UNIVERSITÉ DU QUÉBEC À MONTRÉAL

IMPACT OF GLYPHOSATE-BASED HERBICIDES AND CROPPING SYSTEMS ON NODULATION AND ATMOSPHERIC NITROGEN FIXATION BY SOYBEAN

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IMPACT DES HERBICIDES À BASE DE GLYPHOSATE ET DES RÉGIES DE CULTURE SUR LA FIXATION DE L'AZOTE ATMOSPHÉRIQUE PAR LE SOYA TRANSGÉNIQUE

MÉMOIRE

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LIST OF ABBREVIATIONS IN THE TEXT

GT: glyphosate-tolerant

GBH: Glyphosate-Based Herbicides

kg a.i. and ton a.i.: kilogram of active ingredient and ton of active ingredient

EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase

ANF: Atmospheric nitrogen fixation

CEROM: Centre de recherche sur les grains

NT2: no-till with 2 crops

NT3: no-till with 3 crops

DMC: direct seeding mulch based cropping systems

Ndfa: nitrogen derived from the atmosphere

RÉSUMÉ

Ce mémoire a été rédigé en anglais sous la forme d'un manuscrit pour soumission au Canadian Journal of Soil Science.

La majorité du soya cultivé au Québec est génétiquement modifié pour tolérer les herbicides à base de glyphosate. Les objectifs de cette étude furent d'examiner les impacts de différentes doses de glyphosate et des régies de culture sur la fixation de l'azote atmosphérique par le soya transgénique. L'étude a été réalisée sur deux sites expérimentaux. Le premier site comportait des parcelles de soya recevant différentes doses d'herbicide à base de glyphosate en deux applications (0.45, 0.90 et 1.79 kg i.a. /ha). Le deuxième site fut utilisé pour observer l'effet de trois régies de cultures sur la fixation d'azote atmosphérique par le soya, soit le semi-direct à deux cultures en rotation (SD2C), le semi-direct à trois cultures en rotation (SD3C) et le semi-direct avec couverture végétale (SCV), et cela avec l'usage d'herbicide à base de glyphosate (HBG). Après la seconde application d'herbicide, des plants de soya furent échantillonnés et une évaluation du statut de la nodulation fut effectuée, suivie d'un comptage des nodules. Le pourcentage d'azote dérivé de l'atmosphère (Ndfa) fut mesuré en utilisant les valeurs isotopiques $\delta^{15}N$ dans la partie aérienne des échantillons. Les différentes doses de HBG n'eurent pas d'effet significatif sur la nodulation et la fixation de l'azote atmosphérique. Les systèmes de culture SD3C et SCV ont eu une Ndfa plus élevée que les plants en SD2C. Les résultats de cette étude suggère donc que le système de culture aurait plus d'impact sur la fixation d'azote atmosphérique par le soya transgénique que les doses de HBG appliqués.

Mots clés: nodulation, glyphosate, plantes de couverture, fixation d'azote atmosphérique , semi-direct avec couverture végétale, nodosités, soya, rhizobia

ABSTRACT

Soybean cultivated in Quebec is mostly genetically modified for tolerating to glyphosate-based herbicides (GBH). The objectives of this study were to examine the impacts of various rates of glyphosate applications and different cropping systems on atmospheric nitrogen fixation in glyphosate-tolerant (GT) soybean. In one experiment, GT soybean received different GBH applications rates in two spreadings (0.45, 0.90 and 1.79 kg a.i. /ha). The other experiment was used to evaluate the effect of three distinct cropping systems (no-till 2 crops NT2, no-till 3 crops NT3 and direct seeding much-based 3 crops DMC) cultivated with GBH. Whole plants were harvested after the second application and the 20/20 Seed Labs Inc. assessment of atmospheric nitrogen fixation potential was made followed by a nodule count of each sample. There was no notable effect of GBH rates or the cropping systems on atmospheric nitrogen fixation potential and the number of nodules. The nitrogen derived from the atmosphere (Ndfa), measured using the $\delta^{15}N$ isotopic values of samples, was not significantly impacted by GBH application rates but was affected by the type of cropping system used. Depending upon the sampling site, atmospheric nitrogen fixation appeared to be most efficient either in one NT3 plot or in one DMC plot. NT2 cropping system had a lower Ndfa in both cases. This study suggests that GBH application rates do not have as much impact on atmospheric nitrogen fixation as cropping systems do.

Key words: nodulation, glyphosate, cover crops, atmospheric nitrogen fixation, direct seeding mulch based cropping systems, nodules, soybean, rhizobia

CHAPTER I

INTRODUCTION

Herbicides are widely used in field crops to control weeds. While the broad application of chemicals is known to have repercussions on human and environmental health, this weed control method is to this day the most efficient and advantageous (Singh and Singh Yadav. 2012). The other generally employed alternative, i.e. mechanical weeding, might not have those drawbacks but has many others. The use of machinery is energy consuming, tedious and the hire of laborers, costly. The mechanical method also causes compaction and erosion of soils in addition to emit GHG. As such, it is more and more replaced in major single-crop farming by herbicide utilization (Singh and Singh Yadav. 2012). Helped by the launch of crops that have been genetically modified in order to be glyphosate-tolerant (GT), glyphosate has been the most used active ingredient in herbicides worldwide for three decades (Székács and Darvas. 2012).

