Dynamics of Glyphosate in the Rhizosphere: A Possible Threat to Crop Plants?

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Overview

- Introduction/Background
- Relevant knowledge on glyphosate
- Waiting times: An important issue?
- The Rhizosphere: Place for possible glyphosate toxicity
- Roots of target plants: Key players in stabilization and toxicity
- Conclusions / Prospects

Symposium on Mineral Nutrition and Disease Problems in Modern Agriculture: Threats to Sustainability?.
Coplacana, Piracicaba, Brazil, 20.-21.Sept. 2007

Universität Hohenheim
University Hohenheim
(founded 1818)

Institut für Pflanzenernährung
Institute of Plant Nutrition
(founded 1923 for Prof. Margarethe v.Wrangell)

(1876 - 1932)

Main research interest:

Role of rhizosphere processes in P acquisition of P-efficient plant species

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A Possible Threat to Crop Plants?

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Introduction/Background

Glyphosate:

- Worldwide the most widely used herbicide (Trade name „Roundup).
- Non-selective, inhibits synthesis of aromatic amino acids via the shikimate pathway.
- Efficient and cheap – low production costs
- General claimed (e.g. by Monsanto) :
  - rapid microbial degradation and / or binding to the soil (= detoxification)
  - no residual effects in soils
  - no negative environmental effects

However, recent observations suggest significant side effects on non-target organisms!!

Observed interactions between glyphosate and crop plants

- Partial desiccation of cover crops after wheat by accidental double application of glyphosate (4L/ha glyphosate) before sowing of cover crops

(Farm near Tübingen, Germany 2006)

- <u>Enhanced drought stress after glyphosate applications</u> (see:glyphosate case between cotton growers in Texas and Monsanto)

  due to strongly inhibited root growth or

  to impeded nutrient acquisition (Mn, Zn, Fe, K) and

  thus due to more heat stress problems.

- Drought stress partially linked with enhanced root diseases

Drought spells in sugar cane due to take-all (Sao Paulo State, 2004)

Greening effect of `Weather Max´, a new formulation of glyphosate by Monsanto, as observed by Myriam Fernandez in the field: a positive effect of glyphosate?

- By `Weather Max´ Myriam observed a better performance of the following crop (taller plants, more green, delayed senescence, higher plant N and lower S concentration)
- (Possible explanation: Release of soluble N from microbial biomass after short-term killing of soil microorganisms by glyphosate; immobilization of S by the flush of easily plant available N). No long-term positive effect for sustainability!?

Observed interactions between glyphosate and micronutrients

In the USA with a high percentage of RR (Roundup-resistant)-crops, there are increasing reports on:

- micronutrient deficiencies induced by glyphosate
- increase in demand for micronutrient foliar fertilizers

(Jurin, 2004; Brown, 2005)

Glyphosate-induced Mn deficiency in soybeans on a low- Mn soil (D. Huber)

Interaction of seed applied Fe and glyphosate application on Fe deficiency chlorosis in soybeans; Minnesota, USA *(Jolley et al., Soil Sci Plant Nutr. 50, 793-981, 2004)*

Treatment	visual chlorosis scrore (1=green to 5 =severe)		grain yield (t/ha)	
	– Fe	+ Fe*	– Fe	+ Fe*
Control (no herbicide)	3.1	2.8	1.01	1.70
Glyphosate	3.7	3.3	0.27	0.61

* 50g Fe/ha as FeEDDHA applied to seeds

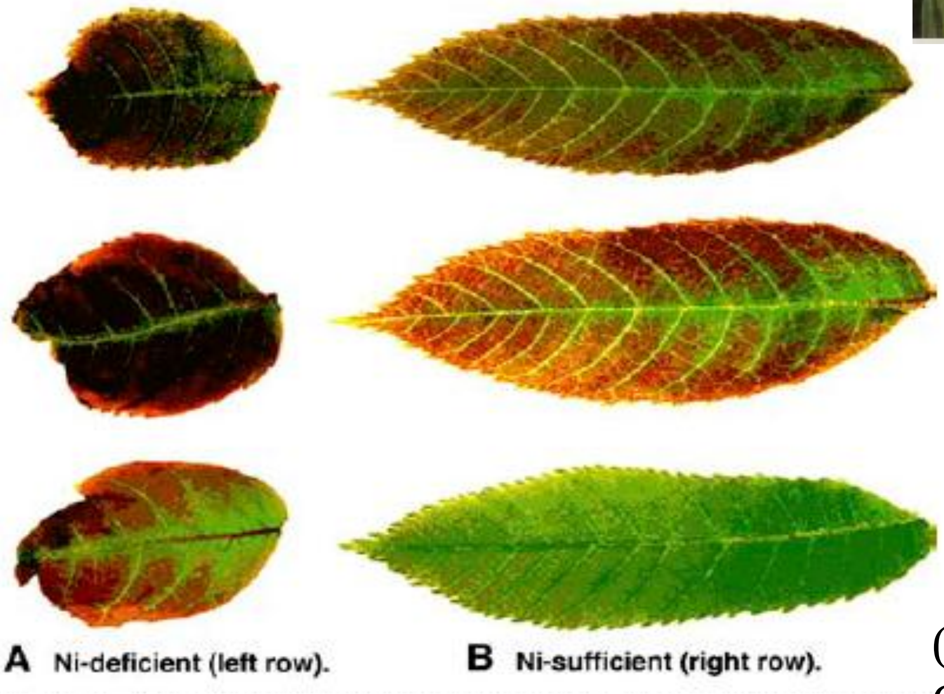
Ni deficiency in pecan trees:

glyphosate-induced similar to Mn- and Fe-deficiency as assumed by Yamada?

-via strongly inhibited root growth by glyphosate,

- via inhibited micronutrient acquisition and thus susceptibility to heat stress,

(- besides high Zn-induced Ni deficiency).

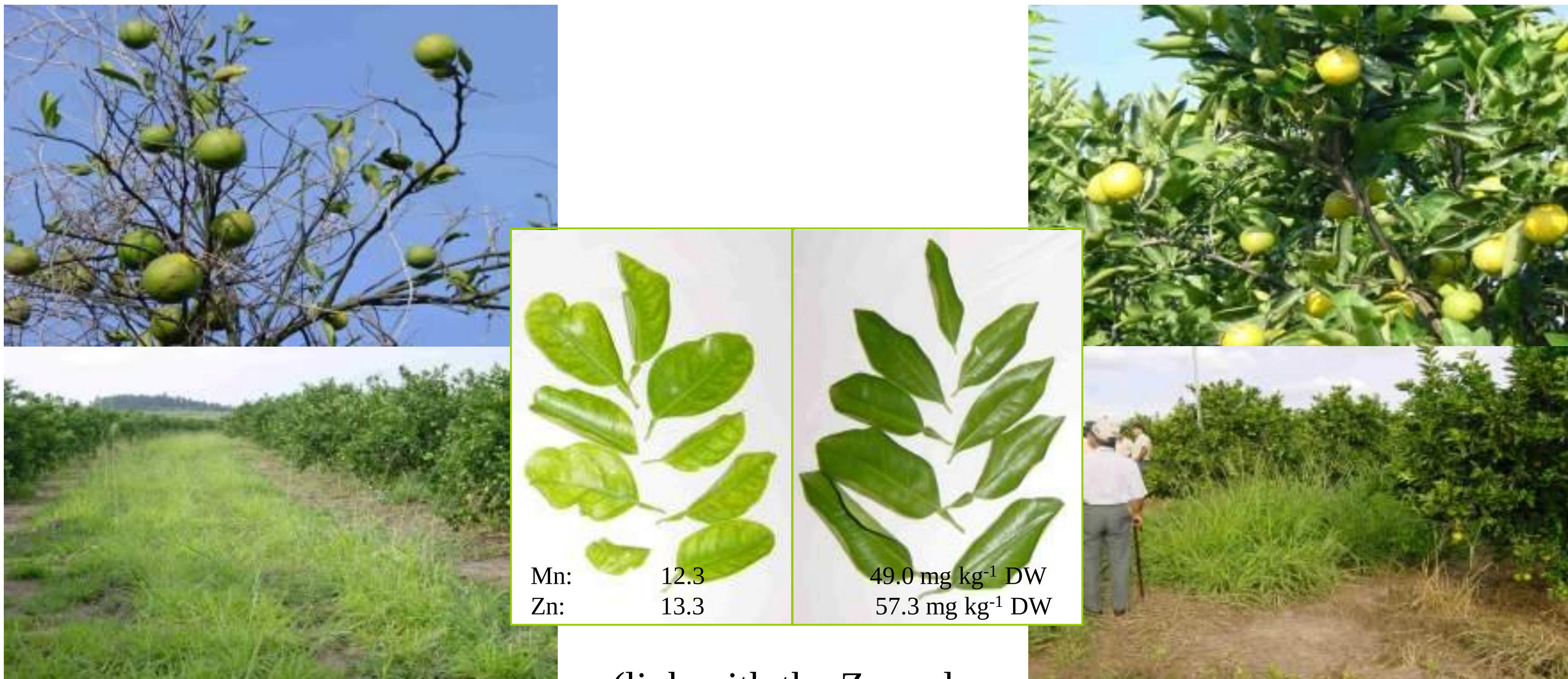


Mouse ear symptoms

(Wood et al. 2003;
Chen Bai et al. 2006)

<u>Observed interactions between glyphosate and diseases</u>

The <u>dieback syndrome (C.V.C.)</u> is particularly expressed in traditional production systems with a high application rate of the herbicide Roundup (Glyphosate), but less in biological production systems with *Brachiaria* mulch for weed control.



(traditional system)

use of Roundup

(link with the Zn and Mn nutritional status)

(biological system)

mulching, no herbicide

<u>High incidence level of *Fusarium* Head Blight (FHB) in wheat in Saskatchewan, Canada</u>

“Risk Production Factors” associated with FHB:

Environment (rainfall, temperature)

Crop Production Factors-

** Roundup applied 18-36 months prior to wheat planting had the <u>most consistent</u> relationship to FHB development across all years studied.

Fernandez et al., 2005; Crop Sci. <u>45</u>, 1908-1916

A wide range of observations believed due to glyphosate applications: How can they all induced by glyphosate or explained?

- What do we know on glyphosate for understanding these various before mentioned observations in fields?

  In discussions with various representatives of Monsanto (e.g. Brazil, Europe, St Louis USA)

  no links between these mentioned observations and glyphosate use!

  Safety, always and everywhere!

- However: What have we to know on glyphosate for a better understanding and possible counteraction against these observed negative effects by management?

- Need for a more integrative or holistic view!

