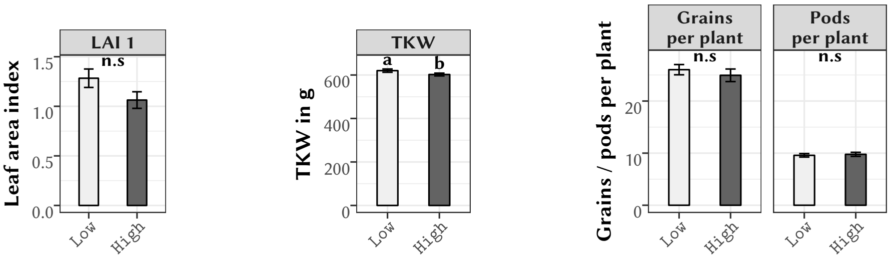
Figure A.5: Effect of oats straw mulch biomass on faba bean shoot height and diameter at site `WG`, evaluated 57 and 89 DAE (BBCH 65 and BBCH 77, respectively). For treatment abbreviations refer to figure 3.12. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Oats straw cutting height



(a) Faba bean shoot height

(b) Faba bean shoot diameter



(c) Faba bean leaf area index

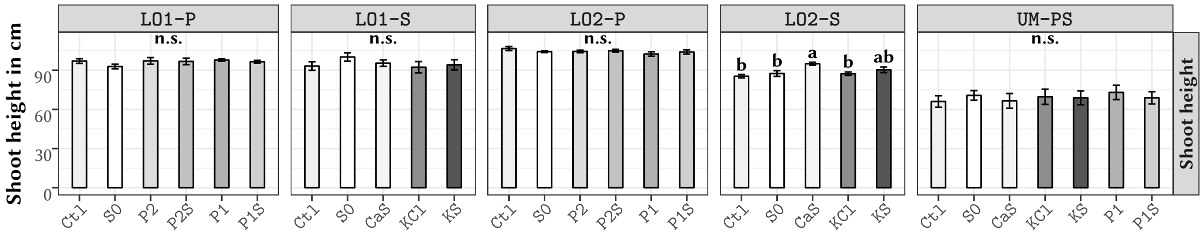
(d) Faba bean TKW

(e) Faba bean grains and pods per plant

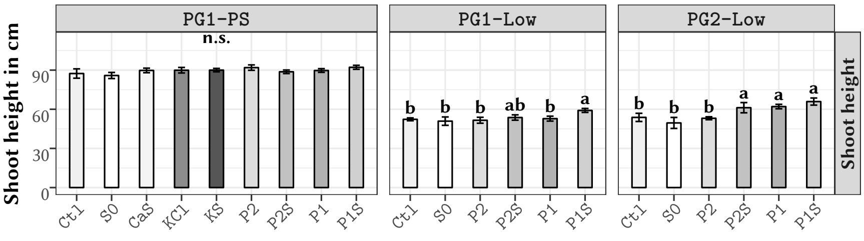
Figure A.6: Effect of oats cutting height on faba bean shoot height and diameter at site `WG`, evaluated 57 and 89 DAE (BBCH 65 and BBCH 77, respectively). Treatments were `Low`: low cut at oats harvest at the base of the plant, and `High`: high cut at oats harvest beneath the panicle. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

B Soybean fertiliser trials (Brazil)