Glyphosate based herbicides (GBH) accounted for 27% of pesticide sales in 2018 in Quebec and are used mainly in agriculture (MELCC. 2018). Glyphosate (N-phosphonomethylglycine) is a phosphonomethyl derivative of glycine that acts as an herbicide by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme in the shikimic acid pathway (Myers *et al.* 2016). This pathway is used by plants, bacteria, and fungi to synthesize aromatic amino acid and makes glyphosate a nonselective herbicide as it acts on a broad variety of organisms. Since the shikimic acid pathway is not present in vertebrates, glyphosate is usually considered to have minimal effect on humans (Székács and Darvas. 2012). It was demonstrated that an acute intoxication to glyphosate causes digestive troubles in mammals but rarely death. That kind of intoxication does not happen often to humans who are more likely to undergo chronic expositions with doses assumed harmless (Portier *et al.*, 2016). The scientific community has not reached a consensus yet on glyphosate toxicity to humans. Some studies reported that exposure to low doses of glyphosate has potential carcinogenetic properties and could induce adverse effects on some mammalian systems like liver and kidneys hinting to other pathways for metabolization of glyphosate (Myers *et al.* 2016). Some scientists are concerned that the unclear risks of glyphosate to human, is no reason to conclude it is safe (Myers *et al.* 2016). However, other scientists consider that faced with little evidence of toxicity to human, glyphosate should be considered a "probable human carcinogen" (Portier *et al*. 2016). But whether or not there are toxic effects on humans, glyphosate affects environmental health in many ways. Amongst some of those impacts are toxicity to aquatic microorganisms and ecosystem interactions between different invertebrates (Portier *et al.*, 2016). Though a helpful agricultural tool, glyphosate also has adverse effects on farming (Mertens *et al.* 2018). Excessive glyphosate use could increase the development of crops diseases such as Fusarium head blight by weakening the crop plants either by directly affecting their fitness or modifying the soil ecosystem that is necessary to crop nutrition (Kanissery *et al.* 2019). Maybe the most acknowledged effect of the broad use of glyphosate is the progressive emergence of glyphosate-resistant weeds (Duke. 2017).

Nitrogen is essential in the biosynthesis of amino acids, proteins, chlorophylls, and other cellular components needed for the plant growth (Morot-Gaudry. 1997). This element is found as inert dinitrogen gas (N_2) in the atmosphere and must undergo ammonification to become bioavailable. The transformation from N_2 to a nitrogen compound is only accomplished by soil microorganisms (Stein and Klotz. 2016). However, some bacteria create a symbiosis with specific plants for which they can fix atmospheric nitrogen. They create nodules, structures on the roots where the symbionts fix nitrogen to provide their plant host with a supply of nitrogen, in addition to that in the soil. In exchange, the bacteria use photosynthesis products as an energy supply (Morot-Gaudry. 1997; Giller *et al.* 2016). There are many species able to achieve such symbiosis but legumes are the most studied. Those plants are crops that are grown for their protein-rich grains, a result of higher nitrogen intake (Giller *and al.* 2016). While biological nitrogen fixation (ANF) by soybean is widely studied, the effects of glyphosate applications on this mechanism are still unclear. Studies run in greenhouses and field alike, came out with contradictory results. Other experiments observed that the impact of GBH on ANF depends on many factors like glyphosate application rates (Zablotowicz and Reddy. 2007; Patil *et al.* 2012), time of application (Zobiole *et al.* 2011) and sampling period (King *et al.* 2001).

While many studies were conducted on glyphosate impact on soybean ANF and associate structures in greenhouses and agricultural fields, few were done in direct seeding mulch-based cropping systems (DMC). This agricultural practice makes use of cover crops to protect soil from erosion and increase plants and soil diversity. It is argued that the main advantages of DMC are a soil richer in nutrients for the crops, a weed control neither mechanic nor chemical, and a reduced dependence on pesticides (Séguy *et al.* 2012; Vincent-Caboud *et al.* 2017).The purpose of this study is to evaluate the impact of GBH on nodulation and ANF by GT soybean *Glycine max*, and the potential of DMC systems to catalyse those processes. This impact was evaluated at different GBH application rates at an experimental field. In addition, samples retrieved from two farmer's fields were used to evaluate the effect of different cropping systems, no-till with a rotation of two different crops (NT2), three crops in rotation (NT3) and DMC, on soybean nodulation and ANF. It was hypothesized that lower applied GBH application would increase the capacity for GT soybeans to produce nodules and to fix atmospheric nitrogen. DMC was expected to be the cropping system where the nodulation and ANF would be the most efficient, while NT2 would be the least productive. In observing the nodulation and fixation of atmospheric nitrogen by soybean at different GBH application rates and in no-till cropping system with and without cover crops, it could give an indication of the sustainability of a DMC system for soybean in Quebec.

