RESEARCH CONCERNING THE IMPACT OF BT TECHNOLOGY APPLIED TO CULTIVATE GENETICALLY MODIFIED POTATOES ON SOIL QUALITY

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Abstract: *Evaluation of genetically modified plant varieties (before placing in culture) is generally focused on aspects of genetic stability of the inserted genes and agronomic aspects of GM varieties. However, in the international scientific community there is concern about the environmental consequences of introducing a functional gene associated with changes in management practices and agricultural systems on the essential functions of the ecosystem, and the fate of the products obtained from genetically modified organisms (GMOs) e.g. persistence in the environment and gene transfer to other organisms. These issues must be included in every study of risk assessment for GMOs. In Romania were not conducted studies and independent researches to assess the impact of cultivation of genetically modified plants on biodiversity, quality and functioning of agro ecosystems. Was studied, however, ecological and economic impact of the introduction into the environment of plants with a single genetically modified character, mainly glyphosate-tolerant soybean, cultivated in Romania until 2007 (Badea et al., 2004, 2006; Otiman et al., 2004). Soil biological communities are among the most diverse biotic groups on the planet. Soil microorganisms are involved in regulating a number of processes in terrestrial ecosystems, which are essential for maintaining its productivity and health. Develop methodology for assessing the Bt biotechnology impact, applied to cultivate genetically modified potato, on soil quality parameters, and particularly, on the microorganisms diversity, was done according to the following objectives: soil type influence (through its defining physical-chemical parameters) and genotypes of cultivated hybrids about insecticide persistence and degradation of proteins, influence of transgenic plants cultivation about microbial diversity, study of transgenic plant cultivars about the main soil chemical properties. The criteria for selection of soil materials on which to conduct the impact study of Bt biotechnology was favorability for species taken in the study and contrasting physical and chemical properties. Thus, were used two soil types: Eutric Fluvisols and Fluvi-Eutric Cambisols. Research developed in greenhouse has pursued: identifying correlations between the main physical-chemical and biological attributes of soil and plant genotypes (GMOs or non-GMOs) cultivation; environmental impact assessment of Bt protein on essential soil biological processes by investigating the biological activities associated with plant debris decomposition and determination of carbon, nitrogen, phosphorus and potassium in the soil; assessing the impact of Bt technology about taxonomic and genetic diversity of soil micro-organisms; assessment of possible modifications of the main physical, chemical and biological properties of soil under the influence of Bt technology.*

Key words: *Bt technology, transgenic plant, soil, impact study*

INTRODUCTION

GMOs (plants, microbes and animals) with useful characters are considered to be a powerful technology for the future development of sustainable agricultural systems. Plants have been genetically modified to resist insect and fungal pathogens, withstand specific

herbicide application (better weed management) or environmental conditions (e.g. water logging), to improve crop quality, for biomolecule production and for bioremediation/phytoremediation of polluted soils. The major limiting factor in growing potato in many areas of the world is the Colorado potato beetle (L. decemlineata), which is resistant to most classes of chemical pesticides (GEORGHIOU and LAGUNES-TEJADA, 1991; METCALF and METCALF, 1993). The first commercial fields of Bt potato, with a number of different Monsanto transformation events expressing the Cry3A protein under the name of New Leaf Potatoes, were grown in the USA in 1996 (SHElton et al., 2002). The Cry3A protein is active against certain species of beetle (*Coleoptera*), and it provides not only excellent control of the larvae—there are essentially no survivors—but it also inhibits reproduction in the adult beetles. However, in response to marketing and political pressures by the public, many food producers have chosen not to use Bt potatoes in their products. As a result, since 2001, Bt potatoes are no longer available (SHELTon et al., 2002; ICOZ & STOTZKY 2008). Red-Sec 7, 9 and 14 are potatoes varieties with a single transformation event, genetically modified to express the Bt insecticidal toxin gene that confers plant resistance to attack by Colorado potatoes beetles, created in Romania. While reduced pesticide/herbicide use associated with genetically modified potatoes is, clearly beneficial, very little is known about potential non-target effects of Bt potatoes plants on the functional groups of biota and biological processes that are critical for plant health, and essential ecosystem functions including ecosystem health. Pre-release evaluation of GM plant varieties is generally concentrated on the genetic stability of gene insertions and agronomic aspects of GM varieties.

