RESEARCH ON THE WEED CONTROL DEGREE AND GLYPHOSATE SOIL BIODEGRADATION IN APPLE PLANTATIONS (PIONEER VARIETY)

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Abstract: In this study we follow control degree of glyphosate herbicide on weeds in apple plantations (Pioneer variety) of the Research Station Timisoara. It was also followed glyphosate biodegradation capacity in the soil by determining the amount of CO_2 released by the action of microorganisms on C14 glyphosate marked isotope. Laboratory analysis of glyphosate residues in soil was made using a Liquid Scintillation TRIATHLER. Glyphosate biodegradation ability in the presence of soil microorganisms is high, so glyphosate residues remaining in soil, in terms of its use in weed combating, are minimal. Study of glyphosate biodegradation capacity in the experimental field indicates that the CO_2 fraction accumulated after 50 days is 28.02% for samples exposed in the experimental field. Weather conditions, especially temperature variations between day and night, influences the activity of soil microorganisms and affect biodegraded glyphosate percentage.

Chemical method of weed control consisted in: herbicide used was Roundup 3 l/ha (glyphosate isopropyl amine salt 360 g/l) and are based on chemical application on weeds, on the rows of trees, on their uptake and translocation in their organs having as principal scope the total destruction of weeds. The experimental results obtained reveal a weed combat degree of 82.98% , in the case of chemical variant, compared with control variant. The species combated mainly due to glyphosate herbicide, which is no longer found in the final mapping are: *Capsella bursa-pastoris, Chenopodium album, Echinochloa crus-galli, Plantago major, Polygonum aviculare.* Total combated weeds /m^2 with glyphosate is 126.67.

Keywords: glyphosate, apple plantation, mineralization, microorganisms, weed control degree

INTRODUCTION

Glyphosate (N-(phosphonomethyl) glycine) is a broad-spectrum systemic herbicide used to kill weeds, especially perennials [15]. Glyphosate is a broad-spectrum, systemic, post-emergence herbicide that is phloem mobile and is readily translocated throughout the plant. Its introduction in agriculture will be of benefit because of the low toxicity and environmental safety of the product. The introduction of genetically modified crops resistant to glyphosate will lead to a significant increase in the use of this herbicide [16].

From the leaf surface, glyphosate molecules are absorbed into the plant cells were they are translocated to meristematic tissues. Glyphosate`s mode of action is to prevent a plant from producing the essential amino acids tryptophan, tyrosine and phenylalanine, which reduces the production of protein within the plant, thereby inhibiting plant growth [9].

Because plants absorb glyphosate it cannot be completely removed by washing or peeling produce or by milling, baking or brewing grains. It has been shown to persist in food products for up to two years [7].

Regarding absorption, distribution, excretion and metabolism of glyphosate in mammals the previous studies shows that the herbicide is: rapidly but only to a limited extent (approx. 30%) absorbed, potential for accumulation is less as 1% after 7 days, rate and extent of excretion in mammals is rapid and nearly complete (approx. 30% via urine) [10].

Glyphosate is the active ingredient of some of the most common herbicides used in farming and gardening. These products have been promoted as quickly biodegradable and non toxic. People believe that they are so safe that you can drink a cup of these herbicides without any ill effect [11].

Glyphosate is water-soluble, but it has an extremely high ability to bind to soil particles. Adsorption of glyphosate increases with increasing clay content, cation exchange capacity, and decreasing soil pH and phosphorous content. Glyphosate is adsorbed to soil particles rapidly during the first hour following application and slowly thereafter. Strong adsorption to soil particles slows microbial degradation, allowing glyphosate to persist in soils and aquatic environments [12].

Consequently, it is sprayed on roadsides while people are driving, on footpaths when people are shopping and in schoolyards and sports fields, exposing children to drift and residues. People buy it from supermarkets or garden shops and use it without any protective clothing because it is deemed 'safe'. It is sprayed in national parks and other environmentally sensitive areas in the belief that it is not toxic and or residual [8].

