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# Effect of Active Ingredients on *Rhizobium* and *Bradyrhizobium* Legume Dinitrogen Fixation

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## Abstract

In field experiment the effect of two active ingredients (imazethapyr and linuron) on symbiotic nitrogen fixation activity and microorganisms under legume crops (pea, horse bean, yellow lupine, white lupine, soybean) was determined.

The studies indicated that both imazethapyr and linuron can cause decrease of root-nodule bacteria nitrogenase activity. They also can stimulate development of bacteria and inhibit growth of fungi.

**Keywords:** root-nodule bacteria, bacteria, fungi, nitrogenase, herbicides, dinitrogen fixation activity.

## Introduction

Use of herbicides for weed control in legume fields has contributed to increased yield and improved quality **[11].** Frequently, herbicides not only affect plant growth but have a detrimental effect on soil microorganisms, growth and metabolism [4, 18, 22]. Some studies have evaluated the effect of different herbicides on *Rhizobium* growth and nitrogen fixation activity. The effect depends on the herbicide, its concetration, and different weather conditions. Applied research methodology also may depend on the *Rhizobium* or *Bradyrhizobium* species and even the strain used [5, 8, 10, 14, 16, 22, 25].

The herbicides have possible targets in both the legumes and symbiotic bacteria, too. There is little information available on imazethapyr and linuron effects on symbiosis.

The objective of this study was to observe the effect of imazethapyr and linuron on soil microflora under legume crops and on dinitrogen fixing activity of nodulating bacteria.

## Material and Methods

Field trials were conducted with pea *(Pisum sativum* L.), horse bean *{Viciafaba* L.), white lupine *(Lupinus albus* L.), yellow lupine *{Lupinus luteus* L.) and soybean *{Glycine max* L.) grown at the Brody Agricultural Experimental Station of Agricultural University of Poznan during 1996 growing season on 25 m x 2.25 m plots.

Seeds were inoculated with effective strains of: *Rhizobium leguminosarum* 312 - pea, *Rhizobium leguminosarum* AS - horse bean, *Bradyrhizobium* sp. NF - white and yellow lupines and *Bradyrhizobium japonicum* 94p - soybean, from the collection of the Department of Microbiology of the Institute of Plant Cultivation, Fertilization and Soil Science in Pulawy, Poland, directly before sowing.

One month after sowing, the crops were fertilized with ammonium nitrate at a rate of 30 kg N • $ha^{-1}$.

Two herbicides were utilized to assess the effect on soil microflora. Pivot 100 - active ingredient imazethapyr was applied preemergence at 90 g • $ha^{-1}$ and postemergence at the same rates (9-10 days after emergence). Afalon - linuron a. i. was applied preemergence at 850 g • $ha^{-1}$. Plots without herbicides were used as the control.

All field experiments were arranged in a randomized complete block design with four replications.

For microbiological analyses soil was collected from under legume crops at five dates: postemergence, in the phase of full development of the plants, in the blooming phase, at the beginning of seed formation, and after harvest. Using the pour plate method [6], the total number of bacteria and actinomycetes [13], the number of proteolytic bacteria [3] and fungi [15], as well as the number of free - living dinitrogen fixing bacteria of *Azotobacter* species [20] were determined in the various soil samples.

At the begining and at the full of plant blooming phases, the activity of $N_2$ - fixation was determined directly on the field using the acetylene reduction assy (ARA) [21].

All microbiological analyses were made in five replications and illustrated as the arithmetic mean.

## Results and Discussion

The total number of microorganisms, especially bacteria, in the soils without herbicides (the control soils) analyzed at different stages of the vegetative season was related to the developmental phase of the legume plants and was greatest at the blooming phase which resulted from the highest photosynthetic activity of these plants at that phase.

But, the mean number of microorganisms during all the vegetation season (in these soils) depended on the crop species (Figures 1-5).

The lowest number of bacteria was noted under pea and the greatest under yellow lupine (Fig. 1). It ranged from $11.64 \cdot 10^6$ to $19.35 \cdot 10^6$ cells per one gram dry weight of soil, respectively. Also, in the control soil under yellow lupine was found the highest number of proteolytic bacteria and actinomycetes (Figures 2 and 3) as compared to the control soils under other legume plants.

But, in the soil under soybean the highest number of fungi ($81.55 \cdot 10^3$ per 1 g dried of soil) and lowest under pea ($50.36 \cdot 10^3$) (Fig.4) was noted.