However, comparatively little experimental (especially quantitative) data are available on: environmental consequences of the introduced gene function and associated changes in management practices/farming systems on essential ecosystem functions and the fate of the products of engineered genes from genetically modified organisms (GMOs) e.g. persistence in the environment and gene transfer to other organisms. This needs to be an essential part of the risk assessment of any GMOs release.

Soil organism's communities, which are among the most diverse groups of earth's biota, regulate a number of processes in terrestrial ecosystems that are not only critical for productivity, but are, also, essential for maintenance of ecosystem health (BRUSSARD, L., et al., 1997). Micro organisms and microbial activity have a key role in stable aggregate formation. Water-stable aggregates are essential for good soil structure in all types of soils. Good soil structure is necessary to reduce soil erosion. Very few biological processes are mediated by individual species of biota; therefore, the successful functioning of most ecosystem processes requires a balance of biota interactions in the complex soil biota community. The availability of energy (carbon), the most important regulating factor of biological activity in soils, affects the composition of the soil biota community and food web structure (ELLIOTT, E.T. and COLEMAN, D.C., 1988; COLEMAN, D.C et al, 1995). In addition, the number of trophic levels in a terrestrial food-web community and the stability of this complex community depend upon the amount and quality of carbon input and the level and type of disturbance (e.g. tillage, GM crops and use of agrochemicals).

Plant residues are one of the primary sources of carbon in soils and the majority of biota populations are concentrated near crop residues and in the plant root rhizosphere (GUPTA, V.V.S.R et al, 2000). Therefore, any change to the quality of crop residue and rhizosphere inputs will potentially modify the dynamics of the soil biota composition and activity. Soil microoorganisms perform a number of key functions essential to plants, organic mater mineralization, nutrient cycling, disease regulation, agrochemical degradation, and the development and maintenance of physical and chemical properties of soil. Therefore, any change to the quality of rhizosphere exudates will potentially modify the dynamics of the soil

biota composition (biodiversity) and activity and may cause changes to both deleterious and beneficial microflora and micro fauna (GUPTA, V.V.S.R. and YEATES, G.W. 1997; GUPTA, V.V.S.R. et al, 1999; BIAO L. et al, 2005).

GM plants, through the products of introduced genes, modified rhizosphere chemistry, or altered crop residue quality, have the potential to significantly change the microbial dynamics and essential ecosystem functions such as nutrient mineralization, disease incidence, and carbon turnover and plant growth (GUPTA, V.V.S.R et al, 2000). For example, a decrease in specific microbial populations would lead to a decrease in decomposition processes, have secondary effects on plant pathogen survival, and build up, as well as soil organic matter level and composition (TERMORSHUIZEN, A.J. and LOTZ, L.A.P., 2002). However, little experimental data are available on the consequences of plant-microbe-soil interactions due to the sustained expression and/or presence of Bt toxin in the rhizosphere. GUPTA et al. (1998; 2002) have found significant changes in the composition of bacteria in the rhizosphere of Bt cotton compared to that of its non-GM parent variety.

There is no ongoing research on the impact of genetically modified potatoes on soil biological processes in Romania. Limited research in Europe and North America suggests significant effects of GM crops on specific soil biota. STOTZKY (2000) in a recent review recommends a thorough evaluation of the persistence of GM products such as Bt toxins in soil and their effects on the inhabitants of soil and other habitats.

Due to the differences in soil and climatic conditions, and the biota composition, the evaluation of GM plant effects on soil biodiversity under Romanian conditions is necessary.

MATERIAL AND METHODS

Develop methodology for assessing the impact of Bt technology applied to potatoes on microbial diversity in soil was done according to the following objectives: study of the soil type influence, due to its physical-chemical parameters, on persistence and degradation of Bt insecticidal protein; study of transgenic crops on microbial diversity and study of transgenic plants cultivation on the main soil chemical properties.