Previous studies regarding glyphosate toxicity no evidence carcinogenicity of this compound. Comprehensive medical database, mainly related to accidental or intentional oral intake of glyphosate products. Toxicological studies on AMPA revealing the metabolite to be less toxic than the parent compound, no evidence of mutagenicity and teratogenicity [14].

Due to the non-extractability of bound residues, radiolabelled pesticides are needed to follow the fate of the active molecule and its degradation products into various soil organic compartments.

In this study we follow control degree of glyphosate herbicide on weeds in apple plantations (Pioneer variety) of the Research Station Timisoara. It was also followed glyphosate biodegradation capacity in the soil by determining the amount of CO_2 released by the action of microorganisms on C14 glyphosate marked isotope.

MATERIAL AND METHODS

Chemical method of weed control consisted in: herbicide used was Roundup 3 l/ha (glyphosate isopropyl amine salt 360 g/l) and are based on chemical application on weeds, on the rows of trees, on their uptake and translocation in their organs having as principal scope the total destruction of weeds.

Herbicide application was performed 1-2 days after the weed mapping, where most of them were in the stage of seedlings or plants without reproductive organs, or depending on the species, the plant with blossoms (grass).

Experience is a single factorial type, following the weed control efficacy of the Pioneer variety.

Weed mapping has been done to determine the quantity and quality of weeding degree of surface studied before and after herbicide application.

Data were obtained using quantitative numerical method, which is counting on the species of weeds in the sample area (0.25 m^2), is an expedient and sufficiently accurate method. Metric frame used had an area of 0.25 m^2, beeing an square with an inner side of 0.5 m.

Besides the actual number of weed species found within the metric frame, was noted the weed development phase that was found to each species using the following scale of assessment: A – seedlings or plant without reproductive organs, B - plant with blossoms or in the case of grass plants, the skin stage, C – plant with flower, D – plant with fruit, E - plant to which the seeds or fruits were spread.

Calculation of analytical data consisted in establishment several indicators and their registration with botanical class and group of weeds that are highlighted in the phase field:

- ***s*****,** sum of individuals of a species found in the observation points, is calculated by summing the values obtained in field after a previous multiplying with 4, to be reported to $1m^2$;

- mean ***m***, which is calculated with relationship ***m* = *s* /*N***, where ***s*** is the sum of individuals of a species found in the observation points, and N is the total number of observation points;

- average number of weeds in 1 m^2 or total species average ***M***, is obtained by adding all ***m*** values;

- participation p% is calculated with relationship p% = m • 100/M, representing percentage expressing of a measure to which a species participate to general weeding;

- constancy k% is calculated with relation k% = n • 100 / N, representing the extent to which a species is found (regardless of the number of individuals) at the point of determination.

Symbols used to class botanical registration were: D.a. - Dicotyledonous annual; D.p. - Dicotyledonous perennial; M.a. - Annual monocotyledon; M.p. - Monocotyledonous perennial.

Calculation of synthetic data consisted in expressing the above mentioned biological categories based on the number of species encountered, the average number of individuals participating in general weeding.

Laboratory analysis of glyphosate residues in soil was made using a Liquid Scintillation TRIATHLER. The soil was treated with cold glyphosate with initial concentration equal to concentration applied in field and estimated at 1.5 ppm.

Radioactivity brought through glyphosate standard C14 marked to fosfonometil group was 37kBq for each soil. Evolution of glyphosate mineralization in the soil was evidenced by capturing $^{14}CO_2$ freed as follow the action of soil microorganisms on herbicide, in 0.2 M NaOH solution at various time intervals. The solution sampled is mixed with 5 ml liquid scintillate (Quicksafe A) in a 20 ml vial and is read the number of disintegration with the help of Thriatler liquid scintillation [1-2]. After each sample is replaced the vial with fresh solution of 0.2 M NaOH. The experiment took place during 50 days, the amount of $^{14}CO_2$ accumulated during the experiment is calculated by adding the amounts of CO_2 released from each sample (Figure 1).