Brazil fertiliser trials: shoot height



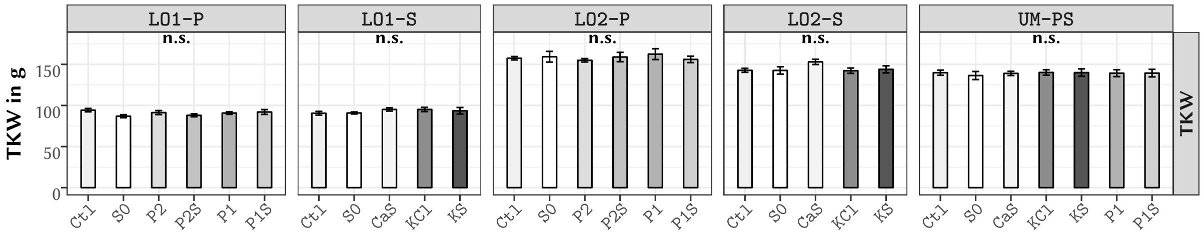
(a) Londrina & Umuarama



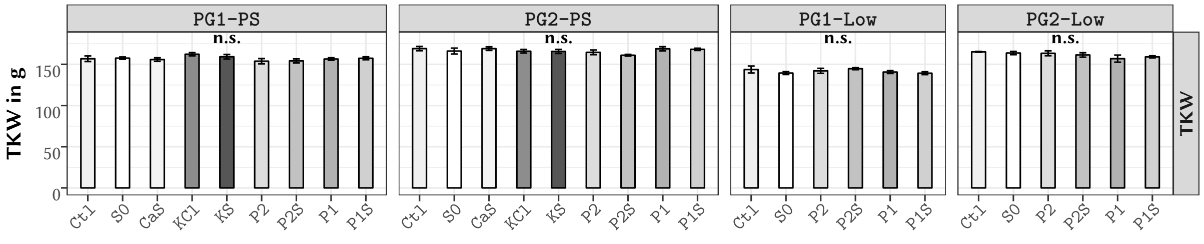
(b) Ponta Grossa

Figure B.1: Shoot height measured at shoot drymass evaluation of all Brazilian fertiliser trials. Treatments were `Ctl`: non-fertilised control, `S0`: elementary sulphur (40 kg ha^{-1} S), `P1`: *Gafsa* rock phosphate (50 kg ha^{-1} P), `P2`: *Alvorada* rock phosphate (50 kg ha^{-1} P), `P1S`: joint application of `S0` and `P1`, `P2S`: joint application of `S0` and `P2`, `CaS`: gypsum ($CaSO_4 \cdot 2\,H_2O$, 40 kg ha^{-1} S) and `KS`: K_2SO_4 (40 kg ha^{-1} S). Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Brazil fertiliser trials: thousand kernel weight