The effects of potatoes genetically modified to express the Cry1Ab crystal toxin protein, on soil microbial communities were assessed in a glasshouse experiment. Soil for the experiment was taken from two field sites. Plants were grown in contrasting soils in terms of clay content, and soil samples taken at the flowering stage and maturity.

Two soil types: Fluvi-eutric Cambisols and Eutric Fluvisols were used. Soil samples were analyzed by ICPA methodology (Florea, N. et al, 1987) developed to assess main physical (particle size) and chemicals soil properties: organic carbon and humus - Walkley-Black method (modified by Gogoasa), total nitrogen content, mobile phosphorus and potassium content - Egner-Riehm-Domingo method, pH (H_2O), ratio soil/water 1/2,5 – electromechanical method using glass electrode. Also, microbiological analyses: quantitative determinations of heterotrophic bacteria (total bacteria number method) using traditional culturing methods and taxonomic determinations by usually identification methods, optical microscopy, determination keys and physiological tests (BERGEY`s 1986; FLORENZANO G., 1983), were carried out.

Data were analyzed using standard analysis of variance (ANOVA) and presented as means with an associated least significant difference (LSD, at the 5% level), using as factors: soil type, and plant type.

RESULTS AND DISCUSSION

Choosing of the two soil types for experimentation was made considering the texture, respectively, different clay content, and reaction. Thus, the first soil type, a Eutric Fluvisols

(FLeu) has a clayey-loamy texture, argyle with Φ <0.002 mm content between 34.0-39.3% and moderate acid reaction and the second soil type, an Fluvi-Eutric Cambisols (CMeu-fv) with low argyle content about 15.1-20.0%, has a sandy-loamy texture and a slightly alkaline reaction.

Soil reaction

Have been recorded relatively minor variations of soil reaction in the experimental variants with GM potatoes, compared with non-GM potatoes, direction and magnitude of these changes being caused by soil type on which plants were grown (Figure 1).

The biggest difference of soil reaction by 0.27 pH units, was recorded in Fluvi-Eutric Cambisols (CMeu-fv) at flowering stage, when soil cultivated with GM Red-Sec 14 potatoes was acidified as compared with soil from the non GM Red-Sec potatoes variant.

Variance analysis showed that there were no very significant variations of soil reaction caused by the crop type (GM or non-GM) in any stage of analysis, but there were significant variations of pH values between the two soils types used for experimentation.

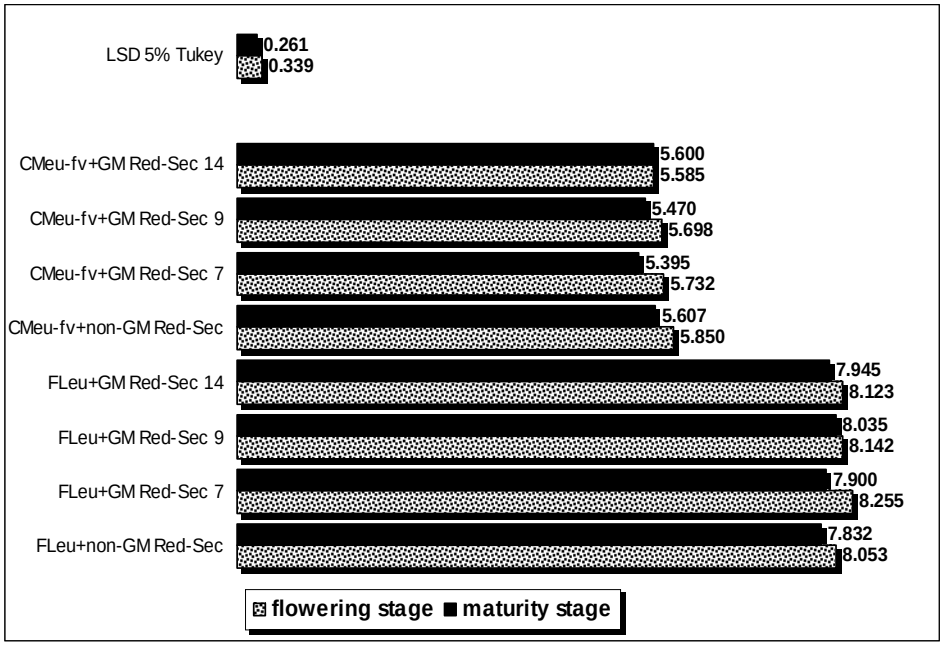

Figure 1. pH variation in two contrasting soils, planted with GM and non-GM Red-Sec variety potatoes