In the soil where herbicides were applied, a general increase of bacteria numbers were observed in most experimental combinations. The only exception was yellow lupine, under which a fall in the mean number of bacteria was found in the presence of herbicides as it compared to the control soil (Fig.l). This was reflected in the mean

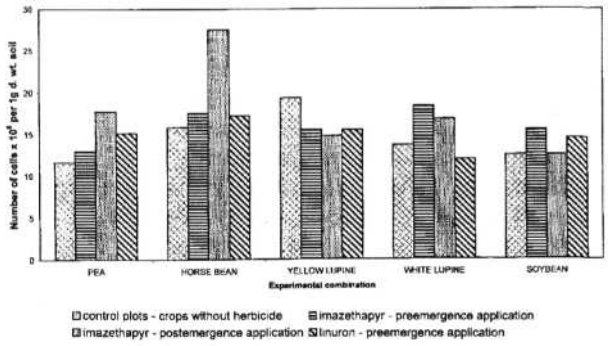


Fig. 1. Mean total of bacteria in soil under legumes during vegetation season.

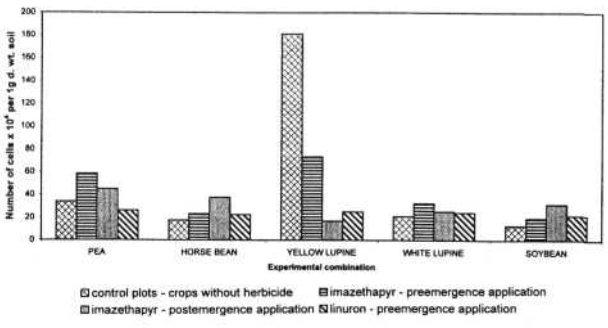


Fig. 2. Mean number of proteolytic bacteria in soil under legumes during vegetation season.

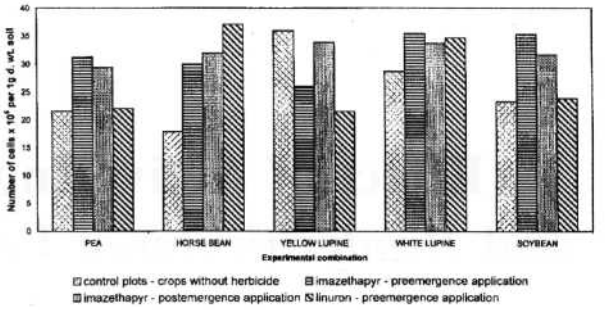


Fig. 3. Mean number of actinomycetes in soil under legumes during vegetation season.

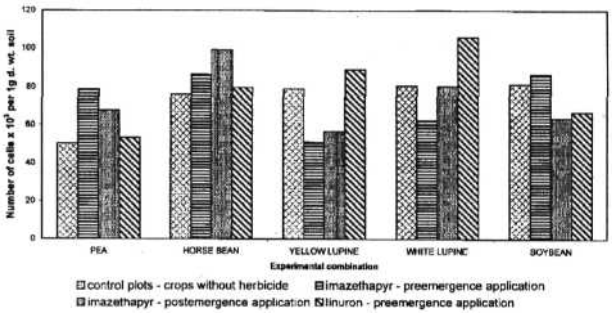


Fig. 4. Mean number of fungi in soil under legumes during vegetation season.

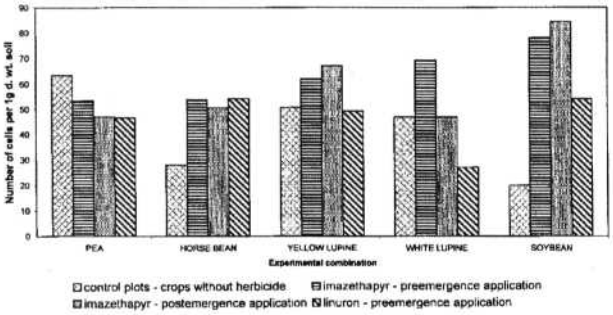


Fig. 5. Mean number of *Azotobacter* in soil under legumes during vegetation season.

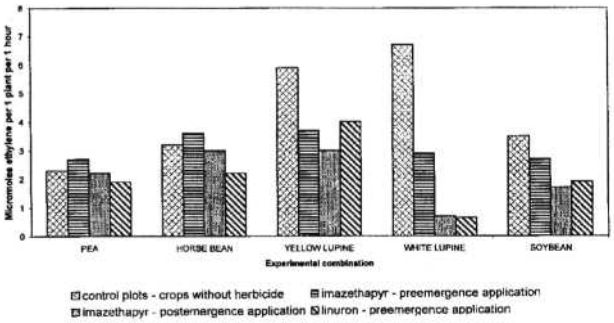


Fig. 6. Nitrogenase activity in the root-nodule bacteria at plant flowering stage.

number of microorganisms from almost all analyzed soils where herbicides were applied (Figures 1-5).

