AG CHEMICAL AND CROP NUTRIENT INTERACTIONS – CURRENT UPDATE

Don M. Huber, Emeritus Professor, Purdue University

ABSTRACT: Micronutrients are regulators, inhibitors and activators of physiological processes, and plants provide a primary dietary source of these elements for animals and people. Micronutrient deficiency symptoms are often indistinct ("hidden hunger") and commonly ascribed to other causes such as drought, extreme temperatures, soil pH, etc. The sporadic nature of distinct visual symptoms, except under severe deficiency conditions, has resulted in a reluctance of many producers to remediate micronutrient deficiency. Lost yield, reduced quality, and increased disease are the unfortunate consequences of untreated micronutrient deficiency. The shift to less tillage, herbicide resistant crops and extensive application of glyphosate has significantly changed nutrient availability and plant efficiency for a number of essential plant nutrients. Some of these changes are through direct toxicity of glyphosate while others are more indirect through changes in soil organisms important for nutrient access, availability, or plant uptake. Compensation for these effects on nutrition can maintain optimum crop production efficiency, maximize yield, improve disease resistance, increase nutritional value, and insure food and feed safety.

INTRODUCTION

Thirty+ years ago, U.S. agriculture started a conversion to a monochemical herbicide program focused around glyphosate (Roundup®). The near simultaneous shift from conventional tillage to no-till or minimum tillage stimulated this conversion and the introduction of genetically modified crops tolerant to glyphosate. The introduction of genetically modified (Roundup Ready®) crops has greatly increased the volume and scope of glyphosate usage, and conversion of major segments of crop production to a monochemical herbicide strategy. Interactions of glyphosate with plant nutrition and increased disease have been previously over looked, but become more obvious each year as glyphosate residual effects become more apparent

The extensive use of glyphosate, and the rapid adoption of genetically modified glyphosate-tolerant crops such as soybean, corn, cotton, canola, sugar beets, and alfalfa; with their greatly increased application of glyphosate for simplified weed control, have intensified deficiencies of numerous essential micronutrients and some macronutrients. Additive nutrient inefficiency of the Roundup Ready® (RR) gene and glyphosate herbicide increase the need for micronutrient remediation, and established soil and tissue levels for nutrients considered sufficient for specific crop production may be inadequate indicators in a less nutrient efficient glyphosate weed management program.

Understanding glyphosate's mode of action and impact of the RR gene, indicate strategies to offset negative impacts of this monochemical system on plant nutrition and its predisposition to disease. A basic consideration in this regard should be a much more judicious use of glyphosate. Glyphosate damage is often attributed to other causes such as drought, cool soils, deep seeding, high temperatures, crop residues, water fluctuations, etc. Table X provides some of the common symptoms of drift and residual glyphosate damage to crops. This paper is an update of information on nutrient and disease interactions affected by glyphosate and the RR gene(s), and includes recently published research in the European Journal of Agronomy and other international scientific publications.

UNDERSTANDING GLYPHOSATE

Glyphosate (N-(phosphomonomethyl)glycine) is a strong metal chelator and was first patented as such by Stauffer Chemical Co. in 1964 (U.S. Patent No. 3,160,632). Metal chelates are used extensively in agriculture to increase solubility or uptake of essential micronutrients that are essential for plant physiological processes. They are also used as herbicides and other biocides (nitrification inhibitors, fungicides, plant growth regulators, etc.) where they immobilize specific metal co-factors (Cu, Fe, Mn, Ni, Zn) essential for enzyme activity. In contrast to some compounds that chelate with a single or few metal species, glyphosate is a broadspectrum chelator with both macro and micronutrients (Ca, Mg, Cu, Fe, Mn, Ni, Zn). It is this strong, broadspectrum chelating ability that also makes glyphosate a broad-spectrum herbicide and a potent antimicrobial agent since the function of numerous essential enzymes is affected (Ganson and Jensen, 1988).

Primary emphasis in understanding glyphosate's herbicidal activity has been on inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) at the start of the Shikimate physiological pathway for secondary metabolism. This enzyme requires reduced FMN as a co-factor (catalyst) whose reduction requires manganese (Mn). Thus, by immobilizing Mn by chelation, glyphosate denies the availability of reduced FMN for the EPSPS enzyme. It also can affect up to 25 other plant enzymes that require Mn as a co-factor and numerous other enzymes in both primary and secondary metabolism that require other metal co-factors (Co, Cu, Fe, Mg, Ni, Zn). Several of these enzymes also function with Mn in the Shikimate pathway that is responsible for plant responses to stress and defense against pathogens (amino acids, hormones, lignin, phytoalexins, flavenoids, phenols, etc.). By inhibiting enzymes in the Shikimate pathway, a plant becomes highly susceptible to various ubiquitous soilborne pathogens (*Fusarium, Pythium, Phytophthora, Rhizoctonia*, etc.). It is this pathogenic activity that actually kills the plant as "the herbicidal mode of action" (Johal and Rahe, 1984; Levesque and Rahe, 1992, Johal and Huber, 2009). <u>If glyphosate is not translocated to the roots because of stem boring insects or other disruption of the vascular system, aerial parts of the plant may be stunted, but the plant is not killed.</u>

Recognizing that glyphosate is a strong chelator to immobilize essential plant micronutrients provides an understanding for the various non-herbicidal and herbicidal effects of glyphosate. Glyphosate is a phloem-mobile, systemic chemical in plants that accumulates in meristematic tissues (root, shoot tip, reproductive, legume nodules) and is released into the rhizosphere through root exudation (from RR as well as non-RR plants) or mineralization of treated plant residues. Degradation of glyphosate in most soils is slow or non-existent since it is not 'biodegradable' and is primarily by microbial co-metabolism when it does occur. Although glyphosate can be rapidly immobilized in soil (also spray tank mixtures, and plants) through chelation with various cat-ions (Ca, Mg, Cu, Fe, Mn, Ni, Zn), it is not readily degraded and can accumulate for years (in both soils and perennial plants). Very limited degradation may be a "safety" feature with glyphosate since most degradation products are toxic to normal as well as RR plants. Phosphorus fertilizers can desorb accumulated glyphosate that is immobilized in soil to damage and reduce the physiological efficiency of subsequent crops. Some of the observed affects of glyphosate are presented in table 1.

TABLE 1. Some things we know about glyphosate that influence plant nutrition and disease.

1. Glyphosate is a strong metal chelator (for Ca, Co, Cu, Fe, Mn, Mg, Ni, Zn) – in the spray tank, in soil and in plants.
2. It is rapidly absorbed by roots, stems, and leaves, and moves systemically throughout the plant (normal and RR).
3. Accumulates in meristematic tissues (root, shoot, legume nodules, and reproductive sites) of normal and RR plants.
4. Inhibits EPSPS in the Shikimate metabolic pathway and many other plant essential enzymes.
5. Increases susceptibility to drought and disease.
6. Non-specific herbicidal activity (broad-spectrum weed control).
7. Some of the applied glyphosate is exuded from roots into soil.
8. Immobilized in soil by chelating with soil cat-ions (Ca, Co, Cu, Fe, Mg, Mn, Ni, Zn).
9. Persists and accumulates in soil and plants for extended periods (years) – it is not 'biodegradable,' but is rapidly immobilized by chelation generally.
10. Desorbed from soil particles by phosphorus and is available for root uptake by all plants.
11. Toxic to soil organisms that facilitate nutrient access, availability, or absorption of nutrients.
12. Inhibits the uptake and translocation of Fe, Mn, and Zn at very low, non-herbicidal rates.
13. Stimulates soilborne pathogenic and other soil microbes to reduce nutrient availability.
14. Reduces secondary cell wall formation and lignin in RR and non-RR plants.
15. Inhibits nitrogen fixation by chelating Ni for ureide synthesis and is toxic to *Rhizoiaceae*.
16. Reduces physiological availability and concentration of Ca, Cu, Fe, K, Mg, Mn, and Zn in plant tissues and seed.
17. Residual soil activity can damage plants through root uptake.
18. Increases mycotoxins in stems, straw, grain, and fruit.
19. Reduces photosynthesis (CO_2 fixation).
20. Causes fruit (bud) drop and other hormonal effects.
21. Accumulates in food and feed products to enter the food chain as an item of food safety.

UNDERSTANDING THE ROUNDUP READY® GENE

Plants genetically engineered for glyphosate-tolerance contain the Roundup Ready® gene(s) that provide an alternate EPSPS pathway (EPSPS-II) that is not blocked by glyphosate. The purpose of these gene inserts is to provide herbicidal selectivity so glyphosate can be applied directly to these plants rather than only for preplant applications. As an additional physiological mechanism, activity of this duplicate pathway requires energy from the plant that could be used for yield. The RR genes are 'silent' in meristematic tissues where glyphosate accumulates so that these rapidly metabolizing tissues are not provided an active alternative EPSPS pathway to counter the physiological effects of glyphosate's inhibition of EPSPS. Meristematic tissues also are areas of high physiologic activity requiring a higher availability of the essential micronutrients needed for cell division and growth that glyphosate immobilizes by chelation.

Residual glyphosate in RR plant tissues can immobilize Fe, Mn, Zn or other nutrients applied as foliar amendments for 8-35 days after it has been applied. This reduces the availability of micronutrients required for photosynthesis, disease resistance, and other critical physiological functions.The presence of the RR gene(s) reduces nutrient uptake and physiological efficiency and may account for some of the 'yield drag' reported for RR crops

when compared with the 'normal' isolines from which they were derived. Reduced physiological efficiency from the RR gene is also reflected in reduced water use efficiency (WUE) and increased drought stress (table 2).

It should be recognized that:

1. **There is nothing in the glyphosate-tolerant plant that operates on the glyphosate applied to the plant.**
2. **All the technology does is insert an alternative enzyme (EPSPS-II) that is not blocked by glyphosate in mature tissue.**
3. **When glyphosate enters the plant, it is not selective; it chelates with a host of elements influencing nutrient availability, disease resistance, and the plant's other physiological functions.**
4. **Glyphosate is present for the life of the plant or until it is exuded into soil or groundwater through the roots. Degradation products are toxic to RR and non-RR plants.**

TABLE 2. Some things we know about the glyphosate-tolerance (RR) gene(s).

1. Provides selective herbicidal activity for glyphosate.
2. Inserts an alternative EPSPS pathway that is not sensitive to glyphosate action in mature tissue.
3. Reduces the plant's physiological efficiency of Fe, Mn, Ni, Zn, etc.
4. Inactive (silent) in meristematic tissues (root and shoot tips, legume root nodules, and reproductive tissues).
5. Reduces nutrient uptake and efficiency.
6. Increases drought stress.
7. Reduces N-fixation.
8. Lowers seed nutrient content.
9. Transferred in pollen to plants, and from degrading plant tissues to microbes.
10. Generally causes a yield 'drag' compared with near-isogenic normal plants from which it was derived.
11. Has greatly increased the application of glyphosate.
12. Permanent in plants once it is introduced.

INTERACTIONS OF GLYPHOSATE WITH PLANT NUTRITION

Glyphosate can affect nutrient efficiency in the plant by chelating essential nutrient co-factors after application since there is many times more 'free' glyphosate in the plant than all of the unbound cat-ions. Chelation of Mn and other micronutrients after application of glyphosate is frequently observed as a 'flashing' or yellowing that persists until the plant can 'resupply' the immobilized nutrients. The duration of 'flashing' is correlated with the availability of micronutrients in soil. Symptom remission indicates a resumption of physiological processes, but is not an indicator of plant nutrient sufficiency since micronutrient deficiencies are commonly

referred to as ‘hidden hunger.’ As a strong nutrient chelator, glyphosate can reduce physiological efficiency by immobilizing elements required as components, co-factors or regulators of physiological functions at very low rates. Thus, plant uptake and or translocation of Fe, Mn and Zn are drastically reduced (up to 80 %) by commonly observed ‘drift’ rates of glyphosate (<1/40 the herbicidal rate). This is reflected in reduced physiological efficiency, lower mineral nutrient levels in vegetative and reproductive tissues, and increased susceptibility to disease. Microbial and plant production of siderophores and ferric reductase in root exudates under nutrient stress are inhibited by glyphosate to exacerbate plant nutrient stress common in low-available micronutrient soils.

Glyphosate is not readily degraded in soil and can probably accumulate for many years chelated with soil cat-ions. Degradation products of glyphosate are as damaging to RR crops as to non-RR crops. Persistence and accumulation of glyphosate in perennial plants, soil, and root meristems, can significantly reduce root growth and the development of nutrient absorptive tissue of RR as well as non-RR plants to further impair nutrient uptake and efficiency. Impaired root uptake not only reduces the availability of specific nutrients, but also affects the natural ability of plants to compensate for low levels of many other nutrients. Glyphosate also reduces nutrient uptake from soil indirectly through its toxicity to many soil microorganisms responsible for increasing the availability and access to nutrients through mineralization, reduction, symbiosis, etc.

Degradation of plant tissues through growth, necrosis, or mineralization of residues can release accumulated glyphosate from meristematic tissues in toxic concentrations to plants. The most damaging time to plant wheat in ryegrass ‘burned down’ by glyphosate is two weeks after glyphosate application to correspond with the release of accumulated glyphosate from decomposing meristematic tissues. This is contrasted with the need to delay seeding of winter wheat for 2-3 weeks after a regular weed burn-down’ to permit time for immobilization of glyphosate from root exudates and direct application through chelation with soil cat-ions. The Roundup® label for Israel lists recommended waiting times before planting a susceptible crop on that soil.

One of the benefits of crop rotation is an increased availability of nutrients for a subsequent crop in the rotation. The high level of available Mn (130 ppm) after a normal corn crop is not observed after glyphosate-treated RR corn. The lower nutrient availability after specific RR crop sequences may need to be compensated for through micronutrient application in order to optimize yield and reduce disease in a subsequent crop.

THE INFLUENCE OF GLYPHOSATE ON SOIL ORGANISMS IMPORTANT FOR ACCESS, MINERALIZATION, SOLUBILIZATION, AND FIXATION OF ESSENTIAL PLANT NUTRIENTS

Glyphosate is a potent microbiocide and is toxic to earthworms, mycorrhizae (P & Zn uptake), reducing microbes that convert insoluble soil oxides to plant available forms (Mn and Fe, *Pseudomonads, Bacillus*, etc.), nitrogen-fixing organisms (*Bradyrhizobium, Rhizobium*), and organisms involved in the ‘natural,’ biological control of soilborne diseases that reduce root uptake of nutrients. Although glyphosate contact with these organisms is limited by rapid chelation-immobilization when applied on fallow soil; glyphosate in root exudates, or from decaying weed tissues or RR plants, contacts these organisms in their most active ecological

habitat throughout the rhizosphere. It is not uncommon to see Cu, Fe, Mg, Mn, Ni, and Zn deficiencies intensify and show in soils that were once considered fully sufficient for these nutrients. Increasing the supply and availability of Co, Cu, Fe, Mg, Mn, Ni, and Zn have reduced some of the deleterious effects of glyphosate on these organisms and increased crop yields.

In contrast to microbial toxicity, glyphosate in soil and root exudates stimulates oxidative soil microbes that reduce nutrient availability by decreasing their solubility for plant uptake, immobilize nutrients such as K in microbial sinks to deny availability for plants, and deny access to soil nutrients through pathogenic activity. Plant pathogens stimulated by glyphosate (table 3) include ubiquitous bacterial and fungal root, crown, and stalk rotting fungi; vascular colonizing organisms that disrupt nutrient transport to cause wilt and die-back; and root nibblers that impair access or uptake of soil nutrients.

TABLE 3. Some plant pathogens stimulated by glyphosate.

Botryospheara dothidea	*Gaeumannomyces graminis*
Corynespora cassicola	*Magnaporthe grisea*
Fusarium species	*Marasmius spp.*
F. avenaceum	*Monosporascus cannonbalus*
F. graminearum	*Myrothecium verucaria*
F. oxysporum f.sp. cubense	*Phaeomoniella chlamydospora*
F. oxysporum f.sp. (canola)	*Phytophthora spp.*
F. oxysporum f.sp. glycines	*Pythium spp.*
F. oxysporum f.sp. vasinfectum	*Rhizoctonia solani*
F. solani f.sp. glycines	*Septoria nodorum*
F. solani f.sp. phaseoli	*Thielaviopsis bassicola*
F. solani f.sp. pisi	*Xylella fastidiosa*

Clavibacter michiganensis subsp. *nebraskensis* (Goss' wilt)

HERBICIDAL MODE OF ACTION OF GLYPHOSATE

As a strong metal micronutrient chelator, glyphosate inhibits activity of EPSPS and other enzymes in the Shikimate metabolic pathway responsible for plant resistance to various pathogens. Plant death is through greatly increased plant susceptibility of non-RR plants to common soilborne fungi such as *Fusarium*, *Rhizoctonia*, *Pythium*, *Phytophthora*, etc. that are also stimulated by glyphosate (Johal and Rahe, 1984; Levesque and Rahe, 1992; Johal and Huber, 2009). It is very difficult to kill a plant in sterile soil by merely shutting down the Shikimate pathway (secondary metabolism) unless soilborne pathogens are also present. It is the increased susceptibility to soilborne pathogens, and increased virulence of the pathogens, that actually kills the plants after applying glyphosate. Disease resistance in plants is manifest through various active and passive physiological mechanisms requiring micronutrients. Those metabolic pathways producing secondary anti-microbial compounds (phytoalexins, flavenoids, etc.), pathogen inhibiting amino acids and peptides, hormones involved in cicatrisation (walling off pathogens), callusing, and disease escape mechanisms can all be compromised by glyphosate chelation of micronutrient co-factors critical for enzyme function. Genetic modification of

plants for glyphosate tolerance partially restores Shikimate pathway function to provide a selective herbicidal effect.

INTERACTIONS OF GLYPHOSATE WITH PLANT DISEASE

Micronutrients are the regulators, activators, and inhibitors of plant defense mechanisms that provide resistance to stress and disease. Chelation of these nutrients by glyphosate compromises plant defenses and increases pathogenesis to increase the severity of many abiotic (bark cracking, nutrient deficiencies) as well as infectious diseases of both RR and non-RR plants in the crop production system (table 4). Many of these diseases are referred to as 'emerging' or reemerging' diseases because they rarely caused economic losses in the past, or were effectively controlled through management practices.

Non-infectious (Abiotic) Diseases: Research at Ohio State University has shown that bark cracking, sunscald, and winter-kill of trees and perennial ornamentals is caused by glyphosate used for under-story weed control, and that glyphosate can accumulate for 8-10 years in perennial plants. This accumulation of glyphosate can be from the inadvertent uptake of glyphosate from contact with bark (drift) or by root uptake from glyphosate in weed root exudates in soil. Severe glyphosate damage to trees adjacent to stumps of cut trees treated with glyphosate (to prevent sprouting in an effort to eradicate citrus greening or CVC) can occur through root translocation and exudation several years after tree removal.

Infectious Diseases: Increased severity of the take-all root and crown rot of cereals (*Gaeumannomyces graminis*) after prior glyphosate usage has been observed for over 20 years and take-all is now a 'reemerging' disease in many wheat producing areas of the world where glyphosate is used for weed control prior to cereal planting. A related disease of cereals, and the cause of rice blast (*Magnaporthe grisea*), is becoming very severe in Brazil and is especially severe when wheat follows a RR crop in the rotation. Like take-all and Fusarium root rot, this soilborne pathogen also infects wheat and barley roots, and is a concern for U.S. cereal production.

Fusarium species causing head scab are common root and crown rot pathogens of cereals everywhere; however, Fusarium head scab (FHB) has generally been a serious disease of wheat and barley only in warm temperate regions of the U.S. With the extensive use of glyphosate, it is now of epidemic proportions and prevalent throughout most of the cereal producing areas of North America. Canadian research has shown that the application of glyphosate one or more times *in the three years previous to planting wheat* was the most important agronomic factor associated with high FHB in wheat, with a 75 % increase in FHB for all crops and a 122 % increase for crops under minimum-till where more glyphosate is used. The most severe FHB occurs where a RR crop precedes wheat in the rotation for the same reason. Glyphosate altered plant physiology (carbon and nitrogen metabolism) increasing susceptibility of wheat and barley to FHB and increased toxin production, is also associated with a transient tolerance of wheat and soybeans to rust diseases.

The increased FHB with glyphosate results in a dramatic increase in tricothecene (deoxynivalenol, nivalenol, 'vomitoxins') and estrogenic (zaeralenone) mycotoxins in grain; however, the high concentrations of mycotoxin in grain are not always associated with *Fusarium*

infection of kernels. Quite often overlooked is the increase in root and crown rot by FHB *Fusaria* with glyphosate and the production of mycotoxins in root and crown tissues with subsequent translocation to stems, chaff and grain. Caution has been expressed in using straw and chaff as bedding for pigs or roughage for cattle because of mycotoxin levels that far exceeded clinically significant levels for infertility and toxicity. This also poses a health and safety concern for grain entering the food chain for humans. The list of diseases affected by glyphosate (see reference No. 18) is increasing as growers and pathologists recognize the cause-effect relationship.

SPECIAL NUTRIENT CONSIDERATIONS IN A GLYPHOSATE-DOMINANT WEED MANAGEMENT ECOLOGICAL SYSTEM

There are two things that should be understood in order to remediate nutrient deficiencies in a glyphosate usage program: 1) the effects of glyphosate on nutrient availability and function and 2) the effect of the RR gene on nutrient efficiency. With this understanding, there are four objectives for fertilization in a glyphosate environment – all of which indicate a more judicious use of glyphosate as part of the remediation process. These four objectives are to:

1. Provide adequate nutrient availability for full functional sufficiency to compensate for glyphosate and RR reduced availability or physiological efficiency of micronutrients (esp. Mn and Zn but also Cu, Fe, Ni).
2. Detoxify residual glyphosate in meristematic and other tissues, in root exudates, and in soil by adding appropriate elements for chelation with the residual glyphosate.
3. Restore soil microbial activity to enhance nutrient availability, supply, and balance that are inhibited by residual glyphosate in soil and glyphosate in root exudates.
4. Increase plant resistance to root infecting and reemerging diseases through physiological plant defense mechanisms dependent on the Shikimate, amino acid, and other pathways that are compromised by micronutrient inefficiency in a glyphosate environment.

Meeting Nutrient Sufficiency: Extensive research has shown that increased levels and availability of micronutrients such as Mn, Zn, Cu, Fe, Ni, etc can compensate for reduced nutrient efficiency and the inefficiency of RR crops. This need may not be manifest in high fertility or nutrient toxic soils for a few years after moving to a predominantly monochemical strategy. The timing for correcting micronutrient deficiencies is generally more critical for cereal plants (barley, corn, wheat) than for legumes in order to prevent irreversible yield and/or quality loss. Nutrient sufficiency levels from soil and tissue analysis that are considered adequate for non-GM crops may need to be increased for RR crops to be at full physiological sufficiency. Since residual 'free' glyphosate in RR plant tissues can immobilize most regular sources of foliar-applied micronutrients for 8-15 days, and thereby reduce the future availability of these materials, it may be best to apply some micronutrients 1-2 weeks after glyphosate is applied to RR crops.

The expense of an additional trip across the field for foliar application frequently deters micronutrient fertilization for optimum crop yield and quality. There are newly available micronutrient formulations (nutrient phosphites) that maintain plant availability without impacting herbicidal activity of the glyphosate in a tank-mix, and plants have responded well from these micronutrient-glyphosate mixes. Simultaneous application of some micronutrients

with glyphosate might provide an efficient means to overcome deficiencies in low fertility soils, as well as mitigate the reduced physiological efficiency inherent with the glyphosate-tolerant gene and glyphosate immobilization of essential nutrients in the plant.

Under severe micronutrient deficiency conditions, selecting seed high in nutrient content or a micronutrient seed treatment to provide early nutrient sufficiency, establish a well-developed root system, and insure a vigorous seedling plant with increased tolerance to glyphosate applied later, has been beneficial even though excess nutrient applied at this time may be immobilized by glyphosate from root exudates and not available for subsequent plant uptake. Micronutrients such as Mn are not efficiently broadcast applied to soil for plant uptake because of microbial immobilization to non-available oxidized Mn, but could be applied in a band or to seed or foliage.

Detoxifying Residual Glyphosate: Some nutrients are relatively immobile in plant tissues (Ca, Mn) so that a combination of micronutrients may be more beneficial than any individual one to chelate with residual glyphosate and 'detoxify' it in meristematic and mature tissues. Thus, foliar application of Mn could remediate for glyphosate immobilization of the nutrient; however, it may be more effective when applied in combination with the more mobile Zn to detoxify sequestered glyphosate in meristematic tissues even though Zn levels may appear sufficient. Gypsum applied in the seed row has shown some promise for detoxifying glyphosate from root exudates since Ca is a good chelator with glyphosate (one of the reasons that ammonium sulfate is recommended in spray solutions with hard water is to prevent chelation with Ca and Mg which would inhibit herbicidal activity).

Although bioremediation of accumulating glyphosate in soil may be possible in the future, initial degradation products of glyphosate are toxic to both RR and non-RR plants. This is an area that needs greater effort since the application of phosphorus fertilizers can desorb immobilized glyphosate to be toxic to plants through root uptake. Micronutrient seed treatment can provide some detoxification during seed germination, and stimulate vigor and root growth to enhance recovery from later glyphosate applications.

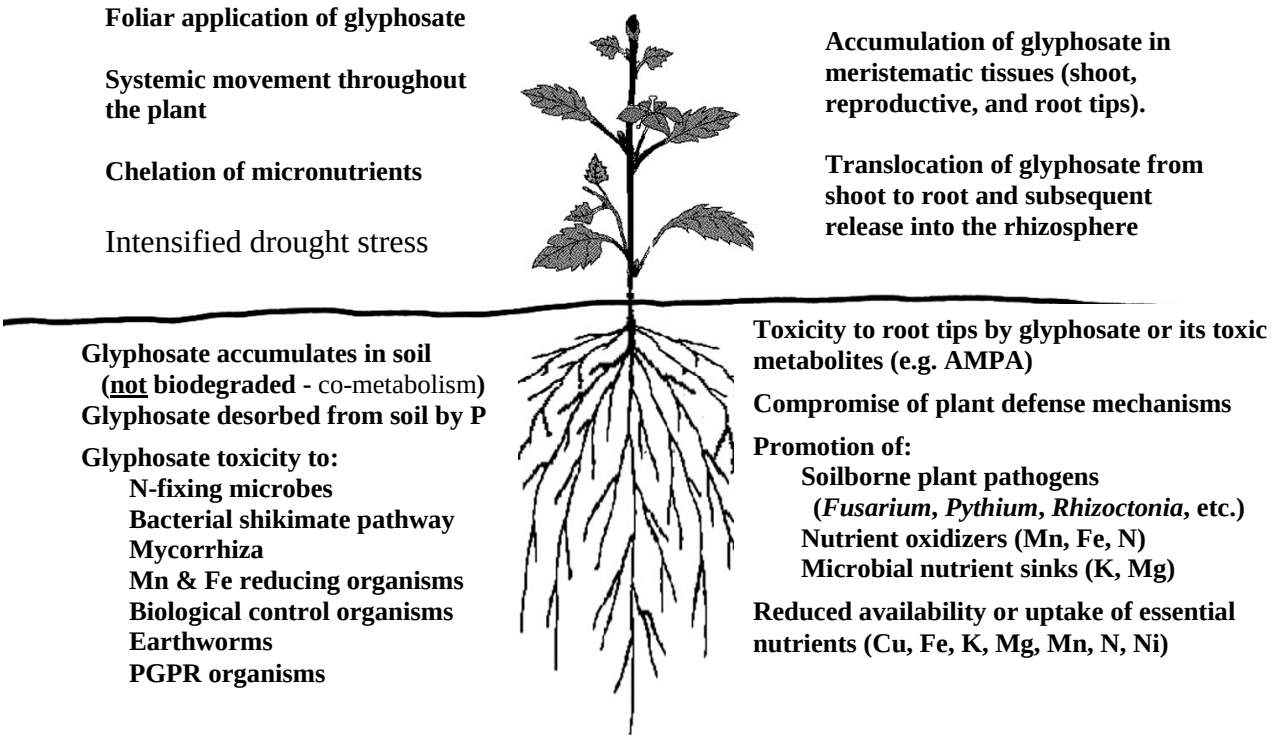
Biological Remediation: The selection and use of plants for glyphosate-tolerance that have greater nutrient efficiency for uptake or physiological function has improved the performance of some RR crops, and further improvements are possible in this area. Enhancing soil microbial activity to increase nutrient availability and plant uptake has been possible through seed inoculation, environmental modification to favor certain groups of organisms, and implementation of various management practices. There are many organisms that have been used to promote plant growth, with the most recognized being legume inoculants (*Rhizobia, Bradyrhizobia* species); however, glyphosate is toxic to these beneficial microorganisms. Continued use of glyphosate in a cereal-legume rotation has greatly reduced the population of these organisms in soil so that annual inoculation of legume seed is frequently recommended.