Humus content

Humus content varied with significant differences between the two types of soil. Fluvi-Eutric Cambisols (CMeu-fv) has more rich humus, compared with the other soil used for experimentation, Eutric Fluvisols (FLeu). Plant type (GM or non-GM hybrid) has not generated considerable variation of humus content in the two soil types. But in Fluvi-Eutric Cambisols, in first stage of soil analysis, humus content increase was very significant and was noted in GM Red-Sec 14 potatoes variant (Fig. 2).

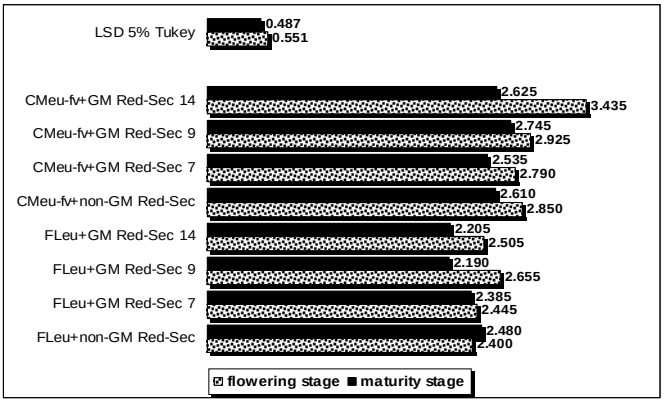

Figure 2. Humus (%) variation in two contrasting soils, planted with GM and non-GM Red-Sec variety potatoes

Total nitrogen content

Total nitrogen content data revealed any important variation in both stages of analysis and plant types: GM or non-GM (figure 3). The only observable differences were those relating to values obtained in the two soils. Total nitrogen values recorded in Fluvi-eutric Cambisols (CMeu-fv) were significant higher than those recorded in Eutric Fluvisols (FLeu) in both determination stages.

Mobile phosphorus and potassium content

Mobile phosphorus content showed important variations between the soil types, variants organized on Eutric Fluvisols (FLeu) recorded values much higher than those on Fluvi-eutric Cambisols (CMeu-fv) both at flowering and at maturity stages (figure 4).

Significant differences spring only from different degrees of initial supply of soil used for experimentation with these nutrients. Thus, Eutric Fluvisols is very well supplied with mobile phosphorus, while Fluvi-eutric Cambisols are significantly low supplied in mobile phosphorus. In terms of mobile potassium content, between the experimental soils, the best supplied is Fluvi-eutric Cambisols, with a high content, while the other soil has medium content (figure 5).

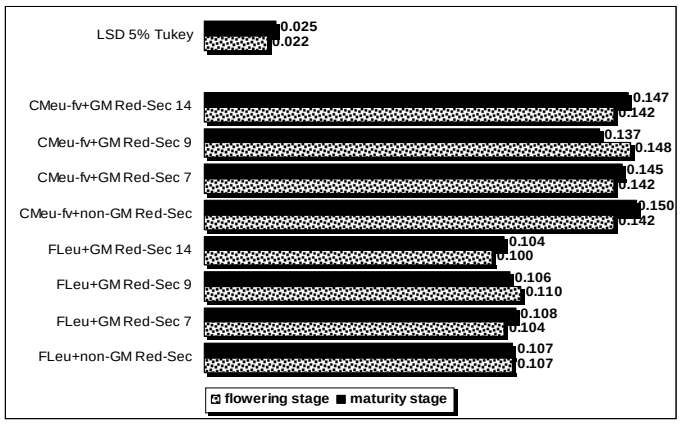

Figure 3. Total nitrogen (%) variation in two contrasting soils, planted with GM and non-GM Red-Sec variety potatoes