CHAPTER II

MATERIAL AND METHODS

2.1 Fields location and experimental systems

Sampling was conducted at the Grain Research Center (CEROM, St Mathieu de Beloeil, Québec, (45˚58' N, 73˚24' W) in July 2019 in a field divided in experimental plots of 9m x 20m, treated with two consecutive glyphosate applications (Annex B). The soil at that location is mainly silty clay loam, which is typical of the agricultural St Lawrence Lowlands (Info-sols, 2021). Samples were collected in 16 plots where transgenic soybean was cultivated. Those 16 plots represented 4 replicates and 4 cultivation systems (NT3, DMC50, DMC100 and DMC200). The GBH application rate on NT3 plots was 0.90 kg a.i./ha of glyphosate. The DMC plots were established on rye and winter wheat as cover plants and were exposed to three distinct GBH application rates: 0.45, 0.90 and 1.79 kg a.i./ha of glyphosate (DMC50, DMC100 and DMC200 respectively). GBH applications were made twice, before sowing and the vegetative stage with three trifoliates (V3 stage).

Samples were also collected in August 2019 in fields belonging to two row crop producers at Sainte-Marthe (45°24' N, 74°20' W) and Montmagny (46°57' N, 70°33' W) (Québec). The first site is situated in the same region than the CEROM, its soil consisting of a silty clay composite (Info-sols. 2021). The soil of the second site is mainly composed of silt loam (Info-sols. 2021). Both sites were divided into 12m x

200m experimental plots (Annex A). Eight transgenic soybean plants were sampled in the NT3 plot, eight in the no-till with two crop rotations (NT2) plot, and eight in the DMC with plot alfalfa as cover plant.

2.2 Sampling

At each sampling site, the soil was excavated in the 0-20cm horizon where roots grow. Three soil cores were sampled at each plot at Sainte-Marthe and Montmagny. At CEROM, two soil samples were taken in each experimental plot and then pooled together. Those samples will be used to calculate nitrogen contents below the ground. Soybean plants were carefully harvested in their entirety to retrieve nodules that could easily be detached from their host. The sod around the plant roots was dug at a depth of 15 cm and a diameter of 25 cm with a trowel and the specimen delicately removed from the soil with its roots to keep the nodules intact (Date and Halliday. 1987). Samples were kept in paper bags to prevent the growth of mold. The shoots were separated from the roots and oven-dried at 50 ˚C until free of their water content (48h or more depending on the water content and size of samples). Shoot samples were ground into a fine powder, the mill cleaned with methanol between each sample to avoid cross-contamination. Though only a very small quantity of tissues powder was necessary for the stable nitrogen isotope analysis, all the shoot was ground in a fine powder and mixed together for each sample to have the greatest homogeneity possible (Herridge and Giller. 2016). The nodulation fitness status of each plant sample was estimated using the 20/20 Seed Labs Inc. assessment grid (2019) (Table 2.1). This allowed a first evaluation of the nodulation and the ANF potential.

Table 2.1 Assessment grid from 20/20 Seed Labs Inc. (2019). The scores are values assigned to chosen plant characteristics. For each sample, three criteria scores are compiled. A score of 1 to 6 is indicative of a plant with poor nodulation status, 7 to 10 is a reduced one, and 11 to 13 is the score of optimal nodulation.

Criteria	Plants appearance	Scores
Plant growth and vigor	• Green and vigorous	5
	green and relatively small	3
	• Slightly chlorotic	2
	• Very chlorotic	1
Nodule position	• Crown and lateral nodulation	3
	• Crown nodulation	2
	• Lateral nodulation	1
Color and number of nodules	• Greater than five clusters of pink pigmented nodules	5
	• Three to five clusters of predominantly pink nodules	3
	• Fewer than three clusters of nodules, or whitish/greenish nodules	1
	• No nodules or white/green nodules	0

2.3 δ^{15}N isotopic values

The soybean shoot samples and soil samples from the interval of 0-20cm depth were weighted in a tin cup to determine the carbon and nitrogen atomic ratio using a Carlo Erba NC2500 Elemental Analyzer. Atomic C/N ratio was measured for the soybean and soil of each plot for the three open-fields and used to determine the quantity of sample needed for the nitrogen stable isotope analysis. In total, 79 shoot and 34 soil samples were weighted using the results from C/N ratio and analyzed with a Micromass model Isoprime 100 isotope ratio mass spectrometer coupled to an Elementar Vario MicroCube elemental analyser in continuous flow mode. The delta units (δ) represent the values of abundance of ^{15}N atoms in percentage (atom%^{15}N):

$$\delta^{15}\mathrm{N} = \left(\frac{\mathrm{Sample\ atom\ \%^{15}N} - 0.3663}{0.3663}\right) \mathrm{x}\ 1000$$

The spectrometer uses three internal references; δ^{15}N = -0.10 ± 0.24‰ & + 14.95 ± 0.09‰, to normalize the results with respect to the air scale (IAEA-N1, IAEA-N2 & IAEA-N3), and δ^{15}N = -0,1 ± 0,15‰ as an unknown to determine the accuracy of normalization. The atmospheric atom%^{15}N value is 0.3663 and is used as the international standard (Herridge and Giller. 2016). The unit of the obtained results is in ‰ vs. air with an uncertainty better than ± 0.2‰.