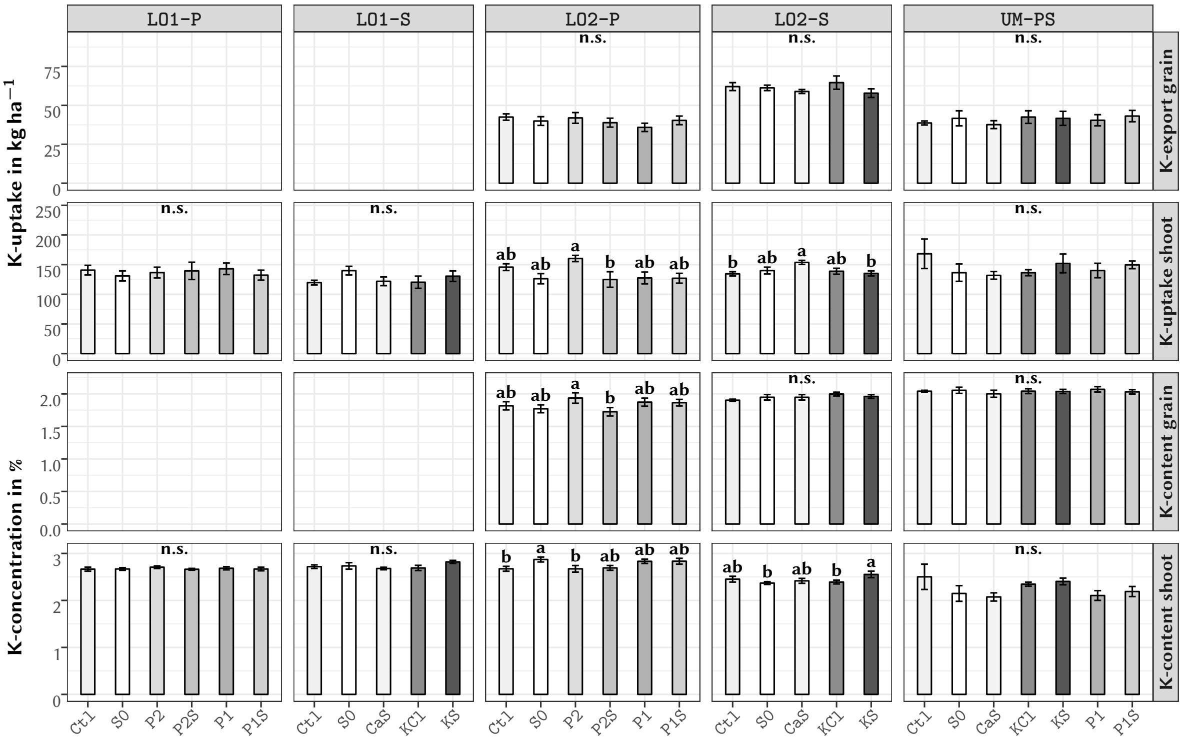
(a) Londrina & Umuarama



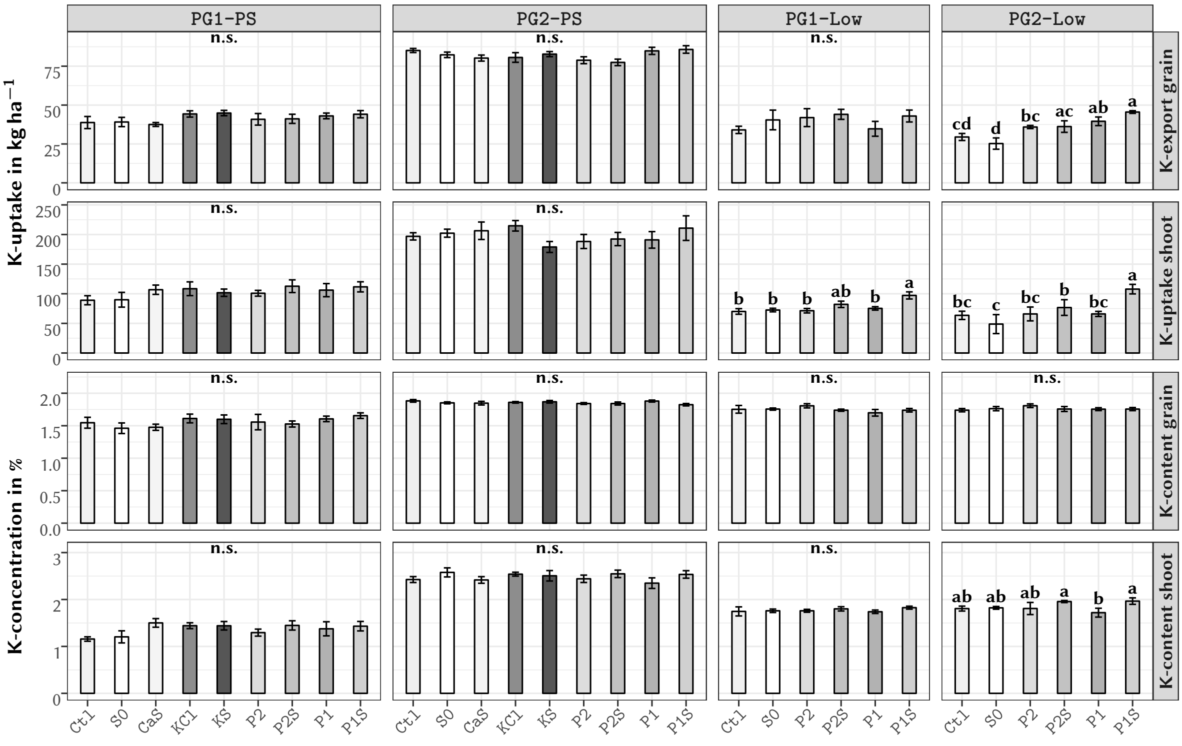
(b) Ponta Grossa

Figure B.3: Thousand kernel weight of all Brazilian fertiliser trials. The low weights in the first year in Londrina were due to dying off of soybean during the grain filling stage. Grain weight is presented with 13 % humidity. For treatment abbreviations refer to figure B.1 on page 130. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Brazil fertiliser trials: shoot and grain K-uptake and -concentration