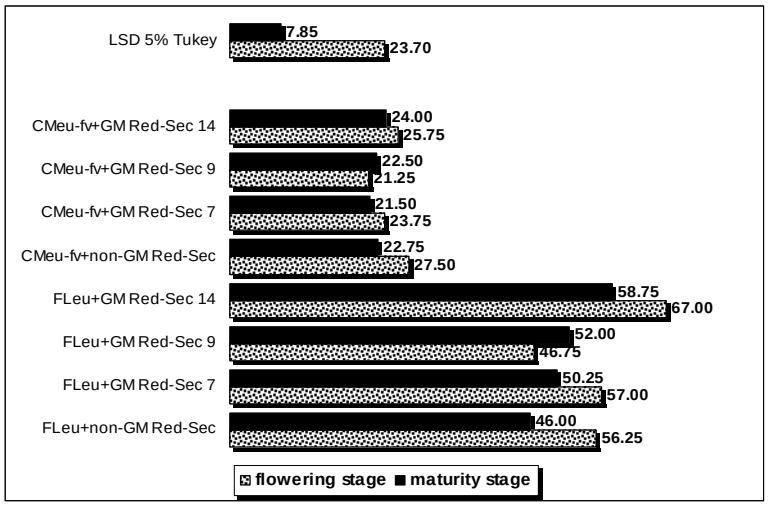

Figure 4. Mobile phosphorus contentent ($mgkg^{-1}$) variation in two contrasting soils, planted with GM and non-GM Red-Sec variety potatoes

Compared with GM varieties, non-GM Red-Sec variety had noticeably increased consumption of mobile phosphorus in both soil types. Mobile potassium highest consumption was recorded for both soils, in case of the non-GM Red-Sec variety, followed by GM Red-Sec 9 hybrid. Mobile potassium lowest consumption was reported to the GM Red-Sec 14 hybrid, also in both soils.

Although it is about preliminary experimental data, these issues might suggest different phosphorus and potassium nutrition needs of the four varieties of potato plants used in this experiment.

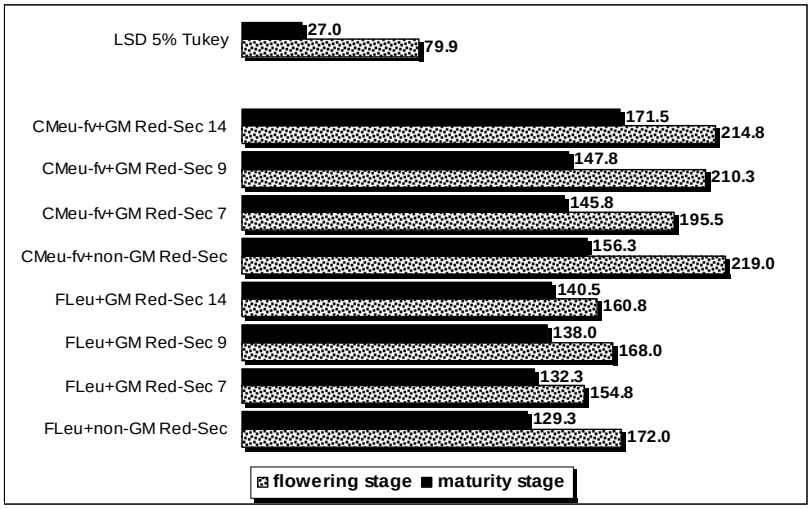

Figure 5. Mobile potasium contentent ($mgkg^{-1}$) variation in two contrasting soils, planted with GM and non-GM Red-Sec variety potatoes

Soil heterotrophic bacteria

Quantitative determinations of heterotrophic bacteria in the soil did not reveal significant differences between variants cultivated with GM potatoes as compared with those cultivated with non-GM hybrid nor in any of the soils, and even between stages of determination (Figure 6).

Differences between the total bacteria number values recorded in variant cultivated with GM potatoes compared with those cultivated with non-GM potatoes on Haplic Chernozems, can be interpreted only as a trend, not statistically assured.