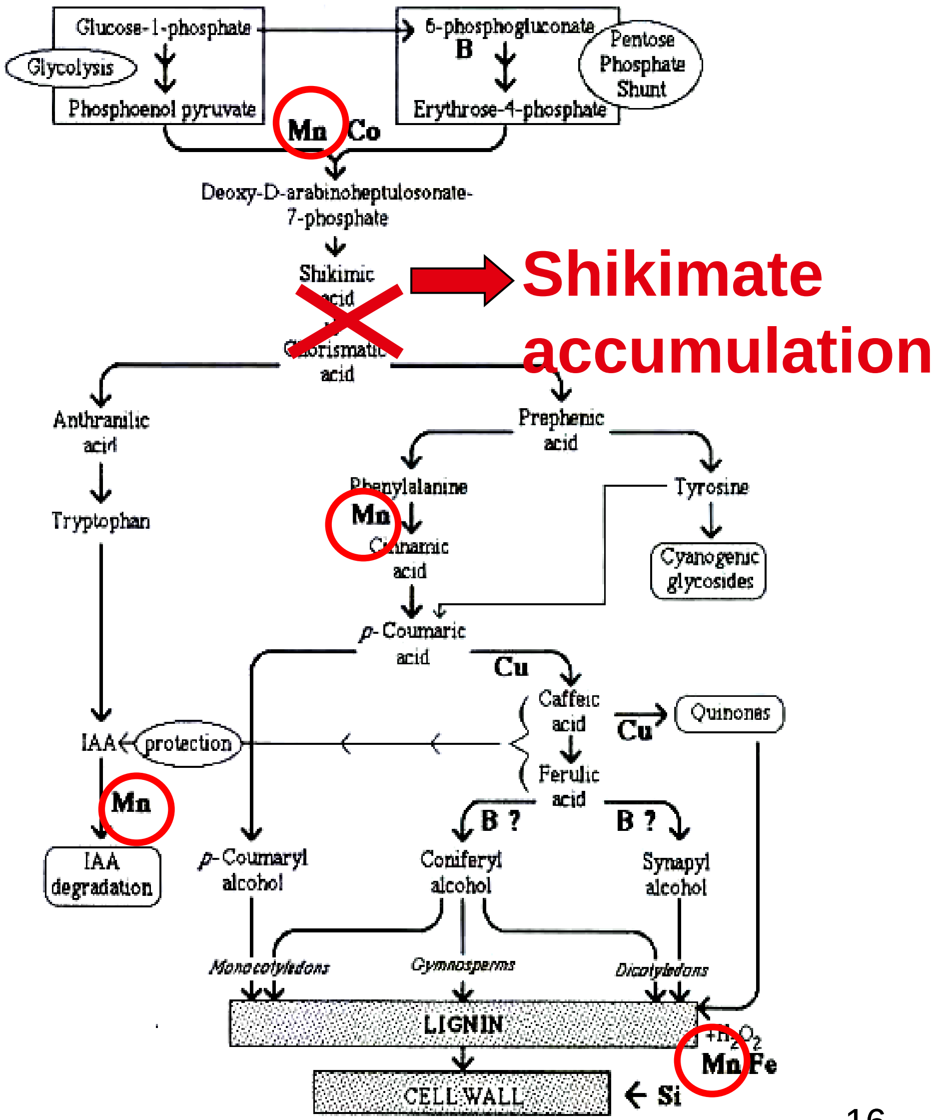
Relevant knowledge on glyphosate

Glyphosate is a non-selective, systemic, phloem-mobile inhibitor of the enzyme EPSPs, disrupting the shikimate pathway for biosynthesis of essential aromatic amino acids such as tryptophan, phenylalanine and tyrosine.

In plants, glyphosate is quite stable, with little detectable degradation occurring over long periods and tends to accumulate in the meristematic regions.

Source: Gruys & Sikorki, (1999).

Shikimate pathway



Relevant knowledge on glyphosate

Strong fixation to soil = immobilization = detoxification

(possible re-mobilization as a phosphoric compound?)

Inhibition of the shikimate pathway (see presentation before!)

Preferential transport within target plants to apical tissue (e.g. root tips)

Release into the rhizosphere (*scheme of overlapping rhizosphere of a target and non-target plant root*!)

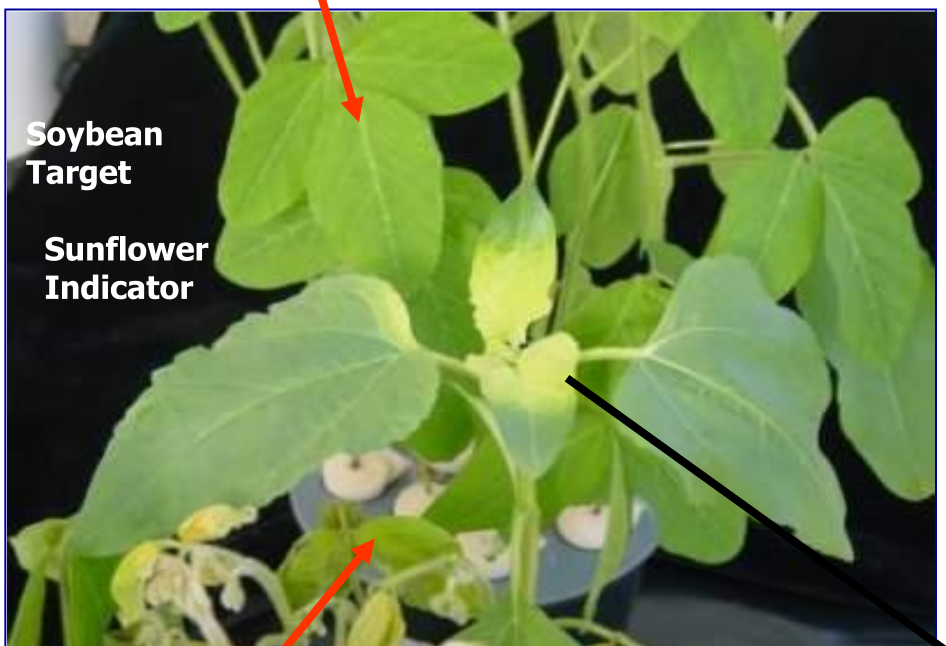
and what is then? What is the mechanism of this release into the rhizosphere and how fast is this release depending on which factors?

(important questions which are not seriously adressed by Monsanto or even by S. O. Duke as a well-known herbologist from USDA, USA)

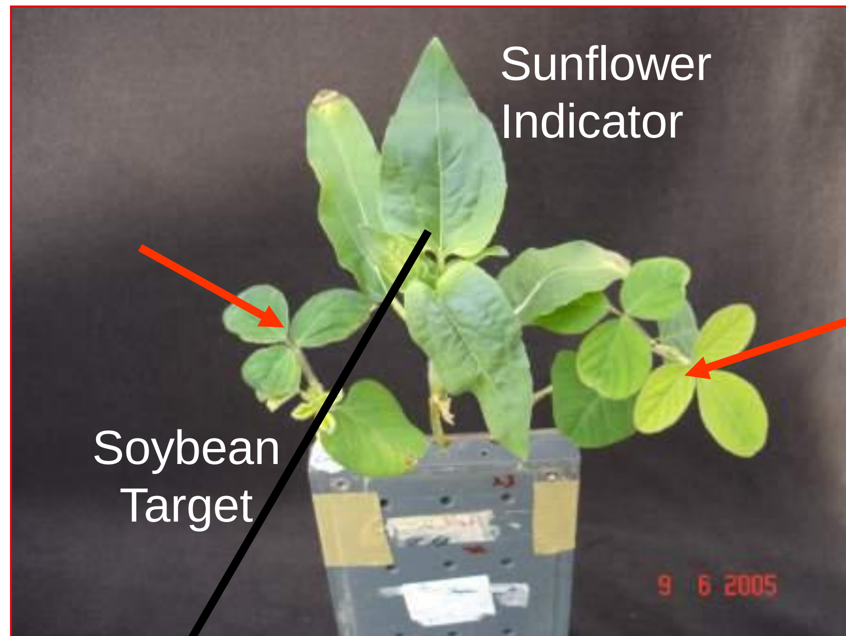
Glyphosate applied to target plants (weed) can be released into the rhizosphere

Induction of Fe deficiency chlorosis in non-target plants (sunflower) induced by glyphosate transfer from foliar treated target plants (soybean)

Nutrient solution experiment



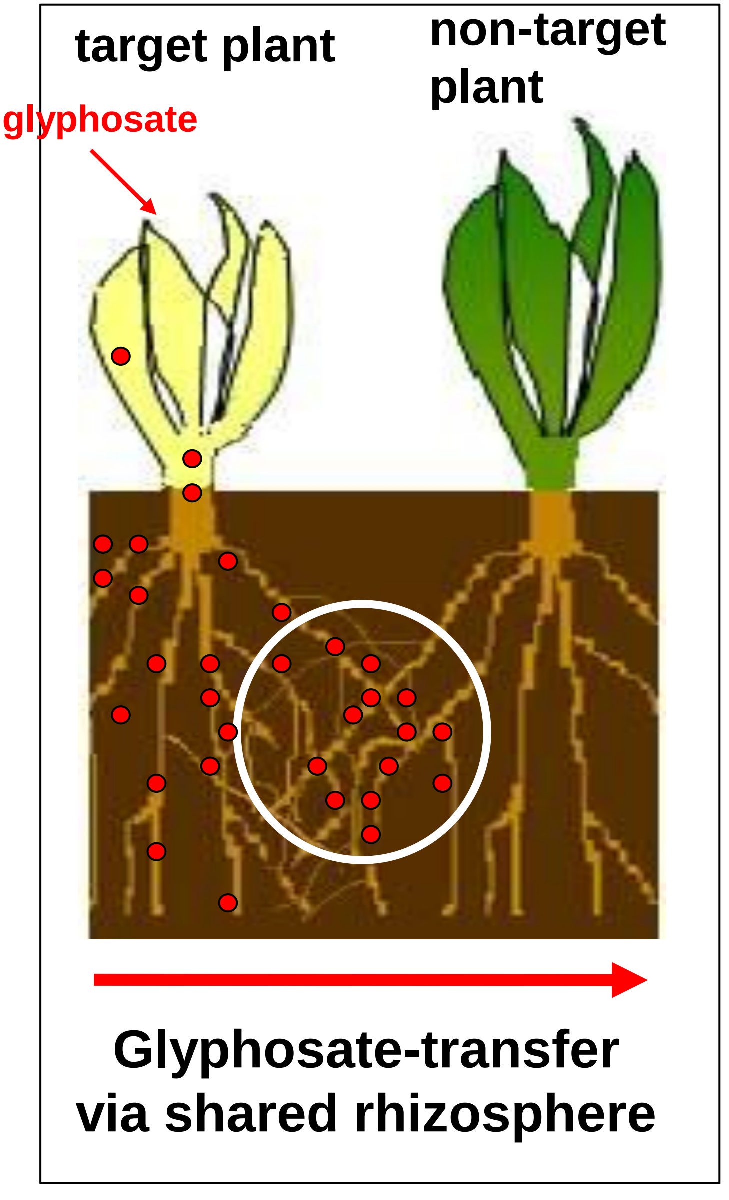
Rhizobox experiment



Glyphosate application to target plants

Fe deficiency symptoms in non-target plants … and accumulation of shikimate!

