NODULATION OF YELLOW LUPIN (Lupinus luteus L.) DEPENDING ON THE FORECROP, SEED INOCULATION WITH Bradyrhizobium lupini AND GENISTEIN

NODULACJA ŁUBINU ŻÓŁTEGO (Lupinus luteus L.) W ZALEŻNOŚCI OD PRZEDPLONU, SZCZEPIENIA NASION Bradyrhizobium lupini I GENISTEINY

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ABSTRACT

A strict two-factor field 'Mister' yellow lupin experiment was made in a split-plot design in poor rye complex soil (soil valuation class IVb) at the Experiment Station of the Faculty of Agriculture and Biotechnology, the University of Technology and Life Sciences at Mochełek over 2008-2011. The experiment design covered 2 factors: growing yellow lupin after intercrops of 'Bardena' white mustard and 'Pastar' winter rye as well as seed inoculation with *Bradyrhizobium lupini* with or without genistein added. Growing intercrops in the poor rye complex soil considerably decreased neither the Nmin content in early spring nor the size of nodulation in yellow lupin. After lupin harvest, slightly more Nmin was noted for the treatments where intercrops were applied, especially rye in the 0-30 cm layer. The weather pattern, especially moisture conditions, pointed to a special sensitivity of nodulation to water deficit or excess in the rhizosphere. The method of preparing seeds for sowing did not play a greater role in developing the Nmin content in both soil profile layers analysed. White mustard showed a negative effect on the dry weight of nodules and the total N content in the plants and the seeds of yellow lupin as well as created the least favourable conditions for the survival and the capacity for symbiosis by symbiotic bacteria remaining in soil after lupin harvest. The state of yellow lupin plant nitrogen nutrition did not depend on the experimental factors.

Keywords yellow lupin, intercrops, seed inoculation, genistein

STRESZCZENIE

Ścisłe dwuczynnikowe doświadczenie polowe nad łubinem żółtym 'Mister' wykonano w układzie losowanych podbloków na glebie kompleksu żytniego słabego (klasa bonitacyjna IVb) w Stacji Badawczej Wydziału Rolnictwa i Biotechnologii UTP w Mochełku w latach 2008-2011. Schemat doświadczenia obejmował 2 czynniki: uprawę łubinu żółtego po międzyplonach z gorczycy białej Bardena i żyta ozimego Pastar oraz szczepienie nasion *Bradyrhizobium lupini* z lub bez dodatku genisteiny. Uprawa międzyplonów na glebie kompleksu żytniego słabego nie przyczyniała się do znaczącego obniżenia zawartości Nmin wczesną wiosną ani rozmiarów brodawkowania łubinu żółtego. Po zbiorze łubinu nieco więcej Nmin stwierdzono na obiektach, gdzie zastosowano międzyplony, szczególnie żyto w warstwie 0-30 cm. Przebieg warunków pogodowych, a zwłaszcza wilgotnościowych wskazał na szczególną wrażliwość nodulacji na niedobór lub nadmiar wody w ryzosferze. Sposób przygotowania nasion do siewu nie odgrywał większej roli w kształtowaniu zawartości Nmin w obu analizowanych warstwach profilu glebowego. Gorczyca biała wpływała negatywnie na suchą masę brodawek oraz na zawartość N ogółem w roślinach i nasionach łubinu żółtego, a także stwarzała najmniej korzystne warunki do przeżycia i zdolności do nawiązywania symbiozy przez bakterie symbiotyczne pozostałe w glebie po zbiorze łubinu. Stan odżywienia roślin łubinu żółtego azotem nie był kształtowany przez czynniki doświadczenia.