2.4 Percentage of nitrogen derived for the atmosphere

The percentage of nitrogen derived from the atmosphere (%Ndfa) was measured using the δ^{15}N isotopic values of samples:

$$\%\mathrm{Ndfa} = \left(\frac{(\delta^{15}\mathrm{N\ of\ soil\ N} - \ \delta^{15}\mathrm{N\ of\ N^{2}fixing\ legume})}{\delta^{15}\mathrm{N\ of\ soil\ N} - \mathrm{B}}\right) x\ 100$$

Since the samples were extracted from open fields, it was presumed that they used a combination of nitrogen from the atmosphere and the soil, and thus δ^{15}N of both a shoot sample and a soil sample taken nearby are used to calculate the %Ndfa (Unkovich *et al*. 2008). The "B" value of soybean shoot ($\delta^{15}N = -1.83‰$) provides an adjustment for the isotopic fractionation within the plant since only the part above ground was used (Herridge and Giller. 2016; Unkovich *et al*. 2008).

2.5 Statistical method

The JMP® Pro 14 software (SAS Institute Inc., Cary, NC, 1989-2019.) provided the statistical comparisons. A one-way ANOVA was used with a Tukey-Kramer test to compare data in each field with a significance level of $p < 0.05$ for the nodule count and Ndfa. Nodulation assessment data were compared with a Chi-squared test with $p < 0.05$.

CHAPTER III

RESULTS

3.1 Relationship between GBH applications and soybean nodulation

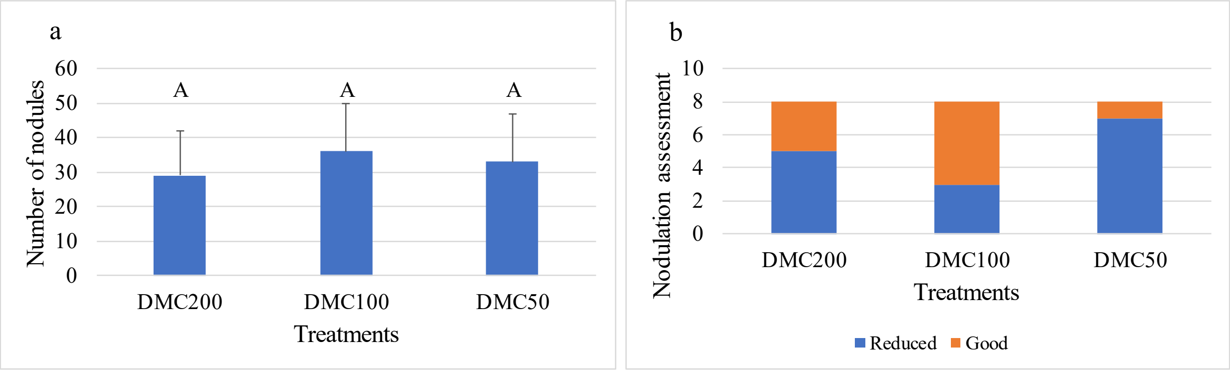


Figure 3.1 Nodulation assessment of soybean at different GBH application rates in DMC cropping systems.
(a) Nodules count for each soybean plots in CEROM with data representing mean ± standard deviation (n = 8). (b) Number of reduced vs good nodulation status by treatment (n = 8).

In the experimental plots at CEROM, the possible impact of GBH on legume nodulation was assessed along with nodule counts and nodulation assessment of 20/20 Seed Labs Inc. (Table 2.1.). No significant difference in nodules count was observed following different GBH application rates. According to the assessment used, there was no poor nodulation in any treatment and all samples had a perfect score for the growth and vigor criteria (Annex C). The proportion of good and reduced nodulation between each treatment was not significant with a *p*-value of 0.1184 (Table 3.1., Figure 3.1).

Table 3.1 Effect of GBH and cropping systems on nodulation and atmospheric nitrogen fixation. Testing was done by One-way ANOVA for nodule count and Ndfa and Chi-Square for the nodulation assessment score. The means for all columns in the three fields are n = 8 except for DMC at Montmagny n = 7. Means within each column for the same field followed by different letters are significantly different at $P < 0.05$.

Treatment	Nodule count	Nodulation assessment score	Ndfa (%)
Sainte-Marthe			
NT2	42 A	Reduced	78 B
NT3	50 A	Good	88 A
DMC	30 A	Reduced	77 B
Std Error	6		3
p-value	0.0724	0.2691	0.0160*
Montmagny			
NT2	68 A	Reduced	80 B
NT3	66 A	Reduced	86 A B
DMC	61 A	Good	100 A
Std Error	9		4 (NT2, NT3), 5 (DMC)
p-value	0.8648	0.8425	0.0150*
CEROM			
DMC200	29 A	Reduced	6 A
DMC100	36 A	Good	32 A
DMC50	33 A	Reduced	30 A
Std Error	5		9
p-value	0.8211	0.2341	0.1665

3.2 Relationship between GBH applications rates and atmospheric nitrogen fixation by soybean

Figure 3.2 shows that there is no significant relationship between GBH application rates and Ndfa (*p* = 0.1492). However, soybean harvested in the DMC200 plots had only 6% of its nitrogen content derived from the atmosphere versus 32% and 30% respectively in the DMC100 and DMC50 plots.