Biological remediation to compensate for glyphosate's impact on soil organisms important in nutrient cycles may be possible if the remediating organism is also glyphosate-tolerant and capable of over coming the soils natural biological buffering capacity. This would be especially important for nitrogen-fixing, mycorrhizae, and mineral reducing organisms, but will be of limited benefit unless the introduced organisms are also tolerant of glyphosate. Modification of the soil biological environment through tillage, crop sequence, or other cultural management practices might also be a viable way to stimulate the desired soil biological activity.

Increasing Plant Resistance to Stress and Root-Infecting Pathogens: Maintaining plant health is a basic requirement for crop yield and quality. Plant tolerance to stress and many pathogens is dependent on a full sufficiency of micronutrients to maintain physiological processes mediated through the Shikimate or other pathways that are compromised in a glyphosate environment. Sequential application(s) of specific micronutrients (esp. Ca, Cu, Fe, Mn, Zn) may be required to compensate for those nutrients physiologically lost through glyphosate chelation. Breeding for increased nutrient efficiency and disease resistance will be an important contributor to this objective.

SUMMARY

Glyphosate is a strong, broad-spectrum nutrient chelator that inhibits plant enzymes responsible for disease resistance so that plants succumb from pathogenic attack. This also predisposes RR and non-RR plants to other pathogens. The introduction of such an intense mineral chelator as glyphosate into the food chain through accumulation in feed, forage, and food, and root exudation into ground water, could pose significant health concerns for animals and humans and needs further evaluation. Chelation immobilization of such essential elements as Ca (bone), Fe (blood), Mn, Zn (liver, kidney), Cu, Mg (brain) could directly inhibit vital functions and predispose to disease. The lower mineral nutrient content of feeds and forage from a glyphosate-intense weed management program can generally be compensated for through mineral supplementation. The various interactions of glyphosate with nutrition are represented in the following schematic:



Schematic of glyphosate interactions in soil

SELECTED REFERENCES

1. Arregui, M.C., Lenardon, A., Sanchez, D., Maitre, M.I., Scotta, R., and Enrique, S. 2003. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. Pest Manag. Sci. 60:163-166.
2. Bernards, M.L. Thelen, K.D., Muthukumaran, R.J. and McCracker, J.L. 2005. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. Weed Sci. 53:787-794.
3. Bellaloui, N., Reddy, K.N., Zablotowicz, R.M., Abbas, H.K., and Abel, C.A. 2009. Effects of glyphosate application on seed iron and root ferric (III) reductase in soybean cultivars. J. Agric. Food Chem. 57:9569-9574.
4. Bott, S., Tesfamariam, T., Candan, H., Cakmak, I., Roemheld, V., and Neumann, G. 2008. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). Plant Soil 312:185-194.
5. Cakmak, I., Yazici, A., Tutus, Y., and Ozturk, L. 2009. Glyphosate reduced seed and leaf concentrations of calcium, magnesium, manganese, and iron in non-glyphosate resistant soybean. European J. Agron. 31:114-119.
6. Comeau, A., Pageau, D., Voldeng, H., and Brunelle, A. 2005. Micronutrients: essential for early canopy establishment in bread wheat. EECCO poster, Ottawa, Canada.
7. Datnoff, L.E., Elmer, W.H., and Huber, D.M. 2007. Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN, 278 pages.
8. Duke, S.O., Rimando, A.M., Pace, P.F., Reddy, K.N., and Smeda, R.J. 2003. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean, J. Agric. Food Chem. 51:340-344.
9. Eker, S., Ozturk, L, Yazici, A., Erenoglu, B., Roemheld, V., and Cakmak, I. 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. J. Agric. Food Chem. 54:10019-10025.
10. Fernandez, M.R., Selles, F., Gehl, D., DePauw, R.M., and Zentner, R.P. 2005. Crop production factors associated with Fusarium head blight in spring wheat in eastern Saskatchewan. Crop Sci. 45:1908-1916.
11. Fernandez, M.R., Zentner, R.P., Basnyat, P., Gehl, D., Selles, F., and Huber, D.M. 2009. Glyphosate associations with cereal diseases caused by *Fusarium* spp. in the Canadian Prairies. European J. Agron. 31:133-143.
12. Ganson, R.J. and Jensen, R.A. 1988. The essential role of cobalt in the inhibition of the cytosolic isozyme of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase from *Nicotiana silvestris* by glyphosate. Arch Biochem. Biophys. 260:85-93.
13. Gordon, W.B. 2007. Does (the) glyphosate gene affect manganese uptake in soybeans? Fluid J. Early Spring:12-13.
14. Hernandez, A., Garcia-Plazaola, J.I., and Bacerril, J.M. 1999. Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L. Merril). J. Agric. Food Chem. 47:2920-2925.
15. Huber, D.M., Leuck, J.D., Smith, W.C., and Christmas, E.P. 2004. Induced manganese deficiency in GM soybeans. North central Fert. Exten. Conf., November 2004, Des Moines, IA.
16. Huber, D.M. and Haneklaus, S. 2007. Managing nutrition to control plant disease. Landbauforschung Volkenrode 57:4:313-322.

17. Johal, G.R. and Rahe, J.E. 1984. Effect of soilborne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology 74:950-955.
18. Johal, G.R. and Huber, D.M. 2009. Glyphosate effects on diseases of plants. European J. Agron. 31:144-152.
19. Johnson, W.G., Davis, V.M., Kruger, G.R., and Weller, S.C. 2009. Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. European J. Agron. 31:162-172.
20. King, C.A., Purcell, L.C., and Vories, E.D. 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. Agron. J. 93:79-186.
21. Kremer, R.J., Means, N.E., and Kim, S. 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. Inter. J. Environ. Anal. Chem. 85:1165-1174.
22. Kremer, R.J. and Means, N.E. 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. European J. Agron. 31:153-161.
23. Laitinen, P., Ramo, S., and Siimes, K. 2005. Glyphosate translocation from plants to soil – does this constitute a significant proportion of residues in soil? Plant Soil 300:51-60.
24. Larsen, R.L., Hill, A.L., Fenwick, A., Kniss, A.R., Hanson, L.E., and Miller, S.D. 2006. Influence of glyphosate on Rhizoctonia and Fusarium root rot in sugar beet. Pest Manag. Sci. 62:1182-1192.
25. Levesque, C.A. and Rahe, J.E. 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Ann. Rev. Phytopathol. 30:579-602.
26. Nilsson, G. 1985. Interactions between glyphosate and metals essential for plant growth. In: Grossbard E. and Atkinson, D. (eds.) The Herbicide Glyphosate. Butterworth, London. Pp 35-47.
27. Ozturk, L., Yazici, A. Eker, S., Gokmen, O., Roemheld, V., and Cakmak, I. 2008. Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots. New Phytol. 177:899-906.
28. Reddy, K.N., Hoagland, R.E., and Zablotowicz, R.M. 2000). Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (*Glycine max*) varieties. J. New Seeds 2:37-52.
29. Reddy, K.N. and Zablotowicz, R.M. 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. Weed Sci. 51:496-502.
30. Reddy, K.N., Rimando, A.M., and Duke, S.O. 2004. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. J. Agric. Food Chem. 52:5139-5143.
31. Reichenberger, L. 2007. Missing micronutrients: Using glyphosate is complicating the uptake of some minor nutrients. The Furrow pp. 22-23.
32. Rodrigues, J.J.V., Worsham, A.D., and Corbin, F.T. 1982. Exudation of glyphosate from wheat (*Triticum aestivum*) plants and its effects on interplanted corn (*Zea mayes*) and soybean (*Glycine max*). Weed Tech. 30:316-320.
33. Tesfamariam, T., Bott, S, Cakmak, I., Roemheld, V., and G. Neumann. 2009. Glyphosate in the rhizosphere – role of waiting times and different glyphosate binding forms in soils for phytoxicity to non-target plants. European J. Agron. 31:126-132.
34. de Vendomois, J.S., Roullier, F., Cellier, D., and Seralini, G-E. 2009. A comparison of the effects of three GM corn varieties on mammalian health. Int. J. Biol. Sci. 5:706-726.

35. Waltz, E. 2009. Battlefield; Papers suggesting that biotech crops might harm the environment attract a hail of abuse from other scientists. Nature 461/3:27-32.
36. Yamada, T., Kremer, R.J., Camargo e Castro, P.R., and Wood, B.W. 2009. Glyphosate interactions with physiology, nutrition, and diseases of plants: Threat to agricultural sustainability? European J. Agron. 31:111-113.
37. Zablotowicz, R.M. and Reddy, K.N. 2007. Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. Crop Prot. 26:370-376.
38. Zobiole, L.H.S., Oliveira, R.S. Jr., Huber, D.M., Constantin, J., Castro, C., Oliveira, F.A., Oliveira, A. Jr. 2010. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. Plant Soil 328:57-69
39. Zobiole, L.H.S., Oliveira, R.S. Jr., Kremer, R.J., Constantin, J., Bonato, C.M., and Muniz, A.S. 2010. Water use efficiency and photosynthesis as affected by glyphosate application to glyphosate-resistant soybean. Pesticide Biochem. Physiol. (In Press).
40. Zobiole, L.H.S., Bonini, E.A., Oliveira, R.S. Jr., Kremer, R.J., and Ferrarese-Filho, O. 2010. Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean. Acta Physiol. Plant. (In Press).
41. Zobiole, L.H.S., Oliveira, R.S., Kremer, R.J., Constantin, J., Yamada, T., Castro, C., Oliveira, F.A., and Oliveira, A. Jr. 2010. Effect of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans. Applied Soil Ecol. 44:176-180.
42. Zobiole, L.H.S., Oliveira, Jr., R.S., Kremer, R.J., Muniz, A.S., and Oliveira Jr., A. 2010. Nutrient accumulation and photosynthesis in glyphosate resistant soybeans is reduced under glyphosate use. J. Plant Nutr. (In Press).
43. Zobiole, L.H.S., Oliveira Jr., Constantin, J., R.S., Kremer, R.J., Biffe, D.F. 2010. Amino application can be an alternative to prevent glyphosate injury. J. Plant Nutr. (In Press).
44. Zobiole, L.H.S., Oliveira Jr., Visentainer, J.V., Kremer, R.J., Yamada, T., Bellaloui, N. 2010. Glyphosate affects seed composition in glyphosate-resistant soybean. J. Agric. Food chem.. (In Press).

Table X. Some symptoms of glyphosate damage to non-target plants.

1. Micronutrient (and often some macronutrient) deficiency
2. Low vigor, slow growth, stunting
3. Leaf chlorosis (yellowing) – complete or between the veins
4. Leaf mottling with or without necrotic spots
5. Leaf distortion – small, curling, strap-like, wrinkling, or ‘mouse ear’
6. Abnormal bud break, stem proliferation – witches broom
7. Retarded, slow regrowth after cutting or running (alfalfa, perennial plants)
8. Lower yields, lower mineral value – vegetative parts and reproductive (grain, seeds)
9. Early fruit, bud, or leaf drop
10. Early maturity, death before physiological maturity, tip die-back
11. Predisposition to infectious diseases and extended infection/susceptible period– numerous
12. Predisposition to insect damage
13. Induced abiotic diseases – drought, winter kill, sun scald, bark cracking (perennial plants)
14. Root stunting, inefficient N-fixation and uptake
15. Poor root nodulation in legumes

Proceedings Fluid Fertilizer Forum, Scottsdale, AZ February 14-16, 2010. Vol. 27. Fluid Fertilizer Foundation, Manhattan, KS.

Europ. J. Agronomy 31 (2009) 133–143

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Glyphosate associations with cereal diseases caused by *Fusarium* spp. in the Canadian Prairies

M.R. Fernandez[a,*], R.P. Zentner[a], P. Basnyat[a], D. Gehl[b], F. Selles[c], D. Huber[d]

[a] *Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, SK S9H 3X2, Canada*
[b] *Indian Head Research Farm, Agriculture and Agri-Food Canada, P.O. Box 760, Indian Head, SK S0G 2K0, Canada*
[c] *Brandon Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1000A, Brandon, MB R7A 5Y3, Canada*
[d] *Purdue University, Botany and Plant Pathology Department, 915 West State Street, West Lafayette, IN 47907-2054, United States*

ARTICLE INFO

Article history:
Received 28 May 2008
Received in revised form 2 July 2009
Accepted 6 July 2009

Keywords:
Fusarium head blight
Fusarium root rot
Fusarium graminearum
Cochliobolus sativus
Wheat
Barley
Herbicide
Glyphosate

ABSTRACT

Fusarium pathogens cause important diseases, such as root/crown rot and Fusarium head blight (FHB), in cereal crops. These diseases can be caused by similar *Fusarium* spp. Common root rot (CRR) is widespread in the western Canadian Prairies, whereas FHB has potential of becoming an important disease in this region. There are no commercially available cereal cultivars with good resistance to these diseases. It is therefore important to identify agronomic practices that could affect levels of *Fusarium* pathogens in cereals. This review deals primarily with the effects of tillage systems and glyphosate use on the development of FHB and CRR in wheat and barley in eastern Saskatchewan. Although the FHB study in 1999–2002 indicated that environment was the most important factor determining FHB development, previous glyphosate use and tillage practice were among the production factors with the greatest association with FHB. Overall, disease was highest in crops under minimum-till management. Previous glyphosate use was consistently associated with higher FHB levels caused by the most important FHB pathogens, *Fusarium avenaceum* and *Fusarium graminearum. Cochliobolus sativus*, the most common CRR pathogen, was negatively associated with previous glyphosate use, while *F. avenaceum*, *F. graminearum*, and other fungi were positively associated, suggesting that glyphosate might cause changes in fungal communities. The occurrence and isolation of *F. avenaceum* from cereal residues were greater under reduced-till than conventional-till while *C. sativus* was most common under conventional-till, and *F. graminearum* was lowest under zero-till. Previous glyphosate applications were again correlated positively with *F. avenaceum* and negatively with *C. sativus*. These observations agreed with results from the FHB and CRR studies. These are the first studies that established a relationship between previous glyphosate use and increased *Fusarium* infection of spikes and subcrown internodes of wheat and barley, or *Fusarium* colonization of crop residues. However, because of the close association between noncereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on *Fusarium* infections. Determining the relative contribution of these popular production trends to the development of diseases caused by *Fusarium* spp. are essential for devising appropriate agronomic recommendations to prevent their further spread in western Canada, and to reduce the impact that these diseases are having in areas where they are already established. The consistent association between previous glyphosate use and *Fusarium* infections also warrants further research to elucidate the nature of this association and the underlying mechanisms determining these effects.

1. Introduction

Fusarium pathogens cause important diseases of cereal crops in western Canada. Root and crown rot (Fernandez and Jefferson, 2004) and Fusarium head blight (FHB), also known as scab or tombstone (Gilbert and Tekauz, 2000), can be especially severe. Common root rot (CRR) is a prevalent disease throughout the western Canadian Prairies (Ledingham et al., 1973). In the province of Saskatchewan, root and crown rot is generally caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur (anamorph *Bipolaris sorokiniana* [Sacc.] Shoemaker) and *Fusarium* spp. (Fernandez and Jefferson, 2004). *Fusarium avenaceum* (Fr.:Fr.) Sacc. (teleomorph *Gibberella avenacea* Cook) is one of such species found in underground and ground-level tissue of common (*Triticum aestivum* L.) and durum (*T. turgidum* L. ssp. *durum* [Desf.] Husn.) wheat (Fernandez and Jefferson, 2004; Fernandez and Zentner, 2005; Fernandez et al., 2007a). This pathogen is also frequently isolated from discoloured roots of noncereal crops, being found at high-

* Corresponding author. Tel.: +1 306 778 7255; fax: +1 306 778 3188.
E-mail address: myriam.fernandez@agr.gc.ca (M.R. Fernandez).

1161-0301/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2009.07.003

est levels in pulse crops (Fernandez, 2007). Many of the *Fusarium* isolates in discolored subcrown internodes or crowns are also associated with FHB in wheat and barley (*Hordeum vulgare* L.) (Fernandez et al., 2002a,b).

Of the several *Fusarium* species that can cause FHB, the most important pathogen in North America is *F. graminearum* Schwabe (teleomorph *G. zeae* [Schwein.] Petch). This pathogen produces mycotoxins harmful to humans and livestock. The most commonly found mycotoxin in infected grain is deoxynivalenol (DON). Tolerance levels for *Fusarium*-damaged kernels (FDK) are very low due to processing problems and potential food safety concerns. For example, FDK greater than 0.25% by weight will cause Canada Western Red Spring (CWRS) class of wheat, to be downgraded from CWRS #1 to CWRS #2. A FDK value of over 1% down grades it to CWRS #3, and over 2% to CWRS #4 (Canadian Grain Commission, 2007). For malting barley, the tolerance for FDK is nil for Super Select and 0.2% for Select, whereas FDK for feed barley is 1%. These low tolerance levels cause significant economic losses to producers in affected areas.

Relative to other *Fusarium* pathogens, *F. graminearum* has been less commonly isolated from infected cereal spikes and kernels in western regions of the Canadian Prairies than in areas where this disease has been historically more prevalent. The other *Fusarium* pathogens commonly found in Saskatchewan are also mycotoxin producers. Among these are *F. avenaceum*, *F. culmorum* (W.G. Smith) Sacc., and *F. poae* (Peck) Wollenw., with *F. avenaceum* reported as the most, or one of the most, common species in infected spikes and kernels of wheat and barley (Clear et al., 2000; Fernandez et al., 2003, 2007d; Pearse et al., 2007b; Turkington et al., 2002). Although neither *F. avenaceum* nor *F. poae* produce DON, they produce other harmful mycotoxins (Abramson et al., 2002).

Unfavourable weather conditions have caused FHB to occur at lower levels in the last few years in Saskatchewan (Pearse et al., 2007a,b) than in the late 1990s and early 2000s when province-wide surveys showed the disease to be well established in wheat and barley in eastern regions of the province and spreading westward (Fernandez et al., 1999, 2000, 2001, 2002a,b; Pearse et al., 2003). Thus, FHB still has potential to spread further west, and adversely impact production and marketing opportunities for wheat and barley throughout the western Prairies when conditions are favourable for its development.

There are no commercially available wheat or barley cultivars with good resistance to FHB registered in western Canada (Fernandez et al., 2005, 2007d). Chemical treatment has proven inconsistent or ineffective in controlling FHB and/or preventing its spread.

Because of the continued importance of FHB in the eastern Prairies, and its potential to spread westward, strategies need to be designed to stop or reduce the rate of spread, and to decrease the damage it causes in areas where it is already well established. Understanding the impact of agronomic practices on disease and inoculum levels should form part of comprehensive strategies aimed at controlling FHB. A comprehensive strategy should also include the role of *Fusarium* infection of crop roots and crowns as sources of fungal inoculum and its potential carryover from one growing season to the next.

The adoption and use of conservation tillage (minimum- till and zero-till) practices have become widespread throughout western Canada (Zentner et al., 2002). These tillage methods are heavily dependent on the use of glyphosate formulations for weed control. Thus, it is also important to determine the possible impact that this increased use of glyphosate might have on the development of FHB.

Several studies have examined the effect of tillage practice on FHB or FDK and associated DON levels in wheat in other regions in North America and elsewhere (Dill-Macky and Jones, 2000; Krebs et al., 2000; Miller et al., 1998; Schaafsma et al., 2001; Yi et al., 2001). These findings vary with respect to the impact that tillage and amount of crop residue have on disease levels, with no difference among tillage systems often observed (Miller et al., 1998; Teich and Nelson, 1984). There are few reported studies on the impact of tillage system on FHB in barley. In studies conducted in Quebec, Rioux et al. (2005) found that DON content was greater in barley grown under minimum till than conventional-till management.

The effect of herbicides on FHB development has not been extensively examined. Teich and Hamilton (1985) and Teich and Nelson (1984) reported that there was no significant difference in disease levels in wheat fields with or without herbicides, but they did not identify the specific herbicides used. Difficulties in evaluating the possible effect of glyphosate or other herbicides on FHB include a lack of information regarding type, time and dose of herbicide applied, and lack of adequate glyphosate-free controls.

Severity of CRR was not affected by tillage method (Bailey et al., 2001; Conner et al., 1987) or declined in reduced tillage systems (Bailey et al., 2000; Tinline and Spurr, 1991). Furthermore, higher levels of *C. sativus*, and lower levels of *Fusarium* spp., in wheat or barley roots were reported with changes from less to more intensive tillage (Bailey et al., 2000, 2001; Windels and Wiersma, 1992). Although the effect of several herbicides on CRR pathogens was examined (Hsia and Christensen, 1951; Isakeit and Lockwood, 1989; Tinline and Hunter, 1982), the impact of glyphosate usage on this disease has not been reported.

The overall objective of our research was to identify agronomic practices associated with the development of high FHB levels in wheat and barley. Because of the possible impact that *Fusarium* spp. in underground plant tissue might have on the development of FHB and persistence of inoculum in the field, the objectives included an examination of the impact of agronomic practices on fungal populations in subcrown internodes. A comprehensive approach to understanding disease development includes examining the role of crop residues as reservoirs of inoculum for infection and fungal carryover from one season to the next, and how pathogen populations on these residues are affected by agronomic practices.

This information should help identify cultural and management practices that might decrease *Fusarium* populations in live and dead crop tissue, and thus lead to recommendations regarding practices that can reduce damage to wheat and barley from FHB on the Canadian Prairies. A better understanding of the factors affecting pathogen inoculum and crop infection is important for devising highly efficacious strategies to reduce inoculum levels, disease development, and further spread of cereal diseases caused by *Fusarium* spp.

The studies reported here were conducted on commercial fields in eastern Saskatchewan. Surveying commercial fields allows examination of plants in a large area, with little or no interference from fields under other agronomic practices, a common concern in experimental plot trials. Although surveys of commercial fields allow examination of a wide range of crops under different combinations of agronomic practices, they can also suffer from confounding effects of the various practices.

This review focuses on the impact of tillage system and glyphosate use on FHB and CRR diseases. Because glyphosate use is dependent on tillage frequency, these two factors are usually confounded. In order to isolate the effects of tillage frequency from those of glyphosate use, the latter was analyzed as an effect nested within tillage system. Only the most important findings concerning tillage and glyphosate associations with FHB, CRR and fungal populations on crop residues are presented here. Comprehensive reports of associations with other agronomic practices, such as cropping sequence, have been previously published (Fernandez et al., 2005, 2007a,c,d, 2008).

2. Materials and methods

Commercial fields (experimental units) were selected randomly within Crop Districts 1B and 5A in south-east and east-central Saskatchewan to represent the most common cropping practices in the area. A description of the study area was provided by Fernandez et al. (2005).

In late July to early August, spikes at the mid-milk to dough stage of plant development (growth stage 75–83, Zadoks et al., 1974) were taken at random from each field and the percentage of spikes with FHB-like symptoms (incidence) determined. Disease severity was estimated visually based on the percentage of spikelets discolored on each spike. Individual spikelets/lemma showing discoloration were removed, surface-disinfested, plated on modified potato dextrose agar (Burgess et al., 1988; Fernandez and Chen, 2005), and incubated for 7 days under fluorescent and near-UV lights at 22 °C day/15 °C night, 16 h photoperiod to confirm infection by *Fusarium* spp. and for species identification. *Fusarium* spp. were identified by colony and spore morphology and reproductive structures (Samson et al., 2002; Watanabe, 2002). All isolates identified as *F. graminearum* produced perithecia. A FHB index was calculated for each of the wheat and barley crops sampled based on the presence of *Fusarium* isolates in the tissue plated and on the percentage isolation of the most common *Fusarium* spp., i.e. *F. avenaceum* (FHB-*Fav*), *F. graminearum* (FHB-*Fg*), *F. poae* (FHB-*Fp*), and *F. sporotrichioides* Sherb. (FHB-*Fspo*).

Grain samples from most of the fields sampled were obtained from cooperating producers in 2000 and 2001. Kernels with FDK-like symptoms were visually identified in a 50 g subsample, removed, and weighed. The percentage of FDK-like symptoms was determined based on total weight of the sample. A subsample of kernels with FDK symptoms was plated as above, and fungi growing out of kernels were identified. A percentage "total FDK" was then calculated based on the percentage isolation of *Fusarium* spp. Percentage FDKs were also calculated based on the percentage isolation of the most common species (FDK-*Fav*, FDK-*Fg*, FDK-*Fp*, and FDK-*Fspo*).

Plants were selected at random in each field surveyed and carefully removed from the soil to determine CRR. Subcrown internodes were rated for extent of brown to black discoloration on a 0–3 scale (Ledingham et al., 1973) and a subcrown internode discoloration index was calculated for each field based on the incidence and severity of discoloration. The most discolored segment of each subcrown internode was then surface-disinfested, plated and incubated as above. Fungi growing out of subcrown internodes were identified and percentage isolation of each fungus calculated.

Residues of crops grown the previous year were sampled to identify and quantify fungal populations in each field. Pieces were cut from each residue sample (cereal pieces included a node), washed thoroughly and air-dried before surface-disinfesting, plated and incubated as described above. The percentage occurrence of each fungus was based on the proportion of fields sampled where the fungus was isolated at least once. The mean percentage isolation of a fungus in a field was the total number of isolates of the fungal species divided by the number of plated residue pieces from that field.

Crops/fields were categorized according to agronomic practice from information provided by cooperating producers. Wheat and barley crops were categorized into FHB "susceptible" and "intermediate" cultivars. Susceptible cultivars were those rated as "poor", and intermediate cultivars were those rated as "fair" or "fair+" in the Saskatchewan Varieties of Grain Crops publication (SAFRR, 2005). For tillage system, fields were categorized by the total number of tillage operations performed in the previous three years. Fields under conventional-till had a total of seven or more tillage operations, and those under minimum-till had one to six operations (i.e., up to two tillage passes per year), while there were no tillage operations performed in fields under zero-till management. Residue cover was not estimated for any field. Fields were also categorized according to whether they received any application of glyphosate herbicide in the previous 18 months. Some of the glyphosate had been applied on glyphosate-tolerant canola (*Brassica* spp.). No other glyphosate-tolerant crop was grown in any of the fields sampled.

The FHB, FDK, CRR severity, and fungal data were analyzed as described by Fernandez et al. (2005, 2007a,c,d, 2008).

3. Results

3.1. Fusarium head blight

Fusarium graminearum was the most common pathogen isolated in all four years of the study (41.3%) from common and durum wheat; however, various other *Fusarium* species were also isolated from spikes or kernels. Tillage significantly affected the proportion of fields with a high FHB index, and/or the mean FHB index in three of four years (Tables 1 and 2), with the mean FHB index generally highest under minimum-till and lowest under zero-till.

Previous glyphosate application, nested within tillage system, was the only agronomic factor significantly associated with higher FHB levels every year of the study (Tables 3 and 4). Glyphosate's effect on the FHB index was not influenced by environmental conditions as much as for other agronomic factors whose effects on disease levels were not consistent from year to year. Under minimum-till, application of glyphosate at least once in the previous 18 months significantly increased the mean FHB index and the

Table 1
Effect of tillage system on the frequency of common and durum wheat crops in high and low classes based on the Fusarium head blight (FHB) index, in eastern Saskatchewan, from 1999 to 2002 (adapted from Fernandez et al., 2005).

Year	No. of fields	FHB class	Tillage system			Chi-square
			Conventional	Minimum	Zero	
1999	33	High	10	15	8	4.03 (0.13)[a]
	55	Low	11	19	25	
2000	55	High	11	28	16	3.20 (0.20)
	62	Low	17	27	18	
2001	95	High	9	64	22	4.72 (0.09)
	93	Low	8	50	35	
2002	47	High	1	29	17	na[b]
	158	Low	5	83	70	

[a] Values in parentheses are probabilities of obtaining a larger value of chi-square by chance alone.

[b] na: chi-square test not performed due to fewer than five observations in one cell.

Table 2
Effect of tillage system on the mean Fusarium head blight (FHB) index in common and durum wheat crops sampled in eastern Saskatchewan, from 1999 to 2002 (adapted from Fernandez et al., 2005).

Year	No. of fields	Tillage system			Probability[a]
		Conventional (%)	Minimum (%)	Zero (%)	
1999	88	0.33 a[b]	0.22 ab	0.09 b	0.07
2000	117	1.99 ab	3.39 a	1.80 b	0.08
2001	188	8.90 ab	9.51 a	6.25 b	0.04
2002	205	0.28	0.52	0.31	0.36
Mean		2.99	4.21	2.10	

[a] Probability of achieving a larger value of *F* by chance alone.

[b] Mean FHB index within a year followed by a different letter are significantly different according to least significant differences.

Table 3
Effect of glyphosate application (previous 18 months) across tillage systems on the mean Fusarium head blight (FHB) index in common and durum wheat crops sampled in eastern Saskatchewan, from 1999 to 2002 (adapted from Fernandez et al., 2005).