A. B.

Figure 1. Study of glyphosate persistence and effectiveness against weeds: **A** - apple plantations in the Research Station Timisoara; **B** – field experiment.

RESULTS

The experimental results regarding the glyphosate effectiveness on the degree of weed control on Pioneer apple variety are presented in Tables 1-3. Mineralization curve obtained which show the amount of $^{14}CO_2$ accumulated during the experiment (expressed as a percentage of initial radioactivity) and degradation rate in the experimental field, is given in Figure 2.

Table 1. The glyphosate efficacy on the weed dregree extent to Pioneer variety.

Variant	No. of existent weed plants /m^2	No. of weed combated/m^2 comparatively with control variant	Weed combat degree (%)
V_1 – without herbicide, spawn	152.85	-	0.00
V_2 – with Roundup herbicide on plantation rows	**26.02**	**126.83**	**82.98**

Table 2. Initial sheet with weeds in Pioneer variety plantation, Variant 2, application of Roundup herbicide on rows, grass was cut on plantation intervals.

No.	Species	Vegetation phase	Sum of individuals/m^2	Mean number of weed./m^2	Participation percentage (%)	Botanical class
1	*Agropyron repens*	A-C	76	25.33	16.67	M.p.
2	*Cirsium arvensis*	A-B	56	18.67	12.28	D.p.
3	*Convolvulus arvensis*	A-C	44	14.67	9.65	D.p.
4	*Capsella bursa-pastoris*	A-C	44	14.67	9.65	D.a.
5	*Cynodon dactylon*	A-C	40	13.33	8.77	M.p.
6	*Veronica hederifolia*	A-B	40	13.33	8.77	D.a
7	*Stellaria media*	A-C	28	9.33	6.14	D.a.
8	*Amaranthus retroflexus*	A-C	28	9.33	6.14	D.a.
9	*Cardaria draba*	C	28	9.33	6.14	D.p.
10	*Chenopodium album*	B	28	9.33	6.14	D.a.
11	*Echinochloa crus-galli*	B	20	6.67	4.39	M.a.
12	*Plantago major*	B	12	4.00	2.63	D.a.
13	*Rubus caesius*	A	4	1.33	0.88	D.p.
14	*Portulaca oleracea*	A-B	4	1.33	0.88	D.a.
15	*Polygonum aviculare*	B	4	1.33	0.88	D.a.
Total			**456**	**152.00**	**100.00**	

Species rapport number (D.a./D.p./M.a./M.p.): 8/4/1/2=15 Mean weed number/m^2 = 152.00

Tabel 3. Final sheet with weeds in Pioneer variety plantation, Variant 2, application of Roundup herbicide on rows, grass was cut on plantation intervals.

No.	Species	Vegetation phase	Sum of individuals/m^2	Mean number of weed./m^2	Participation percentage(%)	Botanical class
1	*Agropyron repens*	A-C	16	5.33	21.05	M.p.
2	*Cirsium arvensis*	A-C	16	5.33	21.05	D.p.
3	*Amaranthus retroflexus*	A-C	8	2.67	10.53	D.a.
4	*Cynodon dactylon*	A-C	8	2.67	10.53	M.p.
5	*Veronica hederifolia*	A-B	8	2.67	10.53	D.a.
6	*Cardaria draba*	A-B	4	1.33	5.263	D.p.
7	*Convolvulus arvensis*	C	4	1.33	5.263	D.p.
8	*Portulaca oleracea*	A-B	4	1.33	5.263	D.a.
9	*Rubus caesius*	A	4	1.33	5.263	D.p.
10	*Stellaria media*	A-C	4	1.33	5.263	D.a.
Total			**76**	**25.33**	**100.00**	

Species rapport number (D.a./D.p./M.a./M.p.): 4/4/0/2=10 Mean weed number / m^2 = 25.33

The experimental results obtained reveal a weed combat degree of 82.98%, in the case of chemical variant, compared with control variant. Combated number of weeds/m^2 V_2 = 126.67

Of obtained weed sheets it can see that species combated mainly due to glyphosate herbicide, which is no longer found in the final mapping are: *Capsella bursa-pastoris*, *Chenopodium album*, *Echinochloa crus-galli*, *Plantago major*, *Polygonum aviculare*.