Relevant knowledge on glyphosate

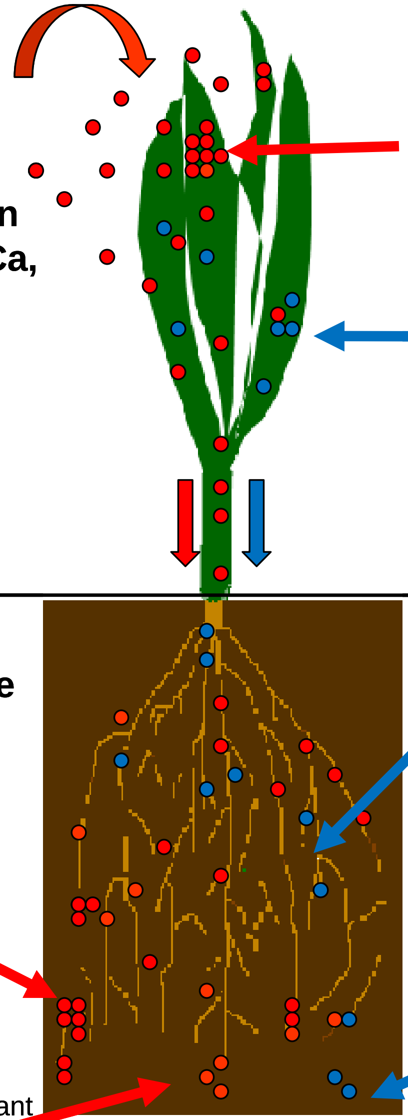


What have we to know?

After accumulation of glyphosate in the roots of target plants (e. g. weed) release into the rhizosphere with possible consequences for a non-target crop plant!

***Glyphosate dynamics in plants*:**

Glyphosate: (red) AMPA: (blue)



foliar application of glyphosate on target-plants (weeds)/ glyphosate-resistant cultivars;uptake by leafs potential influenced by composition of spray solution (e.g. addition of Ca, Fe, Mn) (Bernards et al. 2005 Weed Sci. 53)

accumulation of glyphosate in meristematic shoot tissue (Hetherington et al. 1999. J. Exp. Bot. 50)

depending on plant species degradation of glyphosate to AMPA in shoots at a lower rate (Nandula et al., 2007 J. Agric. Food Chem. 55)

rapid translocation of glyphosate from shoots to roots (Hetherington et al. 1999. J. Exp. Bot. 50)

Intermediate storage of glyphosate in roots (Laitinen et al., 2007 unpubl.)

translocation of AMPA from shoots to roots and/ or <u>formation</u> of AMPA in roots at a lower rate

accumulation of glyphosate in meristematic root tissue (Hetherington et al. 1999. J. Exp. Bot. 50)

release of AMPA in the rhizosphere or formation in the rhizosphere

release of glyphosate in the rhizosphere (Neumann et al., 2006. J. of Plant Diseas. and Proct. 20)

<u>Open questions:</u> What is the mechanism of this release into the rhizosphere and how fast is this release depending on which factors?

Relevant knowledge on glyphosate

- How long this toxic glyphosate or AMPA can be stored in roots of target plants……. depending on which soil and management factors?

Important questions for the issue of waiting times after glyphosate use by farmers!

Waiting times: An important issue?

Regarding Monsanto's representatives (2006) there is no need for waiting times to be considered! No need for such an indication on package label for directions for use by farmers!

Even advertisement for an use of glyphosate till one week after sowing in Germany or Brazil!

Is this general statement of Monsanto responsible to farmers and in agreement with increasing observations by farmers and research result during the last years?

Effects of timing of cover crop desiccation on RR soybean yield

Time of desiccation	Cover crop		
	Black oat	Ryegrass	Fallow
21 dbp	(100)	(100)	(100)
14 dbp	-2.1	-7.3	-3.7
7 dbp	-6.8	-18.5	-12.3
0	-11.2	-23.4	-17.2
7 dap	-17.4	-25.9	-21.2

dbp = days before planting; dap = days after planting (Aroldo Marochi, 2006)

Clearly, best time for glyphosate application 2-3 weeks before sowing the following crop (even for RR soybeans) in Brazil on low buffered soils!

Results by POTAFOS, Brazil showing the need of waiting times

dap = day after planting, dbp = days before planting

“best plant development when sowing soybean 14-21 days after desiccation by glyphosate”

Relevance of waiting times after weed glyphosate desiccation (model green house experiment) :

Luvisol

Arenosol

Sunflower plants grown on a Luvisol (subsoil) sown 0, 7, 14, 21DAA (after glyphosate application) to weed or mechanical weeding (-Gly).

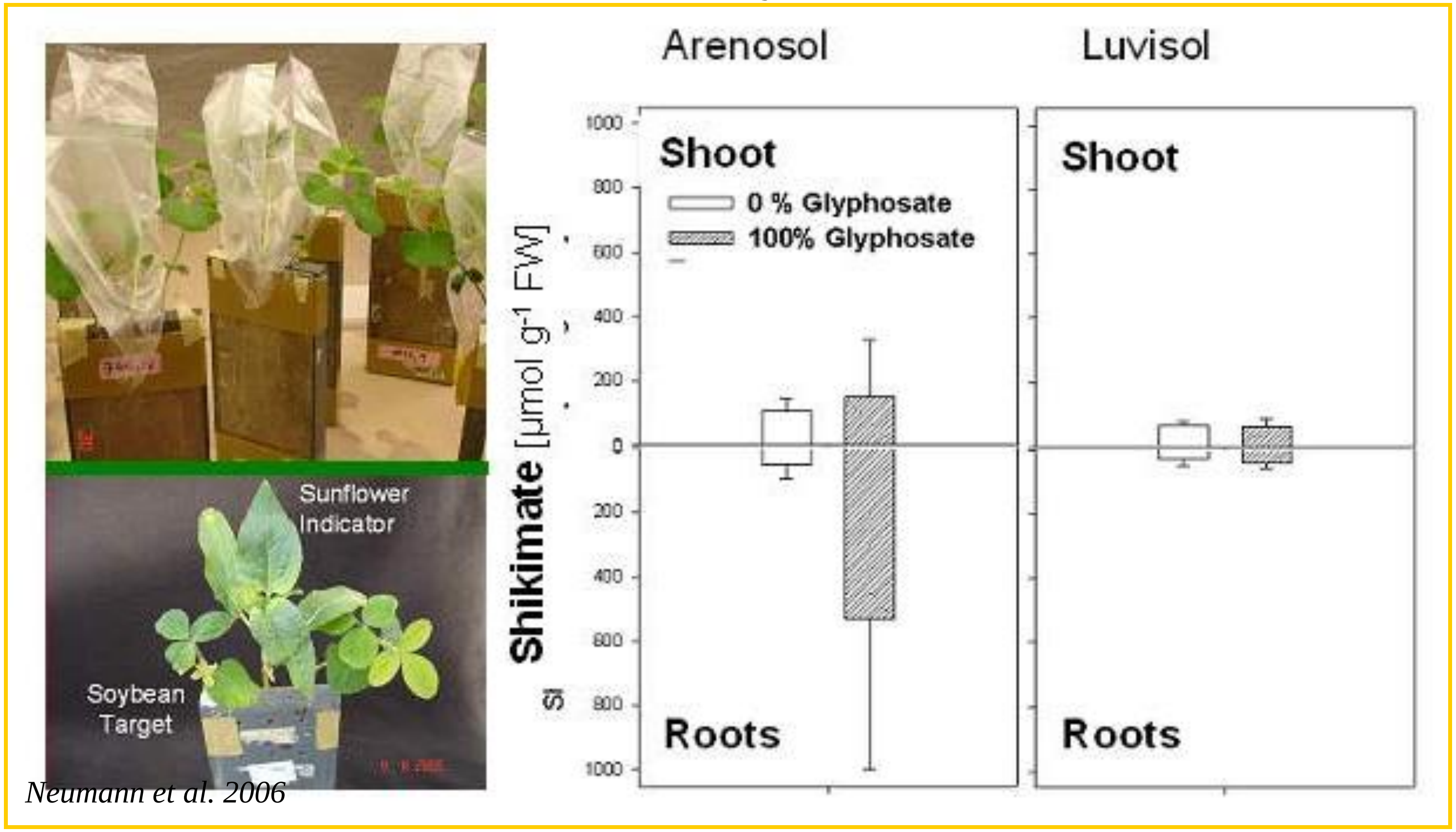
Sunflower plants grown on an Arenosol sown 0, 7, 14, 21DAA (after glyphosate application) to weed or mechanical weeding (-Gly).

Sever plant growth inhibition if waiting time is less than 21 days and a stronger observed toxicity if buffering capacity of the soil is low.

➡ This indicates relevance of waiting time in glyphosate use and the consideration of the soil type!

<u>Soil type dependent **Short-term rhizosphere transfer** of glyphosate from glyphosate-treated RR soybean (recommended dosage) to simultaneously cultivated, untreated sunflower.</u>