(a) Londrina & Umuarama



(b) Ponta Grossa

Figure B.5: K-uptake and -concentration in shoot and grain at trial sites in *Londrina* (`LO`), *Ponta Grossa* (`PG`) and *Umuarama* (`UM`). Shoot values were determined 73 days after emergence (`LO1`), 71 DAE (`LO2-P`), 74 DAE (`LO2-S`), 78 DAE (`UM-PS`), 82 DAE (`PG1-PS`) and 85 DAE (`PG2-PS`). `LO1-P` grain concentration was not determined due to stand dying off at early grain filling stage 101 DAE. For treatment abbreviations refer to figure B.1 on page 130. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Londrina 2012, first evaluation: shoot dry mass, height and diameter

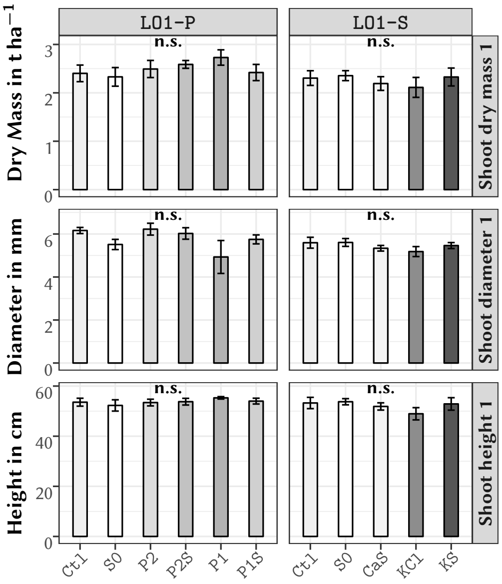


Figure B.7: Shoot drymmass, shoot height and shoot diameter of `L01` trials at the first shoot dry mass evaluation at 53 DAE. For treatment abbreviations refer to figure B.1 on page 130. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Londrina 2012, first evaluation: nutrient uptake

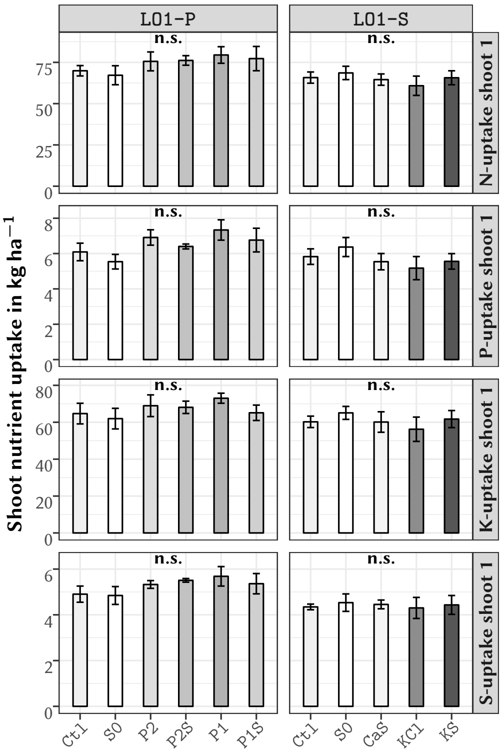


Figure B.8: Soybean shoot nutrient uptake in `L01` trials, determined at the first shoot dry mass evaluation at growth stage BBCH 72 / R2. For treatment abbreviations refer to figure B.1 on page 130. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Londrina 2012: yield components