Year	No. of crops	Glyphosate use	Tillage system		
			Conventional (%)	Minimum (%)	Zero (%)
1999	39	Yes	0.71 (<0.01)[a]	0.27 (0.08)	0.10 (ns)
	28	No	0.15	0.12	0.00
2000	80	Yes	2.93 (ns)	4.30 (<0.01)	1.82 (ns)
	47	No	1.55	1.92	0.42
2001	143	Yes	8.28 (ns)	11.53 (<0.01)	6.36 (ns)
	45	No	9.45	5.00	0.42
2002	133	Yes	0.14 (ns)	0.75 (<0.01)	0.23 (ns)
	74	No	0.36	0.19	0.52

[a] Values in parentheses are probabilities of achieving a larger value of *F* by chance alone; ns indicates probability >0.10.

proportion of fields in the high FHB index class every year. Previous glyphosate application did not always cause a significant ($P > 0.10$) increase in the mean FHB index under conventional-till or zero-till management although the mean FHB index was consistently higher and statistically significant in 1999 for conventional-till ($P \leq 0.01$) wheat grown on fields previously treated with glyphosate.

Tillage system did not affect the mean percentage FDK of common and durum wheat in 2000 or 2001, whereas previous glyphosate use significantly increased the proportion of fields with a high percentage FDK, and/or the mean percentage FDK (Fernandez et al., 2005).

For two-rowed and six-rowed barley, *Fusarium* species other than *F. graminearum* were most common. The percentage FHB attributed to *F. graminearum* was 29%, 47% for *F. sporotrichioides*, 35% for *F. poae*, and 38% for *F. avenaceum*. In most cases, the interaction of tillage with cultivar susceptibility was significant. For total FHB index, FHB-*Fg* and FHB-*Fspo*, susceptible cultivars had the lowest disease levels under conventional-till, whereas cultivars with intermediate resistance had the lowest levels under zero-till; this resulted in barley grown under minimum-till having similar or higher disease levels than when grown under the other tillage systems (Table 5). Barley grown under minimum-till management had a significantly higher percentage total FDK, FDK-*Fg*, and FDK-*Fp* than barley grown under zero till and conventional-till combined (Fernandez et al., 2007d).

The use of glyphosate was associated with increased levels of all *Fusarium* pathogens in barley, although effects varied with tillage system (Table 6). Glyphosate use was associated with a significantly

Table 4
Effect of glyphosate application (previous 18 months) across tillage systems on the frequencies of common and durum wheat crops in high and low classes based on the Fusarium head blight (FHB) index, in eastern Saskatchewan, from 1999 to 2002 (adapted from Fernandez et al., 2005).

Year	No. of crops	Glyphosate use	Tillage system								
			Conventional			Minimum			Zero		
			FHB class			FHB class			FHB class		
			High	Low	Chi-square	High	Low	Chi-square	High	Low	Chi-square
1999	39	Yes	5	2	na[a]	13	9	na	8	2	na
	28	No	5	9		2	9		0	3	
2000	80	Yes	5	4	na	28	12	5.70 (0.02)[b]	14	17	na
	47	No	6	13		10	15		2	1	
2001	143	Yes	4	4	na	53	26	12.53 (<0.01)	22	34	na
	45	No	5	4		11	24		0	1	
2002	133	Yes	0	2	na	22	44	4.64 (0.03)	14	51	na
	74	No	1	3		7	39		5	19	

[a] na: chi-square test not performed due to fewer than five observations in one or more cells.
[b] Values in parentheses are probabilities of obtaining a larger value of chi-square by chance alone.

Table 5
Effect of tillage system on total Fusarium head blight (FHB) index, and on that attributed to *F. avenaceum* (FHB-*Fav*), *F. graminearum* (FHB-*Fg*), *F. poae* (FHB-*Fp*), and *F. sporotrichioides* (FHB-*Fspo*) in barley crops sampled in eastern Saskatchewan, 1999–2002 (adapted from Fernandez et al., 2007d).

Effect/contrast	No. of crops	FHB-total	FHB-*Fav*	FHB-*Fg*	FHB-*Fp*	FHB-*Fspo*
				P-value[a]		
Crop susceptibility[b] × tillage system[c]		0.020	0.853	0.049	0.066	0.003
Crop susceptibility × CT vs. MT, ZT		0.006	0.630	0.014	0.311	0.001
				Mean % (SE)[d]		
Susceptible cultivars						
CT	20	1.1 (0.3)	0.5 (0.2)	0.1 (0.1)	0.4 (0.3)	0.4 (0.1)
MT	47	2.1 (0.5)	0.4 (0.1)	0.7 (0.3)	0.1 (<0.1)	0.9 (0.2)
ZT	18	2.2 (0.6)	0.4 (0.1)	0.5 (0.3)	0.3 (0.2)	1.2 (0.5)
Intermediate cultivars						
CT	13	1.9 (0.4)	0.3 (0.1)	0.3 (0.1)	0.1 (<0.1)	1.3 (0.3)
MT	65	1.4 (0.3)	0.2 (<0.1)	0.2 (<0.1)	0.2 (0.1)	0.7 (0.2)
ZT	24	0.5 (0.1)	0.2 (0.1)	<0.1 (<0.1)	0.1 (<0.1)	0.2 (0.1)

[a] Probability of having a larger difference by chance alone.
[b] Categorization of cultivars into "susceptible" ("poor") and "intermediate" ("fair" or "fair+") is based on data presented in Varieties of Grain Crops (Saskatchewan Agriculture, Food and Rural Revitalization, 2005) for each of the cultivars.
[c] CT: conventional-till; MT: minimum-till; ZT: zero-till.
[d] Standard error of the mean.

Table 6
Effect of glyphosate use (previous 18 months) on total Fusarium head blight (FHB) index, and FHB index attributed to *F. avenaceum* (FHB-*Fav*), *F. graminearum* (FHB-*Fg*), *F. poae* (FHB-*Fp*), and *F. sporotrichioides* (FHB-*Fspo*), of barley crops within each tillage system, sampled in eastern Saskatchewan, 1999-2002 (adapted from Fernandez et al., 2007d).

Tillage system[a]	Glyphosate use[b]	No. of crops	FHB-total	FHB-*Fav*	FHB-*Fg*	FHB-*Fp*	B-*Fspo*
					P-value[c]		
CT			0.017	0.841	0.121	0.071	0.001
MT			0.465	0.010	0.375	0.585	0.801
ZT			0.015	0.604	0.100	0.378	0.025
					Mean % (SE)[d]		
CT	No	14	0.8 (0.3)	0.4 (0.2)	0.1 (0.0)	0.0 (0.0)	0.4 (0.2)
CT	Yes	7	2.8 (0.7)	0.4 (0.2)	0.4 (0.2)	0.6 (0.3)	1.5 (0.4)
MT	No	47	1.4 (0.3)	0.1 (0.0)	0.2 (0.1)	0.2 (0.0)	0.7 (0.3)
MT	Yes	76	1.7 (0.3)	0.3 (0.1)	0.4 (0.2)	0.2 (0.1)	0.7 (0.1)
ZT	No	7	0.5 (0.3)	0.3 (0.3)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)
ZT	Yes	36	1.3 (0.3)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.7 (0.2)

[a] CT: conventional-till; MT: minimum-till; ZT: zero-till.
[b] No: no glyphosate applied; Yes: glyphosate applied at least once in the previous 18 months.
[c] Probability of having a larger difference by chance alone.
[d] Standard error of the mean.

Table 7
Correlation between number of glyphosate applications in previous 18 months and Fusarium head blight index attributed to *F. avenaceum* (FHB-*Fav*) and *F. graminearum* (FHB-*Fg*), for all barley crops, and for susceptible cultivars and cultivars with intermediate resistance, grown under minimum-till, sampled in eastern Saskatchewan, 2000–2002 (adapted from Fernandez et al., 2007d).

	No. of crops	FHB-*Fav*	FHB-*Fg*
All crops	112	0.234 (0.019)[a]	0.146 (0.151)
Susceptible[b]	47	0.115 (0.456)	0.163 (0.289)
Intermediate	62	0.439 (0.000)	0.347 (0.005)

[a] Correlation coefficients (r) values in parentheses are probabilities of obtaining a higher value of r by chance alone.
[b] Categorization of cultivars into "susceptible" ("poor") and "intermediate" ("fair" or "fair+") is based on data presented in Varieties of Grain Crops (SAFRR, 2005) for each of the cultivars.

higher level of FHB-*Fav* in barley grown under minimum-till compared with barley grown in untreated fields. Similarly, barley grown under zero-till had a significantly higher total FHB index, FHB-*Fg*, and FHB-*Fspo* after previous applications of glyphosate. Barley grown under conventional-till also had significantly higher FHB-total, FHB-*Fp*, and FHB-*Fspo* in fields that had received glyphosate compared with those that had not.

Correlations between the number of glyphosate applications applied to barley fields in the previous 18 months and FHB caused by *F. graminearum* (FHB-*Fg*) and *F. avenaceum* (FHB-*Fa*) (Table 7) showed that the impact of this herbicide on disease levels was greater for barley cultivars with intermediate resistance than for susceptible cultivars.

3.2. Common root rot

The most commonly isolated fungus from wheat and barley subcrown internodes was *C. sativus*, followed by the genus *Fusarium*. Most of the *Fusarium* spp. isolated from subcrown internodes were also isolated from spikes and kernels.

Levels of most fungi in common wheat were affected by tillage, although not in a consistent manner. Significant tillage effects for *F. culmorum* and *F. graminearum* were only observed in wheat planted after another cereal crop, where *F. graminearum* was favoured by minimum-till and *F. culmorum* was lowest under zero-till management (Table 8). Reduced tillage had a positive effect on *F. avenaceum* populations in subcrown internodes of wheat, although this tillage effect was only observed in wheat grown after an oilseed crop.

Table 8
Effect of tillage system by previous crop/summerfallow on the common root rot index (CRRI), and mean percentage isolation of fungi from discoloured subcrown internodes of common wheat crops sampled in eastern Saskatchewan, in 1999–2001 (adapted from Fernandez et al., 2007a).

Effect/contrast	No. of crops	CRRI	*Cs*[a]	*Fav*	*Fc*	*Fg*
				P-value[b]		
Summerfallow: CT vs. MT[c]		0.049	0.253	0.781	0.311	0.179
Cereal: CT vs. MT, ZT		0.166	0.602	0.836	0.129	0.010
Cereal: MT vs. ZT		0.198	0.284	0.144	0.062	0.010
Oilseed: CT vs. MT, ZT		0.127	0.000	0.011	0.749	0.205
Oilseed: MT vs. ZT		0.446	0.027	0.259	0.476	0.911
Pulse: MT vs. ZT		0.077	0.144	0.888	0.984	0.608
				Mean % (SE)[d]		
Summerfallow (CT)	18	1.5 (0.1)	66.3 (3.7)	1.9 (1.1)	0.2 (0.2)	1.3 (0.9)
Summerfallow (MT)	14	1.2 (0.1)	59.0 (5.1)	2.4 (1.5)	0.0 (0.0)	0.0 (0.0)
Cereal (CT)	10	1.1 (1.6)	40.7 (8.5)	3.6 (1.5)	1.7 (1.6)	0.0 (0.0)
Cereal (MT)	52	1.3 (<0.1)	39.1 (3.6)	5.0 (1.1)	1.5 (0.6)	1.3 (0.5)
Cereal (ZT)	17	1.4 (0.1)	32.8 (4.5)	2.9 (1.0)	0.3 (0.2)	0.0 (0.0)
Oilseed (CT)	31	1.3 (0.1)	50.4 (3.5)	2.4 (0.9)	0.7 (0.5)	1.7 (0.6)
Oilseed (MT)	84	1.3 (<0.1)	37.1 (2.3)	4.6 (0.8)	1.2 (0.6)	0.8 (0.3)
Oilseed (ZT)	54	1.2 (0.1)	28.7 (2.9)	6.2 (1.1)	1.0 (0.4)	0.8 (0.5)
Pulse (MT)	34	1.3 (<0.1)	38.4 (3.9)	8.1 (1.4)	1.2 (0.7)	1.7 (0.8)
Pulse (ZT)	25	1.2 (0.1)	29.1 (4.9)	8.4 (2.0)	1.2 (0.6)	1.1 (0.5)

[a] *Cs*: *Cochliobolus sativus*; *Fav*: *F. avenaceum*; *Fc*: *F. culmorum*; *Fg*: *F. graminearum*.
[b] Probability of having a larger difference by chance alone.
[c] CT: conventional-till; MT: minimum-till; ZT: zero-till.
[d] Standard error of the mean.

Table 9
Effect of tillage system on the common root rot index (CRRI) and percentage isolation of the most common fungi isolated from subcrown internodes, for all barley crops and for those preceded by a cereal or an oilseed crop, sampled in eastern Saskatchewan, 1999–2001 (adapted from Fernandez et al., 2007c).

Effect/contrast	No. of crops	CRRI	*Cs*[a]	*Fus* spp.	*Fav*	*Fc*	*Fe*	*Fg*
					P-value[b]			
All previous crops		0.074	0.001	0.011	0.026	0.663	0.445	0.008
CT vs. MT, ZT[c]		0.016	0.000	0.006	0.012	0.907	0.187	0.005
					Mean % (SE)[d]			
CT	28	1.9 (0.1)	62.6 (3.2)	14.6 (2.3)	3.4 (0.9)	3.0 (1.3)	6.3 (1.7)	0.2 (0.2)
MT	82	1.6 (<0.1)	49.0 (2.1)	20.5 (1.7)	4.6 (0.7)	3.6 (0.9)	8.5 (1.2)	2.1 (0.6)
ZT	23	1.6 (0.1)	45.8 (3.9)	25.7 (2.6)	7.2(1.4)	2.2 (1.9)	11.7 (2.2)	2.0 (1.0)
					P-value			
Cereal		0.138	0.045	0.000	0.114	0.004	0.258	0.178
CT vs. MT, ZT		0.513	0.032	0.001	0.090	0.227	0.070	0.058
					Mean % (SE)			
CT	11	1.9 (0.2)	65.9 (5.5)	8.4 (1.8)	1.9 (0.9)	0.6 (0.4)	5.1 (1.5)	0.0 (0.0)
MT	31	1.6 (0.1)	50.6 (3.6)	16.0 (1.8)	4.1 (0.9)	3.3 (1.1)	6.5 (1.4)	1.3 (0.5)
ZT	8	1.7 (0.1)	48.9 (6.4)	19.0 (2.2)	3.8 (1.5)	0.0 (0.0)	12.3 (2.6)	0.0 (0.0)
					P-value			
Oilseed		0.904	0.003	0.551	0.014	0.025	0.401	0.700
CT vs. MT, ZT		0.553	0.001	0.411	0.007	0.518	0.892	0.491
					Mean % (SE)			
CT	9	1.6 (0.1)	63.8 (5.5)	17.5 (5.2)	3.6 (1.7)	5.0 (3.5)	6.1 (3.8)	0.6 (0.6)
MT	39	1.6 (0.1)	44.4 (3.1)	21.9 (3.0)	5.0 (1.1)	3.0 (1.1)	8.5 (1.8)	2.6 (1.0)
ZT	10	1.6 (0.1)	42.0 (6.2)	27.0 (4.6)	8.7 (2.0)	0.2 (0.2)	13.8 (4.0)	2.2 (1.5)

[a] *Cs*, *Cochliobolus sativus*; *Fus* spp., total *Fusarium* spp.; *Fav*, *F. avenaceum*; *Fc*, *F. culmorum*; *Fe*, *F. equiseti*; *Fg*, *F. graminearum*.
[b] Probability of having a larger difference by chance alone.
[c] CT, conventional-till; MT, minimum-till; ZT, zero-till.
[d] Standard error of the mean.

While CRRI and *C. sativus* isolations from subcrown internodes of barley were favoured by conventional-till management, colonization by *Fusarium* spp., especially *F. avenaceum* and *F. graminearum*, increased under reduced tillage (Table 9).

The effect of glyphosate on fungi isolated from subcrown internodes of wheat and barley varied with tillage system. Previous glyphosate applications were associated with a significantly ($P \leq 0.05$) lower percentage isolation of *C. sativus* in wheat grown after a crop under minimum-till (44% and 35%, for unsprayed fields, $n = 51$, and for sprayed fields, $n = 110$, respectively). In contrast, there was a higher level of *F. avenaceum* in wheat grown in glyphosate-sprayed than glyphosate-free fields under conventional-till and zero-till, although this was only significant ($P \leq 0.01$) for the latter (for fields under conventional-till: 2% for unsprayed, $n = 26$, and 5% for sprayed, $n = 14$; for fields under zero-till: 1%, $n = 6$, for unsprayed, 7%, $n = 81$, for sprayed).

Glyphosate was associated with higher levels of total *Fusarium* spp., *F. culmorum* and *F. graminearum*, but lower levels of *C. sativus,* on barley grown under minimum-till (Table 10). Levels of *F. avenaceum* also tended to be higher in glyphosate-sprayed than in glyphosate-free fields. The same effects of glyphosate on fungal isolations observed under minimum-till were generally observed under conventional-till and/or zero-till; although they were not always significant ($P > 0.10$). The exception was for *F. avenaceum* in barley which was present at higher levels in the glyphosate-sprayed than the glyphosate-free zero-till fields.

3.3. Crop residues

A variety of fungal species were isolated from crop residues, including the same *Fusarium* spp. found in discolored underground tissue and spikes affected by FHB, although at different relative frequencies.

Table 10
Effect of glyphosate use (previous 18 months) on the common root rot index (CRRI) and percentage isolation of fungi within each tillage system, for barley crops sampled in eastern Saskatchewan, 1999–2001 (adapted from Fernandez et al., 2007c).

Tillage system[b]	Glyphosate use[c]	No. of crops	CRRI	*Cs*[a]	*Fus* spp.	*Fav*	*Fc*	*Fe*	*Fg*
						P-value[d]			
CT			0.165	0.125	0.229	0.448	0.923	0.472	1.000
MT			0.934	0.033	0.027	0.296	0.056	0.667	0.092
ZT			0.494	0.219	0.765	0.021	0.325	0.377	0.815
						Mean % (SE)[e]			
CT	No	9	2.0 (0.2)	59.6 (6.1)	16.2 (4.7)	4.0 (1.9)	4.5 (3.4)	5.8 (3.1)	0.0 (0.0)
CT	Yes	7	1.8 (0.2)	51.5 (4.0)	24.4 (4.5)	5.4 (1.7)	5.2 (2.9)	11.2 (4.9)	0.0 (0.0)
MT	No	26	1.7 (0.1)	56.3 (3.0)	15.5 (2.3)	3.4 (0.9)	1.5 (0.5)	8.3 (2.1)	0.9 (0.4)
MT	Yes	55	1.6 (0.1)	46.2 (2.6)	23.0 (2.3)	5.1 (0.9)	4.6 (1.3)	8.8 (1.5)	2.7 (0.8)
ZT	No	2	2.0 (0.1)	61.0 (8.2)	26.8 (8.0)	4.1 (0.1)	0.0 (0.0)	18.5 (4.8)	2.1 (1.6)
ZT	Yes	19	1.6 (0.1)	43.8 (3.5)	25.9(2.8)	7.9 (1.5)	2.6 (2.3)	10.8 (2.5)	2.1 (1.1)

[a] CT, conventional-till; MT, minimum-till; ZT, zero-till.
[b] No, no glyphosate applied; Yes, glyphosate applied at least once in previous 18 months.
[c] *Cs*, *Cochliobolus sativus*; *Fus* spp., total *Fusarium* spp.; *Fav*, *F. avenaceum*; *Fc*, *F. culmorum*; *Fe*, *F. equiseti*; *Fg*, *F. graminearum*.
[d] Probability of having a larger difference by chance alone.
[e] Standard error of the mean.

Table 11
Correlations between mean percentage isolation of *F. graminearum* (*Fg*) and *F. avenaceum* (*Fav*) from various crop residues compared with Fusarium head blight (FHB) severity or percent *Fusarium*-damaged kernels (FDK) caused by these fungi in common and durum wheat and barley crops categorized by FHB reaction, sampled in eastern Saskatchewan, 2000 and 2001 (adapted from Fernandez et al., 2008).

Crop residue	Current crop	FHB reaction[a]	*Fg* vs. FHB-*Fg*			*Fgv* vs. FDK-*Fg*			*Fav* vs. FHB-*Fav*			*Fav* vs. FDK-*Fav*		
			No. of fields	*r*[b]	*P*[c]	No. of fields	*r*	*P*	No. of fields	*r*	*P*	No. of fields	*r*	*P*
Cereal	Barley	Susceptible	15	0.412	(0.127)	13	0.713	(0.006)	15	0.308	(0.265)	13	0.069	(0.824)
Cereal	Barley	Intermediate	15	0.366	(0.180)	13	0.878	(0.000)	15	0.213	(0.446)	13	0.027	(0.931)
Oilseed	Barley	Susceptible		na[d]			na		20	0.496	(0.026)	20	0.457	(0.043)
Oilseed	Barley	Intermediate		na			na		19	0.270	(0.263)	19	0.195	(0.423)
Cereal	Wheat	Susceptible	16	0.342	(0.195)	15	0.875	(0.000)	16	0.224	(0.404)	15	0.111	(0.694)
Cereal	Wheat	Intermediate	32	0.789	(0.000)	26	0.610	(0.001)	32	0.125	(0.495)	26	−0.160	(0.435)
Oilseed	Wheat	Susceptible		na			na		33	−0.084	(0.643)	32	−0.150	(0.414)
Oilseed	Wheat	Intermediate		na			na		66	0.095	(0.447)	64	0.099	(0.436)
Pulse	Wheat	Susceptible		na			na		19	0.427	(0.068)	16	0.227	(0.398)
Pulse	Wheat	Intermediate		na			na		26	−0.194	(0.342)	25	0.026	(0.901)

[a] Categorization of wheat and barley crops according to their reactions to FHB (Fernandez et al., 2005, 2007d).
[b] Correlation coefficient.
[c] Probability of having a higher r value by chance alone.
[d] na: not applicable.

The FHB index and percentage FDK caused by *F. graminearum* or *F. avenaceum*, obtained from the FHB studies of wheat and barley presented above, were correlated with the mean percentage isolation of each of these pathogens from crop residues (Table 11). For *F. graminearum*, the FHB index and percentage FDK in wheat and barley were positively correlated with its mean percentage isolation from cereal residues, although for the FHB index this was significant only for wheat cultivars with intermediate resistance. For *F. avenaceum*, there were significant positive correlations between its mean percentage isolation from oilseed residues and the FHB index in susceptible barley cultivars grown on oilseed stubble, and between its mean percentage isolation from pulse residues and percentage FDK in common wheat cultivars with intermediate resistance grown on pulse stubble.

The *Fusarium* species isolated from crop residues varied depending on tillage method. The percentage occurrence of *F. avenaceum* was highest under zero-till in cereal residues with another cereal as the current crop (Table 12). *Fusarium culmorum* in cereal residues had the lowest percentage occurrence under zero-till when the current crop was an oilseed; whereas, under these same residue conditions, *F. graminearum* had the lowest percentage occurrence under conventional-till. In contrast, the percentage occurrence of *C. sativus* decreased as the intensity of tillage decreased.

Generally, the mean percentage isolation of the various fungal species among tillage systems was similar to those observed for their percentage occurrence (Table 12). For cereal residues with another cereal as the current crop, the mean percentage isolation of *F. graminearum* was lowest under zero-till, whereas the opposite was true for *F. avenaceum*. In contrast, *C. sativus* had the highest mean percentage isolation under conventional-till and lowest under zero-till, whereas the mean percentage isolation of *F. culmorum* was highest under minimum-till. For cereal residues with an oilseed or pulse as the current crop, the mean percentage isolation of *F. culmorum* was lowest under zero-till, whereas for cereal residues with a pulse as the current crop, *F. graminearum* had again the lowest mean percentage isolation under zero-till.

The close association between tillage operations and glyphosate applications made it difficult to separate their effects on the fungal colonization of residues; however, correlations between glyphosate use and the mean percentage isolation of some fungi under zero-till reflect a negative or positive association with glyphosate, independent of potential tillage intensity effects. There was a significant positive correlation between previous glyphosate applications and *F. avenaceum* ($r = 0.563$, $P = 0.015$) in cereal residues under zero-till, and a significant negative correlation between glyphosate applications and *C. sativus* ($r = -0.589$, $P = 0.010$) when an oilseed was the current crop. The observation that the percentage isolation of *F. avenaceum* and *C. sativus* in crop residues was positively and negatively correlated, respectively, with the number of glyphosate applications under zero-till management suggests that glyphosate might play a role in fungal growth and competition among fungal species in crop residues.

The association of glyphosate with lower *C. sativus* and higher *F. avenaceum* in crop residues is consistent with results from the present CRR studies of wheat and barley, while the positive association of glyphosate use with some of the *Fusarium* pathogens, such as *F. avenaceum*, is also consistent with results from the present FHB studies.

4. Discussion

The prevalence of *Fusarium* pathogens responsible for FHB and FDK development was different in wheat than in barley. *Fusarium graminearum* was the most commonly isolated species only in common and durum wheat.

Our results from these FHB studies agree with those showing that average DON levels in wheat were higher under minimum-till than under either zero-till or conventional-till management (Schaafsma et al., 2001). Other studies have indicated that tillage had no effect on FHB (Teich and Nelson, 1984; Miller et al., 1998), or that the incidence of *F. graminearum*-infected wheat grain and DON content (Krebs et al., 2000) or FHB levels (Yi et al., 2001) were lowest under conditions of high disturbance tillage. For barley, Rioux et al. (2005) reported that barley grown under minimum-till had a higher DON content than when grown under conventional-till.

The significant positive association of previous glyphosate application with FHB development suggests that the lower disease levels observed under zero-till compared to minimum-till management were not related to previous glyphosate application, but to factors intrinsic to zero-till management such as the lack of disturbance of residues which appears to have impacted inoculum levels and/or its availability for head infection.

Our observations on tillage effects on the relative prevalence of these pathogens in subcrown internodes of wheat and barley agree with studies conducted elsewhere. The higher CRRI and *C. sativus* levels with increasing tillage intensity observed after some cropping sequences are similar to results reported by Bailey et al. (2000,

Table 12
Effect of tillage system by cropping sequence on percentage occurrence and mean percentage isolation of fungi from various crop residues categorized by the crop grown in the sampling year, sampled in eastern Saskatchewan, 2000 and 2001 (adapted from Fernandez et al., 2008).

Contrast	Crop residue	Current crop	Tillage system[b]	No. of fields	Percentage occurrence				Mean percentage isolation (SE)[a]			
					Fav[c]	*Fc*	*Fg*	*Cs*	*Fav*	*Fc*	*Fg*	*Cs*
									%			
	Cereal	Cereal	CT	11	82	18	46	91	14.8 (2.9)	0.5 (0.3)	10.5 (4.7)	26.9 (6.6)
			MT	56	95	38	57	77	21.9 (2.3)	5.7 (1.5)	7.1 (1.2)	11.3 (1.5)
			ZT	19	100	32	37	58	37.0 (5.4)	1.5 (0.5)	3.1 (1.3)	6.3 (1.7)
									P-value[d]			
CT vs. MT, ZT					0.199	0.226	0.926	0.033	0.001	0.001	0.274	0.009
MT vs. ZT					0.078	0.639	0.124	0.144	0.015	0.011	0.033	0.033
									%			
	Cereal	Oilseed	CT	9	22	56	11	67	4.6 (3.5)	8.3 (3.2)	2.8 (2.6)	19.4 (6.7)
			MT	41	44	46	39	78	7.9 (2.0)	7.6 (1.7)	5.9 (1.5)	16.5 (2.8)
			ZT	33	42	15	36	49	8.1 (2.2)	1.3 (0.5)	6.8 (2.1)	10.6 (2.7)
									P-value			
CT vs. MT, ZT					0.170	0.157	0.030	0.840	0.379	0.247	0.229	0.403
MT vs. ZT					0.900	0.002	0.814	0.009	0.964	0.001	0.723	0.137
									%			
	Cereal	Pulse	MT	30	33	27	27	70	3.9 (1.2)	4.2 (1.5)	7.8 (3.2)	15.0 (2.5)
			ZT	12	17	8	8	67	3.5 (2.7)	0.7 (0.7)	1.4 (1.3)	9.7 (2.6)
									P-value			
MT vs. ZT					0.244	0.120	0.120	0.838	0.892	0.046	0.085	0.154
									%			
	Oilseed	Cereal	CT	22	91	0	1	1	24.8 (3.9)	0.0 (0.0)	0.4 (0.4)	0.2 (0.2)
			MT	86	94	2	9	2	22.2 (1.7)	0.1 (0.1)	0.6 (0.3)	0.2 (0.2)
			ZT	46	89	4	7	4	29.4 (3.4)	0.3 (0.2)	0.4 (0.2)	0.2 (0.1)
									P-value			
CT vs. MT, ZT					0.911	0.054	0.505	0.801	0.820	0.082	0.775	0.920
MT vs. ZT					0.337	0.556	0.564	0.557	0.075	0.408	0.449	0.796

[a] Value in parentheses are standard errors of the mean.
[b] CT: conventional-till; MT: minimum-till; ZT: zero-till.
[c] *Fav*: *F. avenaceum*; *Fc*: *F. culmorum*; *Fg*: *F. graminearum*; *Cs*: *Cochliobolus sativus*.
[d] Probability of having a larger difference by chance alone.