Sandy soil Calcareous sub-soil



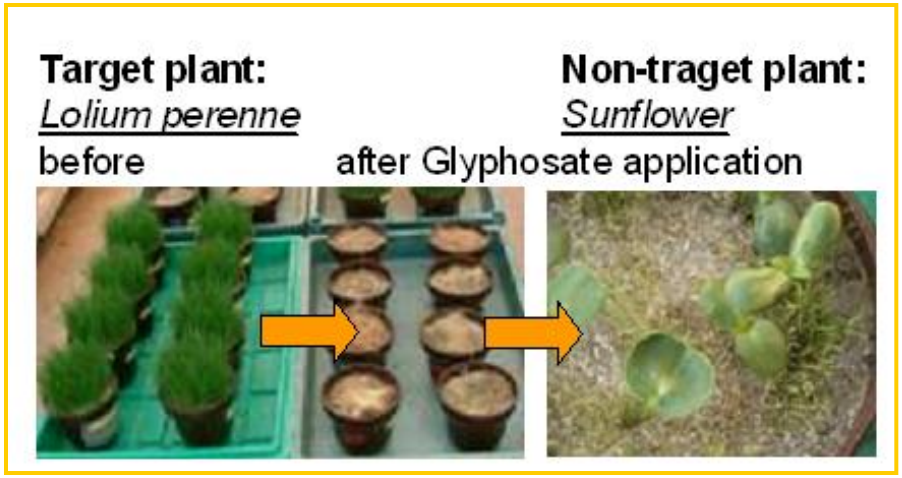
Neumann et al. 2006

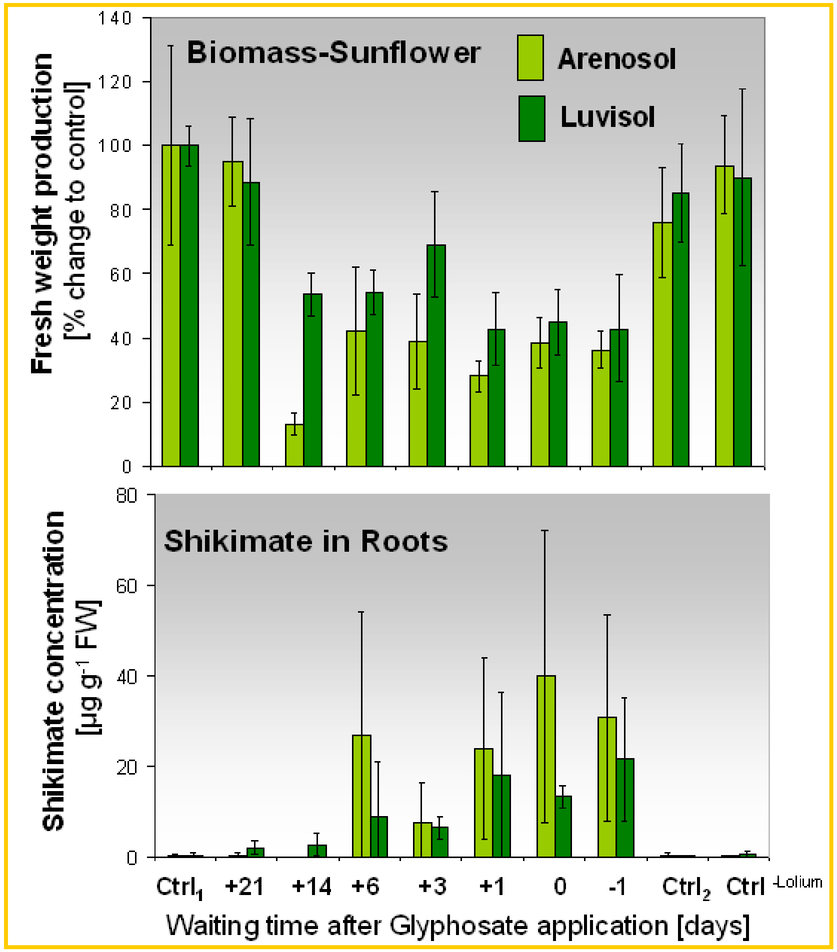
Shikimate accumulation (indicator for glyphosate toxicity) in sunflower **<u>7 days after glyphosate application</u>** to soybean

Glyphosate-induced shikimate accumulation in non-target sunflower plants on the Arenosol, <u>but not</u> on the calcareous soil (rapid immobilisation of glyphosate on the calcareous soil as Ca-salts ???)

Root to Root transfer of glyphosate from target (*Lolium perenne*) to non-target plants (sunflower) depending on waiting time after glyphosate application

Plant growth and intracellular shikimate accumulation as physiological indicator for glyphosate toxicity .



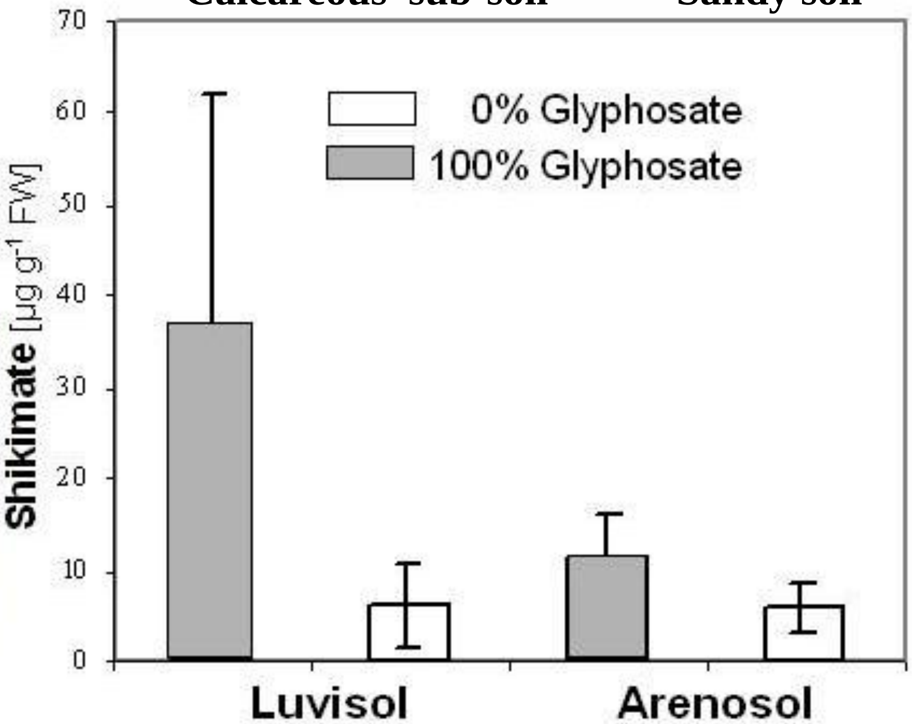


By waiting time of less than 14 days inhibited shoot growth and shikimate accumulation in roots!

Long-term rhizosphere transfer from glyphosate-treated *Lolium perenne* to simultaneously cultivated untreated soybean.

Pre-culture:

Following crop:



Shikimate accumulation (indicator for glyphosate toxicity) in soybean **<u>8 weeks after glyphosate application</u>** to *Lolium perenne*

Glyphosate-induced shikimate accumulation in non-target plants on the calcareous soil (re-mobilisation of fixed glyphosate?) **<u>but not</u> on the Arenosol with low glyphosate immobilisation** (complete microbial degradation within 8 weeks?)

ראונדאפ® ROUNDUP®

הדברת עשבים לקראת גידולי שדה ובשטחי כרב

הגידול	העשבים המודברים	המינון (סמ"ק/ד')	מועד הטיפול
לקראת גידולי קיץ: כותנה, מקשה, אגוזי אדמה, תירס וירקות.	א. נבטי חד-שנתיים עד גובה 10 ס"מ, לרבות נבטי דורת ארם-צובא שהציצו מזרעים.	100 בתוספת 0,5% משטח DX	לפני הזריעה או לאחריה, וכן לקראת שתילה. אין לרסס לאחר השקיית ההנבטה. **למעט בקרקעות חוליות המכילות פחות מ־10% חרסית.**
	ב. חד-שנתיים 10-40 ס"מ, יבלית ודורת ארם-צובא מקני שורש ייצרבו וידוכאו זמנית.	150 בתוספת 0.5% משטח DX	
לקראת זריעת גידולי קיץ: כותנה, מקשה, אגוזי אדמה, תירס וירקות.	עשבים רב-שנתיים: יבלית, דורת ארם-צובא, חבלבל, גומא הפקעים-בעלי עלווה מפותחת.	500 (במוקדים 5%)	במצע מוכן לפני הזריעה ולאחריה (לא לשתילה). אין לרסס לאחר השקיית ההנבטה. **למעט בקרקעות חוליות המכילות פחות מ־10% חרסית.**
	דורת ארם-צובא בלבד.	300 (במוקדים 3%)	
לקראת כותנה בדו-גידול בזריעה למישרין (לאחר חיטה, שעורה, שחת).	שלף מתחדש ועשבים חד-שנתים קיימים, לרבות דורת ארם-צובא שהציץ מזרעים.	150 עד 200 בהתאם לגובה העשבים, בתוספת 0.5% משטח DX	על שלף ועשבים מלבלבים לאחר השקית יסוד וזריעת הכותנה, לא יאוחר מיומיים לפני הצצתה.
טיפול קיצי לקראת דגני חורף בלבד.	עשביה רב-שנתית הכוללת יבלית, גומא הפקעים, דורת ארם-צובא, ינבוט, חבלבל, הגא.	300 עד 500 בתוספת 100-150 טומהוק (מינון ראונדאפ הגבוה להדברת יבלית וגומא הפקעים)*	על כרב או לאחר פנוי פני הקרקע, משאריות הגידול הקודם, כל עוד העשבים בצימוח פעיל.
	יבלית בעלת עלווה מפותחת וכן עשביה אחרת הקיימת בזמן הריסוס.	500 (במוקדים 5%)	

* בריסוס בתערובת ראונדאפ עם אלבר סופר או טומהוק, עלול רחף התרסיס לגרום לפגיעה חמורה בגידולים רגישים לתכשירים אלה, לכן השימוש בשילוב זה כפוף להנחיות ולאמצעי הזהירות בעת הריסוס המפורטים בתויות, לרבות ההוראות לניקוי המרסס לאחר הטיפול.

נפח התרסיס:
בריסוס קרקע 10-15 ל'/ד'.
בריסוס אוירי 5 ל'/ד' (ראה תוית).

טיפול בראונדאפ להקמלת הגידול

הגידול	המינון (סמ"ק/ד')	מועד הטיפול	נפח התרסיס
חיטה, שעורה, בקיה, שיש, תירס, פנסילריה-לשחת או לתחמיץ	100-300	עד 7 ימים לפני הקציר	ריסוס קרקע: 10-20 ל'/ד' ריסוס אויר: 5-7 ל'/ד'

הערה: אין לטפל בגידול המיועד לזרעים.

In Israel: Glyphpsate use on dry and sandy soils forbidden as mentioned on the package label for farmers use.

The results by Myriam Fernandez on negative effects of glyphosate on FHB incidence in Canada even 18-36 months after glyphosate application might indicate <u>even longer waiting times</u> in distinct situations with a long lasting glyphosate effect!

In conclusion, waiting times after weed control with glyphosate might be

0 - 3 weeks for wet, light soils with a fast turn-over of weed roots (e.g. in Brazil),

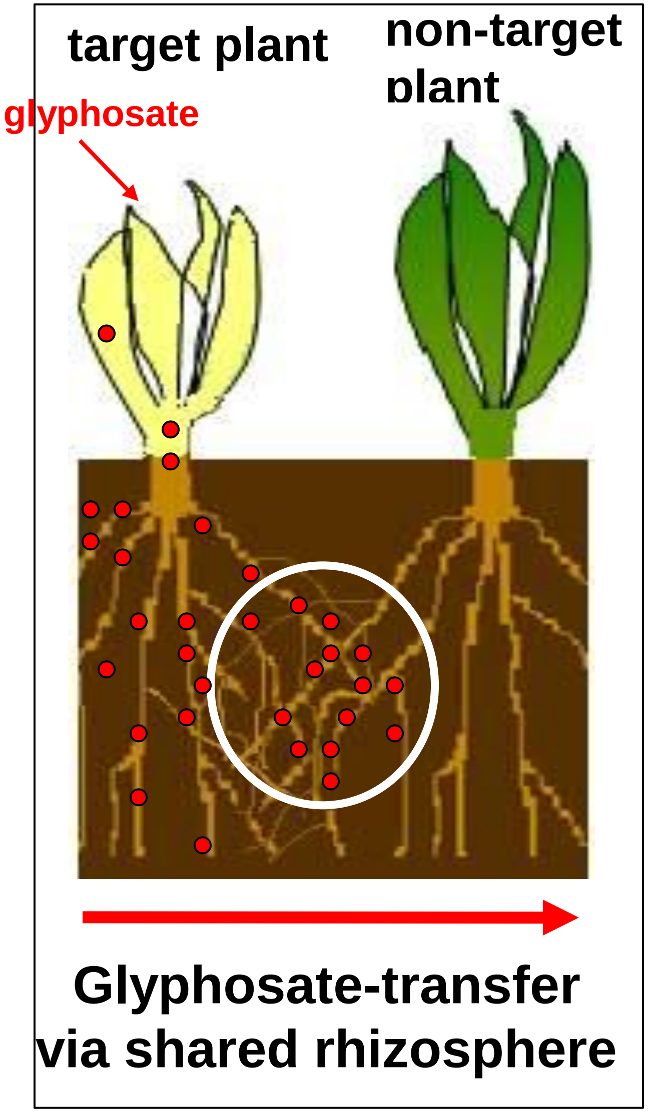
4 - 8 weeks for wet, heavy calcareous soils with a slower turn-over of weed roots,

but might be up to

1 year for dry sandy soils as wide-spread in Israel,

1.5 - 3.0 years for cold soils with an impeded turn-over of weed roots as in some regions of Canada.