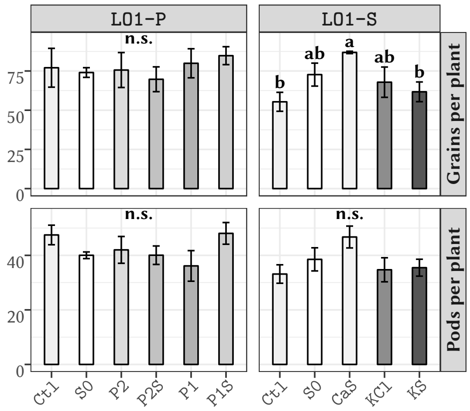


Figure B.9: Effect of fertiliser treatments on the yield components grains per plant and pods per plant of `L01` trials. For treatment abbreviations refer to figure B.1 on page 130. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

Ponta Grossa 2012: LAI and yield components

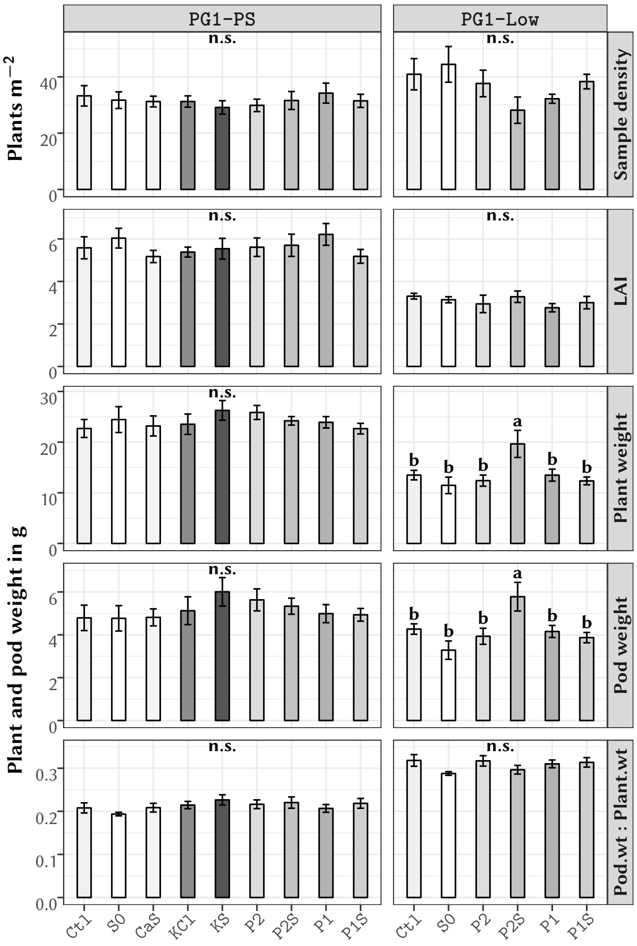


Figure B.10: Effect of fertiliser treatments on leaf area index (LAI), weight per plant, weight of pods per plant and the ratio of pod to whole-plant weight, determined in `PG1` trials. The plant number of the area sampled in each plot was determined at cutting. Of a subsample of ten plants from each plot the parameters presented in this plot were determined. Apart from LAI and the pod : plant weight ratio, all other parameters in `PG1-Low` correlate strongly with the crop density in the sampled area ($r = -0.738$ and $R^2 = 0.54$ for plant weight and $r = -0.71$ and $R^2 = 0.50$ for pod weight). Therefore, the significant treatment effects were rather due to a heterogeneous stand than to treatment effects. For treatment abbreviations refer to figure B.1 on page 130. Error bars: SE. *n.s.*: not significant. Different letters denote significant differences (TukeyHSD, $\alpha < 0.05$).

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This thesis, containing 52 figures, 15 tables, and 46 boxes, was typeset with LaTeX using Linux Libertine serif and sans-serif as well as Computer Modern typewriter type faces. Walter & Lieth climatic diagrams were created with R package `iki.dataclim` and all other diagrams with the `ggplot2` plotting system for R.