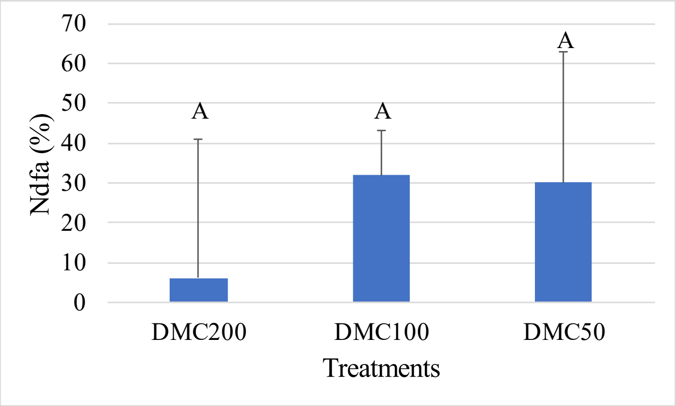


Figure 3.2 Percentage of nitrogen derived from the atmosphere (± standard deviation, n=8) in soybean as a function GBH doses.

3.3 Relationship between cropping systems and soybean nodulation

The cropping systems had no significant impact on nodule count at Sainte-Marthe (*p* = 0724; Figure 3.3). The lowest count was observed in the DMC plot with an average of 30 nodules while NT3 had the highest one (50). The difference between treatments was even less at Montmagny (*p*-value of 0.8648) with an average count of 61, 66 and 68 nodules in DMC, NT3 and NT2 plots respectively.

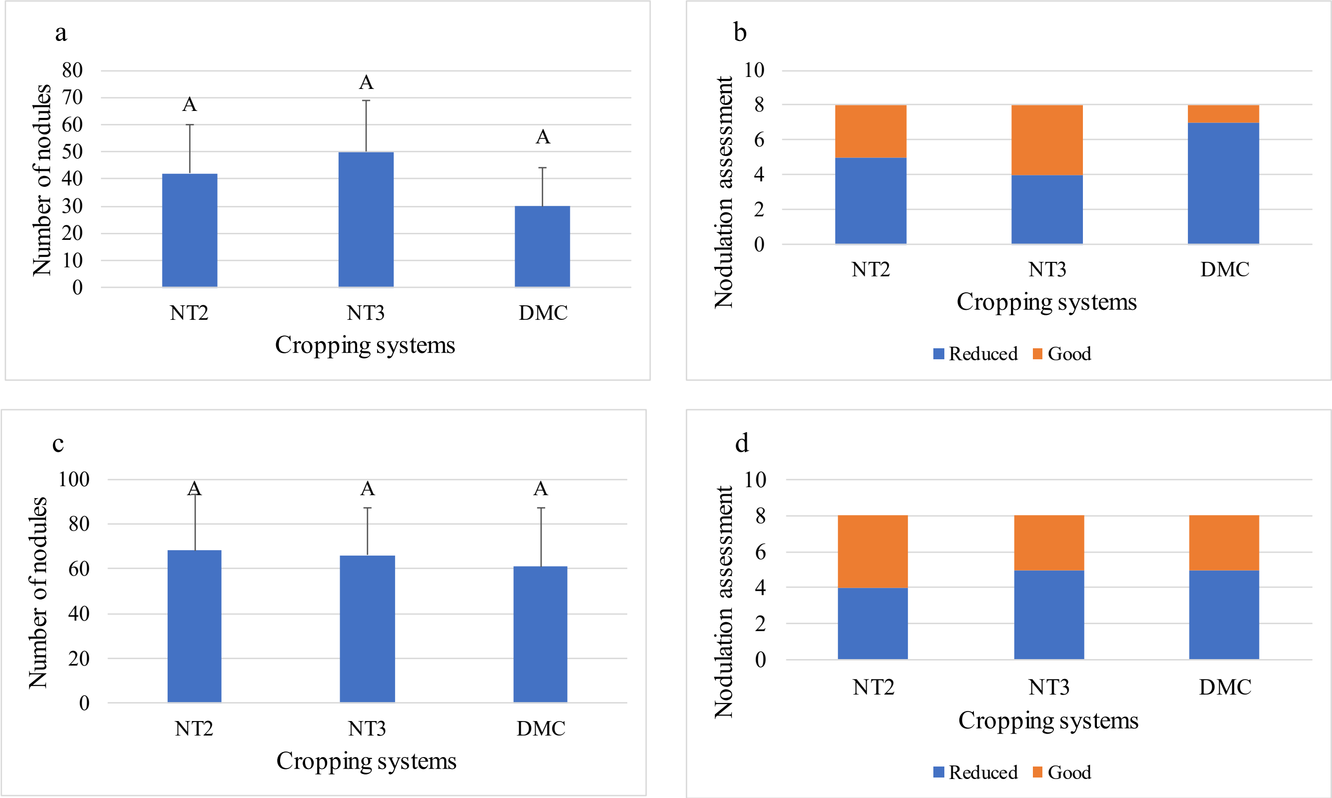


Figure 3.3 Nodulation assessment of soybean at different GBH application rates in different cropping systems.
(a) Nodules count for each soybean plots at Sainte-Marthe with data representing mean ± standard deviation (n = 8).
(b) Number of reduced vs good nodulation status by treatment by cropping system at Sainte-Marthe (n = 8).
(c) Nodules count for each soybean plots at Montmagny with data representing mean ± standard deviation (NT2 and NT3: n = 8, DMC: n = 7).
(d) Number of reduced vs good nodulation status by treatment by cropping system at Montmagny (NT2 and NT3: n = 8, DMC: n = 7).