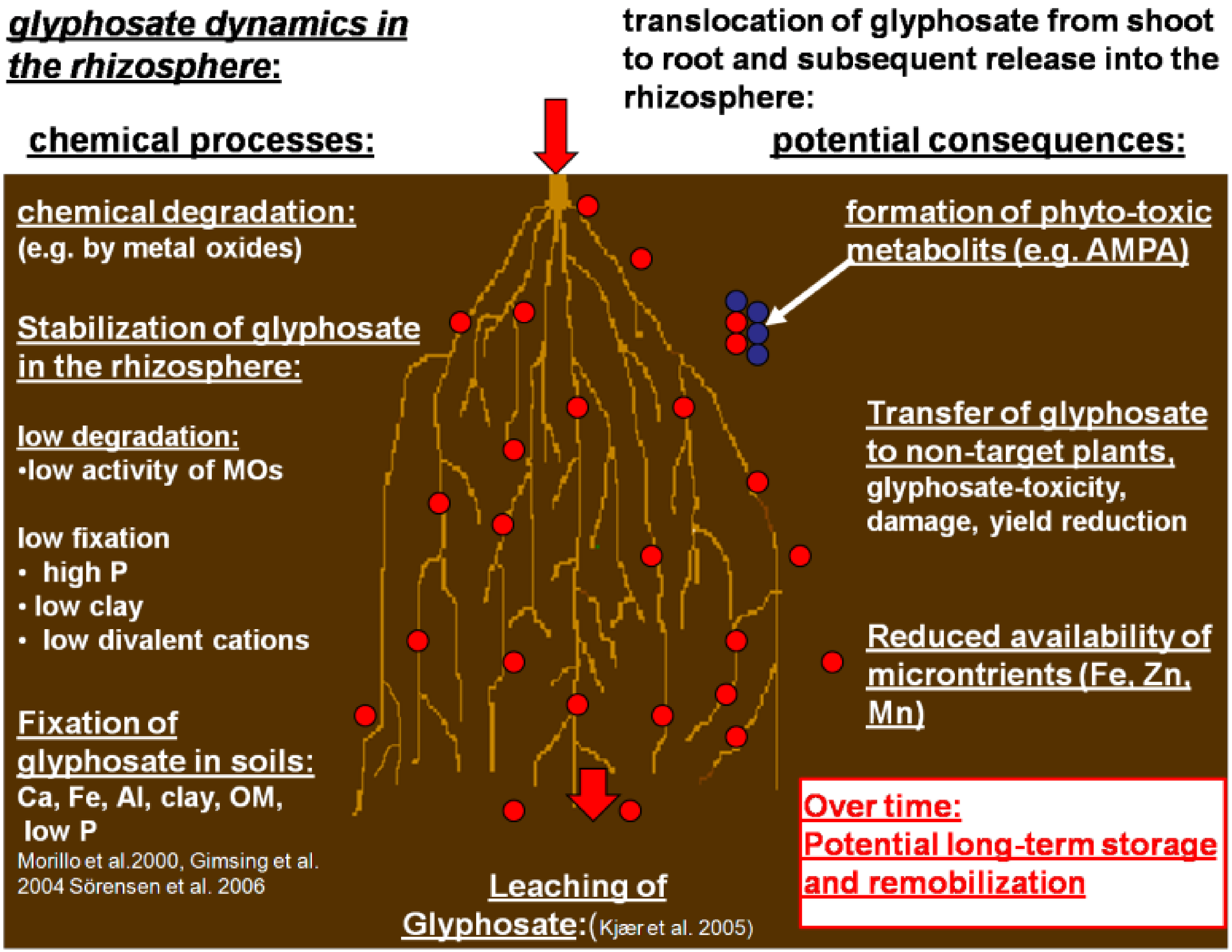
The Rhizosphere: An important place for possible glyphosate toxicity



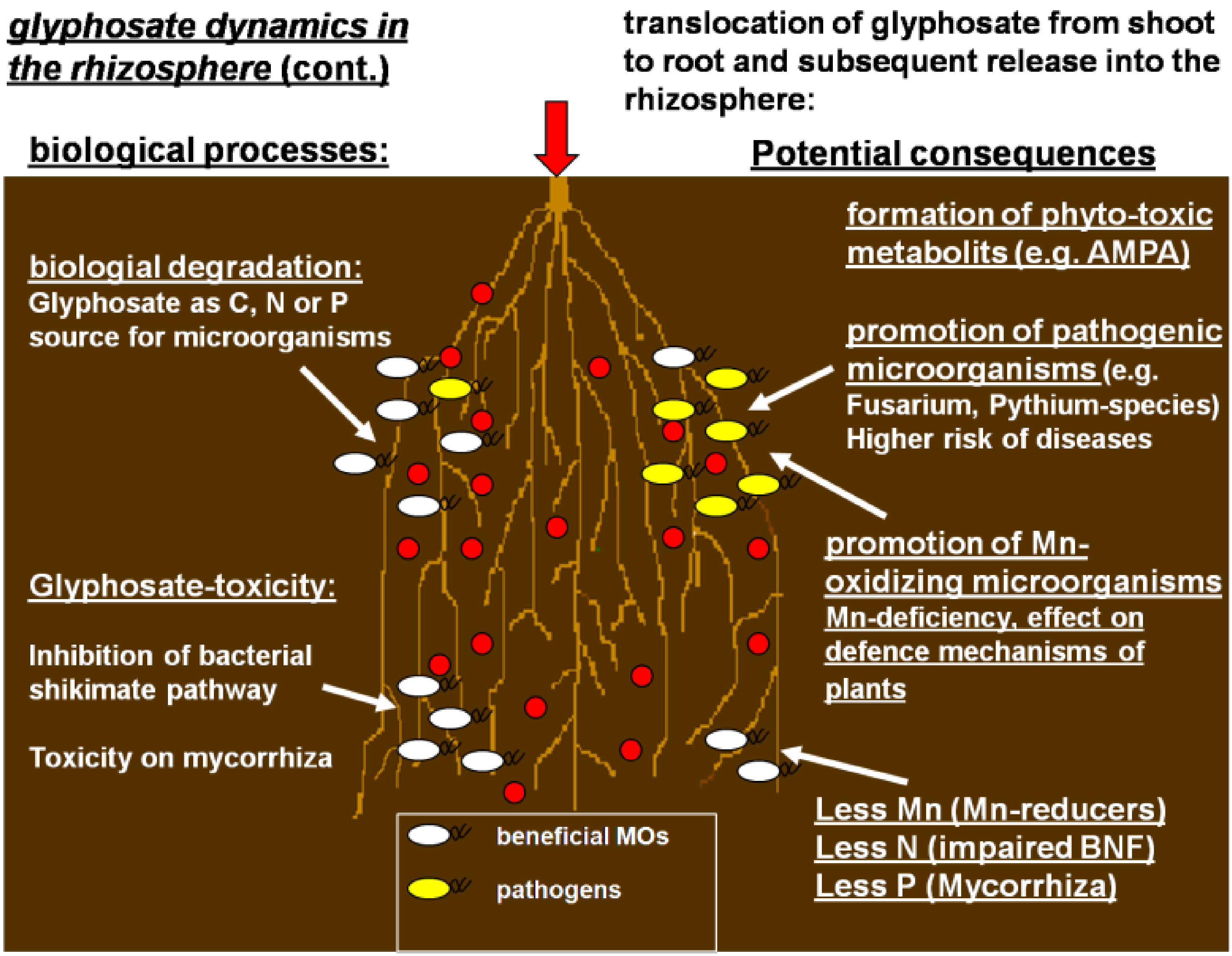
Obviously, various processes of glyphosate dynamics take part in the immediate vicinity of roots, the so-called rhizosphere.

What are these various processes of importance for glyphosate toxicity?