As noted in our experiment, and many other studies [1, 12], soil microorganisms generally react to herbicides by increasing their biomass and activity, although inhibitory effects have also been noted [22, 24].

Table 1. Dinitrogen fixation rates in legume crops (kg N • season$^{-1}$)

| Crop combinations | At the beginning of flowering | At full flowering |
|---|---|---|
| **PEA** | | |
| Control plot (crop without herbicide) | 123.00 | 104.40 |
| Crop + imazethapyr (preemergence application) | 65.80 | 124.80 |
| Crop + imazethapyr (postemergence application) | 58.50 | 114.70 |
| Crop + linuron (preemergence application) | 76.10 | 93.10 |
| **HORSE BEAN** | | |
| Control plot (crop without herbicide) | 78.00 | 43.40 |
| Crop + imazethapyr (preemergence application) | 88.00 | 80.30 |
| Crop + imazethapyr (postemergence application) | 82.60 | 61.60 |
| Crop + linuron (preemergence application) | 35.50 | 40.10 |
| **YELLOW LUPINE** | | |
| Control plot (crop without herbicide) | 129.20 | 216.30 |
| Crop + imazethapyr (preemergence application) | 135.00 | 147.70 |
| Crop + imazethapyr (postemergence application) | 41.30 | 74.70 |
| Crop + linuron (preemergence application) | 81.00 | 126.80 |
| **WHITE LUPINE** | | |
| Control plot (crop without herbicide) | 85.10 | 130.90 |
| Crop + imazethapyr (preemergence application) | 64.00 | 27.70 |
| Crop + imazethapyr (postemergence application) | 10.50 | 15.30 |
| Crop + linuron (preemergence application) | 41.70 | 12.70 |
| **SOYBEAN** | | |
| Control plot (crop without herbicide) | 17.10* | 12.20 |
| Crop + imazethapyr (preemergence application) | 11.90* | 99.90 |
| Crop + imazethapyr (postemergence application) | 10.50* | 70.00 |
| Crop + linuron (preemergence application) | 8.80* | 73.20 |

Explanation:* - measurement was carried out at the beginning of root nodulation (before flowering).

Also concerning the activity of dinitrogen fixation in the legume crops, the effect of herbicides can have great importance. In our experiment distinct adverse effect of herbicides concerned dinitrogen fixation by the root —noduling bacteria (Fig. 6, Tab. 1). Imazethapyr applied postemergence and linuron lowered nitrogenase activity in all experimental combinations. Particuraly strong fall, by about 90% as compared to the control was noted for *Bradyrhizobium* - white lupine dinitrogen fixation.

Imazethapyr applied preemergence slightly stimulated pea and horse bean ARA rates and decreased them in lupines and soybeans. These results do not fully conform to earlier studies [22], where a clear decrease in nitrogenase activity due to herbicides under pea was noted - especially with imazethapyr preemergence applied. These differentiations may result from different conditions and imperfection of the applied research methodology. The effect of imazethapyr on pea - *Rhizobium* symbiosis was studied by Gonzales et al. [9]. They suggested that imazethapyr inhibits the growth of the symbiotic plant rather than having a direct effect on the bacteria.

Nitrogen fixation by root-noduling bacteria *Rhizobium* and *Bradyrhizobium* results in a significant input to many types of farming systems. For instance, even 80% of total N in legume plants can be supplied by root-nodule bacteria [21]. Therefore, the effect of herbicides on legume nitrogen ($N_2$) fixation is of great importance. Many studies have indicated differentiated effect of herbicides on nodulation and dinitrogen fixation. In most cases it was found that applied herbicide doses are safe both for nodulation and maintaining full dinitrogen fixation activity although Bollich et al. [2] reported that metribuzin reduced soybean nodulation and $N_2$ fixation rate (by ARA) by 50%. Rennie and Dubetz [19] found that linuron did not decrease the $N_2$ fixation rate. It was found, too, that postemergence herbicides also affect nodulation and $N_2$ fixation [17, 23, 26]. In our experiment white lupine reacted strongly to linuron lowering $N_2$ fixation rate. De Felipe et al. [7] showed that white lupine had little tolerance to simazine and strongly reduced the $N_2$ fixation rate.

Our study indicated that not only the kind and concentration of a herbicide decide its effects on microorganisms and their activity, but also the crop itself.

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