Glyphosate mineralization curve reveals two phases of CO_2 release, the first rapid phase, followed by a slow phase, when the mineralization curve reaches a steady plateau (Figure 2). Initial rapid phase covers a period of approximately 20 days from the beginning of the experiment and is attributed to the action of microorganisms on free glyphosate from soil, while the second phase is attributed to slow action of microorganisms on glyphosate adsorbed on soil components.

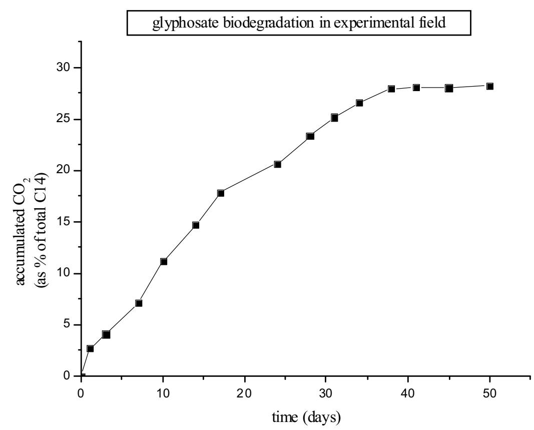

Figura 2. Biodegradation curve of glyphosate in soil made under experimental field conditions

DISCUSSIONS

Study of glyphosate biodegradation capacity in the experimental field indicates that the CO_2 fraction accumulated after 50 days is 28.02% for samples exposed in the experimental field. Glyphosate biodegradation ability in the presence of soil microorganisms is high, so glyphosate residues remaining in soil, in terms of its use in weed combating, are minimal. Glyphosate degradation by microorganisms has been widely tested in a variety of field and laboratory studies [1-6]. The obtained results complete previous research made on soil types: chernozem, phaeozem, gleyosol and vertisol which indicate a high microbial degradation of the glyphosate herbicide [13].

Weather conditions, especially temperature variations between day and night influences the activity of soil microorganisms and affect biodegraded glyphosate percentage. Previous studies conducted by research team on the same soil in the laboratory, indicate that the percentage of CO_2 accumulated as a result of glyphosate biodegradation under the action of micro organisms is higher in the case of laboratory experiment comparatively with the experiment conducted in field. $C0_2$ fraction accumulated after 50 days, in the case of experiment performed in laboratory is 73.42% versus 28.02% for samples exposed in the experimental field. Glyphosate shows low mobility in soil, extractable residues can be found in quantities above the minimum detection limit of 0.1 ppm in the soil, exclusive in soil layer ranging from 0-10 cm. [3].

Glyphosate is the active ingredient in 53 of herbicides formulation and is used to kill a variety of broadleaf weeds and grasses [15]. Sheets with weed number made in case of using glyphosate in weed control in apple plantations, indicating good efficiency of this herbicide for the Pioneer apple, both regarding weeds species and their combated number. Total combated weeds /m^2 with glyphosate is 126.67. Number of combated weeds per square, respectivelly the percentage of control for each species is: *Agropyron repens* – 20 (78.95%), *Cirsium arvensis* – 14.67 (78.57%) *Amaranthus retroflexus* – 6.66 (71.38%), *Cynodon dactylon* – 10.66 (79.96%), *Veronica hederifolia* – 10.66 (79.96%), *Cardaria draba* – 8 (85.74%), *Convolvulus arvensis* – 13.34 (90.93%), *Portulaca oleracea* – 0 (0 %), *Rubus caesius* – 0 (0%), *Stellaria media* – 8 (85.74%), *Capsella bursa-pastoris* – 14.67 (100%), *Chenopodium album* – 9.33 (100%), *Echinochloa crus-galli* – 6.67 (100%), *Plantago major* – 4 (100%), *Polygonum aviculare* – 1.33 (100%).