2001), Mathieson et al. (1990), and Tinline and Spurr (1991). The higher levels of *C. sativus* and lower levels of *F. avenaceum* in more intensive tillage systems are similar to observations by Windels and Wiersma (1992); however, they did not observe a tillage effect on *F. acuminatum* or *F. culmorum*. In other regions of Saskatchewan, *F. avenaceum* in underground wheat tissue was also associated with reduced tillage management and continuously cropped diversified rotations (Fernandez et al., 2007b). In studies of noncereal crops conducted in eastern Saskatchewan, tillage operations were also positively associated with the occurrence of *C. sativus*, and negatively associated with *F. avenaceum* in roots of lentil (*Lens culinaris* Medik.), flax (*Linum usitatissimum* L.) and canola plants grown in rotation with wheat or barley (Fernandez, 2007).

Our observation that tillage system effects were dependent on the previous cropping practices suggests that tillage effects on CRR, or the fungi isolated from discoloured subcrown internodes, might be attributed to different factors across the different practices.

The lower population of *F. graminearum* under zero-till than conventional-till, in cereal residues when the current crop was another cereal, is consistent with observations that *F. graminearum* developed in parallel with the mineralization of residues (Yi et al., 2002). The lower recovery of *F. culmorum* from cereal residues under zero-till than conventional-till (when the current crop was an oilseed) agrees with its lower occurrence reported in culm bases of winter wheat in ploughless than in conventional tillage treatments by Weber et al. (2001), and with its greater isolation from barley subcrown internodes in our study as tillage intensity increased. In contrast to the above, *F. avenaceum* was favoured under reduced tillage. Tillage system effects on this fungus are similar to those observed in the subcrown internodes of barley and common wheat in the present studies, although the tillage effect on wheat depended on the cropping sequence.

These studies document the positive association of glyphosate with pathogenic *Fusarium* spp., including *F. avenaceum*, *F. culmorum* and *F. graminearum*, in spikes/kernels and subcrown internodes of wheat and/or barley, and in residues of these crops almost a year after harvest. The exact nature of these associations was not determined.

Previous research has shown that herbicides, including glyphosate, can inhibit or stimulate the growth of fungal pathogens, and can either increase or decrease disease development through direct or indirect means (Altman, 1993; Levesque and Rahe, 1992). Levesque and Rahe (1992) showed evidence that herbicides can have a direct effect on various components of the soil microflora, such as plant pathogens, antagonists, or mycorrhizae, which can potentially increase or decrease the incidence of plant disease. Pathogens able to infect weeds can also increase their inoculum potential after weeds have been sprayed with herbicides, which could subsequently affect host crops.

The observation that *Fusarium* infections increased in fields previously sprayed with glyphosate agrees with reports of the association of glyphosate with *Fusarium* colonization of other crops. Although no previous studies examined the effect of glyphosate on FHB or *F. graminearum* in cereals, several studies have shown a

stimulatory effect of glyphosate on *Fusarium* populations (Kawate et al., 1997; Kremer et al., 2005; Levesque and Rahe, 1992; Rahe et al., 1990; Sanogo et al., 2001), including *F. avenaceum* and *F. culmorum* (Brown and Sharma, 1984; Levesque et al., 1987). For example, Levesque et al. (1987) reported that glyphosate increased root colonization of various treated weeds by *F. avenaceum* and *F. oxysporum* Schltdl.:Fr., as well as increasing the propagule density of these species in soil. Johal and Rahe (1984) and Rahe et al. (1990) showed that the glyphosate-induced root colonization by *Fusarium* spp. and other pathogens was the cause, and not the result, of plant death following application of certain doses of glyphosate, and that the efficacy of glyphosate depended on the synergistic action of these species and others in the soil. Kawate et al. (1997) reported that *Fusarium* populations were greater in the rhizosphere soil from glyphosate-treated than from untreated henbit (*Lamium amplexicaule* L.), and suggested that weed control with glyphosate in the spring may provide *Fusarium* pathogens an energy source for survival and proliferation. Glyphosate-treated quackgrass (*Elymus repens* [L.] Gould) was also rapidly colonized by *F. culmorum*, which subsequently caused damage to the following barley crop (Lynch and Penn, 1980). Brown and Sharma (1984) reported that flax plants treated with glyphosate were rapidly colonized by several species of fungi, including *F. culmorum*. Glyphosate could also act directly on plants by inhibiting their phenolic metabolism which could potentially affect plant resistance; thus, causing them to be more susceptible to pathogenic organisms. Sub-lethal doses of glyphosate induced susceptibility to *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker in two resistant tomato cultivars whose root tissue was invaded by the pathogen soon after glyphosate treatment (Brammal and Higgins, 1988).

Glyphosate applied to glyphosate-tolerant soybean significantly increased the isolation frequency of the causal agent of sudden death syndrome, *F. solani* (Mort.) Sacc. f. sp. *glycines* Roy (Sanogo et al., 2001), and *Fusarium* populations on roots and in the rhizosphere of plants (Kremer et al., 2005). Glyphosate applications could cause increased disease levels in soybean through enhanced pathogen activity in the rhizosphere of treated plants caused by glyphosate or plant metabolites in root exudates (Kremer et al., 2005).

Laboratory studies have also shown stimulatory activity of glyphosate on *Fusarium*. Krzysko-Lupicka and Orlik (1997) reported that *Fusarium* spp. grew out of soil suspensions only when these were plated on nutrient media in which glyphosate had been used as the sole source of C or P, but not on nutrient media alone.

Glyphosate was also shown to have a differential effect on fungi, thus potentially altering the outcome of competition between them (Wardle and Parkinson, 1992). Our studies showed a significant negative association of previous glyphosate use with *C. sativus*, suggesting changes in populations of the most common root rot fungi associated with the use of this herbicide. The observation of negative associations of previous glyphosate use with *C. sativus* under conventional-till and zero-till management systems suggests that changes in population of this pathogen might be due to the herbicide and unrelated to tillage management.

We could not determine if the higher *Fusarium* levels in subcrown internodes associated with previous glyphosate use was due to effects on fungal inoculum, host susceptibility, or the lower levels of *C. sativus* with which the *Fusaria* might be competing. There are no previous reports of glyphosate effects on infection of underground tissue of wheat or barley by *C. sativus*. Furthermore, these studies could not determine how much of the increased levels of *Fusarium* might be due to other factors, such as microenvironment, in these systems or other factors associated with tillage frequency. Increases in populations of *F. avenaceum* and *F. graminearum* might cause more severe crown and root rot as well as spike infection in subsequently grown cereal crops. Because *Fusarium* infections in crown/roots would be less affected by environmental conditions than spike infections, they might maintain high levels of inoculum in years not conducive to FHB development and thus contribute to the further spread of this disease in the Canadian Prairies.

These are the first studies showing a significant association of previous glyphosate application with FHB and underground tissue infections by *C. sativus* and *Fusarium* spp. Based on the observations made across four years in eastern Saskatchewan, growing susceptible crops under minimum-till management in fields where glyphosate has been previously applied, resulted in the most damage from FHB in years conducive to disease development. The barley study also showed that the greatest FHB levels occurred in continuous cropping systems than when summerfallow was included in the rotation (Fernandez et al., 2007d). For CRR, we conclude that growing wheat or barley under reduced tillage systems that include glyphosate applications will likely increase infection by *Fusarium* spp. These studies also show that the highest *Fusarium* populations occur when noncereal crops are grown in rotation with wheat or barley (Fernandez et al., 2007a,c).

Thus, current production practices relying on reduced tillage management and increased glyphosate use, and continuous cropping sequences that include noncereal crops, are potentially associated with increases in cereal diseases caused by *Fusarium* spp. The similar impact of production factors on FHB and CRR points to the importance of agronomic practices vis-a-vis the environment in the development of wheat and barley diseases. The observation that similar crop production factors are associated with common pathogenic *Fusarium* spp. in subcrown internodes and spikes or kernels of barley suggests that measures aimed at reducing crown/root rot caused by *Fusarium* spp. might also help reduce FHB development in cereal crops.

4.1. Recommendations for future research

The FHB and FDK levels observed in our studies varied from low to high for this region, but on average, disease levels were lower than those normally found in eastern Canada and the eastern Prairies where FHB recently occurred in epidemic proportions. It is unknown if cereal crops, grown in areas with traditionally higher FHB levels and where *F. graminearum* is the predominant pathogen, are likely to be impacted by the same crop production factors, including previous glyphosate use.

The nature of these studies also does not permit separation of the role of tillage intensity from glyphosate use on relative levels of fungal pathogens in cereal spikes and underground tissue. It is also not possible to completely separate the role of tillage system/glyphosate use versus cropping sequence on *Fusarium* populations in cereal tissue. Since correlations between the isolation frequency of fungi in crop residues and tillage operations were often of opposite sign to correlations between their isolation frequency and number of previous glyphosate applications, it is not possible to determine which factor(s) played the most important role in the tillage system effects on fungal colonization of residues.

Determining the relative contribution of tillage, herbicide application, and cropping sequence on FHB and CRR development in wheat and barley should help in understanding the role that each of these plays in disease levels and the relative frequency of the various pathogens. It should also help elucidate the mechanisms responsible for the changes in *Fusarium* populations under different tillage/input systems. Further research on the associations observed in these studies is warranted considering the continuing adoption of conservation tillage practices and glyphosate use, and the increased development of important wheat diseases caused by *Fusarium* spp. The increasing importance of noncereal crops in cereal-based systems in western Canada and other parts of the world, and the importance of *F. avenaceum* as a crown/root pathogen of cereal and noncereal crops and a FHB pathogen of

cereal crops, indicates that the impact of noncereal crops on diseases caused by *Fusarium* spp. merits further investigation.

Determining the mechanism(s) responsible for the associations of previous glyphosate applications with spike and root infections by two of the most important FHB pathogens, *F. graminearum* and *F. avenaceum*, will likely help in the control of these important crop diseases. This is especially relevant considering that increased incorporation of glyphosate-tolerant crops in rotations with cereal crops increases the use of glyphosate. Further information might allow for improved recommendations for lowering populations of *Fusarium* pathogens in affected areas, as well as preventing their further spread in western Canada.

Acknowledgements

This work was conducted with financial support from the Saskatchewan Agriculture Development Fund, and Agriculture and Agri-Food Canada. We thank Y. Chen, S. Stolhandske-Dale, M. Boire and D. Kern for their technical assistance.

References

Abramson, D., McCallum, B., Smith, D.M., Tekauz, A., 2002. Moniliformin in barley inoculated with *Fusarium avenaceum*. Food Addit. Contam. 19, 765–769.

Altman, J., 1993. Pesticide-pathogen interactions in plant disease. In: Altman, J. (Ed.), Pesticide Interactions in Crop Production, Beneficial and Deleterious Effects. CRC Press, Inc., Boca Raton, FL, pp. 315–332.

Bailey, K.L., Johnston, A.M., Kutcher, H.R., Gossen, B.D., Morrall, R.A.A., 2000. Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. Can. J. Plant Sci. 80, 169–175.

Bailey, K.L., Gossen, B.D., Lafond, G.P., Watson, P.R., Derksen, D.A., 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: univariate and multivariate analysis. Can. J. Plant Sci. 81, 789–803.

Brammal, R.A., Higgins, V.J., 1988. The effect of glyphosate on resistance of tomato to Fusarium crown and root rot disease on the formation of host structural defensive barriers. Can. J. Bot. 66, 1547–1555.

Brown, A.E., Sharma, H.S.S., 1984. Production of polysaccharide-degrading enzymes by saprophytic fungi from glyphosate-treated flax and their involvement in retting. Ann. Appl. Biol. 105, 65–74.

Burgess, L.W., Liddell, C.M., Summerell, B.A., 1988. Laboratory Manual for Fusarium Research, 2nd ed. University of Sydney, Sydney, Australia.

Canadian Grain Commission, 2007. Official Grain Grading Guide. http://www.grainscanada.gc.ca/pubs/GGG/2007.

Clear, R.M., Patrick, S.K., Gaba, D., 2000. Prevalence of fungi and fusariotoxins on barley seed from western Canada 1995 to 1997. Can. J. Plant Pathol. 22, 44–50.

Conner, R.L., Lindwall, C.W., Atkinson, T.G., 1987. Influence of minimum tillage on severity of common root rot in wheat. Can. J. Plant Pathol. 9, 56–58.

Dill-Macky, R., Jones, R.K., 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84, 71–76.

Fernandez, M.R., 2007. *Fusarium* populations in roots of oilseed and pulse crops grown in eastern Saskatchewan. Can. J. Plant Sci. 87, 945–952.

Fernandez, M.R., Basnyat, P., Zentner, R.P., 2007a. Response of wheat root pathogens to crop management in eastern Saskatchewan. Can. J. Plant Sci. 87, 953–963.

Fernandez, M.R., Chen, Y., 2005. Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. Plant Dis. 89, 164–169.

Fernandez, M.R., Holzgang, G., Celetti, M.J., Hughes, G., 1999. The incidence of Fusarium head blight in barley, common wheat and durum wheat grown in Saskatchewan during 1998. Can. Plant Dis. Surv. 79, 79–82.

Fernandez, M.R., Huber, D., Basnyat, P., Zentner, R.P., 2008. Impact of agronomic practices on populations of *Fusarium* and other fungi in cereal and noncereal crop residues on the Canadian Prairies. Soil Till. Res. 100, 60–71.

Fernandez, M.R., Jefferson, P.G., 2004. Fungal populations in roots and crowns of common and durum wheat in Saskatchewan. Can. J. Plant Pathol. 26, 325–334.

Fernandez, M.R., Pearse, P.G., Holzgang, G., Hughes, G., 2000. Fusarium head blight in common and durum wheat in Saskatchewan in 1999. Can. Plant Dis. Surv. 80, 57–59.

Fernandez, M.R., Pearse, P.G., Holzgang, G., Hughes, G., 2001. Fusarium head blight in common and durum wheat in Saskatchewan in 2000. Can. Plant Dis. Surv. 81, 83–85.

Fernandez, M.R., Pearse, P.G., Holzgang, G., Hughes, G.R., 2002a. Fusarium head blight in barley in Saskatchewan in 2001. Can. Plant Dis. Surv. 82, 32–33.

Fernandez, M.R., Pearse, P.G., Holzgang, G., Hughes, G.R., 2002b. Fusarium head blight in common and durum wheat in Saskatchewan in 2001. Can. Plant Dis. Surv. 82, 36–38.

Fernandez, M.R., Selles, F., Gehl, D., DePauw, R.M., Zentner, R.P., 2003. Identification of crop production factors associated with the development of Fusarium head blight in spring wheat in southeast Saskatchewan. Report to Saskatchewan Agriculture Development Fund, Project No. 98000306, December 2003.

Fernandez, M.R., Selles, F., Gehl, D., DePauw, R.M., Zentner, R.P., 2005. Crop production factors associated with Fusarium head blight in spring wheat in eastern Saskatchewan. Crop Sci. 45, 1908–1916.

Fernandez, M.R., Ulrich, D., Sproule, L., Brandt, S.A., Thomas, A.G., Olfert, O., Zentner, R.P., McConkey, B.G., 2007b. Impact of crop management systems on diseases of spring wheat on the Canadian Prairies. In: Buck, H.T., Nisi, J.E., Salomon, N. (Eds.), Wheat Production in Stressed Environments. Proceedings of the 7th International Wheat Conference, 27 November–2 December, 2005. Mar del Plata, Argentina. Developments in Plant Breeding, vol. 12. Springer, The Netherlands, pp. 265–271.

Fernandez, M.R., Zentner, R.P., 2005. The impact of crop rotation and N fertilizer on common root rot of spring wheat in the brown soil zone of western Canada. Can. J. Plant Sci. 85, 569–575.

Fernandez, M.R., Zentner, R.P., DePauw, R.M., Gehl, D., Stevenson, F.C., 2007c. Impacts of crop production factors on common root rot of barley in eastern Saskatchewan. Crop Sci. 47, 1585–1595.

Fernandez, M.R., Zentner, R.P., DePauw, R.M., Gehl, D., Stevenson, F.C., 2007d. Impacts of crop production factors on Fusarium head blight of barley in eastern Saskatchewan. Crop Sci. 47, 1574–1584.

Gilbert, J., Tekauz, A., 2000. Review: recent developments in research on Fusarium head blight of wheat in Canada. Can. J. Plant Pathol. 22, 1–8.

Hsia, Y., Christensen, J.J., 1951. Effect of 2, 4-D on seedling blight of wheat caused by *Helminthosporium sativum*. Phytopathology 41, 1011–1020.

Isakeit, T., Lockwood, J.L., 1989. Lethal effect of atrazine and other triazine herbicides on ungerminated conidia of *Cochliobolus sativus* in soil. Soil Biol. Biochem. 21, 809–817.

Johal, G.S., Rahe, J.E., 1984. Effect of soilborne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology 74, 950–955.

Kawate, M.K., Colwell, S.G., Ogg Jr., A.G., Kraft, J.M., 1997. Effect of glyphosate-treated henbit (*Lamium amplexicaule*) and downy brome (*Bromus tectorum*) on *Fusarium solani* f. sp. *pisi* and *Pythium ultimum*. Weed Sci. 45, 739–743.

Krebs, H., Streit, B., Forrer, H.-R., 2000. Effect of tillage and preceding crops on Fusarium infection and deoxynivalenol content of wheat. In: Alfoldi, T., Lockeretz, W., Niggli, U. (Eds.), IFOAM 2000 the World Grows Organic: Proceedings, 13th International IFOAM Scientific Conference, Convention Center Basel, 28–31 August 2000. IOS, Amsterdam, The Netherlands, p. 13.

Kremer, R.J., Means, N.E., Kim, S.J., 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. Int. J. Environ. Anal. Chem. 85, 1165–1174.

Krzysko-Lupicka, T., Orlik, A., 1997. The use of glyphosate as the sole source of phosphorus or carbon for the selection of soil-borne fungal strains capable to degrade this herbicide. Chemosphere 34, 2601–2605.

Ledingham, R.J., Atkinson, T.G., Horricks, J.S., Mills, J.T., Piening, L.J., Tinline, R.D., 1973. Wheat losses due to common root rot in the Prairie provinces of Canada 1969–71. Can. Plant Dis. Surv. 53, 113–122.

Levesque, C.A., Rahe, J.E., 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Ann. Rev. Phytopathol. 30, 579–602.

Levesque, C.A., Rahe, J.E., Eaves, D.M., 1987. Effects of glyphosate on *Fusarium* spp.: its influence on root colonization of weeds, propagule density in the soil, and crop emergence. Can. J. Microbiol. 33, 354–360.

Lynch, J.M., Penn, D.J., 1980. Damage to cereals caused by decaying weed residues. J. Sci. Food Agric. 31, 321–324.

Mathieson, J.T., Rush, C.M., Bordovsky, D., Clarke, L.E., Jones, O.R., 1990. Effects of tillage on common root rot of wheat in Texas, USA. Plant Dis. 74, 1006–1008.

Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Seaman, W.L., Seifert, K.A., Turkington, K., Voldeng, H., 1998. Effect of tillage practice on Fusarium head blight of wheat. Can. J. Plant Pathol. 20, 95–103.

Pearse, P.G., Holzgang, G., Harris, C.L., Fernandez, M.R., 2003. Fusarium head blight in barley in Saskatchewan in 2002. Can. Plant Dis. Surv. 83, 48–49.

Pearse, P.G., Holzgang, G., Weitzel, C.N., Fernandez, M.R., 2007a. Fusarium head blight in barley and oat in Saskatchewan in 2006. Can. Plant Dis. Surv. 87, 61–62.

Pearse, P.G., Holzgang, G., Weitzel, C.N., Fernandez, M.R., 2007b. Fusarium head blight in common and durum wheat in Saskatchewan in 2006, with comments on irrigated corn. Can. Plant Dis. Surv. 87, 92–93.

Rahe, J.E., Levesque, C.A., Johal, G.S., 1990. Synergistic role of soil fungi in the herbicidal 7 efficacy of glyphosate. In: Hoagland, R.E. (Ed.), Biological weed control using microbes and 8 microbial products as herbicides. Symposium, 9–14 April, 1989. American Chemical 9 Society, Washington, D.C., pp. 260–275.

Rioux, S., Pageau, D., Lajeunesse, J., Lafond, J., Savard, M.E., 2005. Previous crop residues and Fusarium head blight on cereals. In: Proceedings of the 4th Canadian workshop on Fusarium head blight, 1–3 November, 2005, Ottawa, ON.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., 2002. Introduction to Food- and Air-Borne Fungi, 6th ed. Centraalbureau voor Schimmelcultures, Utrecht.

Sanogo, S., Yang, X.B., Lundeen, P., 2001. Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. Plant Dis. 85, 773–779.

Saskatchewan Agriculture, Food and Rural Revitalization, 2005. Saskatchewan Varieties of Grain Crops 2005. Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK.

Schaafsma, A.W., Tamburic-Ilinic, L., Miller, J.D., Hooker, D.C., 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. Can. J. Plant Pathol. 23, 279–285.

Teich, A.H., Hamilton, J.R., 1985. Effect of cultural practices, soil phosphorus, potassium, and pH on the incidence of Fusarium head blight and deoxynivalenol levels in wheat. Appl. Environ. Microbiol. 49, 1429–1431.

Teich, A.H., Nelson, K., 1984. Survey of Fusarium head blight and possible effects of cultural practices in wheat fields in Lambton County in 1983. Can. Plant Dis. Surv. 64, 11–13.

Tinline, R.D., Hunter, J.H., 1982. Herbicides and common root rot of wheat in Saskatchewan. Can. J. Plant Pathol. 4, 341–348.
Tinline, R.D., Spurr, D.T., 1991. Agronomic practices and common root rot in spring wheat: effect of tillage on disease and inoculum density of *Cochliobolus sativus* in soil. Can. J. Plant Pathol. 13, 258–266.
Turkington, T.K., Clear, R.M., Burnett, P.A., Patrick, S.K., Orr, D.D., Xi, K., 2002. Fungal plant pathogens infecting barley and wheat seed from Alberta 1995–1997. Can. J. Plant Pathol. 24, 302–308.
Wardle, D.A., Parkinson, D., 1992. The influence of the herbicide glyphosate on interspecific interactions between four soil fungal species. Mycol. Res. 96, 180–186.
Watanabe, T., 2002. Pictorial Atlas of Soil And Seed Fungi. Morphologies of Cultured Fungi and Key to Species, 2nd ed. CRC Press, Boca Raton, FL.
Weber, R., Hrynczuk, B., Runowska-Hrynczuk, B., Kita, W., 2001. Influence of the mode of tillage on diseases of culm base in some winter wheat varieties, oats and spring wheat. J. Phytopathol. 149, 185–188.
Windels, C.E., Wiersma, J.V., 1992. Incidence of *Bipolaris* and *Fusarium* on subcrown internodes of spring barley and wheat grown in continuous conservation tillage. Phytopathology 82, 699–705.
Yi, C., Kaul, H.P., Kubler, E., Schwadorf, K., Aufhammer, W., 2001. Head blight (*Fusarium graminearum*) and deoxynivalenol concentration in winter wheat as affected by pre-crop, soil tillage and nitrogen fertilization. Z. Pflanzenkr. Pflanzensch. 108, 217–230.
Yi, C., Kaul, H.P., Kubler, E., Aufhammer, W., 2002. Populations of *Fusarium graminearum* on crop residues as affected by incorporation depth, nitrogen and fungicide application. Z. Pflanzenkr. Pflanzensch. 109, 252–263.
Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421.
Zentner, R.P., Wall, D.D., Smith, D.G., Young, D.L., Miller, P.R., Campbell, C.A., Lafond, G.P., Brandt, S.A., Johnston, A.M., 2002. Economics of crop diversification opportunities for the northern Great Plains. Agron. J. 94, 216–230.

Europ. J. Agronomy xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Glyphosate effects on diseases of plants

G.S. Johal*, D.M. Huber[1]

Department of Botany & Plant Pathology, Purdue University, 915 West State Street, West Lafayette, IN 47907, USA

ARTICLE INFO

Article history:
Received 5 June 2008
Received in revised form 24 February 2009
Accepted 20 April 2009

Keywords:
Glyphosate
Plant defense
Disease
Manganese
Micronutrient
Transgenic
Soil microflora

ABSTRACT

Glyphosate, N-(phosphonomethyl)glycine, is the most extensively used herbicide in the history of agriculture. Weed management programs in glyphosate resistant (GR) field crops have provided highly effective weed control, simplified management decisions, and given cleaner harvested products. However, this relatively simple, broad-spectrum, systemic herbicide can have extensive unintended effects on nutrient efficiency and disease severity, thereby threatening its agricultural sustainability. A significant increase in disease severity associated with the wide spread application of the glyphosate herbicide can be the result of direct glyphosate-induced weakening of plant defenses and increased pathogen population and virulence. Indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients involved in physiological disease resistance. Strategies to ameliorate the predisposing effects of glyphosate on disease include judicious selection of herbicide application rates, micronutrient amendment, glyphosate detoxification in meristematic tissues and soil, changes in cultural practices to enhance micronutrient availability for plant uptake, and biological amendment with glyphosate-resistant microbes for nitrogen fixation and nutrient availability. Given that recommended doses of glyphosate are often many times higher than needed to control weeds, we believe the most prudent method to reduce the detrimental effects of glyphosate on GR crops will be to use this herbicide in as small a dose as practically needed. Such a frugal approach will not only curtail disease predisposition of GR crops, but will also benefit the grower and the environment.

1. Introduction

Changes in agricultural practices such as crop rotation, crop sequence, tillage, and fertility that affect the soil microflora or nutrient availability generally result in changes in disease expression (Datnoff et al., 2007; Englehard, 1989; Huber and Graham, 1999). This is commonly observed for soilborne diseases where only limited innate resistance is available in commercial cultivars so that cultural controls become important management practices to minimize the impact of these diseases. Threatening to make things worse in this regard is the introduction of herbicide-resistant crops (canola, corn, cotton, soybeans, alfalfa, etc.) that are now grown extensively throughout the world. This new trend in agriculture has increased the usage and intensity of specific herbicides while limiting genetic diversity in the specific crops that have been genetically modified.

Herbicides are known to increase specific plant diseases (Altman and Campbell, 1977; Hornby et al., 1998; Mekwatanakarn and Sivasithamparam, 1987), and several are reported to influence micronutrient availability (Evans et al., 2007; Huber et al., 2004, 2005). Micronutrients are the activators or inhibitors of many critical physiological functions. Thus, a deficiency or change in availability of these regulatory elements can greatly affect plant growth and resistance to diseases and pests (Datnoff et al., 2007). The virulence mechanism of some pathogens such as *Gaeumannomyces*, *Magnaporthe*, *Phymatotrichum*, *Corynespora*, and *Streptomyces* involves Mn oxidation at the infection site to compromise the plant's resistance mechanisms involving the shikimate pathway (Thompson and Huber, 2007). Isolates of these pathogens that cannot oxidize physiologically available Mn^{2+} to the non-available Mn^{4+} are avirulent and not able to cause significant tissue damage (Roseman et al., 1991). Production of the Mn oxidizing enzyme(s) occurs soon after spore germination and during epiphytic growth (Cheng, 2005; Schulze et al., 1995; Thompson et al., 2005). Environmental conditions that reduce the availability of micronutrients for plant uptake also predispose plants to disease (Huber and McCay-Buis, 1993; Huber and Graham, 1999; Thompson and Huber, 2007).

* Corresponding author. Tel.: +1 765 494 4448; fax: +1 765 494 0363.
E-mail addresses: gjohal@purdue.edu (G.S. Johal), huberd@purdue.edu (D.M. Huber).
[1] Tel.: +1 765 714 6825.

1161-0301/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2009.04.004

Table 1
Some diseases increased in glyphosate weed control programs.