Soil microbial communities are very plastic in their species composition and structure and change constantly in different root zones, agricultural practices, and with respect to various other environmental variables (Buckley, D.H and T.M. Schmidt, 2003; Lipson D.A. and SK Schmidt, 2004).

In terms of genus and species diversity of soil bacteria, no major differences were recorded between species composition of bacterial communities in soil cultivated with non-GM hybrid compared to soil cultivated with transgenic hybrid, the number of bacterial strains being quite close in samples analyzed (Table 1).

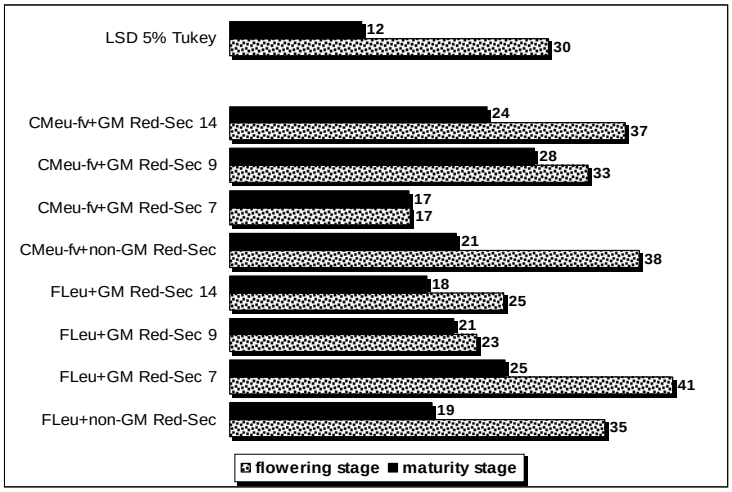

Figure 6. Total bacteria number (colony forming units$x10^6$/g dry soil) variation in two contrasting soils, planted with GM and non-GM Red-Sec variety potatoes

CONCLUSIONS

Research on the impact of Bt technology applied to transgenic potatoes Red-Sec 7, Red-Sec 9 and Red-Sec 14 on the main soil parameter quality were conducted in green house, using two contrasting soils in terms of clay content and reaction: Eutric Fluvisols and Fluvi-eutric Cambisols.

Soil chemical parameters: pH, humus and total nitrogen contents, the contents of mobile phosphorus and mobile potassium revealed significant differences between soil types only, not between the types of potatoes varieties, GM and non-GM, used in experiment.

Also, quantitative determinations of heterotrophic bacteria in the soil did not reveal significant differences between variants cultivated with GM potatoes as compared with those cultivated with non-GM variety nor in any of the soils, and even between stages of determination.

No major differences were recorded between species composition of bacterial communities in soil cultivated with non-GM variety compared to soil cultivated with transgenic hybrids, the number of bacterial strains being quite close in samples analyzed.

Table 1.

Diversity of bacterial communities in two contrasting soils, planted with GM and non-GM potatoes