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REFERCENCES

[1] Alexa, E., Bragea, M., Sumalan, R., Lazureanu, A., Negrea, M., Iancu, S., (2009): Dynamic of glyphosate mineralization in different soil types. Romanian Agricultural Research, no.26, pp. 57-60.

[2] Alexa, E., Bragea, M., Sumalan, R., Negrea, M., Lazureanu, A., Alda, S., (2009): Degradation of 14C-Glyphosate in compost amended soil. Communications In Agricultural And Applied Biological Sciences, Ghent University, 74(2),pp. 197-204.

[3] Alexa, E., Bragea, M., Sumalan, R., Negrea, M., (2009): Glyphosate mineralization and adsorbtion in different agricultural soils. Proceedings of the second International Conference, Research people and actual tasks on multidisciplinary sciences, Bulgaria, Vol. 1, pp. 372-375.

[4] Alexa E., Lazureanu A., Alda S., Negrea M., Iordanescu O, (2008): Researches regarding extractable Glyphosate residues from different soils, Communications In Agricultural And Applied Biological Sciences, Ghent University, 73(4),pp. 861 – 870.

[5] Alexa E., Lazureanu A., Alda S., Negrea M., Bulmaga A., (2008): Researches regarding elutration capacity of glyphosate herbicide and of his methabolit AMPA in different types of soils, Lucrari Stiintifice Agronomie Bucuresti, Seria A LI, 2008, pp. 501-506.

[6] Alexa E., Lazureanu A., Alda S., Negrea M., Bulmaga A., (2008): Extraction and identification of the herbicide glyphosate from natural water with different characteristics, The 51th Scientific Conference, Lucrări Ştiinţifice – vol.51, Seria Agronomie, Iasi, pp.45-49

[7] Braja, B., Afonso, D., (2005): AMPA and glifosat adsorbtion, a comparative study. Environmental Science & Technology, 39: pp. 205-209.

[8] Charnay, M.P., Mougin, C., Farrugia, A., Barriuso, E., (2004): Incorporation of pesticides by soil micro-organisms as a way of bound residues formation. Environment Chemical Letters, 2(1): pp. 27–30.

[9] Cox, C., (2004): Glifosat herbicide factsheet. Journal of pesticide reform, 24(4), pp.10–14.

[10] Kiely T., Donaldson, D (2004): U.S. Environmental Protection Agency-EPA, Pesticides Industry Sales and Usages: 2000/2001, Market Estimates, pp. 14-16

[11] Leu, A., (2007): Monsanto's Toxic Herbicide Glyphosate: A Review of its Health and Environmental Effects. Organic Producers Association of Queensland, pp. 12-16.

[12] Snapp, S.S., Borden, H., (2005): Enhanced nitrogen mineralization in mowed or glyphosate treated cover crops compared to direct incorporation Plant and Soil, 270: 101–112.

[13]. Sumalan R, Alexa E., Negrea M., Schmidt B. (2009), Study regarding the structure and activity of edaphic microflora community in conditions of glyphosate addition, Journal of Horticulture, Forestry and Biotechnology, vol.13, pp. 260-263,

[14] Tu, M., Hurd, C., Robison, R. Randal, M.J., (2001): Glyphosate, Weed Control Methods Handbook, The Nature Conservancy. pp. 7e1-7e10,

[15]*** http://en.wikipedia.org/wiki/Glyphosate, downloaded in March 2010

[16]*** http://www.mindfully.org/Pesticide/Roundup-Glyphosate-Factsheet-Cox.htm, downloaded in March 2010

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