Nodulation was not influenced by the cropping system at Sainte-Marthe (Table 3.1). The same trend, while not significative, is observed at NT2 plots that had less efficient nodulation than NT3 plots but higher than DMC ones with only 13% of good nodulation in its samples (Figure 3.3). At Montmagny, the NT2 system had the best nodulation assessment score but it was not significantly different from NT3 or DMC ones (Table 3.1, Figure 3.3).

3.4 Relationship between cropping systems and atmospheric nitrogen fixation

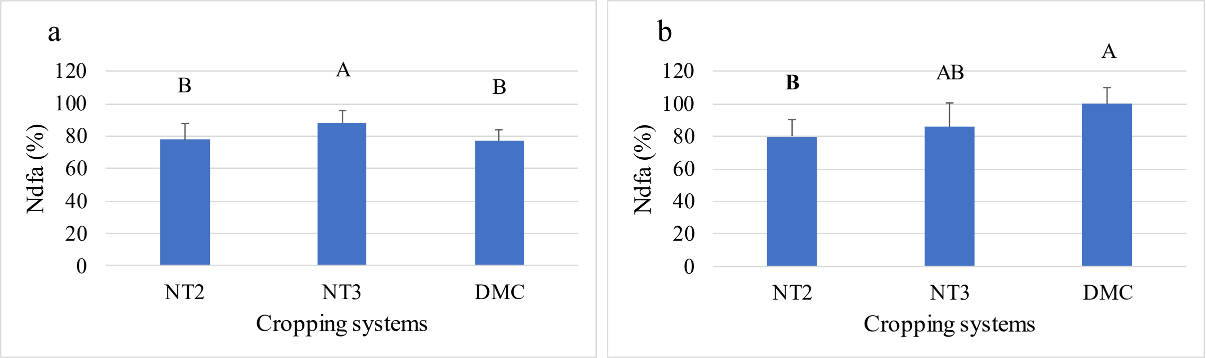


Figure 3.4 Percentage of nitrogen derived from the atmosphere in soybean in function of the cropping system. (a) Percentage of nitrogen derived from the atmosphere at Sainte-Marthe ± standard deviation (n = 8).
(b) Percentage of nitrogen derived from the atmosphere at Montmagny ± standard deviation (NT2 and NT3: n = 8, DMC: n = 7).

There was a significant effect on the percentage of atmospheric nitrogen fixed by the soybean rhizobium between the treatments at Sainte-Marthe ($p = 0.0160$*) (Figure 3.4). The DMC and NT2 plots both have a Ndfa of 77-78% and there is no difference between them (Table 3.1). Figure 4 shows that NT3 plot has significantly 10% more nitrogen fixed from the atmosphere than the two other cropping systems (Ndfa = 88%). At Montmagny, there is a relationship observed between the cropping system and the percentage of nitrogen fixed from the atmosphere by soybean plants. All nitrogen in plants at the DMC plot is derived from the atmosphere while the Ndfa of the NT3 plot is 86%, and 80% for the NT2 plot. The Ndfa of the DMC plot was significantly higher than that of NT3 plot while the latter was significantly more efficient in fixing N_2 than the NT2 plot.

CHAPTER IV

DISCUSSION

4.1 Relationship between GBH application rates and atmospheric nitrogen fixation by soybean

Nodule number was not affected by GHB application rates at R2 stage, which is consistent with the findings of Zablotowicz and Reddy (2003), and Bohm *et al.* (2015). In a field study in Stoneville (Mississippi), Zablotowicz and Reddy (2007) observed abundant nodulation in GT soybean treated with GBH even at application rates exceeding the manufacturer's recommendations. There was no significative difference in nodule number between untreated soybean and glyphosate treated soybean grown in ideal greenhouse-controlled conditions (Fan *et al.* 2017). According to Zablotowicz and Reddy (2003), glyphosate does not affect the nodulation starting phase but its further development, so a high nodule count might not indicate an efficient ANF. However, other field studies found that the number of nodules decreased with the increasing GBH application rates especially when the herbicide was applied at the V2 stage (Zobiole *et al.* 2010b; Zobiole *et al.* 2011; Zobiole *et al.* 2012). In the laboratory, nodule count is reduced with GBH applications up until a certain rate when there is no nodule at all (Shankar *et al.* 2012). The contradictory results could be due to the response variability between cultivars. Soybean nodule number is affected in less than half the genotypes and more severely when GBH is sprayed in one application rather than in sequential applications (Oliveira Jr *et al.* 2008).