Soil type/ Hybrid type	**Bacteria genus and species (in order of frequency)**		
	Before planting	**Flowering stage**	**Maturity stage**
FLeu+ non-GM Red-Sec potatoes	*Pseudomonas sp***** *Mycobacterium roseum*** *Bacillus sphaericus*** *Bacillus megaterium**** *Bacillus circulans*** *Arthrobacter globiformis** *Arthrobacter citreus***** *Actinomyces sp.****	*Pseudomonas sp**** *Mycobacterium roseum** *Bacillus megaterium***** *Bacillus circulans** *Arthrobacter globiformis** *Arthrobacter citreus***** *Actinomyces sp**	*Pseudomonas sp***** *Bacillus megaterium***** *Bacillus circulans** *Arthrobacter globiformis** *Arthrobacter citreus**
FLeu+ GM Red-Sec 7 hybrid potatoes	*Pseudomonas sp***** *Mycobacterium roseum** *Bacillus sphaericus** *Bacillus megaterium***** *Bacillus cereus** *Arthrobacter globiformis***** *Arthrobacter citreus*** *Actinomyces sp*****	*Pseudomonas sp*** *Bacillus sphaericus** *Bacillus megaterium***** *Bacillus cereus** *Arthrobacter globiformis***** *Arthrobacter citreus** *Actinomyces sp**	*Pseudomonas sp**** *Bacillus megaterium***** *Bacillus circulans** *Arthrobacter globiformis***** *Arthrobacter citreus***
FLeu+ GM Red-Sec 9 hybrid potatoes	*Pseudomonas sp***** *Mycobacterium roseum** *Bacillus megaterium***** *Bacillus circulans*** *Arthrobacter citreus*** *Actinomyces sp****	*Pseudomonas sp**** *Mycobacterium roseum** *Bacillus megaterium***** *Bacillus circulans***	*Pseudomonas sp***** *Mycobacterium roseum** *Bacillus megaterium****,* *Bacillus circulans ***
FLeu+ GM Red-Sec 14 hybrid potatoes	*Pseudomonas sp***** *Mycobacterium roseum** *Bacillus sphaericus** *Bacillus megaterium***** *Bacillus cereus** *Arthrobacter globiformis**** *Arthrobacter citreus*** *Actinomyces sp*****	*Pseudomonas sp**** *Bacillus megaterium***** *Bacillus cereus** *Arthrobacter globiformis*** *Arthrobacter citreus** *Actinomyces sp**	*Pseudomonas sp**** *Bacillus megaterium***** *Bacillus circulans*** *Arthrobacter globiformis*** *Arthrobacter citreus **
CMeu-fv+ non-GM Red-Sec potatoes	*Pseudomonas sp***** *Bacillus megaterium***** *Bacillus circulans*** *Arthrobacter globiformis** *Arthrobacter citreus***** *Actinomyces sp.***	*Pseudomonas sp**** *Bacillus megaterium***** *Bacillus circulans*** *Arthrobacter globiformis** *Arthrobacter citreus***	*Pseudomonas sp**** *Bacillus megaterium***** *Bacillus circulans**** *Arthrobacter globiformis*** *Arthrobacter citreus**
CMeu-fv+ GM Red-Sec 7 hybrid potatoes	*Pseudomonas sp.***** *Bacillus sphaericus*** *Bacillus megaterium**** *Bacillus cereus** *Arthrobacter globiformis*** *Arthrobacter citreus*** *Actinomyces sp.****	*Pseudomonas sp***** *Bacillus sphaericus** *Bacillus megaterium**** *Bacillus circulans** *Bacillus cereus** *Arthrobacter globiformis***	*Pseudomonas sp***** *Bacillus sphaericus** *Bacillus megaterium***** *Arthrobacter globiformis**** *Actinomyces sp **
CMeu-fv+ GM Red-Sec 9 hybrid potatoes	*Pseudomonas sp.***** *Mycobacterium roseum** *Bacillus sphaericus*** *Bacillus megaterium***** *Arthrobacter globiformis***** *Arthrobacter citreus**** *Actinomyces sp.****	*Pseudomonas sp***** *Mycobacterium roseum** *Bacillus sphaericus** *Bacillus megaterium***** *Arthrobacter globiformis**** *Arthrobacter citreus**	*Pseudomonas sp***** *Bacillus sphaericus** *Bacillus megaterium***** *Arthrobacter globiformis**** *Arthrobacter citreus**
CMeu-fv+ GM Red-Sec 14 hybrid potatoes	*Pseudomonas sp.***** *Bacillus megaterium***** *Bacillus circulans*** *Bacillus cereus** *Arthrobacter globiformis*** *Arthrobacter citreus*** *Actinomyces sp.****	*Pseudomonas sp***** *Bacillus megaterium***** *Bacillus circulans*** *Bacillus cereus** *Arthrobacter globiformis*** *Arthrobacter citreus** *Actinomyces sp**	*Pseudomonas sp***** *Bacillus megaterium***** *Bacillus circulans *** *Arthrobacter globiformis***

Research will be continued and data from chemical and biological analysis of soil will be correlated with determinations regarding the amount of insecticidal toxin Cry1Ab released into soil (through root exudates or plant debris along with the remaining plants in the soil after harvesting) and its persistence in the soils chosen to investigate the impact of Bt technology on soil as a major component of the environment.

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