Słowa kluczowe łubin żółty, międzyplony, szczepienie nasiona, genisteina

DETAILED ABSTRACT

Ścisłe dwuczynnikowe doświadczenie polowe nad łubinem żółtym ‘Mister’ wykonano w układzie losowanych podbloków na glebie kompleksu żytniego słabego (klasa bonitacyjna IVb) w Stacji Badawczej Wydziału Rolnictwa i Biotechnologii UTP w Mochełku w latach 2008-2011. Schemat doświadczenia obejmował 2 czynniki: A – uprawa łubinu żółtego po międzyplonach: kontrola – bez międzyplonu, międzyplony z: gorczycy białej Bardena zebranej z pola przed nastaniem mrozów, z gorczycy białej Bardena pozostawionej na zimę w formie mulczu, z żyta ozimego Pastar zaoranego wiosną; czynnik B – szczepienie nasion przed siewem: kontrola – nasiona zaprawiane Sarfunem 50 WP, nie szczepione bakteriami symbiotycznymi; nasiona zaprawiane Sarfunem 50 WP i szczepione agarową szczepionką zawierającą *Bradyrhizobium lupini* oraz nasiona zaprawiane Sarfunem 50 WP i szczepione agarową szczepionką zawierającą *Bradyrhizobium lupini* z dodatkiem genisteiny. Nawożenia azotowego nie stosowano; pozostałe zabiegi agrotechniczne – standardowe dla łubinu. Próby glebowe do oznaczenia zawartości Nmin pobierano 3-krotnie za pomocą próbnika gruntu typu KRET: przed siewem międzyplonów (sierpień w latach 2008-2010) – z każdego pola z głębokości 0-30 i 30-60 cm, przed siewem łubinu żółtego – z podbloków A po każdym międzyplonie oraz po zbiorze łubinu żółtego (na każdym obiekcie czynnika A i B). Najbardziej korzystne i wyrównane warunki wilgotnościowe i termiczne dla wzrostu i rozwoju roślin stwierdzono w 2011 roku. Susza kwietniowa w latach 2009 i 2011 opóźniła wschody polowe i rozmiary brodawkowania w fazie kwitnienia, jednak dalsza wegetacja roślin przebiegała w sprzyjających warunkach termicznych i wilgotnościowych, a bardzo wysokie opady w lipcu opóźniły ich dojrzewanie. W Stacji Badawczej WRiB w Mochełku od ponad 60-lat prowadzi się z różnym natężeniem doświadczenia nad łubinami, a dawki N stosowane na polach wyrównawczych pod zboża nie przekraczają 100-120 $kg \cdot ha^{-1}$. Stąd zapewne przeciętna zawartość Nmin w obu analizowanych warstwach profilu glebowego i brak znaczącego efektu zastosowania międzyplonów do obniżenia jego zawartości na wiosnę, przynajmniej w strefie nodulacji. W warunkach polowych zawartość Nmin wczesną wiosną w warstwie 0-30 cm (tam gdzie następuje nodulacja) nie była na tyle wysoka, żeby hamować lub ograniczać brodawkowanie i istotnie kształtować liczbę brodawek na korzeniach łubinu. Gorczyca biała pozostawiona na polu przez okres zimy w formie mulczu wpływała negatywnie na suchą masę brodawek łubinu żółtego oraz na zawartość N w suchej masie roślin i nasion, być może z powodu swoistych wydzielin korzeniowych do ryzosfery, w tym glukozynolanów oraz związków fenolowych charakterystycznych dla wielu gatunków z rodziny *Brassicaceae*. Sposób przygotowania nasion do siewu nie ogrywał większej roli w kształtowaniu zawartości Nmin w obu analizowanych warstwach profilu glebowego. Słaba reakcja roślin bobowatych na mineralne nawożenia azotowe oznacza, że są one w stanie korzystać z obu form azotu – mineralnego i symbiotycznego w zależności od ich dostępności w glebie. Przebieg warunków pogodowych, a zwłaszcza wilgotnościowych wskazał na szczególną wrażliwość brodawek na niedobór (2010) lub nadmiar (2011) wody w ryzosferze – w obu tych przypadkach notowano drastyczne zmniejszanie się liczby i suchej masy brodawek lub nawet ich zanikanie już w 28-35 dni po wschodach, najpewniej z powodu silnej reakcji nitrogenezy na takie warunki wynikającej z niedostatku C lub ograniczonej dostępności tlenu w brodawkach. Uprawa międzyplonów w celu zmniejszenia zawartości Nmin nie przynosiła spodziewanego obniżenia zawartości Nmin w glebie, którego zawartość okazuje się, że nie jest zbyt wysoka do efektywnej symbiozy. Mimo niewielkiego też udziału roślin bobowatych w strukturze zasiewów w naszym kraju i obaw, że w glebach Polski może nie być zdolnych do asymilacji N_2 bakterii brodawkowych, zastosowane metody zwiększenia nodulacji łubinu żółtego nie przyniosły spodziewanych rezultatów.

INTRODUCTION

The experiments made by the Institute of Soil Science and Plant Cultivation all across the country show that the mean Nmin content in early spring in the soil profile in the 0-30 cm layer, being the source of nitrogen in the initial stages of growth and development, is 33-49 kg Nmin·ha^{-1} (Fotyma and Pietruch 2000). Having applied the so-called start rate under legumes of 20-40 kg N·ha^{-1}, its content in the nodulation zone gets much bigger. The nitrogen nutrition in legumes includes mineral nitrogen only which is mostly used to build vegetative plant parts. With an excessively high Nmin content we observe difficulties in the root infection by rhizobia and no nodulation occurs at all, or the nodules get formed on the roots with much delay, only after a decrease in its content in soil (Buttery and Gibson 1990) and show a lower effectiveness in fixing N_2 (Kipe-Nolte et al., 1993). On the other hand, the results reported by Marek-Kozaczuk et al. (2006) demonstrate that the sizes of nodulation depend on neither the N level in soil nor the population of autochthonic rhizobia specific for a given species. However, a Nmin content in soil can enhance nodulation and effectiveness of fixing nitrogen from the air by stimulating the nodules formation and increasing the activity of nitrogenase and plant growth (Tsai et al., 1993).
The intercrops harvested in autumn accumulating in plant aboveground parts in the yield from 68.3 to 80.5% of the nitrogen uptaken remove 20 do 126 kg N z ha from the soil profile (Duer 1996, Dzienia and Boligłowa 1992, Jaskulski and Jaskulska 2004, Wilczewski 2009). According to some authors, growing intercrops decreases the Nmin content in autumn and increases in spring (Płaza et al., 2009).