The efficiency of ANF was measured in this experiment using $\delta^{15}N$ isotopic values to calculate the percentage of nitrogen derived from the atmosphere. There was no significant effect observed between the GBH application rates and the soybean ANF at CEROM experimental field. However, the high standard error might indicate that the results would be different with a greater number of samples. Similar results were found in an experiment in Brazil where there was no negative impact of GBH on soybean ANF (Bohm *et al.* 2015). It has been observed that glyphosate does not affect ANF under field conditions if the soils have adequate water supplies. The same observation was also made in a controlled environment, which might indicate that ANF is more sensitive to water deficit than to GBH applications rates (Zablotowicz and Reddy. 2007; King *et al.* 2001). Still, Fan et al. (2017) reported contradictory results to those presented in this study. In a greenhouse experiment, they found that glyphosate lowers nitrogenase activity, which could indicate a decrease of ANF efficiency, but the resulting amount of nitrogen in soybean was not measured.

4.2 Relationship between cropping system and atmospheric nitrogen fixation

The study of the influence of cropping system on soybean ANF was realized on samples taken in NT2, NT3 and DMC plots at Sainte-Marthe and Montmagny. At either location, the cropping system had no significative impact on the nodule count or the assessment of nodulation fitness. Those findings are not consistent with studies showing that nodulation should be more efficient in NT3 and DMC plots than in NT2 ones. It was observed that the nodule number was higher in legumes grown in intercropping systems, than sown alone (Hu *et al.* 2017). While not exactly a DMC system, it could indicate that a diversity of crops would increase the formation of nodules. Nodulation is also improved by increasing the number of crops in rotation in a field as it lowers rot damage on nodules and increases recovery from weed pressure (Li *et al.* 2018; Li *et al.* 2019).

At Sainte-Marthe, the soybean ANF was more efficient in the NT3 plot than in the NT2 and the DMC systems while it was not significantly different between the last two. The effect of cropping systems on ANF was also observed at Montmagny with soybean in the DMC plot having the most efficient fixation, followed by NT3 and NT2 plots with less successful BNF. Even if different in either two fields, the results are consistent with those from previous studies that showed that crop diversification improves the general robustness of a cropping system (Li *et al.* 2019). In a system with either a soybean-corn or a soybean-corn-oat-sorghum rotation, the latter had a consistently higher atmospheric nitrogen uptake than the system with two crop rotations (Sindelar *et al.* 2016). After 7 years of intercropping wheat-corn-fava bean, Cong *et al.* (2014) showed that the soil had a lower soil $\delta^{15}N$ than in a system with sole fava bean indicating that ANF was more efficient when the legume was sown with other crops. Hu *et al.* (2017) also concluded that crop diversity increased Ndfa significantly in an experiment comparing a system with corn-pea and one with sole pea.

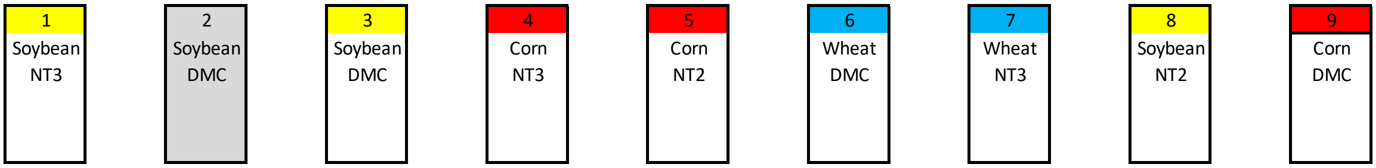
CHAPTER V

CONCLUSION

Recent studies on glyphosate impact on atmospheric nitrogen fixation by transgenic soybean have shown contradictory results. Current findings suggest that GBH does not affect nodulation and the subsequent ANF significantly. However, our results have shown a relationship between the chosen cropping system and the soybean ANF efficiency, which supports the argument that the key to more robust agriculture lies in plant diversity. Further research should be pursued in experimental fields with cover crops established for at least a decade.