Plant	Disease	Pathogen	References
Apple	Canker	*Botryosphaeria dothidea*	Rosenberger and Fargione (2004)
Banana	Panama disease	*Fusarium oxysporum* f. sp. *cubense*	Harper (2007)
Barley	Root rot	*Magnaporthe grisea*	Smiley et al. (1992)
Bean	Anthracnose	*Colletotrichum lindemuthianum*	Johal and Rahe (1984, 1988, 1990)
Bean	Damping off, root rot	*Pythium* spp.	Johal and Rahe (1984)
Bean	Root rot	*Fusarium solani* f. sp. *phaseoli*	Harper (2007)
Bean	Hypocotyl rot	*Phytophthora megasperma*	Keen et al. (1982)
Canola	Crown rot	*Fusarium* spp.	Harper (2007)
Canola	Wilt	*Fusarium oxysporum*	Harper (2007), Large and McLaren (2002)
Citrus	Citrus variegated chlorosis	*Xylella fastidiosa*	Yamada (2006)
Citrus	Crown rot	*Phytophthora* spp.	Yamada (2006)
Cotton	Damping off	*Pythium* spp.	Harper (2007)
Cotton	Bunchy top	Manganese deficiency	Harper (2007)
Cotton	Wilt	*F. oxysporum* f. sp. *vasinfectum*	Harper (2007)
Grape	Black goo	*Phaeomoniella chlamydospora*	Harper (2007)
Melon	Root rot	*Monosporascus cannonbalus*	
Soybeans	Root rot	*Corynespora cassiicola*	Huber et al. (2005)
Soybeans	Target spot	*Corynespora cassiicola*	Huber et al. (2005)
Soybeans	Sudden Death Syndrome	*Fusarium solani* f. sp. *glycines*	Keen et al. (1982)
Soybeans	Root rot	*Phytophthora megasperma*	Keen et al. (1982)
Soybeans	Cyst nematode	*Heterodera glycines*	Geisler et al. (2002), Kremer et al. (2000)
Soybeans	White mold	*Sclerotinia sclerotiorum*	Harper (2007)
Sugar beet	Yellows	*Fusarium oxysporum* f. sp. *betae*	Larson et al. (2006)
Sugar beet	Root rot	*Rhizoctonia solani*	Larson et al. (2006)
Sugarcane	Decline	*Marasmius* spp.	Huber (unpublished)
Tomato	Crown root rot	Fusarium	Bramhall and Higgins (1988)
Tomato	Wilt	*Fusarium oxysporum* f. sp. *pisi*	Harper (2007)
Various	Canker	*Phytophthora* spp.	Harper (2007)
Weeds	Biocontrol	*Myrothecium verrucaria*	Boyette et al. (2006)
Wheat	Bare patch	*Rhizoctonia solani*	Harper (2007)
Wheat	Glume blotch	*Septoria* spp.	Harper (2007)
Wheat	Root rot	*Fusarium* spp.	Fernandez et al. (2005, 2007), Harper (2007)
Wheat	Head scab	*Fusarium graminearum*	Fernandez et al. (2005)
Wheat	Take-all	*Gaeumannomyces graminis*	Hornby et al. (1998)

The herbicide glyphosate, N-(phosphonomethyl)glycine, is a strong systemic metal chelator and was initially patented for that purpose (Bromilow et al., 1993). Its herbicidal action is by chelating with Mn, a cofactor for the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme in the shikimate pathway, to inhibit this metabolic pathway of plants and many microorganisms (Cerdeira and Duke, 2006; Grossbard and Atkinson, 1985; Jaworski, 1972). Many cations chelate with glyphosate, thus reducing its herbicidal efficacy (Bernards et al., 2005; Hickman et al., 2002). Plants with a compromised shikimate metabolism are predisposed to various plant pathogens (Johal and Rahe, 1988; Rahe et al., 1990), and glyphosate is patented as a synergist for mycoherbicides to enhance the virulence and pathogenicity of organisms used for biological weed control (Boyette et al., 2006; Duke and Cerdeira, 2005). The synergistic activity of glyphosate weed control in predisposing plants to infectious organisms has been observed for many diseases (Table 1), and the extensive use of glyphosate in agriculture is a significant factor in the increased severity or "reemergence" of diseases once considered efficiently managed.

The extensive adoption of Roundup Ready® crops such as soybeans, canola, cotton, and corn has intensified the application of glyphosate in these production systems. The applied glyphosate is readily translocated to roots and released throughout the rhizosphere in root exudates of Roundup Ready® plants as well as glyphosate-sensitive plants (Bromilow et al., 1993; Grossbard and Atkinson, 1985). The toxic microbial effects of glyphosate are cumulative with continued use so that Mn deficiency is now observed in areas that were previously considered Mn sufficient because of reduced populations of Mn-reducing soil organisms (Huber, unpublished). The presence of the glyphosate-resistance gene in corn and soybeans also reduces Mn uptake and physiological efficiency (Dodds et al., 2002a,b,c; Gordon, 2006; Reichenberger, 2007). Along with glyphosate-induced Mn deficiency, there has been a gradual recognition of increased disease severity (Harper, 2007; Larson et al., 2006). A few examples are presented to illustrate this relationship.

2. Some diseases increased by glyphosate

2.1. Corynespora root rot of soybean

The damage from Corynespora root rot, previously considered minor, may become economically damaging in Roundup Ready® soybeans since application of glyphosate to Roundup Ready® soybeans greatly increases severity of this disease (Fig. 1). This fungal root rot is more severe when glyphosate is applied to soybeans under weedy conditions even though the weeds may not be hosts for *Corynespora cassiicola*. The weeds serve to translocate and release more glyphosate into the rhizosphere environment to reduce the population of Mn-reducing organisms and increase Mn-oxidizing organisms. This change in soil biology limits manganese availability for plant uptake and active defense reactions, and acts synergistically with *Corynespora* to increase disease (Huber et al., 2005).

2.2. Take-all of cereal crops

The most comprehensive understanding of the interaction of micronutrients influenced by glyphosate and disease is with the take-all disease of cereals. Increased take-all of cereals after a preplant "burn-down" use of glyphosate has been recognized for over 15 years (Hornby et al., 1998). Take-all is also increased when glyphosate is applied to Roundup Ready® soybeans the preceding year compared with the use of a non-glyphosate herbicide (Fig. 2). All of the conditions known to affect Mn availability are inversely related to the severity of take-all (and other diseases, Table 2) so that

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

Fig. 1. Increased severity of Corynespora root rot after glyphosate application to Roundup Ready® soybeans. Non-inoculated control (left), inoculated plants (center), inoculated plants sprayed with glyphosate (right).

Fig. 2. More severe take-all root rot of wheat grown following Roundup Ready® soybeans sprayed with glyphosate (left) than following Roundup Ready® soybeans grown with a non-glyphosate herbicide (right).

Table 2
Some conditions affecting the form of nitrogen, manganese availability, and severity of take-all, rice blast, potato scab, *Phymatotrichum* root rot, and corn stalk rot (after Thompson and Huber, 2007).

Soil factor or cultural practice	Favored N form (NH_4 vs. NO_3)	Manganese availability	Severity of these diseases
Low soil pH	NH_4	Increase	Decrease
Green manures (some)	NH_4	Increase	Decrease
Ammonium fertilizers	NH_4	Increase	Decrease
Irrigation (some)	NH_4	Increase	Decrease
Firm seed bed	NH_4	Increase	Decrease
Nitrification inhibitors	NH_4	Increase	Decrease
Soil fumigation	NH_4	Increase	Decrease
Metal sulfides	NH_4	Increase	Decrease
High soil pH	NO_3	Decrease	Increase
Lime	NO_3	Decrease	Increase
Nitrate fertilizers	NO_3	Decrease	Increase
Manure	NO_3	Decrease	Increase
Low soil moisture	NO_3	Decrease	Increase
Loose seed bed	NO_3	Decrease	Increase

those conditions that increase the availability of Mn for plant uptake generally reduce take-all, and those that reduce Mn availability increase take-all (Huber and McCay-Buis, 1993). Microorganisms proposed for biological control of this disease such as *Bacillus cereus* and *Trichoderma konigii* are all strong Mn reducers that increase Mn availability in the rhizosphere (Huber and McCay-Buis, 1993; McCay-Buis, 1998; Rengel et al., 1996). In contrast, the addition of Mn-oxidizing organisms increases take-all (Crowley and Rengel, 1999; McCay-Buis, 1998; Rengel, 1999; Thompson et al., 1998). *Gaeumannomyces graminis* is a strong Mn oxidizer in soil and as it grows externally along plant roots (Thompson et al., 2000, 2005). Isolates of *Gaeumannomyces* that cannot oxidize Mn are avirulent, and isolates that oxidize Mn only at certain temperatures are virulent only at temperatures where they can oxidize Mn (Roseman et al., 1991).

Species of *Gramineae* such as rye (*Secale cereale* L.) that are efficient in Mn uptake are resistant to take-all compared with the relatively inefficient, highly susceptible wheat (*Triticum aestivum* L.) (Hornby et al., 1998). In contrast, resistance of oats to take-all is associated with glycocyanide root exudates that are toxic to Mn-oxidizing organisms in the rhizosphere. Oats, as a precrop for wheat, provide effective control of take-all in many areas because of the induced shift in soil biological activity that is less favorable for Mn oxidation. The biological activity favoring Mn availability reduces take-all severity for two or more subsequent wheat crops even though there is little change in the pathogen population (Huber and McCay-Buis, 1993). Glyphosate, in contrast to oats, is toxic to Mn-reducing and N-fixing organisms in soil so that the availability of nitrogen and Mn in soil may be markedly compromised (Huber et al., 2004). Low levels of residual glyphosate in soil also reduce root uptake and translocation of Fe, Mn, and Cu (Eker et al., 2006; Ozturk et al., 2008). Increased take-all root, crown, and foot rot of cereals following glyphosate applications (Hornby et al., 1998; Huber and McCay-Buis, 1993) may be the result of reduced resistance from induced Mn deficiency, inhibited root growth from glyphosate accumulation in root tips, modified virulence of the pathogen, or an increase in synergistic Mn-oxidizing organisms in the rhizosphere.

2.3. Diseases caused by *Xylella fastidiosa*

Various diseases caused by *X. fastidiosa* are referred to as "emerging" or "reemerging" diseases as glyphosate weed management

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

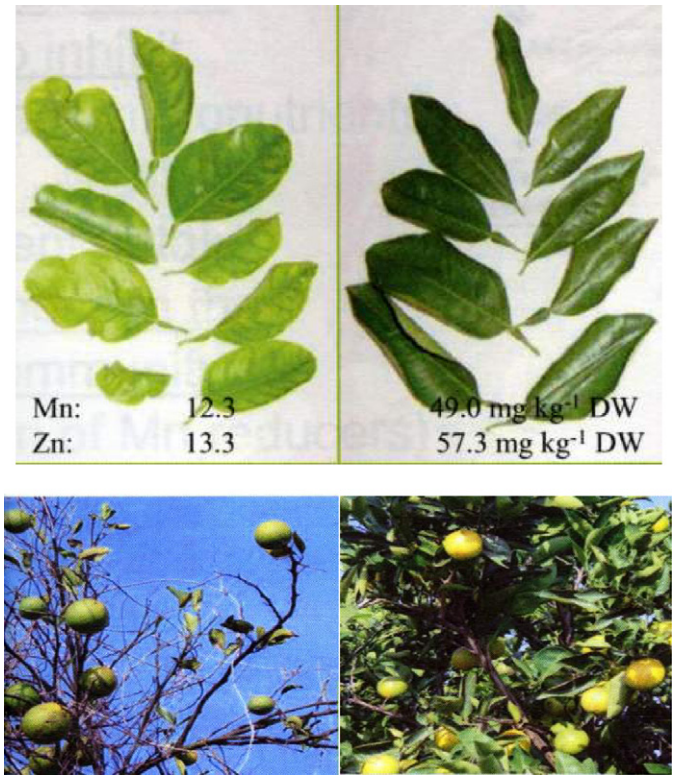


Fig. 3. Expression of citrus variegated chlorosis under glyphosate (left) compared with an alternative mulch (right) weed control program. All trees are infected with the CVC pathogen, *Xylella fastidiosa* (after Yamada, 2006). Left: severe Mn and Zn deficiency, and eventual severe decline in vigor with the glyphosate weed management program. Right: restoration of tissue nutrient levels and productivity of *X. fastidiosa* infected trees under the non-glyphosate mulch system.

programs for their respective crops have intensified. These diseases (Pierce's disease of grapevine, plum scorch, almond scorch, citrus variegated chlorosis, coffee blight, citrus blight, alfalfa dwarf, pecan decline, etc.) are characterized by a loss of vigor, slow decline, micronutrient deficiency, and reduced productivity. The pathogen is an endophytic bacterium that colonizes xylem tissues and restricts nutrient translocation when plants are stressed. Citrus variegated chlorosis (CVC) was first described on oranges in Brazil in 1987 and is also recognized in Puerto Rico. An early symptom of this disease is a variegated chlorosis of foliage (Fig. 3) similar to a deficiency of, and associated with, a drop in tissue levels of Mn and Zn (Li et al., 1996). Normal flushes of new growth are sparse or absent, fruit is small, "skirts" of trees move up, and trees enter a serious decline in growth and productivity. A similar disease referred to as "citrus blight" occurs worldwide and causes the death of several hundred thousand citrus trees annually in the United States (Derrick and Timmer, 2000; Timmer, 2000). Yamada (2006) developed the only known control for CVC, and properly managed trees return to full productivity even though the pathogen may still be present. Control of CVC emphasizes elimination of glyphosate and adoption of an alternative grass mulch weed control program for citrus orchards in Brazil (Yamada and Castro, 2005). This control strategy uses optimally fertilized *Brachiaria* grass grown between the tree rows. The grass is mowed twice a year to provide a 10–15 cm mulch under the citrus trees for weed control and nutrition. Natural mineralization of this mulch inhibits nitrification to provide an ammonium source of nutrition for the citrus trees, Mn and Zn tissue levels are restored to sufficiency levels, and trees in early to mid-decline produce a new flush of growth. Full productivity is restored within a few years. Removing glyphosate from the citrus production system also has significantly reduced the occurrence of *Phytophthora* crown rot.

2.4. Fusarium diseases

Various diseases caused by *Fusarium* spp. are increased by glyphosate (Fernandez et al., 2005; Sanogo et al., 2000, 2001). Glyphosate has made crops susceptible to normally non-pathogenic isolates of *Fusarium*, and the population of *Fusarium* increases in soil after glyphosate application (Levesque et al., 1987; Kremer et al., 2000). Glyphosate predisposes tomato to *Fusarium* crown and root rot by inhibiting the plant's structural and defense barriers (Bramhall and Higgins, 1988). Cotton growers in Australia and the Western United States have seen a resurgence of Fusarium wilt since the introduction of Roundup Ready® cotton, and previously high levels of wilt resistance appear to be less effective under glyphosate management programs (Harper, 2007). Glyphosate also breaks resistance to cyst nematodes in soybeans (Geisler et al., 2002). The increased Fusarium yellows and *Rhizoctonia solani* diseases of Roundup Ready® sugar beets prompted Larson et al. (2006) to comment that "precautions need to be taken when certain soilborne diseases are present if weed management for sugar beet is to include post-emergence glyphosate treatments." These authors also reported that the sugar beet variety resistant to *Rhizoctonia* was as susceptible to this pathogen as the susceptible variety after glyphosate application regardless of the time of inoculation.

Fusarium head scab of cereals and other diseases caused by *Fusarium* spp. increase following glyphosate applications (Fernandez et al., 2005; Larson et al., 2006), and previously established "cardinal" conditions (precipitation, flowering, and temperatures above 26 °C) for head scab are modified when glyphosate is applied prior to a susceptible cereal crop (Fernandez et al., 2005, 2007). Glyphosate modifies plant nitrogen metabolism similar to high temperature-induced changes that provide susceptibility to head scab (Huber, unpublished) so that head scab and the mycotoxins produced by the causal fungi are now prevalent in cooler areas where they were rarely observed before the extensive use of glyphosate (Fernandez et al., 2005, 2007). Similar changes in nitrogen and carbohydrate metabolism provide transient resistance of wheat and soybeans to rust after glyphosate application (Anderson and Kolmer, 2005; Feng et al., 2005, 2007).

The Palouse area of Washington, Idaho, and Oregon in the United States has had a long history of pea, lentil, and wheat production on the deep loess soils characteristic of the area; however, pea and lentil yields have been in slow decline as symbiotic nitrogen fixation is reduced and Fusarium diseases increased commensurate with the extensive use of glyphosate for no-till wheat production. Pea and lentil production are now uneconomical in some areas, and production is rapidly moving from the Palouse to Montana where glyphosate usage has been more limited. The loss of legumes in crop rotations in the Palouse area can result in serious degradation of these once highly productive soils with few economical, alternative crops available as replacements. A new Fusarium wilt of canola caused by *F. oxysporum* and *F. avenaceum* has caused severe yield reductions in nutrient poor soils of Alberta and Saskatchewan, Canada since 2000, but has not yet become a problem in the Mn-rich soils in the Red River valley (Lange and McLaren, 2002).

3. Predisposition to disease underlies the herbicidal efficacy of glyphosate

Inhibition of EPSP synthase initially was considered to be the sole target of glyphosate in plants. It was believed that this mode of action would kill plants by starving them of aromatic amino acids through deregulation of the shikimate pathway (Cerdeira

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

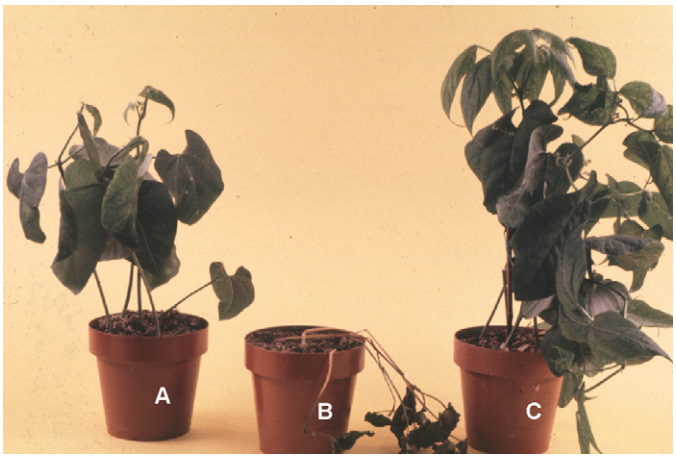


Fig. 4. Fate of glyphosate-treated (10 μg plant^{-1}) bean plants grown in (A) vermiculite and (B) field soil 20 days after glyphosate treatment, and (C) non-glyphosate treated control plants. Glyphosate treated plants in field soil (B) collapsed 10 days after glyphosate treatment from *Pythium* infection.

and Duke, 2006; Grossbard and Atkinson, 1985; Jaworski, 1972). This, however, did not explain some aspects of the death caused by glyphosate. For instance, glyphosate must be translocated to roots to be effective, although growth of the plant stops soon after application of the herbicide. In addition, effects of sublethal doses of this herbicide on perennial plants sometimes appear a year after exposure and persist for two or more years (Rahe et al., 1990). These characteristics of glyphosate-induced injury suggest that the herbicidal action of glyphosate was more than simply the starvation of treated plants of aromatic amino acids as assumed initially (Rahe et al., 1990).

Intrigued by these observations and the possibility that something about the root environment may contribute to the herbicidal action of glyphosate, a systematic research effort was launched in the early 1980s that led to the following findings (Levesque and Rahe, 1992; Rahe et al., 1990):

(1) The herbicidal efficacy of glyphosate is largely due to colonization of roots of affected plants by soil-borne pathogens (Fig. 4) (Johal and Rahe, 1984).
(2) Two pathogens that are most important in this regard are *Pythium*, an oomycete, and *Fusarium*, an ascomycete. Both of these pathogens are ubiquitous in agricultural and other soils.
(3) Plants growing in sterile medium do not die even though their growth is temporarily inhibited by glyphosate.
(4) Amending sterile media with *Pythium* or *Fusarium* restores the ability of glyphosate to kill plants.
(5) Both *Pythium* and *Fusarium* begin to colonize plants within a day or two of glyphosate application to foliar parts of the plant (Fig. 4) (Johal and Rahe, 1984; Levesque et al., 1993).
(6) The amount of glyphosate needed to kill plants in natural soils is much lower than the recommended dose.

These results suggested that glyphosate was somehow compromising the ability of plants to defend against rhizosphere-inhabiting pathogens.

4. Mechanisms of predisposition to disease

Plants rely on multiple components of defense to deter pathogens following infection (Hammond-Kosack and Jones, 2000). Many of these active resistance components are derived from the phenylpropanoid pathway, which acquires almost all of its precursors (notably phenylalanine and chorismate) from the shikimic acid pathway (Hammond-Kosack and Jones, 2000; Dixon et al., 2002). A key inducible defense component associated with the shikimic acid pathway is the production of antimicrobial phytoalexins that accumulate rapidly at the site of infection. Lignification of cell walls at and around the infection site is another shikimate-derived component that functions to fortify cells and ensure isolation of the pathogen at the infection site. Production of salicylic acid (SA) following infection represents another component of inducible defense. SA is synthesized either directly from chorismic acid or indirectly through phenylalanine. Although SA is not antimicrobial per se, it functions to signal and coordinate various defenses following challenge by a pathogen; however, its direct role in plant–pathogen interactions involving root tissue remains unclear. Another defense component that relies on three final products of the shikimic acid pathway – tryptophan, tyrosine and phenylalanine – is the production of a diverse variety of pathogenesis-related (PR) proteins that function to curtail the advance of a pathogen. Many kinds of PR proteins have been identified (Hammond-Kosack and Jones, 2000).

Given the reliance of many plant defenses on the shikimic acid pathway, and the fact that glyphosate blocks this pathway, it is not surprising that this herbicide would render plants more susceptible to pathogens. Keen et al. (1982) were the first to show that by inhibiting the phytoalexin glyceollin, glyphosate was able to compromise resistance of soybeans to *Phytophthora megasperma* f. sp. *glycines*. Using the bean-anthracnose pathosystem, Johal and Rahe (1988, 1990) demonstrated that, while glyphosate did not interfere with the hypersensitive reaction (HR) of incompatible interactions, it suppressed significantly the production of all four of the bean phytoalexins. As a result, the pathogen was able to kill the plant if it escaped the localized HR, a situation that occurred only with glyphosate-treated plants (Fig. 5). The effect of glyphosate on the compatible bean anthracnose interaction was even more dramatic (Johal and Rahe, 1990). Glyphosate almost completely suppressed the production of phytoalexins associated with susceptible lesion containment and permitted the pathogen to invade unimpededly until the entire hypocotyl collapsed (Figs. 6 and 7). As little as 2% of the recommended herbicidal rate of glyphosate was enough to transform normally delimited lesions typical of anthracnose into constantly expanding lesions (Johal and Rahe, 1990).

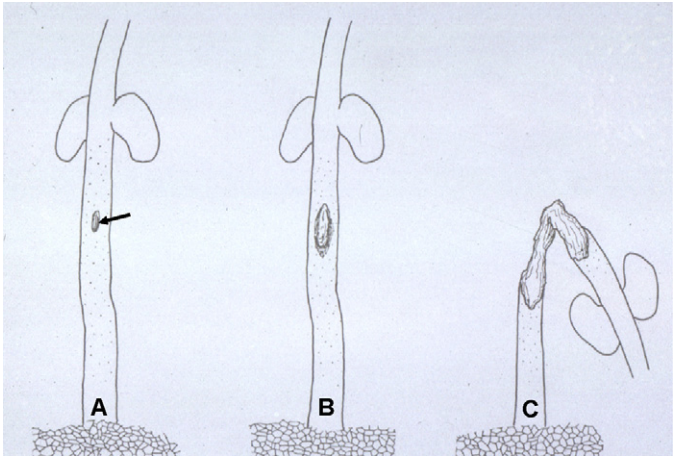


Fig. 5. Diagramatic representation of glyphosate-treated bean seedlings following inoculation with an incompatible race of *Colletotrichum lindemuthianum*. (A) Dots all over the hypocotyl represent hypersensitive reaction (HR) sites (cells) incited by the pathogen on spray inoculation. Arrow indicates the site where a drop of glyphosate (10 μg) was placed. (B) The fungus normally contained inside HR cells sometimes escapes near the glyphosate treatment site and results in a susceptible lesion (arrow). (C) The lesion continues to expand to kill the plant after glyphosate treatment.

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

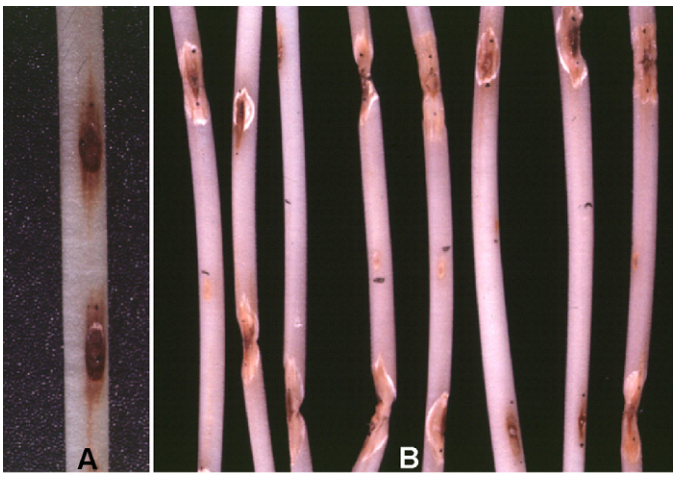


Fig. 6. Anthracnose lesions on bean hypocotyls in the (A) absence of glyphosate and (B) presence of glyphosate. A 10 μg drop of glyphosate (black dot) was placed near the center of the hypocotyls one day after inoculation with *C. lindemuthianum* in (B). Note the loss of lesion delimitation and collapse of tissue seven days after glyphosate treatment.

The defense studies mentioned above were confined largely to diseases of aerial parts of host plants. There are indications that defense components may vary significantly in root tissue that are in intimate and continuous contact with potential pathogens (Hammond-Kosack and Jones, 2000). For instance, roots do not rely on HR-mediated defense to contend with pathogens, although the exact defense components that keep roots pathogen-free are only partially understood. To gain an insight into what contributes to glyphosate-induced susceptibility of French beans (*Phaseolus vulgaris*) to *Pythium*, Liu et al. (1995, 1997) assessed phytoalexins as well as lignification of root tissue in response to glyphosate treatment. By comparing phytoalexins in roots of bean seedlings grown in different media, they concluded that phytoalexins were induced by soil microorganisms. Interestingly, while phytoalexin accumulation was affected only modestly by glyphosate in response to exposure to *Pythium*, lignification (a process requiring Mn) was suppressed significantly. Thus, enhanced colonization by *Pythium* in roots of bean seedlings treated with foliar applied glyphosate occurs as a result of glyphosate interference with lignin-based defense mechanisms (Liu et al., 1997). However, these results also suggest that sustained production of phytoalexins in response to *Pythium* infection is maintained temporarily following glyphosate treatment, whereas lignification is not.

5. Roundup Ready® plants and disease predisposition

Given that the herbicidal activity of glyphosate is mediated largely by its ability to lower plant immunity to pathogens, the status of Roundup Ready® plants with regard to such predisposition following glyphosate treatment becomes a serious consideration. For reasons that were not explained, Cerdeira and Duke (2006) contended that reduced resistance to pathogens in response to glyphosate treatment should not occur in Roundup Ready® plants. This is a misconception that can hold true only if the Roundup Ready® transgene following glyphosate treatment operates and behaves in exactly the same manner as the native EPSP synthase gene does in the absence of glyphosate. Such a scenario is possible only if the Roundup Ready® transgene is completely insensitive to glyphosate and is also as efficacious as the native EPSP synthase gene is in the absence of glyphosate. In addition, the Roundup Ready® gene has to match exactly the transcriptional activity of the native gene in every tissue of the plant and under all conditions, both normal and stressful. This is a tall order of requirements that is unlikely to be fulfilled by the present day Roundup Ready® transgenics, thus making it highly probable that our Roundup Ready® crops are vulnerable to glyphosate toxicity under at least some conditions. One such condition could arise when the level of glyphosate exceeds the ability of the transgenic enzyme to tolerate it, and yet another may develop if the transgene fails to match the transcriptional activity and profile of the native gene under conditions of biotic stress. Both of these scenarios are possible and, if they develop, it is very likely they would enhance the vulnerability of Roundup Ready® plants to fungal diseases following Roundup application.

Glyphosate treatment of transgenic crops to manage weeds can also promote disease damage indirectly by impacting the inoculum potential of pathogens. Shortly after soilborne fungi's causative role was revealed in the herbicidal efficacy of glyphosate (Johal and Rahe, 1984), Levesque et al. (1987) documented a significant, albeit temporary, spike in the level of fungal pathogens in the rhizosphere following glyphosate application to weeds. This prompted the speculation that such a buildup of pathogen load could have ill effects for subsequent crop plants. This, indeed, was found to be the case in barley fields in which significant yield reductions were witnessed if the crop was planted within a few days after glyphosate application (Smiley et al., 1992). Although the latter study was conducted on non Roundup Ready® barley, it is likely that a similar boost in the inoculum potential of pathogens in the rhizosphere (also called 'green bridge') could lead to enhanced root rot problems in Roundup Ready® crops as well.