□ The Rhizosphere: An important place for possible glyphosate toxicity



☐The Rhizosphere: An important place for possible glyphosate toxicity

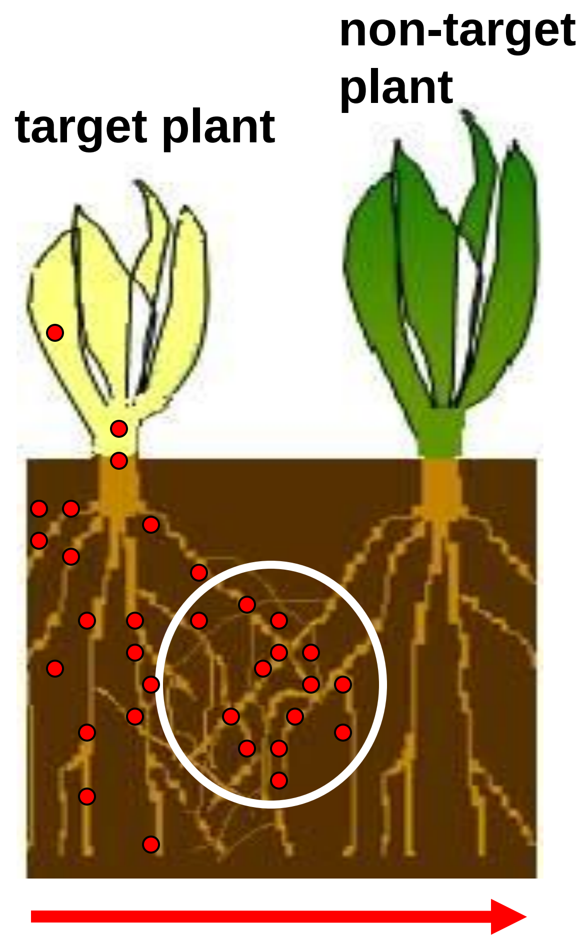


☐ The Rhizosphere: An important place for possible glyphosate toxicity

<u>These processes and their interdependencies may change with:</u>

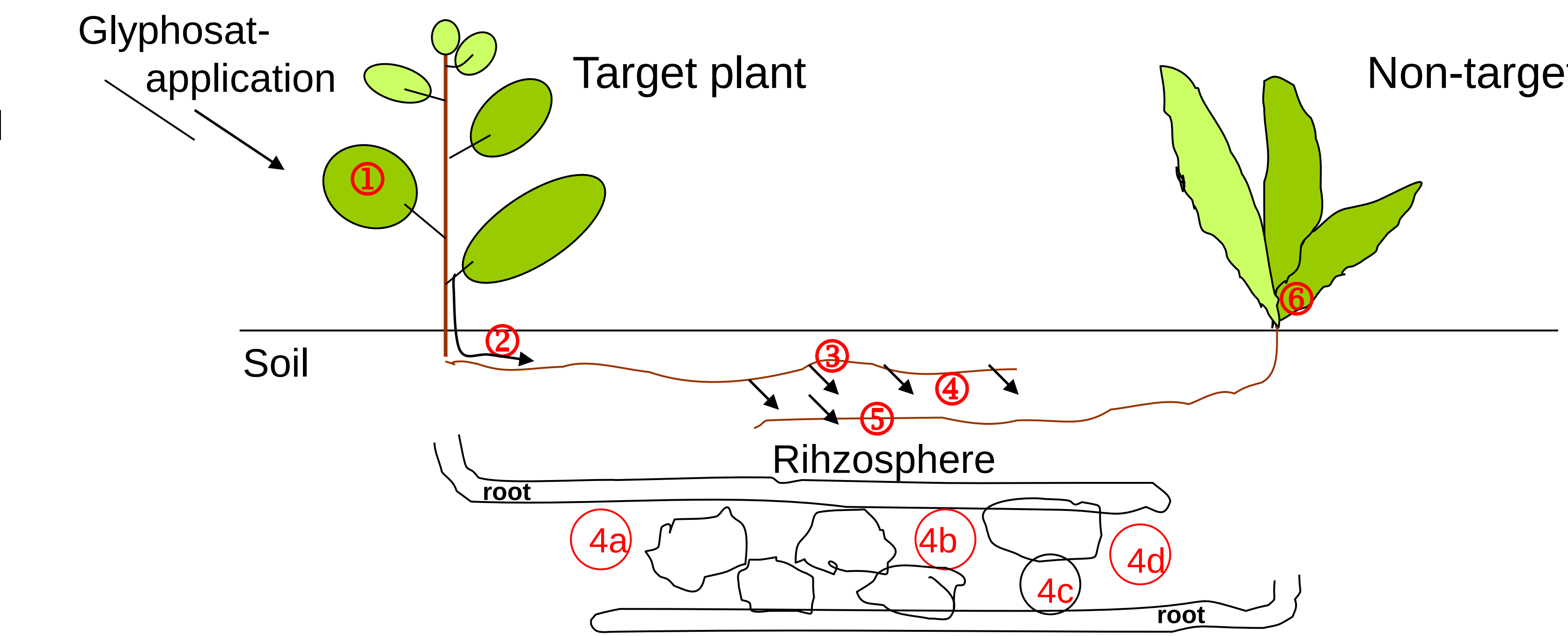
- **soil chemical properties (pH, redox)**
- **microbial population**
- **application frequency**
- **application time**
- **plant species**
- **over time**

The role of the rhizosphere as place for glyphosate toxicity may drastically increase in case of a shared rhizosphere between glyphosate treated and non-treated plants



glyphosate – transfer via shared rhizosphere

Dynamics of Glyphosate/AMPA in the Rhizosphere (Model)



① foliar uptake of glyphosate

② transfer of glyphosate into apical root zones

③ release of glyphosate and possible metabolites (AMPA) into the rhizosphere of target plants or degradation of root residues

④ glyphosate dynamics in the rhizosphere

⑤ uptake of glyphosate by non-target plants

⑥ translocation of glyphosate/AMPA into the shoot of non-target plants and induction of disorders

④ glyphosate/ AMPA dynamics in the rhizosphere

a) extent of interactions between root system of target and non-target plants (intermingled roots)

b) glyphosate immobilization in the rhizosphere

c) glyphosate remobilization by root-induced changes in the rhizosphere of non-target plants

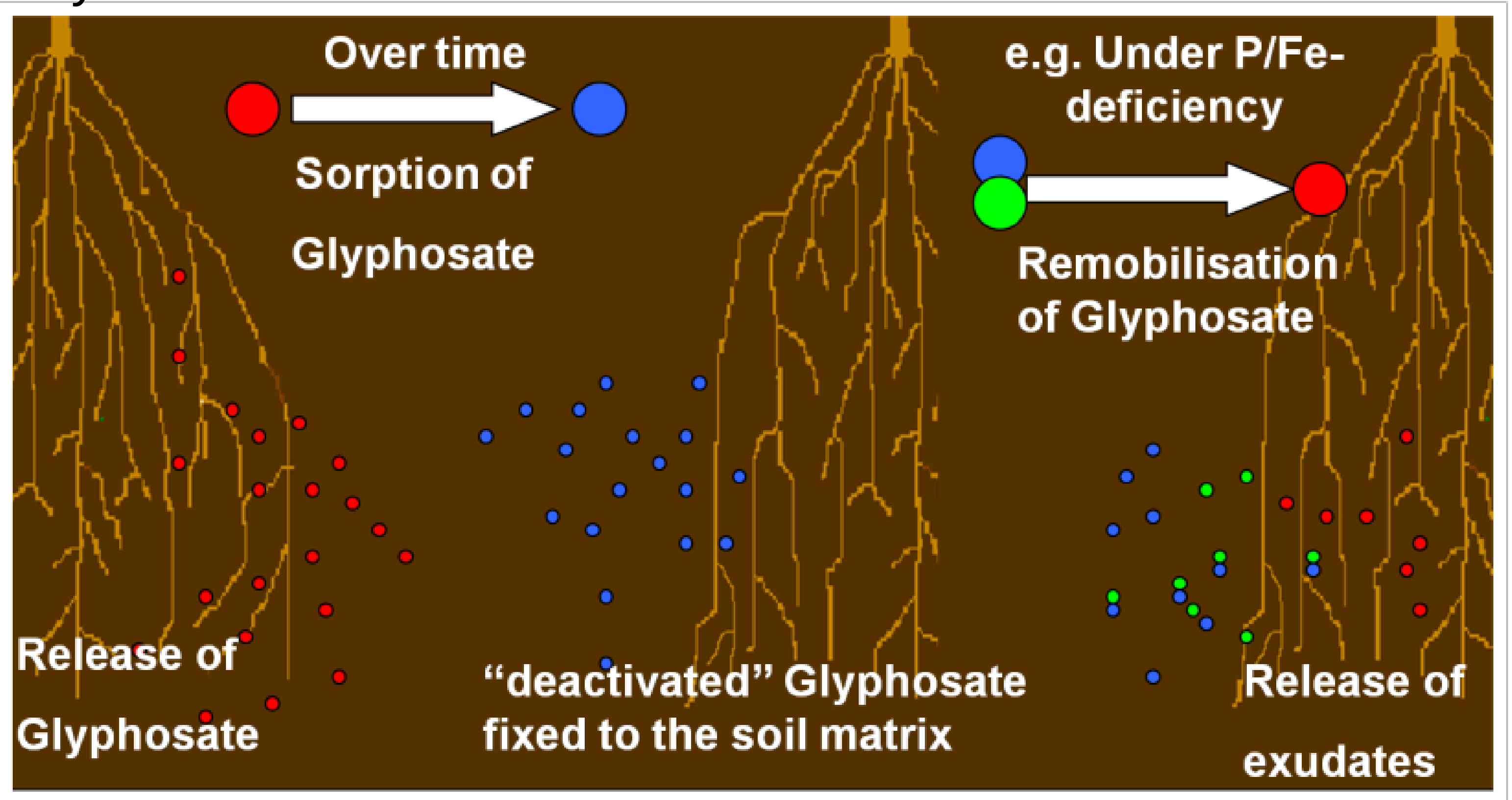
d) interaction of glyphosate with Mn-reducing/oxidizing rhizosphere microorganisms

e) effect of glyphosate on mycorrhizae and microbial diversity

Remobilization of glyphosate from soils: a possible reason for prolonged glyphosate-toxicity in soils?

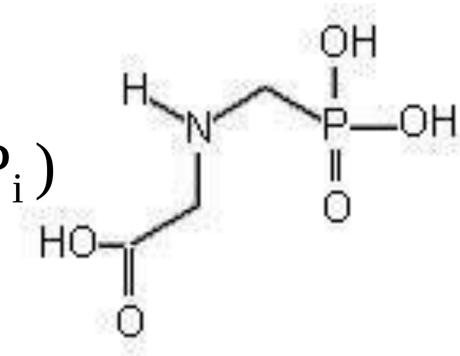
In soils, glyphosate behaves similar to P by strong adsorption to Fe, Al, Ca, organic matter and clay minerals (Morillo et al., 2000, Gimsing et al., 2004, Sörensen et al., 2006)

BUT: a remobilization e.g. by carboxylates released under nutrient deficiency has to be considered!



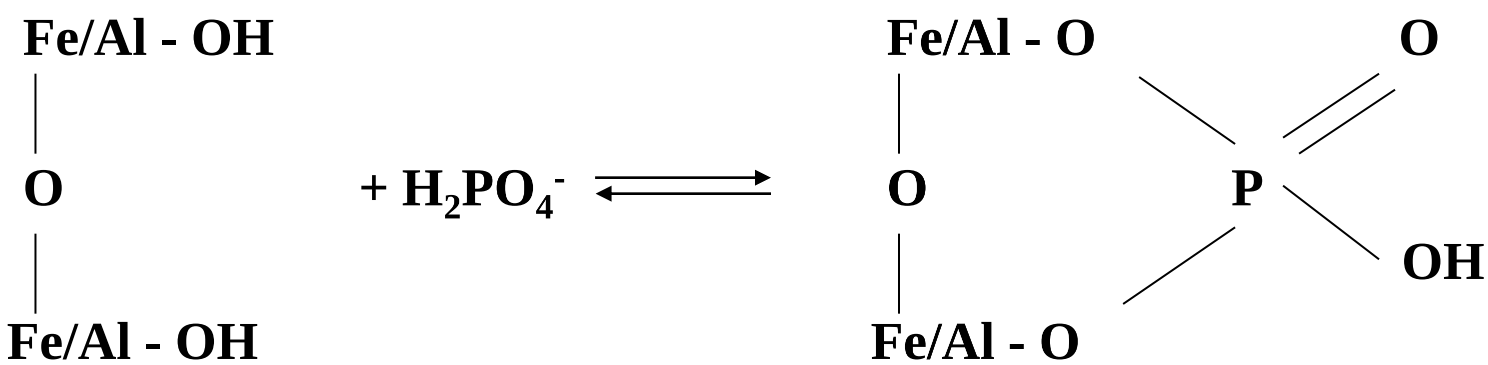
. <u>In the rhizosphere accumulated and stabilized glyphosate can be remobilized and take up by non-target plants</u>

<u>Gyphosate: n-Phosphonomethyl glycine</u>



Structural similarities with inorganic phosphate (P_i)

Adsorption characteristics in soils similar to P_i



From these consideration it can be concluded that P-efficient plant species will mobilize glyphosate more efficient under low P status and that measures for a better P fertilizer use (e.g. pH lowering, silicate or water-soluble humic substances) will also enhance a remobilization of glyphosate !