ANNEX A

EXPERIMENTAL DESIGN OF FARMERS FIELDS IN 2019



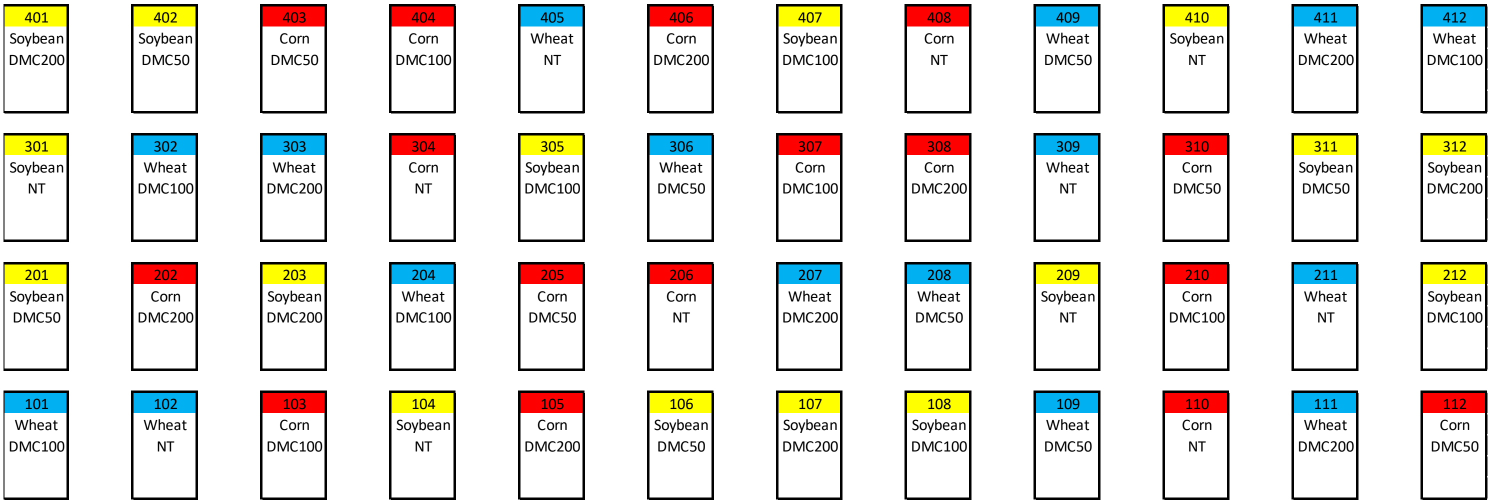
LEGEND

🟨	Soybean plots
🟥	Corn plots
🟦	Wheat plots
▨	Not sampled
NT2	Direct seeding, 2 crops rotations
NT3	Direct seeding, 3 crops rotations
DMC	Direct seeding with cover crops

Legend: Experimental plots were established in 2016 with soybean in plot 1 (NT3) and in plot 3 (DMC) and corn in plot 8 (NT2). In 2017 cash crops were wheat in plot 1 (NT3) and plot 3 (DMC) and soybean in plot 8 (NT2). In 2018 cash crops were corn in plot 1 (NT3) and plot 3 (DMC), and corn in plot 8 (NT2). At Sainte-Marthe, glyphosate was applied as the active ingredient of HBG WeatherMax at a rate of 3L/ha in all soybean plots (08-06-2019). At Montmagny WeatherMax was applied at a rate of 1.25L/ha in all soybean plots (17-07-2019). In both fields, alfalfa is the only cover crop used in the DMC soybean plot.

ANNEX B

EXPERIMENTAL DESIGN OF CEROM EXPERIMENTAL FIELD IN 2019



401 Soybean DMC200	402 Soybean DMC50	403 Corn DMC50	404 Corn DMC100	405 Wheat NT	406 Corn DMC200	407 Soybean DMC100	408 Corn NT	409 Wheat DMC50	410 Soybean NT	411 Wheat DMC200	412 Wheat DMC100
301 Soybean NT	302 Wheat DMC100	303 Wheat DMC200	304 Corn NT	305 Soybean DMC100	306 Wheat DMC50	307 Corn DMC100	308 Corn DMC200	309 Wheat NT	310 Corn DMC50	311 Soybean DMC50	312 Soybean DMC200
201 Soybean DMC50	202 Corn DMC200	203 Soybean DMC200	204 Wheat DMC100	205 Corn DMC50	206 Corn NT	207 Wheat DMC200	208 Wheat DMC50	209 Soybean NT	210 Corn DMC100	211 Wheat NT	212 Soybean DMC100
101 Wheat DMC100	102 Wheat NT	103 Corn DMC100	104 Soybean NT	105 Corn DMC200	106 Soybean DMC50	107 Soybean DMC200	108 Soybean DMC100	109 Wheat DMC50	110 Corn NT	111 Wheat DMC200	112 Corn DMC50

Plot size : 9m x 20m

LEGEND

🟨	Soybean plots
🟥	Corn plots
🟦	Wheat plots
NT	Direct seeding with glyphosate at 3.34 L a.i./ha
DMC200	Direct seeding with cover crops with glyphosate at 3.34 L a.i./ha
DMC100	Direct seeding with cover crops with glyphosate at 1.66 L a.i./ha GP
DMC50	Direct seeding with cover crops with glyphosate at 0.84 L a.i./ha GP

Legend: The experimental plots were established in 2018 with corn in all plots where soybean was cultivated in 2019 (yellow plots). WeatherMax was applied on all soybean plots in two applications (03-06-2019, 27-06-2019). Rye and winter wheat were used as the cover crops of the DMC soybean plots.

ANNEX C

NODULATION ASSESSMENT OF SOYBEAN IN 2019

Results from samples within the assessment grid from 20/20 Seed Labs Inc.

Treatment	Plant growth and vigor	Nodule position	Color and number of nodules	Total score	Nodulation fixation potential
Sainte-Marthe					
NT2	5	3	2	10	Reduced
NT3	5	3	3	11	Good
DMC	5	3	1	9	Reduced
Montmagny					
NT2	5	3	2	10	Reduced
NT3	5	3	2	10	Reduced
DMC	5	3	3	11	Good
CEROM					
DMC200	5	2	2	9	Reduced
DMC100	5	3	3	11	Good
DMC50	5	3	2	10	Reduced

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