A prudent way to avoid disease enhancement is to decrease the concentration of glyphosate applied to Roundup Ready® crops. Many studies have documented that the levels of glyphosate necessary to kill or compromise the health of many weeds are several fold lower than the generally recommended application rates (Rahe et al., 1990). An alternative to using insensitive EPSP synthase genes to generate glyphosate-resistant plants might be to use genes that degrade glyphosate. Three such genes which inactivate glyphosate by oxidation (the *Gox* gene), acetylation (the *Gat* gene) or decarboxylation (the *Gdc* gene) have become available in recent years (Cerdeira and Duke, 2006; Dill, 2005). If the problem persists, there is also the possibility of stacking a resistant EPSP synthase gene with a glyphosate metabolism gene as has been done in canola (Dill, 2005). A major disadvantage of this strategy is that it may encourage the application of higher levels of glyphosate than needed. In turn, this would not only impact the environment negatively but also would hasten the evolution of resistant weeds and thereby further threaten sustainability of this herbicide.

6. Strategies to ameliorate glyphosate predisposition to disease

Several strategies may be deployed to reduce glyphosate-induced predisposition to disease. These strategies primarily focus

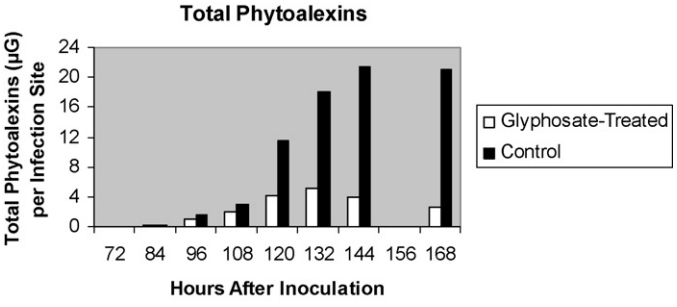


Fig. 7. Glyphosate suppression of phytoalexins in compatible bean anthracnose lesions by 10 μg glyphosate (after Johal and Rahe, 1990).

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

on four aspects of the glyphosate-disease-environment interaction, i.e.:

(1) minimizing non-target exposure to glyphosate by limiting the rates of glyphosate used,
(2) enhancing micronutrient sufficiency to maintain optimum plant physiological function and resistance,
(3) detoxifying accumulated glyphosate in root tips and other meristematic tissues to restore growth potential, and
(4) moderating glyphosate toxicity to rhizosphere microbes or restoring critical microbial components damaged by glyphosate released in root exudates.

6.1. Minimizing non-target exposure to glyphosate by limiting the rates of glyphosate used

As stated earlier, the rates of glyphosate generally recommended for herbicide use are far in excess of the amount required to kill most weeds. Excess application has occurred primarily as a result of advertising promotions, ease of application, increasing weed resistance, low cost of the product, and apathy towards the extensive non-target environmental effects of glyphosate. Very low levels of residual glyphosate in soil can greatly impede the availability and uptake of Mn, Fe, Cu, and Zn with subsequent translocation to vegetative tissues also impeded (Eker et al., 2006; Ozturk et al., 2008). This limitation in uptake and translocation can greatly impede the "replenishing" of these critical micronutrients and restoration of physiological resistance mechanisms dependent on them after nutrient immobilization in tissues by the applied glyphosate. A more judicious use of glyphosate would appear essential to maintain sustainable crop production efficiency.

6.2. Enhancing micronutrient sufficiency to maintain optimum plant physiological function and resistance

Most micronutrients are readily absorbed after foliar application, a common method of fertilization; however, some micronutrients, such as Mn, are relatively immobile and are not basipitally translocated to roots where soilborne root, hypocotyl, crown and vascular pathogens are established (Marschner, 1995; Thompson and Huber, 2007). Thus, although foliar application of Mn can provide nutrient sufficiency to foliar tissues for this essential element, it would not be effective in detoxifying accumulated glyphosate in root tip meristematic tissues or maintaining physiological resistance dependent on the shikimate pathway in root tissues because it is relatively immobile in the plant and does not move downward in the phloem.

A combination of foliar applied Mn with more mobile elements such as Cu or Zn could be more effective in detoxifying glyphosate in root tissues than Mn alone. Difficulties in meeting plant needs for Mn are further compounded since soil-applied Mn can be readily oxidized by soil organisms to the Mn^{4+} form that is not available for plant uptake (Marschner, 1995; Thompson and Huber, 2007). Reduced physiological efficiency of Roundup Ready® crops (Dodds et al., 2002a,b,c; Gordon, 2006; Zobiole et al., 2009) require higher levels of Mn to achieve nutrient sufficiency and comparable productivity as their non-genetically modified isolines (Reichenberger, 2007). Rates of Mn applied to Roundup Ready® soybeans required for comparable yield with non-RR soybean approached toxicity when applied to the isogenic non-Roundup Ready® soybean (Gordon, 2006; Reichenberger, 2007). The simultaneous application of many nutrients with glyphosate ("tank mixes") results in their immobilization and non-availability for plant physiological functions. Full physiological efficiency from nutrient application may not be achieved unless the micronutrients are applied eight to fifteen days after the glyphosate is applied. This is necessary to prevent chelation and immobilization by residual glyphosate in tissues that renders them physiologically unavailable (Huber et al., 2004; Severson, 2006), although earlier applications may be more effective in detoxifying tissue-bound glyphosate.

6.3. Detoxifying accumulated glyphosate in meristematic tissues

Reduced root growth from the accumulation of glyphosate in root tips results in less contact of the roots with dispersed nutrients in the soil profile and may negate tolerance of plants to soilborne pathogens based on their ability to "outgrow" the damage caused from loss of root tissue. Likewise, glyphosate accumulates in active meristematic tissue in shoots and developing fruits to inhibit growth of these tissues. Calcium, Mg, and micronutrients that chelate with glyphosate can reduce its biological activity and restore some of the potential physiological activity in these tissues. These "detoxifying" elements can come from within the plant or from further uptake from the soil. Thus, it is important to maintain mineral sufficiency in plant tissues and their ready availability in soil for plant uptake. This may be achieved by soil or foliar applied nutrients (Bernards et al., 2005; Huber et al., 2004; Reichenberger, 2007) if other environmental restraints are considered.

6.4. Eliminating glyphosate toxicity to rhizosphere microbes or restoring critical microbial components damaged by glyphosate released in root exudates

Detoxifying glyphosate in root exudates may occur in highly calcareous soils or soils with high levels of soluble metal nutrients through chelation to reduce its impact on soil organisms. Toxicity of glyphosate to Mn-reducing and synergistic nitrogen-fixing organisms in the rhizosphere can have serious consequences for sustainability of legume production. Regular inoculation of legume crops with synergistic nitrogen-fixing organisms may be required in many areas for maximal productivity where extended applications of glyphosate have eliminated them from the soil profile. Development of glyphosate-tolerant nitrogen-fixing and Mn-reducing organisms would be beneficial in many of these situations, and especially for perennial Roundup Ready® legume crops such as alfalfa.

7. Summary

Extended use of glyphosate can significantly increase the severity of various diseases by impacting all four of the interacting components of the "plant disease diamond" comprised of the plant, abiotic and biotic environments, and pathogen (Fig. 8). Reduced growth, impaired defenses, impaired uptake and translocation of nutrients, and altered physiology of plants by glyphosate can affect susceptibility or tolerance to various diseases. Glyphosate chelation of nutrients in the plant and soil can render those nutrients immobile and unavailable for plant use or uptake, while toxicity to essential synergistic and beneficial soil organisms (Purcell, 2001) further reduces availability of nutrients that are critical for a plant's physiological defense to disease. Glyphosate stimulation of fungal growth and enhanced virulence of pathogens such as *Fusarium*, *Gaeumannomyces*, *Phytophthora*, *Pythium*, and *Xylella* can have serious consequences for sustainable production of a wide range of susceptible crops and lead to the functional loss of genetic resistance that is dependent on metabolites through the shikimate pathway (Larson et al., 2006). Nutrient balance is important because each element functions as part of a delicately balanced, interdependent physiological system with the plant's genetics and the environment. Maximal utilization of cultural and management practices that increase the availability of nutrients (Table 2) to negate the deleterious effects of glyphosate should be incorporated

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

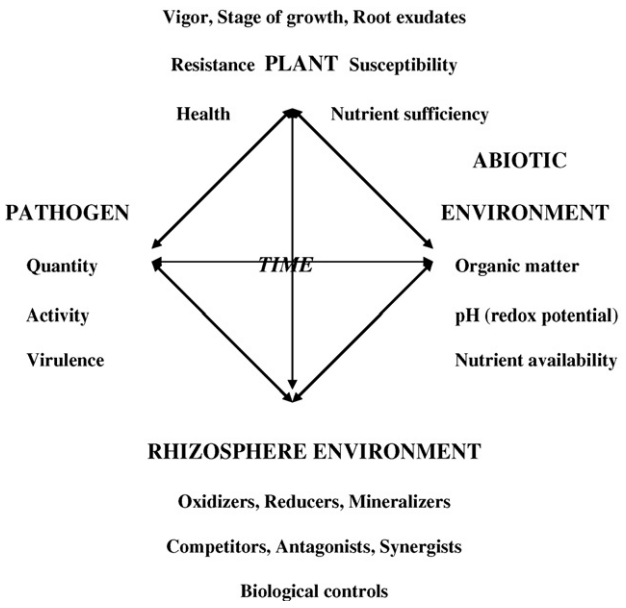


Fig. 8. The four primary interacting factors influencing nutrient availability and disease that are affected by glyphosate.

into crop production programs to facilitate optimal production efficiency and sustainable disease control. It is important to understand the effect of glyphosate on the chemical and biological properties of soils and its overall effects on the agricultural production system to permit its judicious use. Ignoring potential non-target detrimental side effects of any chemical, especially used as heavily as glyphosate, may have dire consequences for agriculture such as rendering soils infertile, crops non-productive, and plants less nutritious (Altman and Campbell, 1977). To do otherwise might well compromise not only agricultural sustainability, but also the health and well-being of animals and humans (Ozturk et al., 2008).

Acknowledgements

We are grateful to Dr. Larry Dunkle for providing a critical review of the manuscript, and to Lynn Johal for editorial comments.

References

Altman, J., Campbell, C.L., 1977. Effect of herbicides on plant diseases. Annu. Rev. Phytopathol. 15, 361–385.
Anderson, J.A., Kolmer, J.A., 2005. Rust control in glyphosate tolerant wheat following the application of the herbicide glyphosate. Plant Dis. 88, 1136–1142.
Bernards, M.L., Thelen, K.D., Muthukumaran, R.J., McCracken, J.L., 2005. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. Weed Sci. 53, 787–794.
Boyette, C.D., Reddy, K.N., Hoagland, R.E., 2006. Glyphosate and bioherbicide interaction for controlling kudzu (*Pueraria lobata*), redvine (*Brunnichia ovata*), and trumpetcreeper (*Campsis radicans*). Biocontrol Sci. Tech. 16, 1067–1077.
Bramhall, R.A., Higgins, V.J., 1988. The effect of glyphosate on resistance of tomato to Fusarium crown and root rot disease and on the formation of host structural defensive barriers. Can. J. Bot. 66, 1547–1555.
Bromilow, R.H., Chamberlain, K., Tench, A.J., Williams, R.H., 1993. Phloem translocation of strong acids – glyphosate, substituted phosphonic, and sulfonic acids – in *Ricinus communis* L. Pestic. Sci. 37, 39–47.
Cerdeira, A.L., Duke, S.O., 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. J. Environ. Qual. 35, 1633–1658.
Cheng, M.-W., 2005. Manganese transition states during infection and early pathogenesis in rice blast. M.S. Thesis. Purdue University, West Lafayette, Ind.
Crowley, D.E., Rengel, Z., 1999. Biology and chemistry of nutrient availability in the rhizosphere. In: Rengel, Z. (Ed.), Mineral Nutrition of Crops: Fundamental Mechanisms and Implications. Food Products Press, London, pp. 1–40 (Chapter 1).
Datnoff, L.E., Elmer, W.H., Huber, D.M., 2007. Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN.
Derrick, K.S., Timmer, L.W., 2000. Citrus blight and other diseases of recalcitrant etiology. Annu. Rev. Phytopathol. 38, 181–205.
Dill, G., 2005. Glyphosate resistant crops: history, status and future. Pest Manag. Sci. 61, 219–224.
Dixon, R.A., Achnine, L., Kota, P., Liu, C.-J., Reddy, M.S., Wang, L., 2002. The phenylpropanoid pathway and plant defense—a genomics perspective. Mol. Plant Pathol. 3, 371–390.
Dodds, D.M., Hickman, M.V., Huber, D.M., 2002a. Micronutrient uptake by isogenic glyphosate tolerant and normal corn. Proc. Proc. Weed Sci. Soc. Am. 42, 2.
Dodds, D.M., Huber, D.M., Hickman, M.V., 2002b. Micronutrient levels in normal and glyphosate-resistant soybeans. Proc. NC-Weed Sci. Soc. Am. 57, 107.
Dodds, D.M., Huber, D.M., Hickman, M.V., Shaw, D.R., 2002c. Hybrid and glyphosate application effects on nutrient uptake in corn. Proc. Weed Sci. Soc. Am. 43, 4.
Duke, S.O., Cerdeira, L., 2005. Potential environmental impacts of herbicide-resistant crops. Collect. Biosaf. Rev. 2, 66–143.
Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V., Cakmak, I., 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. J. Agric. Food Chem. 54, 10019–10025.
Englehard, A.W. (Ed.), 1989. Management of Diseases with Macro and Microelements. American Phytopathological Society, St. Paul, MN.
Evans, I.R., Solberg, E., Huber, D.M., 2007. Copper and plant disease. In: Datnoff, L.E., Elmer, W.H., Huber, D.M. (Eds.), Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN, pp. 177–188 (Chapter 12).
Feng, P.C.C., Baley, J., Clinton, W.P., Bunkers, G.J., Alibhai, M.F., Paulitz, T.C., Kidwell, K.K., 2005. Glyphosate inhibits rust diseases in glyphosate-resistant wheat and soybean. Proc. Natl. Acad. Sci. U.S.A. 102, 17290–17295.
Feng, P.C.C., Clark, C., Andrade, G.C., Baibl, M.C., Caldwell, P., 2007. The control of Asian rust by glyphosate in glyphosate-resistant soybeans. Pest Manag. Sci. 63, 1526.
Fernandez, M.R., Selles, F., Gehl, D., DePauw, R.M., Zentner, R.P., 2005. Crop production factors associated with Fusarium head blight in spring wheat in eastern Saskatchewan. Crop Sci. 45, 1908–1916.
Fernandez, M.R., Zentner, R.P., DePauw, R.M., Gehl, D.T., Stevenson, F.C., 2007. Impacts of crop production factors on Fusarium head blight in barley in eastern Saskatchewan. Crop Sci. 47, 1574–1584.
Geisler, L., Graef, G., Wilson, J., Schimelfenig, J., 2002. Interaction of glyphosate tolerance with soybean cyst nematode resistance. Phytopathology 92, S529.
Gordon, B., 2006. Manganese nutrition of glyphosate-resistant and conventional soybeans. Better Crops 91, 12–13.
Grossbard, E., Atkinson, D., 1985. The Herbicide Glyphosate. Butterworths, London.
Hammond-Kosack, K., Jones, J.D.G., 2000. Responses to plant pathogens. In: Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds.), Biochemistry and Molecular Biology of Plants. ASPP, Rockville, MD, pp. 1102–1156.
Harper, M., 2007. The Review of the Moratorium on GM Canola. Australia. http://www.dpi.vic.gov.au/dpi/nrenfa.nsf/LinkView/5477226A88881F86CA-2572E300074EEF89E6C67B4668BD2A7CA256FB70001BAB8.
Hickman, M.V., Dodds, D.M., Huber, D.M., 2002. Micronutrient interactions reduce glyphosate efficacy on tall fescue. Proc. Weed Sci. Soc. Am. 42, 18.
Hornby, D., Bateman, G.L., Gutteridge, R.J., Lucas, P., Osbourn, A.E., Ward, E., Yarham, D.J., 1998. Take-all Disease of Cereals: A Regional Perspective. CAB International, Wallingford, UK.
Huber, D.M., McCay-Buis, T.S., 1993. A multiple component analysis of the take-all disease of cereals. Plant Dis. 77, 437–447.
Huber, D.M., Graham, R.D., 1999. The role of nutrition in crop resistance and tolerance to diseases. In: Rengel, Z. (Ed.), Mineral Nutrition of Crops: Fundamental Mechanisms and Implications. Food Products Press, London, pp. 169–204.
Huber, D.M., Leuck, J.D., Smith, W.C., Christmas, E.P., 2004. Induced manganese deficiency in GM soybeans. In: Northcentral Fert. Extension Conf., Des Moines, IA, November 2004.
Huber, D.M., Cheng, M.W., Winsor, B.A., 2005. Association of severe Corynespora root rot of soybean with glyphosate-killed giant ragweed. Phytopathology 95, S45.
Jaworski, E.G., 1972. Mode of action of N-phosphonomethyl-glycine: inhibition of aromatic amino acid biosynthesis. J. Agric. Food Chem. 20, 1195–1198.
Johal, G.S., Rahe, J.E., 1984. Effect of soilborne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology 74, 950–955.
Johal, G.S., Rahe, J.E., 1988. Glyphosate, hypersensitivity and phytoalexin accumulation in the incompatible bean anthracnose host–parasite interaction. Physiol. Mol. Plant Pathol. 32, 267–281.
Johal, G.S., Rahe, J.E., 1990. Role of phytoalexins in the suppression of resistance of *Phaseolus vulgaris* to *Colletotrichum lindemuthianum* by glyphosate. Can. J. Plant Pathol. 12, 225–235.
Keen, N.T., Holliday, M.J., Yoshikawa, M., 1982. Effects of glyphosate on glyceollin production and the expression of resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 72, 1467–1470.
Kremer, R.J., Donald, P.A., Keaster, A.J., Minor, H.C., 2000. Herbicide impact on *Fusarium* spp. and soybean cyst nematode in glyphosate-tolerant soybean. Agron. Abstr., p257.
Lange, R., McLaren, D., 2002. Fusarium Wilt—A New Disease of Canola. http://www.umanitoba.ca/afs/agronomists conf/2002/pdf/lange.pdf.
Larson, R.L., Hill, A.L., Fenwick, A., Kniss, A.R., Hanson, L.E., Miller, S.D., 2006. Influence of glyphosate on Rhizoctonia and Fusarium root rot in sugar beet. Pest Manag. Sci. 62, 182–192.
Levesque, C.A., Rahe, J.E., et al., 1987. Effects of glyphosate on *Fusarium* spp.: its influence on root colonization of weeds, propagule density in the soil, and on crop emergence. Can. J. Microbiol. 33, 354–360.
Levesque, C.A., Rahe, J.E., 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu. Rev. Phytopathol. 30, 579–602.

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

Levesque, C.A., Rahe, J.E., Eaves, D.M., 1993. Fungal colonization of glyphosate treated seedlings using a new root plating technique. Mycol. Res. 97, 299–306.
Li, W.B., Donadio, L.C., Sempionato, O.R., Miranda, V.S., 1996. Resistance or tolerance of citrus species and cultivars to citrus variegated chlorosis. Proc. Int. Soc. Citricult. 1, 216–218.
Liu, L., Punja, Z.K., Rahe, J.E., 1995. Effect of *Pythium* spp. and glyphosate on phytoalexin production and exudation by bean (*Phaseolus vulgaris* L.) roots grown in different media. Physiol. Mol. Plant Pathol. 47, 391–405.
Liu, L., Punja, Z.K., Rahe, J.E., 1997. Altered root exudation and suppression of induced lignification as mechanisms of predisposition by glyphosate of bean roots (*Phaseolus vulgaris* L.) to colonization by *Pythium* spp. Physiol. Mol. Plant Pathol. 51, 111–127.
Marschner, H., 1995. Mineral Nutrition of Higher Plants. In: second ed. Academic Press, London, 889 pp.
McCay-Buis, T.S., 1998. Ramifications of microbial interactions conditioning take-all of wheat. Ph.D. Thesis. Purdue University, West Lafayette, Indiana.
Mekwatanakarn, P., Sivasithamparam, K., 1987. Effect of certain herbicides on soil microbial populations and their influence on saprophytic growth in soil and pathogenicity of the take-all fungus. Biol. Fertil. Soils 5, 175–180.
Ozturk, L., Yazici, A., Eker, S., Gokmen, O., Romheld, V., Cakmak, I., 2008. Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots. New Phytol. 177, 899–906.
Purcell, L.C., 2001. Physiological determinants of soybean yield limitations. USDA-CRIS Accession No.: 0164131; project No.: ARK01559, Univ. Arkansas, Fayetteville.
Rahe, J.E., Levesque, C.A., Johal, G.S., 1990. Synergistic role of soil fungi in the herbicidal efficacy of glyphosate. In: Hoagland, R.E. (Ed.), Biological Weed Control Using Microbes and Microbial Products as Herbicides. Symposium, April 9–14, 1989. American Chemical Society, Washington, DC, pp. 260–275.
Reichenberger, L., 2007. Missing micronutrients: using glyphosate is complicating the uptake of some minor nutrients. In: The Furrow, pp. 22–23.
Rengel, Z. (Ed.), 1999. Mineral Nutrition of Crops: Fundamental Mechanisms and Implications. Food Products Press, London.
Rengel, Z., Gutteridge, R., Hirsch, P., Hornby, D., 1996. Plant genotype, micronutrient fertilization and take-all infection influence bacterial populations in the rhizosphere of wheat. Plant Soil 183, 269–277.
Roseman, T.S., Graham, R.D., Arnott, H.J., Huber, D.M., 1991. The interaction of temperature with virulence and manganese oxidizing potential in the epidemiology of *Gaeumannomyces graminis*. Phytopathology 81, S1215.
Rosenberger, D., Fargione, M., 2004. Apple Pest Report 12, 6. University of Maine Cooperative Extension Service.
Sanogo, S., Yang, X.B., Scherm, H., 2000. Effects of herbicides on *Fusarium solani* f. sp. *glycines* and development of sudden death syndrome in glyphosate-tolerant soybean. Phytopathology 90, 57–66.
Sanogo, S., Yang, X.B., Lundeen, P., 2001. Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. Plant Dis. 85, 773–779.
Schulze, D.G., McCay-Buis, T.S., Sutton, S.R., Huber, D.M., 1995. Manganese oxidation states in *Gaeumannomyces*-infested wheat rhizospheres probed by micro-XANES spectroscopy. Phytopathology 85, 990–994.
Severson, R., 2006. Influence of Roundup Herbicide on Manganese Nutrition of Soybean. http://www.nwroc.umn.edu/Cropping_Issues/NW_Crop_trials/2005/Sybn_glyph_+_manganese.pdf.
Smiley, R.W., Ogg, A.G., Cook, R.J., 1992. Influence of glyphosate on *Rhizoctonia* root rot, growth, and yield of barley. Plant Dis. 76, 937–942.
Thompson, I.A., Huber, D.M., 2007. Manganese and plant disease. In: Datnoff, L.E., Elmer, W.H., Huber, D.M. (Eds.), Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN, pp. 139–153 (Chapter 10).
Thompson, I.A., Guest, C.A., Schulze, D.G., Huber, D.M., 1998. Manganese reduction and uptake in wheat rhizospheres as influenced by manganese reducing and oxidizing bacteria. Phytopathology 88, S118.
Thompson, I.A., Huber, D.M., Schulze, D.G., 2000. *In situ* oxidation and accumulation of manganese by the causal agent of the take-all disease on wheat (*Gaeumannomyces graminis*). Phytopathology 90, S77.
Thompson, I.A., Huber, D.M., Guest, C.A., Sculze, D.G., 2005. Fungal manganese oxidation in a reduced soil. Environ. Microbiol. 7, 1480–1487.
Timmer, L.W., 2000. Blight. In: Compendium of Citrus Diseases. APS Press, St. Paul, MN, pp. 66–67.
Yamada, T., 2006. Informações Agronomicas 116. IPNI-International Plant Nutrition Institute, Piracicaba-SP, Brazil. http://www.ipni.org.br/ppiweb/pbrazil.nsf/$webindex/article=93F35608032570B5004E419AD0187284!opendocument.
Yamada, T., Castro, P.R., 2005. Glifosato, herbicida com singular modo de ação: Efeitos secundários e implicações fisiológicas e agronômicas. http://www.ipni.org.br/ppiweb/pbrazil.nsf/$webcontentsbydate!OpenView&Start=1&Count=60&Expand=1.1#1.1.
Zobiole, L.H.S., deOliveira, R.S., Huber, D.M., Constantin, J., Castro, C, deOliveira, F.A., de Oliveira, A., 2009. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. Plant Soil (In Press).

Please cite this article in press as: Johal, G.S., Huber, D.M., Glyphosate effects on diseases of plants. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.04.004

Europ. J. Agronomy xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms

Robert J. Kremer[a], Nathan E. Means[b,*]

[a] USDA-ARS Cropping Systems & Water Quality Research Unit, 302 Natural Resources Bldg., University of Missouri, Columbia, MO 65211, USA
[b] Science Department, Columbia College, 1001 Rogers St., Columbia, MO 65216, USA

ARTICLE INFO

Article history:
Received 18 February 2009
Received in revised form 22 June 2009
Accepted 29 June 2009

Keywords:
Genetically modified crop
Polyphasic microbial analyses
Fusarium spp.
Glycine max (L.) Merr.
Soybean
Zea mays L.
Maize
Microbial ecology
Mn transformation
Pseudomonad

ABSTRACT

Current crop production relies heavily on transgenic, glyphosate-resistant (GR) cultivars. Widespread cultivation of transgenic crops has received considerable attention. Impacts of glyphosate on rhizosphere microorganisms and activities are reviewed based on published and new data from long-term field projects documenting effects of glyphosate applied to GR soybean and maize. Field studies conducted in Missouri, U.S.A. during 1997–2007 assessed effects of glyphosate applied to GR soybean and maize on root colonization and soil populations of *Fusarium* and selected rhizosphere bacteria. Frequency of root-colonizing *Fusarium* increased significantly after glyphosate application during growing seasons in each year at all sites. Roots of GR soybean and maize treated with glyphosate were heavily colonized by *Fusarium* compared to non-GR or GR cultivars not treated with glyphosate. Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen–antagonistic bacterial interactions; and reduction in nodulation. Root-exuded glyphosate may serve as a nutrient source for fungi and stimulate propagule germination. The specific microbial indicator groups and processes were sensitive to impacts of GR crops and are part of an evolving framework in developing polyphasic microbial analyses for complete assessment of GR technology that is more reliable than single techniques or general microbial assays.

1. Introduction

Glyphosate [*N*-(phosphomonomethyl)glycine] is a water-soluble, non-selective herbicide applied to foliage resulting in death of most herbaceous plants. The mode of action of glyphosate is inhibition of the enzyme 5-enolpyruvyl-shikimate-3-phosphatase synthase (EPSPS) in the shikimic acid pathway blocking the synthesis of essential aromatic amino acids and precursors of other critical aromatic compounds including plant growth regulators and phytoalexins (Duke et al., 2003a). Glyphosate is an extremely effective herbicide because the compound remains intact in the plant with little degradation and is systemically transported to metabolically active sites throughout the plant before inducing symptoms (Cerdeira and Duke, 2006). Glyphosate is often described as exhibiting little or no activity in soil due to potential rapid adsorption on soil inorganic and organic particles (Duke and Powles, 2008).

1.1. Glyphosate and soil microorganisms

In contrast to generalizations that glyphosate is tightly bound and inactivated in soil, numerous studies show that glyphosate is available to soil and rhizosphere microbial communities as a substrate for direct metabolism leading to increased microbial biomass and activity (Haney et al., 2000; Wardle and Parkinson, 1990). Indeed, Simonsen et al. (2008) recently demonstrated that agricultural soils amended with phosphorus fertilizers are high in unbound glyphosate because soil sorption sites are occupied by competing phosphate ions; thus, glyphosate remaining in the soil solution is vulnerable to potential uptake by plant roots, microbial metabolism, or leaching into groundwater. The main degradation product, aminomethylphosphonic acid (AMPA), is frequently detected in soils subjected to frequent glyphosate applications (Fomsgaard et al., 2003). Reports on impacts of glyphosate and its degradation products on specific microbial species inhabiting non-rhizosphere soil are limited.

Response to glyphosate appears to vary among soil bacteria based on sensitivity of intracellular EPSPS to the herbicide. Screening assays with glyphosate in minimal medium identified five pseudomonad species (*P. maltophilia*, *P. putida*, *P. aeruginosa* [two strains], *Pseudomonas* sp.) that were not growth inhibited due to a 'glyphosate-resistant EPSPS' (Schulz et al., 1985). Interestingly,

* Corresponding author.
E-mail address: nemeans@ccis.edu (N.E. Means).

1161-0301/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2009.06.004

the ubiquitous environmental bacterium, *Pseudomonas fluorescens*, exhibited a 'glyphosate-sensitive EPSPS' and was inhibited by glyphosate. Using enrichment culture, several bacterial strains isolated from both untreated and glyphosate-treated soils metabolized glyphosate using an initial cleavage of the carbon–phosphorus bond to yield sarcosine (Dick and Quinn, 1995). A majority of the isolates was classified as *Pseudomonas* and *Arthrobacter* species. Liu et al. (1991) also demonstrated that several members of *Rhizobiaceae* including *Rhizobium* spp. and *Agrobacterium* spp. grew on glyphosate and the glyphosate metabolite, AMPA, as the sole source of P under P-limiting conditions in liquid culture medium. Mineralization of glyphosate to CO_2 in five agricultural soils in a microcosm study was highly correlated with *Pseudomonas* spp. population in the soils, suggesting that the mineralizing activity of specific pseudomonads was a major factor controlling the fate of glyphosate in soil (Gimsing et al., 2004).