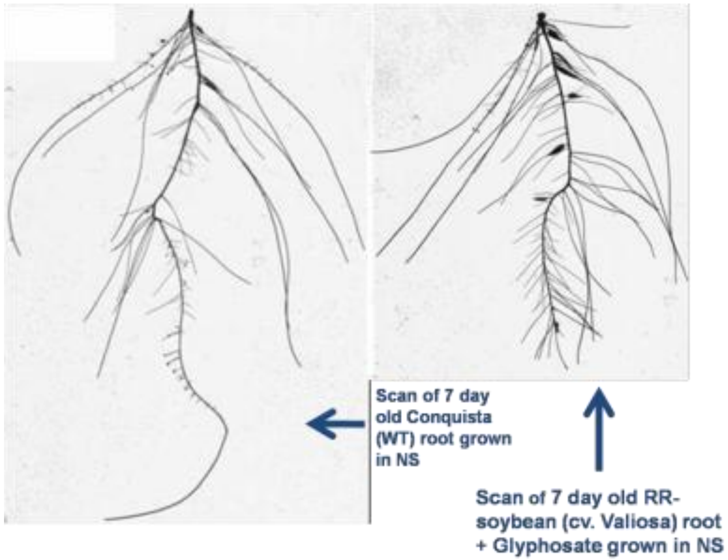
▪ Roots of non-target plants as prime victims of glyphosate residual toxicity:

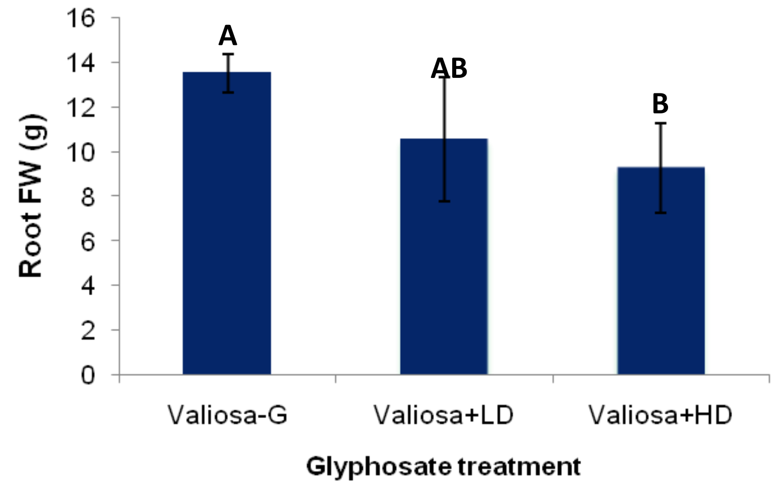
Sunflower seedlings grown on an acidic Arenosol 14 days after glyphosate weed (*Lolium perenne*) desiccation.

Sunflower roots grown on an acidic Arenosol (top) and calcareous Luvisol sub soil (bottom) at 0 days waiting time after glyphosate desiccation of pre-cultivated weed

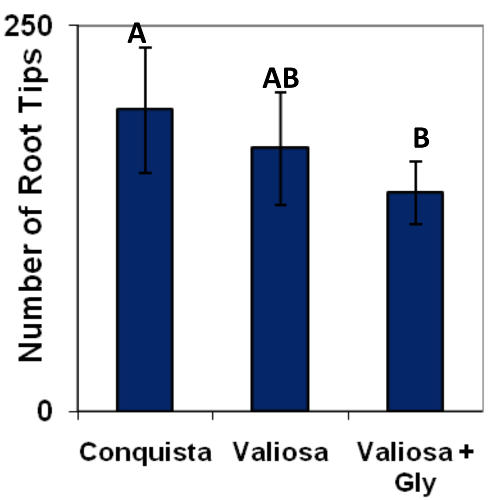
➡ Inhibited root growth of non-target plants after weed glyphosate desiccation if required waiting time is not considered!

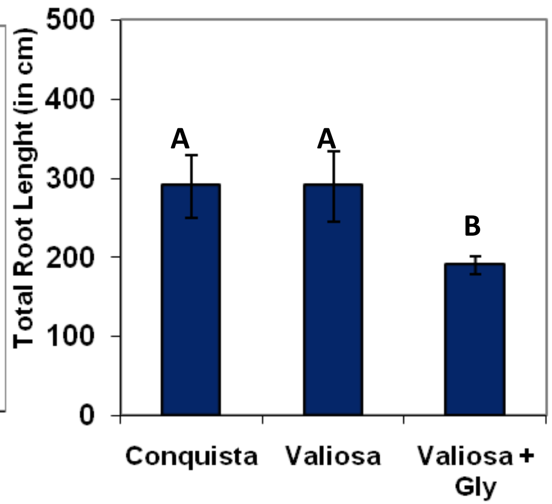
Glyphosate effect on root morphology and -growth of RR soybean plants (cv. Valiosa) grown in soil and nutrient solution cultures.





Inhibition of root biomass of RR soybean (cv. Valiosa) grown on calcareous soil due to glyphosate application at lower (LD i.e.2L/ha) and higher (HD i.e. 4L/ha) range of recommended dosage proposed by the producer company.



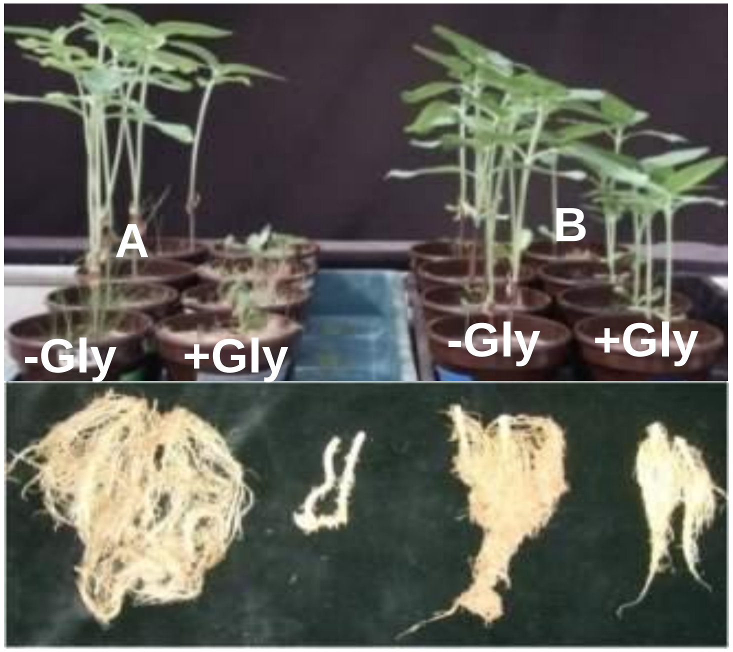


Reduced root elongation 4 days after 28.4mM Glyphosate (recommended rate) application to RR soybean (cv.Valiosa) grown in hydroponics (formation of shorter and reduced number of roots).

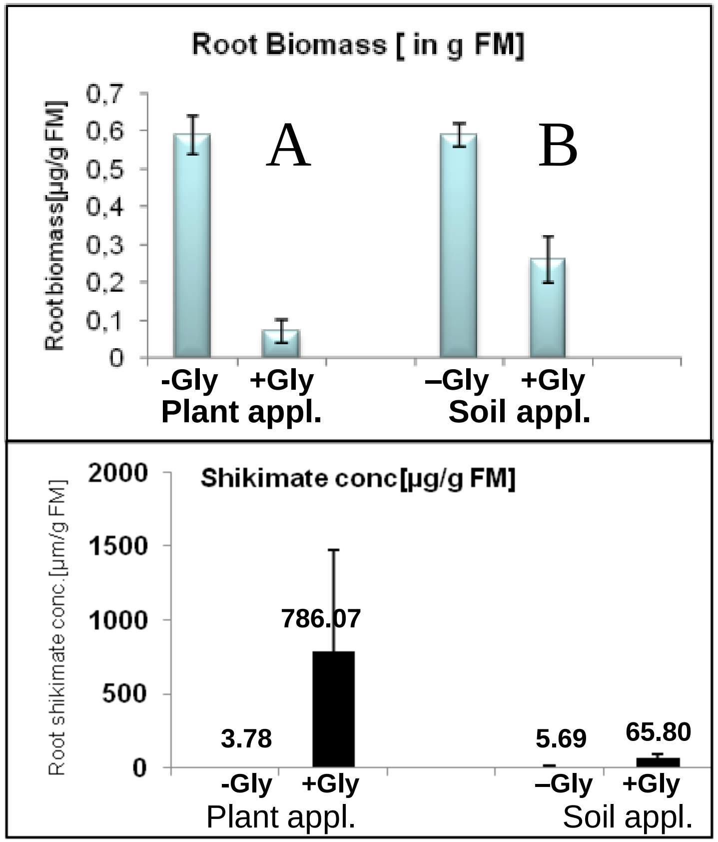
Results on root growth and morphology of non-target and RR-plants highlight risk of increased drought stress by glyphosate use.

Roots of target plants: Key players in affecting stabilization and toxicity of glyphosate

(Green-house model experiment)

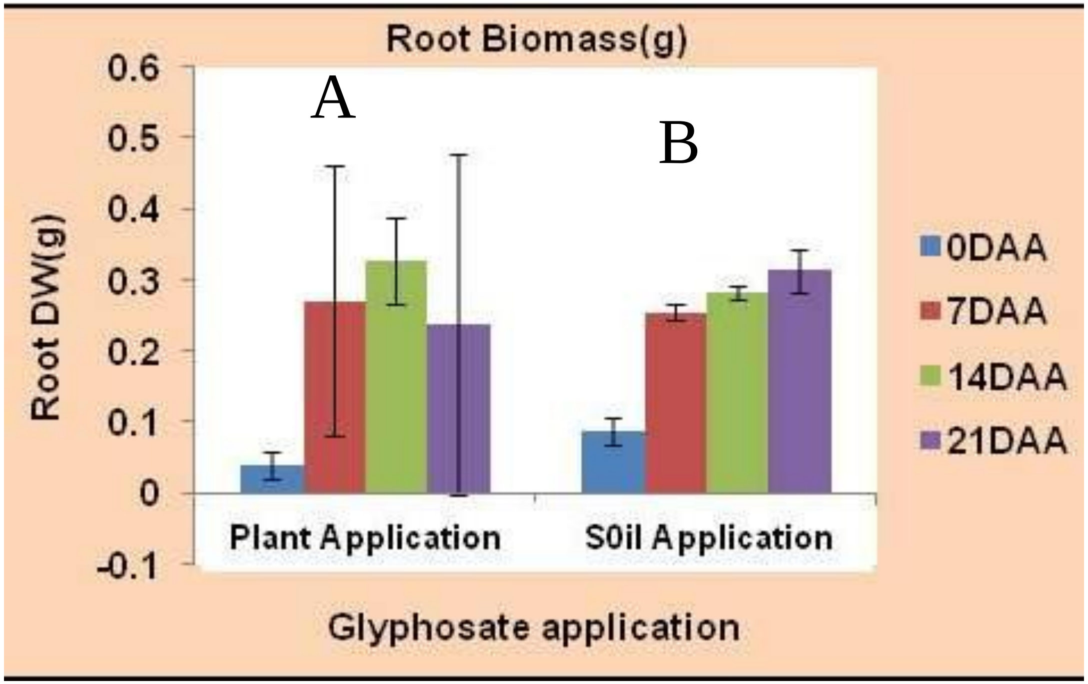


Inhibited sunflower seedling growth (both shoot and root) sown zero days after glyphosate desiccation of pre-cultivated Lolium perenne as weed(A) and direct soil application(B). Stronger effect in weed (A) than soil application (B)!



Root biomass and intracellular shikimate accumulation of sunflower seedlings grown 0 days after Lolium perenne weed glyphosate desiccation (plant appl.) and direct soil incorporation (soil appl.). Stronger residual toxic effect in plant (A) than soil application (B).

Differential pattern of glyphosate residual toxicity between target plant application and direct soil application



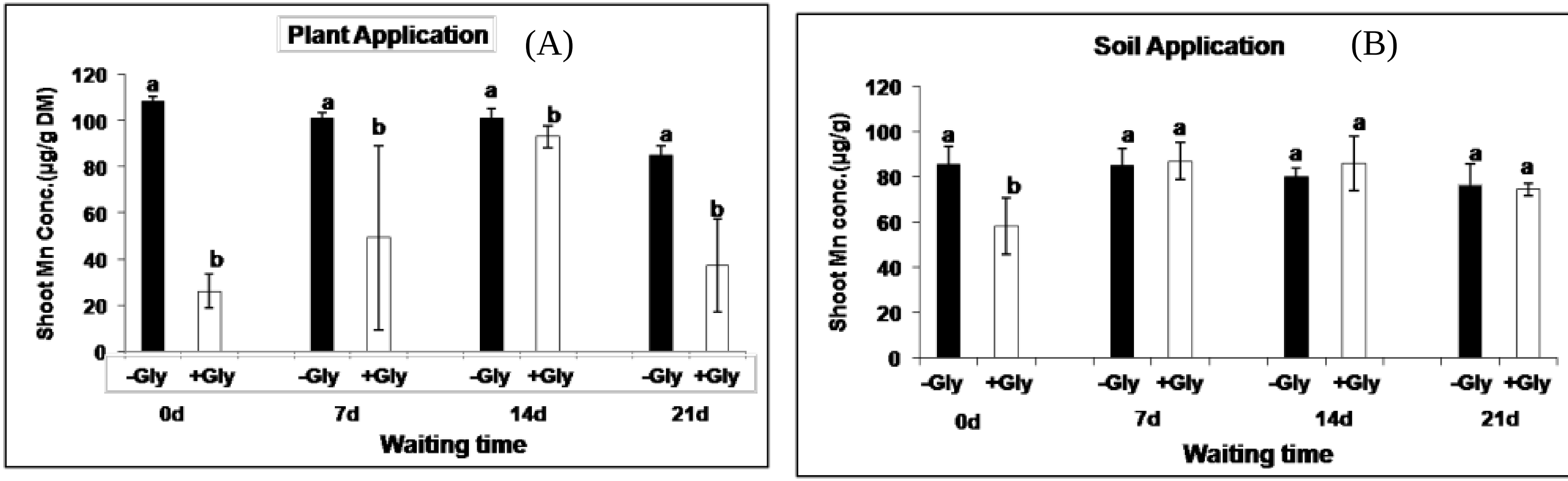
Root biomass of sunflower plants grown on acidic Arenosol after glyphosate Lolium perenne weed desiccation or direct soil application.

Extended glyphosate residual toxicity after plant application compared to soil incorporation.

Note: The big standard errors in plant application (A) seem to represent hot spot glyphosate pool formation in the rhizosphere rather than due to random sampling variability, as there were similar high differences in plant growth within the same pot.

Similar hot spot effects of gyphosate were observed in Ni deficiency of pecan trees by Wood et al. and Bai et al. (see before).

Soil type and application mode dependent inhibition of Mn acquisition by glyphosate: Arenosol



Mn concentration of sunflower plants grown on acidic Arenosol I with low buffering capacity at different waiting times after *Lolium perenne* weed glyphosate desiccation (plant application) and direct soil incorporation (soil application).

In soils with a low buffering capacity, glyphosate residual toxicity can be extended up to 21 days waiting time!!

Soil type dependent role of roots in stabilization process of glyphosate in a soil!!.

Roots of target plants: Key players in affecting stabilization and toxicity of glyphosate

Clear indications for roots as key players in stabilization of glyphosate in pot experiments with sunflower, BUT:
These findings of the model pot experiment need further confirmation by

- further distinct pot experiments and
- field experiments with different crops (on-going!)

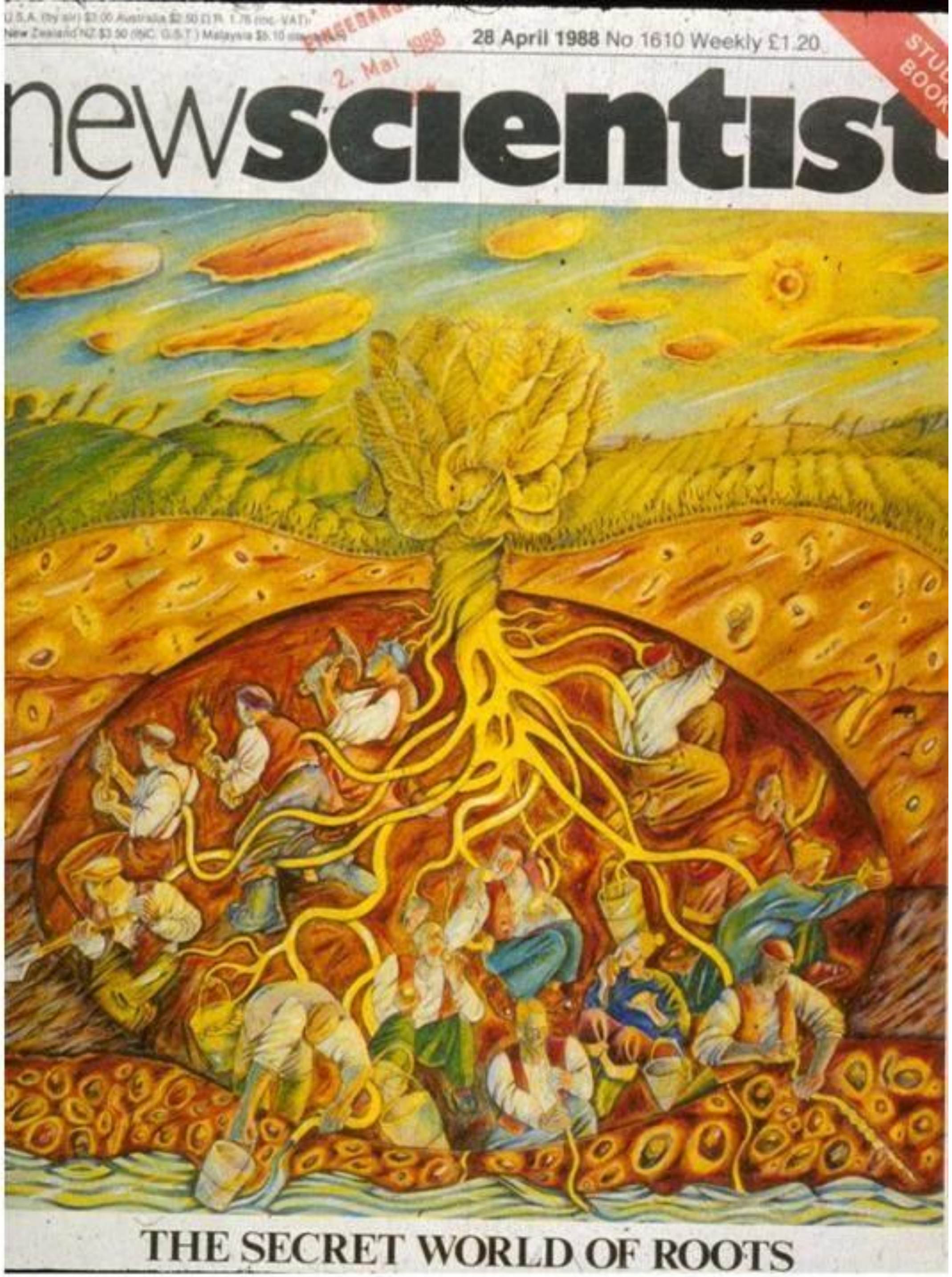
Further, the research of the Italian group (Senesi et al. 199x) on the stabilization of glyphosate on organic matter in the rhizosphere (root exudates?) has to get re-examined including the turn-over of weed roots, high in accumulated glyphosate.

Conclusions / Prospects

- Farmers of non-till practice in Brazil are in favor of glyphosate.
- However, they recognize increasing problems with micronutrient deficiencies, drought and disease problems.
- With innovative rotations (including black oat), higher micronutrient fertilization and more pesticide application they try to counteract at least partially these problems.
- For a better understanding of the non-foreseen negative side-effects of glyphosate by Monsanto the rhizosphere as the immediate vicinity of roots has to be taken into consideration.
- Obviously, in the earlier studies with a rapid detoxification or immobilization of glyphosate in soils, the rhizosphere of target (weed) plants was not properly considered.
- Glyphosate and its high toxic metabolite AMPA (amino-ethylphosphonic acid), released into the rhizosphere of target plants are long enough stable to be taken up by following crop plants (non-target plants) with detrimental effects if waiting times are not considered.

Conclusions / Prospects (continuation)

- Roots of target (weed) plants are the key players affecting stabilization and toxicity of glyphosate depending on the conditions of degradation of the glyphosate containing root residues (soil type and weather dependent).
- A possible re-mobilization of soil-adsorbed glyphosate in the rhizosphere of non-target plants after repeated application of the herbicide over the years, particularly under non-till practice, is not seriously considered up till now.
- A new risk assessment for glyphosate including the rhizosphere processes with stabilized glyphosate in root residues is urgently claimed, in particular if the expected increasing use of Roundup-resistant (RR) cultivars world-wide is considered.
- To avoid negative effects of glyphosate on plant growth and micronutrient acquisition and thus on disease resistance of the following crop, the turnover of glyphosate in the rizosphere via an adequate waiting-time for different soil types and weather conditions have to get elaborated.
- For all the above mentioned requests a stop of the highly polarized or black and white discussion of the glyphosate issue is urgently needed!



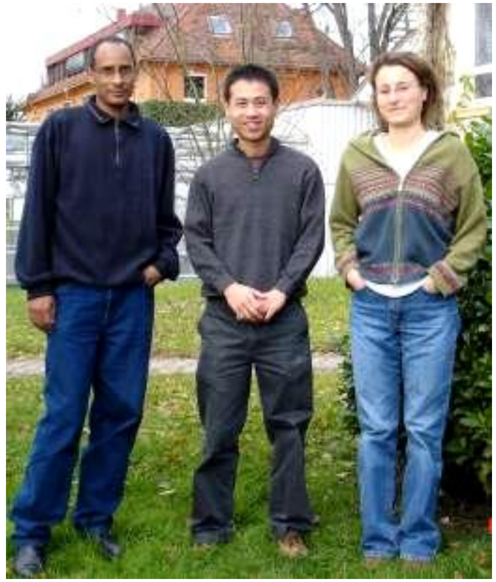
A modeling approach might help to predict the needed waiting time to avoid negative side-effects of glyphosate

depending on conditions for degradation and thus release of stabilized glyphosate in roots of target (weed) plants as key players in glyphosate toxicity in the rhizosphere.

Muito obrigado!
Thank you for your kind attention!

"The Glyphosate Research Team"

M. Guldner



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