Several studies document effects of glyphosate on soil fungal community response and function. Wardle and Parkinson (1990) found that glyphosate applied to soil amended with wheat (*Triticum aestivum* L.) straw and incubated with fungi significantly enhanced straw colonization by *Trichoderma harzianum*, *Mortierella alpina*, and *Arthrinium sphaerospermum*. Subsequent research revealed that antagonistic interactions between the fungal species were eliminated by glyphosate suggesting that the herbicide might influence overall soil fungal community structure (Wardle and Parkinson, 1992). Glyphosate added to sandy clay with a history of repeated glyphosate treatment appeared to select for specific fungal species that were able use the herbicide as a nutrient source (Krzysko-Lupicka and Orlik, 1997). A follow-up study verified that long-term exposure of soil microorganisms to glyphosate led to a fungal community dominated by *Fusarium* spp. (Krzysko-Lupicka and Sudol, 2008). Similarly, Means (2004) detected a significant increase in soil *Fusarium* within 2 weeks after glyphosate was applied at recommended rates in the field. In culture-based studies, five strains of *Fusarium* spp. isolated from soil were able to metabolize glyphosate and use it as a phosphorus source (Castro et al., 2007). Population growth and sporulation of soil *Fusarium* spp. increased after glyphosate application to soils containing maize (*Zea mays* L.) or peanut (*Arachis hypogeae* L.) crop residues compared with lower *Fusarium* populations in similar fallowed soils lacking crop residues (Meriles et al., 2006).

1.2. Glyphosate and rhizosphere microorganisms

Because the rhizosphere is rich in organic substances and high microbial activity with fluctuating soil moisture (Kennedy, 2005), the fate of glyphosate entering this environment is difficult to predict although microbial metabolism, movement through soil pores or root channels, absorption by living plant roots, and soil sorption are all possible. Early research showed that glyphosate absorbed through plant foliage after application was transported systemically toward roots and was eventually released into the rhizosphere (Coupland and Casely, 1979). Indeed, Neumann et al. (2006) demonstrated that glyphosate released through the roots of dying plants was transferred to living plants (not treated with glyphosate) via root absorption, and suggested that glyphosate applied to vegetation in orchard alleys may be similarly transferred to trees causing disease and yield losses. Microbial activity may increase in rhizospheres of glyphosate-treated plants through translocation and release from roots, where it is immediately metabolized resulting in stimulation of activity and changes in functional diversity of the heterotrophic microbial community (Mijangos et al., 2009).

Glyphosate applied to susceptible plants resulted in heavy colonization of roots by soilborne fungi, primarily *Fusarium* and *Phytophthora* (Johal and Rahe, 1984; Lévesque and Rahe, 1992). Lévesque et al. (1987) observed that glyphosate not only increased root colonization of various susceptible weeds by *F. oxysporum* but also increased propagule density of the fungus in soil. Severity of root and crown rot diseases was highest on cereal crops planted immediately after glyphosate treatment of weeds and volunteer cereal plants in which facultative (opportunistic) pathogens rapidly built up to provide inocula for subsequent colonization of seedling roots of the cereal crop (Smiley et al., 1992). Glyphosate treatment of bean (*Phaseolus vulgaris* L.) plants caused heavy root colonization by *Pythium* spp., significantly building up the soil populations of *Pythium* spp. as the dying roots provided substrate increasing inocula levels of the phytopathogen (Descalzo et al., 1998). The severity of damping-off disease of sunflower (*Helianthus annuus* L.) seedlings growing in the colonized soil significantly correlated with *Pythium* population density. Bacterial endophytic communities in soybean [*Glycine max* (L.) Merr.] can also be altered by pre-plant applications of glyphosate (Kuklinshy-Sobral et al., 2005).

These studies suggest that glyphosate exhibits non-herbicidal effects manifested by enhancement or suppression of activity of latent pathogenic and/or plant growth-promoting bacteria and fungi, which may subsequently impact growth of non-target plants. Because glyphosate blocks the shikimate pathway and subsequent synthesis of aromatic compounds including phytoalexins, pathogen defense mechanisms are suppressed, as shown in early studies in which glyphosate contributed to infection of soybean by *Phytophthera megasperma* f. sp. *glycines* (Keen et al., 1982) and of tomato (*Solanum lycopersicon* L.) by crown and root rot *Fusarium* spp. (Brammall and Higgins, 1988). In addition to suppression of phytoalexin synthesis, glyphosate is implicated in immobilization of micronutrients including Mn and Fe, essential in many metabolic pathways (Eker et al., 2006; Jolley et al., 2004; Neumann et al., 2006; Ozturk et al., 2007), and increased excretion of substrates from roots that may be selectively metabolized by pathogens (Lévesque and Rahe, 1992). Regarding the latter, Liu et al. (1997) showed that roots of bean seedlings were vulnerable to infection by *Pythium* spp. 2 days after glyphosate application to the foliage. Increased amino acid contents as well as suspected translocated glyphosate released in root exudates apparently enhanced germination and growth of *Pythium* propagules which, combined with suppression of plant cell lignification defense responses to pathogen attack, rapidly infected and colonized roots (Liu et al., 1997). Glyphosate applied to the winter annual weeds henbit (*Lamium amplexicaule* L.) and downy brome (*Bromus tectorum* L.) significantly increased rhizosphere populations of *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* (Kawate et al., 1997). Proliferation of these phytopathogens on winter annual weeds may boost soil populations suggesting that weed control with a burndown application of glyphosate could lead to disease incidence in subsequent leguminous crops planted into residues of the killed vegetation.

These reports show that glyphosate imposes diverse effects on the biology and ecology of rhizosphere microorganisms and on their interactions with plant roots when released into the rhizosphere. Although the interactions suggest a "secondary mode of action" of glyphosate by pre-disposing susceptible plants to microbial infection (Johal and Rahe, 1984), the potential for developing critical pathogen inocula levels in soils that affect crop health, altering rhizosphere microbial communities involved in nutrient transformations, and shifting the balance of beneficial and detrimental plant-associated microorganisms are legitimate concerns regarding the impact of glyphosate on crop productivity and environmental sustainability. This is especially significant with consideration to the current widespread use of glyphosate in glyphosate-resistant (GR) cropping systems.

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

1.3. Rhizosphere microorganisms and glyphosate-resistant crops

One of the most significant advancements in intensive agriculture was the introduction of GR crops in the mid-1990s. By 2007 the global area planted to all genetically modified crops exceeded 113 million ha on which GR soybean occupied 58.6 million ha (52% of the global area planted to biotech crops), followed by GR maize (35.2 million ha at 31% of global area), and GR cotton (13.4 million ha at 12% of global area) (James, 2007). Indeed, based on average application rate and frequency and area treated, the amount of glyphosate applied to GR soybean in the U.S. increased by 52 million kg from 1997 to 2004 (Benbrook, 2004). The GR cropping system provided a more cost-effective option for farmers, allowing them flexibility in weed management to spray a broad spectrum of weeds with glyphosate on an "as needed" basis and reducing the need for pre- and post-emergence herbicides. Production of transgenic crops, resistant to glyphosate, represents a drastically new approach in weed management that allows broad-spectrum weed control without crop injury, may reduce preemergence herbicide use, and better conserve soils by increasing the use of no-tillage (Zablotowicz and Reddy, 2004; Locke et al., 2008). Because current soybean production relies heavily on use of transgenic, GR cultivars (Roundup Ready™), considerable interest has developed concerning the impacts of widespread cultivation of genetically modified crops and use of one herbicide class on agroecosystems, especially on the potential effects on biological processes including phytopathogenic activity and disease incidence. Glyphosate-resistant soybean was developed by insertion of a transgene (*cp4*) from an *Agrobacterium* species that codes for an insensitive version of EPSPS (Franz et al., 1997). The limited research conducted to date suggests that, in GR soybean, very little glyphosate is degraded within the plant (Arregui et al., 2003) with most of the herbicide translocated to active metabolic sinks including seeds (Duke et al., 2003b), nodules (Reddy and Zablotowicz, 2003), and roots (Duke, 1996). Nodulation and nitrogen fixation are reduced in some early-season GR soybean cultivars receiving glyphosate applications at field rates (King et al., 2001).

Since the introduction of GR crops in the mid- to late-1990s many consequences associated with production of GR crops were soon reported based on field observations of apparent increased disease and nutritional deficiencies relative to conventional or non-transgenic cultivars. A limited number of studies attempted to quantify or repeat anecdotal observations of glyphosate influences on crop-microorganism interactions, yielding variable results. In a controlled field study, increased susceptibility to sudden death syndrome (SDS) in soybean caused by *F. solani* pv. *glycines* [reclassified as *F. virguliforme* (Aoki et al., 2003)] was reported for GR soybean treated with glyphosate compared with no herbicide treatment (Sanogo et al., 2000). However, subsequent research (Njiti et al., 2003) failed to reproduce SDS in other GR soybean cultivars. *Corynespora cassiicola* may cause severe root rot on GR soybean in proximity to giant ragweed (*Ambrosia trifida* L.) treated with glyphosate (Huber et al., 2005). Altered root exudates and/or glyphosate released from roots of dying ragweed plants appeared to modify the rhizosphere environment and predispose adjacent GR soybean roots to severe *Corynespora* root rot. Research with *Sclerotinia sclerotiorum* (Sclerotinia root rot) was inconsistent in describing a definite relationship between susceptibility and glyphosate use on GR soybean, leading to the conclusion that effects of the GR trait or glyphosate use on disease susceptibility may be cultivar specific (Lee et al., 2002). Disease severity caused by *F. oxysporum* and *Rhizoctonia solani* in GR sugarbeet [*Beta vulgaris* (*saccharifera*) L.) increased with glyphosate application (Larson et al., 2006).

Studies on glyphosate interactions with microbial communities in GR cropping systems reveal that the most pronounced effects are detected with specific genera or species of microorganisms (i.e., *Fusarium*, *Pythium*) rather than with broader measurements of soil microbial diversity and functions. In a review on impacts of disturbance on soil microbial communities, Wardle (1995) suggests that measurement of response by functional groupings may be problematic because individual taxonomic species are likely to be more sensitive to disturbance rather than an entire functional group comprised of multiple taxonomic groups. If herbicide application is considered an external ecosystem disturbance, it is not surprising that glyphosate is often reported to have no effect on soil microbial biomass and diversity and broad functional activities such as soil respiration and soil enzymatic activity in GR cropping systems (Liphadzi et al., 2005; Lupwayi et al., 2007; Means et al., 2007; Hart et al., 2009). Based on numerous studies of *Fusarium* spp., Powell and Swanton (2008) proposed possible mechanisms involved in promoting the proliferation of an individual taxonomic group by glyphosate in GR crops including direct stimulation of fungal growth, indirect stimulation due to alteration of root exudate components, increase in host susceptibility to pathogens, and decrease in effectiveness of pathogen antagonists.

Glyphosate and root exudates released into the rhizosphere from GR soybean influence microbial populations and/or activity in the rhizosphere. Previous findings that glyphosate and high concentrations of soluble carbohydrates and amino acids exude from roots of glyphosate-treated GR soybean (Kremer et al., 2005) suggested that impacts on root microbial interactions and micronutrient uptake might mirror those described for glyphosate interactions in non-transgenic cropping systems.

Based on the previous research on glyphosate-induced microbial root infection of susceptible weeds and crops and limited reports for GR crops, we hypothesized GR soybean and maize might be more susceptible to root infection by fungi than conventional, non-transgenic cultivars. Our research was justified because of the numerous reports by producers of seemingly more frequent incidence of diseases appearing in GR soybean than that observed previously with conventional soybean (Sanogo et al., 2000; Njiti et al., 2003). A primary focus of our long-term field studies is documenting effects of glyphosate applied to GR soybean and maize cultivars on root colonization and soil populations of *Fusarium* spp. *Fusarium* spp. were selected as indicators of the microbial ecology of the soybean rhizosphere because they exist in soil saprophytically and are prevalent in rhizospheres of many plants where they may dominate microbial communities and become pathogenic, often in response to root exudation (Nelson, 1990). Additional potential consequences of GR cropping systems are alterations in the microbial ecology and biological processes carried out in the crop rhizosphere environment (Lupwayi et al., 2009). Several functions are affected including nutrient cycling and plant availability; potential phytopathogen and antagonist interactions; and activities and composition of beneficial microorganisms including plant-growth promoting rhizobacteria (Johal and Huber, this issue). Due to the complexity of soil and microbial communities, such plant-associated components are often neglected in evaluations of crop productivity or risk assessments of GM crops (Azevedo and Araujo, 2003). Our objective is to present a research summary to illustrate approaches for understanding the impacts of GR crops on microbiological interactions in the rhizosphere.

2. The glyphosate-resistant crop production and rhizosphere microbial ecology project

Field trials with soybean and maize were conducted at the University of Missouri Bradford Research and Extension Center (Boone County; 38°53′N, 92°12′W), the Delta Center (Pemiscot County; 36°23′N, 89°36′W), and six mid-Missouri on-farm sites

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

from 1997 through 2007. Soils are representative of the Central (U.S.) Claypan Region, developed in loess overlain on glacial till, and contain a claypan that limits water percolation and promotes surface runoff. The soils are generally classified as aeric, vertic Epiaqualfs, and are located on landscapes of 1–2% slopes. The experimental design consisted of crop variety + herbicide combination treatments, each replicated four times: GR variety + glyphosate, GR variety + non-glyphosate herbicide, GR variety + no herbicide, non-GR variety + non-glyphosate herbicide, and non-GR variety + no herbicide. Plot size area varied from 3.7×6.1 m to 3.7×24.4 m depending on availability of field space each year. All treatment-combinations were not established every year; however, GR variety + glyphosate and GR variety + no herbicide treatments were evaluated every year. Field plots were prepared using minimum tillage consisting of one or two passes of a disk-harrow as necessary to incorporate crop residues before planting. Soybean and maize were planted in 76-cm rows from mid-May to mid-June depending on the annual weather conditions. Supplemental fertilization was consistent with recommended management practices. Glyphosate at 0.84 kg a.e. ha^{-1} was applied to GR soybean and GR maize at the V4–V5 and V5–V6 growth stages, respectively. Non-glyphosate herbicides included post-emergence application of clethodim (0.42 kg a.i. ha^{-1}) + fomesafen (0.175 kg a.i. ha^{-1}) on GR and conventional soybean and pre-plant incorporation of atrazine at 2.24 kg a.i. ha^{-1} on GR maize. All herbicides were applied to the appropriate crop variety-herbicide combination plots at a 130 L ha^{-1} spray volume at a pressure of 138 kPa using 11,003 spray nozzles. Plots receiving no herbicides were hand-weeded periodically during the season.

Intact soybean and maize plants were randomly sampled immediately prior to and periodically (up to 42 days) after herbicide application. All nodules present on the roots were removed for fresh and dry mass measurements; a subsample of roots were cleaned of soil and fresh and dry biomass. *Fusarium* colonization was assessed on surface-sterilized root segments following the root plating procedures of Lévesque et al. (1993) and using *Fusarium*-selective agar medium (Nash and Snyder, 1962). After a 7-day incubation, numbers of fungal colonies developing on root segments were recorded. *Fusarium* colonies were randomly selected from colonized roots, subcultured on potato dextrose agar, and tentatively identified using descriptions of cultural and microscopic morphologies (Nelson et al., 1983). Identification was confirmed by molecular analysis using partial translation elongation factor sequences (Skovgaard et al., 2001).

Soil tightly adhered to roots was rigorously removed using a sterile camel-hair brush, suspended in buffer and appropriate dilutions plated on S1 agar medium selective for fluorescent pseudomonads (Gould et al., 1985) and on Gerretsen's agar medium for detecting Mn-oxidizing and -reducing microorganisms (Huber and Graham, 1992). After incubation, colonies developing on agar were recorded and represented an estimate of colony-forming units in the rhizosphere. Mn-transforming bacterial components were further expressed as ratios of Mn reducers to Mn oxidizers to provide a means of detecting potential effects of microbial activity on plant available Mn (Rengel, 1997). Representative bacterial colonies from the pseudomonad and Mn-transformer assays were selected and subcultured on S1 and tryptic soy agars to obtain pure, single-colony isolates. These isolates were further assayed for in vitro antagonism of *Fusarium* (Kremer et al., 1990), extracellular protease production (Harley and Prescott, 1999), and extracellular polysaccharide (EPS) production (Kelman, 1954). Isolates were characterized for colony morphology and fluorescent pigment production; gram stain and oxidase reactions; and classified taxonomically by determining cellular fatty acid profiles using gas chromatography-fatty acid methyl ester analysis (Kennedy, 1994). Rhizobial-induced nodules on soybean root samples were removed and weighed for estimation of relative symbiotic nitrogen fixation.

Statistical analyses were performed with the general linear model procedure; analysis of variance and, where F values were significant ($\alpha = 0.05$), mean separations were conducted using Fisher's protected least significance difference (LSD) test (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Fusarium root colonization of glyphosate-resistant crops

Fusarium colonization was higher on GR soybean treated with glyphosate throughout the growing season, generally two to five times higher compared with soybean receiving no herbicide or a conventional (non-glyphosate) herbicide. The frequency of root-colonizing *Fusarium* increased significantly within 1 week after glyphosate application throughout the growing season in each year at all sites (Fig. 1A). *Fusarium*-like colonies developed on surface-sterilized soybean root segments indicating colonization or infection of root tissue by the fungi. Populations of *Fusarium* in rhizosphere soil from soybean receiving glyphosate were also significantly increased during each growing season at all locations (Means, 2004). Several studies report rhizosphere and soil *Fusarium* spp. increase in response to glyphosate addition (Lévesque et al., 1987; Powell and Swanton, 2008) including infection and disease severity by *F. solani* f. sp. *glycines*, which increased with GR soybean treated with glyphosate compared to no herbicide treatment (Sanogo et al., 2000). *Fusarium* colonization of roots of GR maize receiving glyphosate was 3–10 times higher colonization levels than for colonization levels for the atrazine treatment, similar to colonization patterns documented for soybean with

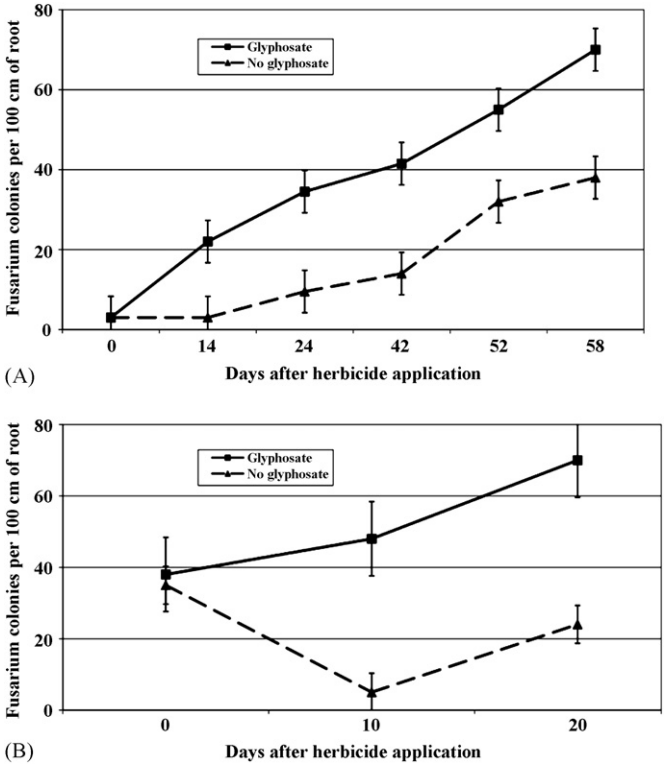


Fig. 1. Relationship of glyphosate application with root colonization of (A) glyphosate-resistant soybean ('Pioneer 94B01') and (B) glyphosate-resistant maize ('DeKalb DKC60') by *Fusarium* spp. Data in (A) based on Kremer (2003); data in (B) based on Means (2004). In both graphs, significant differences (*$P < 0.05$) between glyphosate and no glyphosate treatments within dates are indicated by the vertical bars representing Fisher's protected LSD.

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

glyphosate treatment (Fig. 1B; Means, 2004). These results suggest that glyphosate induces fungal colonization of both soybean and maize rhizospheres via similar mechanisms. Taxonomic diagnosis of representative cultures collected from annual field studies has been distributed among the following groupings: 70% *Fusarium oxysporum* complex, 18% *Fusarium solani* complex, and 10% *Fusarium equiseti*.

A summary of root colonization results of field-grown soybean roots during the period 1997–2007 demonstrates consistently higher colonization of GR cultivars by *Fusarium* spp., especially when glyphosate is applied (Fig. 2). Soybean roots from plants receiving no or conventional post-emergence herbicides exhibited low *Fusarium* colonization; non-transgenic (non-GR) cultivars always showed lowest root colonization. Rhizosphere-inhabiting *Fusarium* may readily metabolize glyphosate in root exudates as a sole source of P and also as a C and energy source (Castro et al., 2007). Also, glyphosate may stimulate propagule germination and early growth by *Fusarium* (Krzysko-Lupicka and Orlik, 1997). When root exudation is excessive, as for glyphosate-treated soybean, root infection by soilborne pathogens is enhanced (Nelson, 1990). Also, Griffiths et al. (1999) indicate that as concentrations of soluble carbohydrates and amino acids increased in root exudates, the proportion of fungi in the rhizosphere community also increased compared with that of bacteria. Therefore, the structure of the microbial community is not solely governed by composition of exudates. Optimal growth of different microorganisms is also related to quantity of available substrates. Thus glyphosate released into the rhizosphere of GR soybean combined with release of high concentrations of carbohydrates and/or amino acids favor increased growth of *Fusarium* spp., illustrated by the results from our repeated field studies with GR soybean (Kremer, 2003; Means, 2004). These findings summarized in Fig. 2 demonstrate that monitoring for effects of glyphosate on environmental processes is prudent especially in light of its current widespread use in agricultural, horticultural, and timber production systems.

Annual variations in weather conditions affected *Fusarium* colonization of soybean roots, accounting for the variable infection levels shown in Fig. 2. For example, low colonization during 2003 was suspected to be related to low rainfall during the growing season, which was about 30% of normal. A greenhouse study in which soil water contents were controlled verified that glyphosate-treated soybean under extreme moisture stress exhibited about 75% reduction in *Fusarium* colonization of roots (Means and Kremer, 2007). Moisture stress reduces activity of other plant-microbial associations, including nitrogen fixation in glyphosate-treated GR soybean (King et al., 2001), considerably more than in untreated plants. These findings partly explain reported problems with soybean productivity and yields experienced by many farmers using the GR cropping system in the Midwestern U.S.

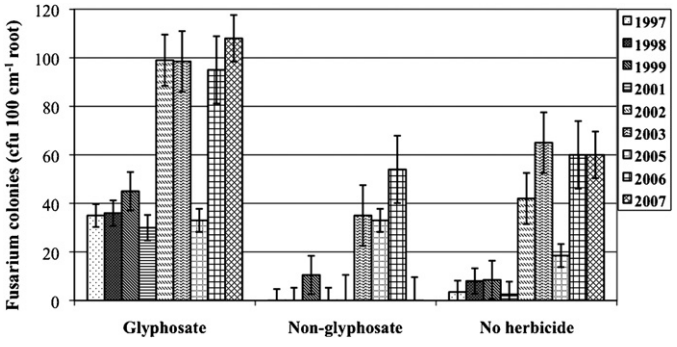


Fig. 2. Average seasonal colonization of glyphosate-resistant soybean roots by *Fusarium* spp. across all sites during 1997–2007, influenced by glyphosate, non-glyphosate, or no herbicide applications. Within year, significant differences (*$P < 0.05$) between colonization of glyphosate-treated roots compared with the non-glyphosate and no herbicide treatments are indicated by the vertical bars representing Fisher's protected LSD. Partly based on data from Kremer (2003).

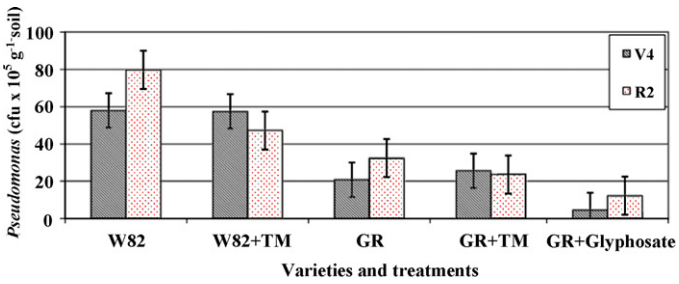


Fig. 3. Populations of rhizosphere *Pseudomonas* spp. detected in 2006 field study on conventional soybean (W82, 'Williams 82') and GR soybean (GR, 'DeKalb DK-838-52') at two growth stages (V4 and R2), influenced by herbicide treatment: TM, tank-mix of fomesafen + clethodim (non-glyphosate); glyphosate; or no designation indicates hand-weeding without herbicide. *Pseudomonas* spp. populations for W82 at both growth stages were significantly higher (*$P < 0.05$) than all GR treatment combinations indicated by the vertical bars representing Fisher's protected LSD.

3.2. Glyphosate and GR soybean interactions with the rhizosphere bacterial community

3.2.1. Fluorescent pseudomonads

Rhizosphere-inhabiting *Pseudomonas* spp. are important multifunctional bacteria in the rhizosphere capable of producing numerous secondary metabolites including siderophores, hydrogen cyanide, extracellular enzymes, and various antibiotics that suppress competing microbial groups (Schroth et al., 2006). A majority of fluorescent pseudomonads is associated with antagonism of fungal pathogens (Schroth and Hancock, 1982), which contribute to Mn transformations, primarily Mn reduction (Rengel, 1997), in plant rhizospheres. Glyphosate and soybean cultivar significantly decreased rhizosphere fluorescent pseudomonads detected using selective culture (Fig. 3). Fluorescent pseudomonads, always higher in non-GR soybean rhizosphere, may be reduced in GR soybean due to their reported sensitivity to glyphosate (Schulz et al., 1985) that is exuded into the rhizosphere or because antagonistic activity is overcome by glyphosate (Wardle and Parkinson, 1992). Also, any negative effects of cultivar on rhizosphere pseudomonads might be further reduced by glyphosate. A negative relationship between population size of fluorescent pseudomonads and *Fusarium* root colonization further demonstrated that GR soybean and/or glyphosate was involved in altering the microbial composition in the rhizosphere (Fig. 4). Bioassays of single cultures of fluorescent pseudomonads confirmed that most (≈85%) were potentially antagonistic toward *Fusarium*; antagonism was also associated with strong extracellular protease activity by many of the isolates. Thus, as suggested by Powell and Swanton

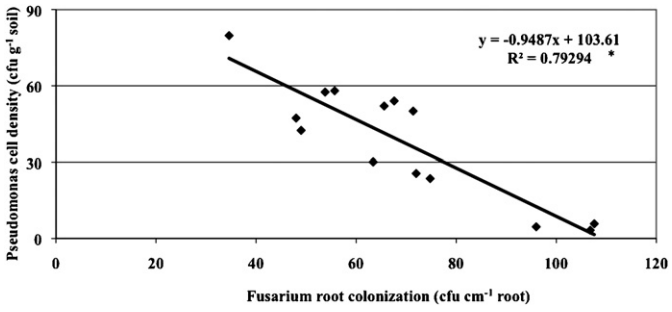


Fig. 4. Relationship between *Pseudomonas* spp. populations and *Fusarium* root colonization on soybean at R2 growth stage based on data collected in 2006 field study. Regression is significant at *$P < 0.05$.

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

(2008), glyphosate and GR soybean may enhance fungal root colonization and potential disease through not only stimulating growth of the fungal pathogen but also by suppressing bacterial antagonists.

3.2.2. Manganese-transforming rhizosphere bacteria

Mn transformations are primarily microbially mediated and thus have a major impact on plant nutrient availability and metabolic processes (Thompson and Huber, 2007). A low ratio of Mn reducers to Mn oxidizers determined for the GR soybean Dekalb 'DK-838-52' treated with glyphosate compared with 'Williams 82' suggested soil Mn was immobilized and not available for plant uptake (Fig. 5). This occurred despite standard chemical analysis showing the soils were Mn sufficient. Glyphosate as a major factor in enhancing Mn-oxidizing bacteria was further strengthened by results determined for both GR and non-GR soybean with or without non-glyphosate herbicides that were significantly different (higher ratio) from glyphosate treatments. Fluorescent pseudomonads are also primarily Mn-reducers involved in fungal suppression (Huber and McCay-Buis, 1993), thus low numbers on GR soybean (Fig. 3) likely further contribute to increased *Fusarium* root colonization due to reduction of this suppressive activity.

Interestingly, a majority of bacterial isolates with Mn-oxidizing ability detected on Gerritsen's medium produced copious amounts of exopolysaccharides (EPS), and was most frequently associated with GR soybean or GR soybean + glyphosate. Many isolates subcultured from this group were phenotypically characterized as *Agrobacterium* spp., which are resident saprophytic bacteria in the soybean rhizosphere (Kuklinsky-Sobral et al., 2004). The consistent detection of agrobacteria producing excessive amounts of EPS suggested that biofilm formation on the soybean root surface may be related Mn-oxidizing agrobacteria, enhanced in the presence of glyphosate. As members of the alpha–proteobacteria, comprised of many Mn-transforming bacteria, agrobacteria are known strong Mn oxidizers (Thompson and Huber, 2007; Johal and Huber, this issue). Agrobacteria also typically form biofilms composed of EPS matrices on the rhizoplane in which many biological functions are mediated (Matthysse, 2006), including biogenic Mn oxidation, reported for many biofilm-forming bacteria that produce precipitated Mn oxides, which are retained within the biofilm (Toner et al., 2005). Thus the frequency of rhizobacteria with both EPS-producing and Mn-oxidizing properties (primarily *Agrobacterium* spp.) in rhizospheres of GR soybean treated with glyphosate suggests that the herbicide alone or in combination with GR soybean enhances or selects this group of bacteria with potential detrimental effects on plant growth through Mn immobilization. Because GR crop cultivars contain the *cp4* gene, derived from an *Agrobacterium* sp., that encodes for the glyphosate-resistant EPSPS (Funke et al., 2006), it is possible that as glyphosate is released through roots of GR crops, genetically similar *Agrobacterium* spp. in soils may be preferentially selected for colonization of the rhizosphere and rhizoplane. The role of glyphosate in biofilm formation and associated Mn transformation by rhizosphere bacteria remains to be clarified.

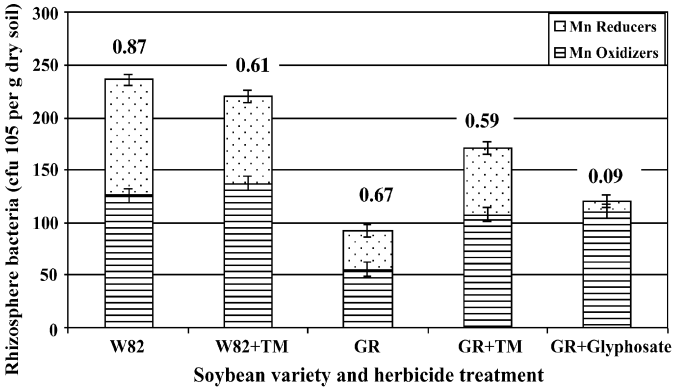


Fig. 5. Populations of rhizosphere Mn-transforming bacteria detected in 2006 field study on conventional soybean (W82, 'Williams 82') and GR soybean ('DeKalb DK-838-52') at growth stage R2 influenced by herbicide treatment (see Fig. 3 for explanation). Ratios of potential Mn-reducing to Mn-oxidizing bacteria are indicated at top of each bar. Significant differences ($^*P < 0.05$) among each bacterial group are indicated by the vertical bars representing Fisher's protected LSD.

3.2.3. Root nodulation

Symbiotic nitrogen fixation may contribute 40–70% of the nitrogen required by soybean during the growing season, thus sustaining this nitrogen input is critical for profitable grain yield and sustaining long-term soil productivity (Zablotowicz and Reddy, 2007). Legume nodules provide the necessary conditions for specialized plant and rhizobial structures to perform nitrogen fixation; thus nodule number and mass on roots qualitatively indicate the status of other parameters including nitrogenase activity, leghemoglobin content, and nitrogen accumulation. In our studies, nodulation was always lower on GR soybean with or without glyphosate compared with conventional varieties (i.e., Williams 82) with non-glyphosate or no herbicide (Fig. 6). These results confirm that glyphosate and perhaps the genetic modification in the GR plant may affect numerous processes associated with the nitrogen fixation symbiosis, including nitrogenase activity and leghemoglobin content (King et al., 2001; Reddy et al., 2000; Reddy and Zablotowicz, 2003; Zablotowicz and Reddy, 2007). A recent field study reporting that glyphosate significantly reduced nodule mass and nitrogen fixation in GR soybean yet did not affect grain yields suggests that an increased dependence of the crop on mineralized N from soil organic matter (Bohm et al., 2009) may contribute to a negative N balance and reduced sustainability of cropping systems based on GR soybean.

4. Recommendation

4.1. Polyphasic microbial analysis to assess impacts of GR crops

Results of our field studies emphasize the necessity for evaluation of the numerous and complex factors in the rhizosphere (root exudation, glyphosate release, microbial activities) to broaden our understanding of glyphosate interactions with root-associated microorganisms. Kowalchuk et al. (2003) recognized the limitations of general measurements such as soil microbial biomass and respiration for describing potential GM crop-induced effects on the microbial community and proposed that a polyphasic microbial analysis comprised of selected indicator groups and activities combined with general assays including microbial diversity analyses would yield a more comprehensive and informative assessment of GM crops.

Initial studies in our GR crop-microbial ecology project focused on rhizosphere *Fusarium* spp. as a key indicator of potential impacts of GR soybean and maize on the rhizosphere ecosystem (Kremer, 2003; Means and Kremer, 2007) because this fungal group readily responds to alterations (i.e., introduction of glyphosate and altered root exudation) in the rhizosphere (Nelson, 1990). However, because of the complexity of the rhizosphere ecosystem, a more comprehensive examination of the structure and functions of the broader microbial community was implemented to provide a more complete assessment of potential effects induced by GR crops.

Our polyphasic microbial analysis entails a multiple assessment of sensitive indicators that provides a more reliable view of GR technology effects relative to any single technique (Kowalchuk et

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

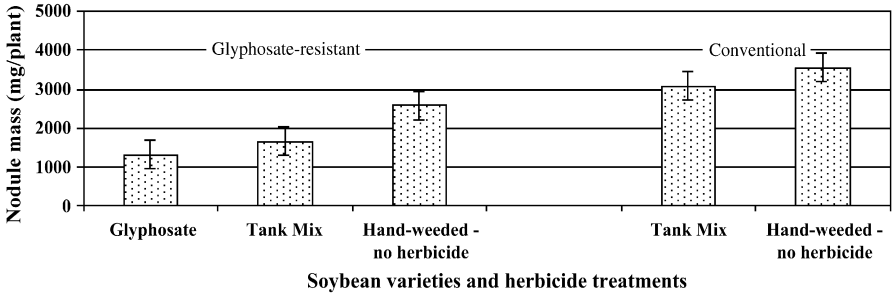


Fig. 6. Relationship of glyphosate application with root nodulation of glyphosate-resistant ('DeKalb DKB38-52') and conventional ('Williams 82') soybean based on 2005 field study. HW, hand-weeded check; TM, conventional herbicide tank-mix. Significant differences (*$P < 0.05$) between treatments are indicated by the vertical bars representing Fisher's protected LSD.

al., 2003; Powell, 2007). The framework for evaluating GR crop effects evolved during the course of our project based on progressive development of information in two areas: (1) non-herbicidal activity of glyphosate including systemic movement and release through GR plant roots; metal chelation within plant and in the rhizosphere; and growth stimulation or suppression of selected microbial groups; and (2) alteration of root exudate composition in GR plants with or without glyphosate treatment (Kremer et al., 2005). These are areas of concern because the structure of the rhizosphere microbial community is determined by the specific types and quantities of component chemicals and signaling compounds in root exudates (Grayston et al., 1998; El-Shatnawi and Makhadmeh, 2001; Broeckling et al., 2008); if the chemical composition of root exudates is drastically altered due to genetic transformation or treatment with glyphosate, it follows that physiological processes, pathogen defense mechanisms, and plant growth could be detrimentally affected. Therefore, our current specific analyses include fungal root colonization, Mn-transforming bacteria, symbiotic nitrogen fixation and nodulation, pseudomonad communities, and arbuscular mycorrhizal fungi (data not reported). We are also linking these key microbial relationships to community structure through analyses of DNA extracted from soybean rhizospheres to yield a molecular fingerprint. Preliminary results indicate that bacterial diversity based on molecular analyses was generally higher for conventional soybean compared with GR soybean treated with or without glyphosate, which agreed with the variations in composition and activities of the key indicators. The decreased microbial diversity in GR soybean rhizospheres is a concern because high diversity is essential in maintaining a stable ecosystem and plant productivity (Grayston et al., 1998).

5. Conclusions

The widespread adoption of transgenic GR crops is largely due to simplified crop management and greater flexibility in weed management offered by the GR cropping system. These crops have been released after assessments based on general soil biological analyses that often lack sensitivity to detect non-target effects (Kowalchuk et al., 2003). A more informative approach to assess the impact of GR crops on the environment is to target sensitive indicator microbial groups and/or processes in addition to the more general tests targeting microbial community diversity. Those microbial groups most neglected in assessments include specific soilborne and plant-associated (rhizosphere) microorganisms (Azevedo and Araujo, 2003; Kowalchuk et al., 2003).

In this overview we have attempted to present the current knowledge regarding impacts of glyphosate and GR crops on plant–microbial interaction. Our current view of the complex interactions in GR soybean and maize rhizospheres illustrates the limited information available and areas where research is desparately needed, such as impacts on mycorrhizal fungi. The report on our GR crop-microbial ecology project presented in this paper supports some of the literature reviewed, especially that dealing with glyphosate-induced *Fusarium* activity in the rhizosphere and negative impacts on soybean nodulation. Additionally, we document new information on changes in microbial components of GR soybean and maize rhizospheres: increases in the proportion of Mn-oxidizing bacteria; decreases in the pseudomonad component that antagonizes fungal pathogens; and increases in agrobacteria that may be involved in Mn oxidation and microbial community shifts due to apparent selection by glyphosate exudation. Analyses of GR soybean root exudates suggest that promotion of rhizosphere and root colonization of GR soybean by specific microbial groups may be a combination of stimulation by glyphosate released through root exudation and altered physiology leading to exudation into the rhizosphere of high levels of carbohydrates and amino acids (Kremer et al., 2005) possibly related to indirect, or pleiotropic, effects of genetic transformation for glyphosate resistance (Powell, 2007). This supports the ecological concept that plants actively modify their rhizospheres through production of specific root exudates that have a profound qualitative and quantitative effect in modifying the microbial communities (Grayston et al., 1998; El-Shatnawi and Makhadmeh, 2001; Broeckling et al., 2008). Overall, exposure of the rhizosphere microbial community to glyphosate and GR crops appeared to cause complex and varied responses; our project demonstrates the importance of using a multiphasic approach to yield a comprehensive analysis of rhizosphere microbial community structure and function in response to GR cropping systems.

It should be noted that the information generated from our project might also be used to improve productivity of GR crops by developing a combination of strategies including soil management for balanced biological activity, proper plant nutrition, and suppression of potential pathogens. Knowledge of the relationships between soil biology, plant nutrition, management factors, and disease potential provide a basis for addressing recurrent productivity problems associated with GR crops integrated in current agricultural production systems.

Acknowledgments

The project was funded by USDA-ARS; Ag Spectrum Co., DeWitt, IA; and Agri-Gro Inc., Doniphan, MO. We thank the technicians and field managers who have been involved during the course of the project including Lynn Stanley, Jenan Nichols, Jim Ortbals, Tim Reinbott, Steve Troesser, and Randy Miles; and the numerous undergraduate and graduate students who provided excellent technical assistance.

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

References

Aoki, T., O'Donnell, K., Homma, Y., Lattanzi, A.R., 2003. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex—*F. virguliforme* in North America and *F. tucumaniae* in South America. Mycologia 95, 660–684.

Arregui, M.C., Lenardon, A., Sanchez, D., Maitre, A.M., Scotta, R., Enrique, S., 2003. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. Pest Manage. Sci. 60, 163–166.

Azevedo, J.L., Araujo, W.L., 2003. Genetically modified crops: environmental and human health concerns. Mutat. Res. 544, 223–233.

Benbrook, C.M., 2004. Genetically engineered crops and pesticide use in the United States: the first nine years. Technical Paper No. 7. BioTech InfoNet.

Bohm, G.M., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Xavier, G.R., Hax, F., Rombaldi, C.V., 2009. Glyphosate- and imazethapyr-induced effects on yield, nodule mass and biological nitrogen fixation in field-grown glyphosate-resistant soybean. Soil Biol. Biochem. 41, 420–422.

Brammall, R.A., Higgins, V.J., 1988. The effect of glyphosate on resistance of tomato to *Fusarium* crown and root rot disease and on the formation of host structural defensive barriers. Can. J. Bot. 66, 1547–1555.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008. Root exudates regulate soil fungal community composition and diversity. Appl. Environ. Microbiol. 74, 738–744.

Castro, J.V., Peralba, M.C.R., Ayub, M.A.Z., 2007. Biodegradation of the herbicide glyphosate by filamentous fungi in platform shaker and batch bioreactor. J. Environ. Sci. Health B 42, 883–886.

Cerdeira, A.L., Duke, S.O., 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. J. Environ. Qual. 35, 1633–1658.

Coupland, D., Casely, J.C., 1979. Presence of ^{14}C activity in root exudates and guttation fluid from *Agropyron repens* treated with ^{14}C-labelled glyphosate. New Phytol. 83, 17–22.

Descalzo, R.C., Punja, Z.K., Lévesque, C.A., Rahe, J.E., 1998. Glyphosate treatment of bean seedlings causes short-term increases in *Pythium* populations and damping off potential in soils. Appl. Soil Ecol. 8, 25–33.

Dick, R.E., Quinn, J.P., 1995. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Appl. Microbiol. Biotechnol. 43, 545–550.

Duke, S.O., 1996. Herbicide Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. CRC Press, Boca Raton, FL.

Duke, S.O., Baerson, S.R., Rimando, A.M., 2003a. Herbicides: glyphosate. In: Plimmer, J.R., Gammon, D.W., Ragsdale, N.N. (Eds.), Encyclopedia of Agrochemicals,. John Wiley & Sons, New York (Accessed September 12, 2008) http://www.mrw.interscience.wiley.com/eoa/articles/agr119/frame.html.

Duke, S.O., Rimando, A.M., Pace, P.F., Reddy, K.N., Smeda, R.J., 2003b. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. J. Agric. Food Chem. 51, 340–344.

Duke, S.O., Powles, S.B., 2008. Glyphosate: a once-in-a-century herbicide. Pest Manage. Sci. 64, 319–325.

Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Römheld, V., Cakmak, I., 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. J. Agric. Food Chem. 54, 10019–10025.

El-Shatnawi, M.K.J., Makhadmeh, I.M., 2001. Ecophysiology of the plant–rhizosphere system. J. Agron. Crop Sci. 187, 1–9.

Fomsgaard, I.S., Spilid, N.H., Felding, G., 2003. Leaching of pesticides through normal-tillage soil—a lysimeter study. II. Glyphosate. J. Environ. Sci. Health B 38, 19–35.

Franz, J.E., Mao, M.K., Sikorski, J.A., 1997. Glyphosate: A Unique Global Herbicide. ACS Monograph 189. American Chemical Society, Washington, DC, USA.

Funke, T., Han, H., Healy-Fried, M.L., Fischer, M., Schönbrunn, E., 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. Proc. Natl. Acad. Sci. U.S.A. 103, 13010–13015.

Gimsing, A.L., Borggaard, O.K., Jacobsen, O.S., Aamand, J., Sørensen, J., 2004. Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. Appl. Soil Ecol. 27, 233–242.

Gould, W.D., Hagedorn, C., Bardinelli, T.R., Zablotowicz, R.M., 1985. New selective medium for enumeration and recovery of fluorescent pseudomonads from various habitats. Appl. Environ. Microbiol. 49, 28–32.

Grayston, S.J., Wang, S.Q., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. 30, 369–378.

Griffiths, B.S., Ritz, K., Ebblewhite, N., Dobson, G., 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biol. Biochem. 31, 145–153.

Haney, R.L., Senseman, S.A., Hons, F.M., Zuberer, D.A., 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Sci. 48, 89–93.

Harley, J.P., Prescott, L.M., 1999. Laboratory Exercises in Microbiology, 4th ed. McGraw-Hill, Boston, MA, pp. 95–98.

Hart, M.M., Powell, J.R., Gulden, R.H., Dunfield, K.E., Pauls, K.P., Swanton, C.J., Klironomos, J.N., Antunes, P.M., Koch, A.M., Trevors, J.T., 2009. Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn. Pedobiologia 52, 253–262.

Huber, D.M., Cheng, M., Winsor, B., 2005. Association of severe *Corynespora* root rot of soybean with glyphosate-killed ragweed. Phytopathology 95, S45.

Huber, D.M., Graham, R.D., 1992. Techniques for studying nutrient–disease interactions. In: Singleton, L.L., Mihail, J.D., Rush, C.M. (Eds.), Methods for Research on Soilborne Phytopathogenic Fungi. APS Press, St. Paul, MN, pp. 204–214.

Huber, D.M., McCay-Buis, T.S., 1993. A multiple component analysis of the take-all disease of cereals. Plant Dis. 77, 437–447.

James, C., 2007. Global status of commercialized biotech/GM crops: 2007. ISAAA Brief No. 37. International Service for the Acquisition of Agri-biotech Associations, Ithaca, NY, USA.

Johal, G.S., Huber, D.M., this issue. Glyphosate effects on diseases of plants. Eur. J. Agron., doi:10.1016/j.eja.2009.04.004.

Johal, G.S., Rahe, J.E., 1984. Effects of soilborne plant-pathogenic fungi on the herbicidal action of glyphosate on bean seedlings. Phytopathology 74, 950–955.

Jolley, V.D., Hansen, N.C., Shiffler, A.K., 2004. Nutritional and management related interactions with iron-deficiency stress response mechanisms. Soil Sci. Plant Nutr. 50, 973–981.

Kawate, M.K., Colwell, S.G., Ogg Jr., A.G., Kraft, J.M., 1997. Effect of glyphosate-treated henbit (*Lamium amplexicaule*) and downy brome (*Bromus tectorum*) on *Fusarium solani* f. sp. *pisi* and *Pythium ultimum*. Weed Sci. 45, 739–743.

Keen, N.T., Holliday, M.J., Yoshikawa, M., 1982. Effects of glyphosate on glyceollin production and the expression of resistance to *Phytophthera megasperma* f. sp. *glycinea* in soybean. Phytopathology 72, 1467–1470.

Kelman, A., 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium chloride medium. Phytopathology 44, 693–695.

Kennedy, A.C., 2005. The rhizosphere. In: Sylvia, D.M., Hartel, P.G., Fuhrmann, J.J., Zuberer, D.A. (Eds.), Principles and Applications of Soil Microbiology, 2nd ed. Pearson-Prentice Hall, Upper Saddle River, NJ, pp. 242–262.

Kennedy, A.C., 1994. Carbon utilization and fatty acid profiles for characterization of bacteria. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis, Part 2—Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 543–556.

King, A.C., Purcell, L.C., Vories, E.D., 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to glyphosate applications. Agron. J. 93, 179–186.

Kowalchuk, G.A., Bruinsma, M., van Veen, J.A., 2003. Assessing responses of soil microorganisms to GM plants. Trends Ecol. Evol. 18, 403–410.

Kremer, R.J., Means, N.E., Kim, S.-J., 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. Int. J. Environ. Anal. Chem. 85, 1165–1174.

Kremer, R.J., 2003. Soil biological processes are influenced by Roundup Ready soybean production. Phytopathology 93, S104.

Kremer, R.J., Begonia, M.F.T., Stanley, L., Lanham, E.T., 1990. Characterization of rhizobacteria associated with weed seedlings. Appl. Environ. Microbiol. 56, 1649–1655.

Krzysko-Lupicka, T., Sudol, T., 2008. Interactions between glyphosate and autochthonous soil fungi surviving in aqueous solution of glyphosate. Chemosphere 71, 1386–1391.

Krzysko-Lupicka, T., Orlik, A., 1997. The use of glyphosate as the sole source of phosphorus or carbon for the selection of soil-borne fungal strains capable to degrade this herbicide. Chemosphere 34, 2601–2605.

Kuklinshy-Sobral, J., Welington, L.A., Mendes, R., Pizzirani-Kleiner, A.A., Azevedo, J.L., 2005. Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 273, 91–99.

Kuklinsky-Sobral, J., Araualjo, W.L., Mendes, R., Geraldi, I.O., Pizzirani-Kleiner, A.A., Azevedo, J.L., 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ. Microbiol. 6, 1244–1251.

Larson, R.L., Hill, A.L., Fenwick, A., Kniss, A.R., Hanson, L.E., Miller, S.D., 2006. Influence of glyphosate on *Rhizoctonia* and *Fusarium* root rot in sugar beet. Pest Manage. Sci. 62, 1182–1192.

Lee, C.D., Penner, D., Hammerschmidt, R., 2002. Influence of formulated glyphosate and activator adjuvants on *Sclerotinia sclerotium* in glyphosate-resistant and susceptible *Glycine max*. Weed Sci. 48, 710–715.

Lévesque, C.A., Rahe, J.E., Eaves, D.M., 1993. Fungal colonization of glyphosate-treated seedlings using a new root plating technique. Mycol. Res. 97, 299–306.

Lévesque, C.A., Rahe, J.E., 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu. Rev. Phytopathol. 30, 597–602.

Lévesque, C.A., Rahe, J.E., Eaves, D.M., 1987. Effects of glyphosate on *Fusarium* spp.: its influence on root colonization of weeds, propagule density in the soil, and crop emergence. Can. J. Microbiol. 33, 354–360.

Liphadzi, K.B., Al-Khatib, K., Bensch, C.N., Stahlman, P.W., Dille, S.J., Todd, T., Rice, C.W., Horak, M.J., Head, G., 2005. Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system. Weed Sci. 53, 536–545.

Liu, C.-M., McLean, P.A., Sookdeo, C.C., Cannon, F.C., 1991. Degradation of the herbicide glyphosate by members of the family *Rhizobiaceae*. Appl. Environ. Microbiol. 57, 1799–1804.

Liu, L., Punja, Z.K., Rahe, J.E., 1997. Altered root exudation and suppression of induced lignification as mechanisms of predisposition by glyphosate of bean roots (*Phaseolus vulgaris* L.) to colonization by *Pythium* spp. Physiol. Mol. Plant Pathol. 51, 111–127.

Locke, M.A., Zablotowicz, R.M., Reddy, K.N., 2008. Integrating soil conservation practices and glyphosate-resistant crops: impacts on soil. Pest Manage. Sci. 64, 457–469.

Lupwayi, N.Z., Harker, K.N., Clayton, G.W., O'Donovan, J.T., Blackshaw, R.E., 2009. Soil microbial response to herbicides applied to glyphosate-resistant canola. Agric. Ecosyst. Environ. 129, 171–176.

Lupwayi, N.Z., Hanson, K.G., Harker, K.N., Clayton, G.W., Blackshaw, R.E., O'Donovan, J.T., Johnson, E.N., Gan, Y., Irvine, R.B., Monreal, M.A., 2007. Soil microbial biomass,

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004

functional diversity and enzyme activity in glyphosate-resistant wheat–canola rotations under low-disturbance direct seeding and conventional tillage. Soil Biol. Biochem. 39, 1418–1427.
Matthysse, A.G., 2006. The genus *Agrobacterium*. Prokaryotes 5, 91–114.
Means, N.E., 2004. Effects of glyphosate and foliar amendments on soil microorganisms in soybean. Doctoral Dissertation. University of Missouri, Columbia, 131 pp.
Means, N.E., Kremer, R.J., 2007. Influence of soil moisture on root colonization of glyphosate-treated soybean by *Fusarium* species. Comm. Soil Sci. Plant Anal. 38, 1713–1720.
Means, N.E., Kremer, R.J., Ramsier, C., 2007. Effects of glyphosate and foliar amendments on activity of microorganisms in the soybean rhizosphere. J. Environ. Sci. Health B 42, 125–132.
Meriles, J.M., Vargas Gil, S., Haro, R.J., March, G.J., Guzmán, C.A., 2006. Glyphosate and previous crop residue effect on deleterious and beneficial soil-borne fungi from peanut–corn–soybean rotations. J. Phytopathol. 154, 309–316.
Mijangos, I., Becerril, J.M., Albizu, I., Epelde, L., Garbisu, C., 2009. Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies. Soil Biol. Biochem. 41, 505–513.
Nash, S.M., Snyder, W.C., 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in the field. Phytopathology 52, 567–572.
Nelson, E.B., 1990. Exudate molecules initiating fungal responses to seeds and roots. Plant Soil 129, 61–73.
Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium* Species, an Illustrated Manual for Identification. Pennsylvania State University Press, University Park, PA.
Neumann, G., Kohls, S., Landsberg, E., Stock-Oliveira Souza, K., Yamada, T., Römheld, V., 2006. Relevance of glyphosate transfer to non-target plants via the rhizosphere. J. Plant Dis. Protect. 20, 963–969 (Special issue).
Njiti, V.N., Myers Jr., O., Schroeder, D., Lightfoot, D.A., 2003. Roundup Ready soybean: glyphosate effects on *Fusarium solani* root colonization and sudden death syndrome. Agron. J. 95, 1140–1145.
Ozturk, L., Yazici, A., Eker, S., Gokem, O., Römheld, V., Cakmak, I., 2007. Glyphosate inhibition of ferric reductase activity in iron deficient sunflower roots. New Phytol. 177, 899–906.
Powell, J.R., 2007. Linking soil organisms within food webs to ecosystem functioning and environmental change. Adv. Agron. 96, 307–349.
Powell, J.R., Swanton, C.J., 2008. A critique of studies evaluating glyphosate effects on diseases associated with *Fusarium* spp. Weed Res. 48, 307–318.
Reddy, K.N., Zablotowicz, R.M., 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. Weed Sci. 51, 496–502.
Reddy, K.N., Hoagland, R.E., Zablotowicz, R.M., 2000. Effect of glyphosate on growth, chlorophyll content and nodulation in glyphosate-resistant soybean (*Glycine max*) varieties. J. New Seeds 2, 37–52.
Rengel, Z., 1997. Root exudation and microflora populations in rhizosphere of crop genotypes differing in tolerance to micronutrient deficiency. Plant Soil 196, 255–260.
Sanogo, S., Yang, X.B., Scherm, H., 2000. Effects of herbicides on *Fusarium solani* f. sp. *glycines* and development of sudden death syndrome in glyphosate-tolerant soybean. Phytopathology 90, 57–66.
Schroth, M.N., Hildebrand, D.C., Panopoulos, N., 2006. Phytopathogenic pseudomonads and related plant-associated pseudomonads. Prokaryotes 6, 714–740.
Schroth, M.N., Hancock, J.G., 1982. Disease-suppressive soil and root-colonizing bacteria. Science 216, 1376–1381.
Schulz, A., Krüper, A., Amrhein, N., 1985. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiol. Lett. 28, 297–301.
Simonsen, L., Fomsgaard, I.S., Svensmark, B., Spliid, N.H., 2008. Fate and availability of glyphosate and AMPA in agricultural soil. J. Environ. Sci. Health B 43, 365–375.
Skovgaard, K., Nireberg, H.I., O'Donnell, K., Rosendahl, S., 2001. Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology 91, 1231–1237.
Smiley, R.W., Ogg Jr., A.G., Cook, R.J., 1992. Influence of glyphosate on Rhizoctonia root rot, growth, and yield of barley. Plant Dis. 76, 937–942.
Toner, B., Fakra, S., Villalobos, M., Warwick, T., Sposito, G., 2005. Spatially resolved characterization of biogenic manganese oxide production within a bacterial biofilm. Appl. Environ. Microbiol. 71, 1300–1310.
Thompson, I.A., Huber, D.M., 2007. Manganese and plant disease. In: Datnoff, L.E., Elmer, W.E., Huber, D.M. (Eds.), Mineral Nutrition and Plant Disease. American Phytopathological Society, St. Paul, MN, pp. 139–153.
Wardle, D.A., 1995. Impacts of disturbance on detritus food webs in agroecosystems of contrasting tillage and weed management practices. Adv. Ecol. Res. 26, 105–185.
Wardle, D.A., Parkinson, D., 1992. The influence of the herbicide glyphosate on interspecific interactions between four fungal species. Mycol. Res. 96, 180–186.
Wardle, D.A., Parkinson, D., 1990. Effects of three herbicides on soil microbial biomass and activity. Plant Soil 122, 21–28.
Zablotowicz, R.M., Reddy, K.N., 2007. Nitrogen activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. Crop Protect. 26, 370–376.
Zablotowicz, R.M., Reddy, K.N., 2004. Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: a minireview. J. Environ. Qual. 33, 825–831.

Please cite this article in press as: Kremer, R.J., Means, N.E., Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. (2009), doi:10.1016/j.eja.2009.06.004