The nodulation of legumes can be limited by an insufficient amount of flavonoids released by the root system (Rengel 2002). With the above in mind, one of the ways to increase infection and the number and the weight of root nodules is the application of e.g. genistein, especially at the soil temperature suboptimal for bacteria (Kosslak et al., 1987).

The working hypothesis assumes that growing intercrops will decrease the Nmin content in soil in early spring, which, in combination with seed inoculation with rhizobia and genistein, will increase the nodulation and the state of yellow lupin plant nutrition with nitrogen.

MATERIAL AND METHODS

A strict two-factor field 'Mister' yellow lupin experiment was made in a split-plot design in poor rye complex soil (soil valuation class IVb) at the Mochełek Experiment Station of the Faculty of Agriculture and Biotechnology, the University of Technology and Life Sciences over 2008-2011. The experiment design involved 2 factors:
A – growing yellow lupin after intercrops:
- a1 – the control; with no intercrop (the forecrop: triticale or winter rye),
- a2 – made up from 'Bardena' white mustard harvested from the field before frosts,
- a3 – made up from 'Bardena' white mustard left for winter in a form of mulch,
- a4 – made up from 'Pastar' winter rye ploughed-in in spring.

B – seed inoculation before sowing:
- b1 – the control; the seeds dressed with Sarfun 50 WP, non-inoculated with symbiotic bacteria,
- b2 – seeds dressed with Sarfun 50 WP and inoculated with agar inoculum containing *Bradyrhizobium lupini*,
- b3 – seeds dressed with Sarfun 50 WP and inoculated with agar inoculum containing *Bradyrhizobium lupini* with genistein added.

The seeds before sowing were dressed with Sarfun 50 WP (a.s. carbendazim) at the dose 200 g·100 kg seed^{-1}. In the combination b2 – agar bacterial suspension of Bradyrhizobium lupini containing 10^8 – 10^{10} bacteria cells in 1 ml prepared by the Department of Agricultural Microbiology of IUNG Puławy was dissolved in distilled water, and then shaken for 24 hours on a laboratory shaker at room temperature. In the combination b3 – to the vaccine thus prepared was added 60µM genistein (SIGMA Aldrich) dissolved beforehand in 1 ml of methanol and again shaken for 24 hours. Seeds sprayed with Bradyrhizobium lupini or Bradyrhizobium lupini and genistein has dried after they were sown on the same day.

The laboratory germinability in yellow lupin seeds in successive years of research was: 84, 86 and 81%, and the weight of 1000 seeds: 132, 129 and 109 g. Prior to sowing lupin in early spring over 2009 – 2011, depending on the soil richness, there was applied mineral fertilisation at the rate of 35-65 kg·ha^{-1} P_2O_5 and 57- 90 kg·ha^{-1} K_2O and ploughing was made (in the control field, the cultivator was used only) 20 cm deep and prior to sowing the field was carefully prepared with the use of cultivator. No nitrogen fertilisation was applied.

In the successive research years yellow lupin was sown on April 7, 8 and 5 with Oyord-type plot seeder 0.03-0.04 cm deep at the 0.20 m row spacing. To determine the seeding norms, the density of 100 plants per 1 m^2 was assumed. The sowing plot area was 16.8 m^2 and the harvest plot area – 13.2 m^2. Right after sowing Afalon Dyspersyjny (a.s. 3 - (3,4-dichlorophenyl)-1-methoxy-methylurea) was applied at the rate of 1.4 dm^3·ha^{-1}, and after emergence – double harrowing was made across the rows. To protect the plantations from monocotyledonous weeds, Fusilade Forte 150 C (a.s. fluazifop present as a butyl ester) was applied at the rate of 1.5 dm^3·ha^{-1}. As a preventive measure against anthracnose, over 2009 and 2011 Sarfun 500 SC was provided. Harvesting was made with the Wintersteiger plot combine harvester in successive research years after 144, 119 and 146 days after sowing.

Dynamics of nodulation as well as the number of nodules were determined seven times on 10 plants collected from each plot as follows: c2 – 14, c3 – 21, c4 – 28, c5 – 35 days after plant emergence and then at full flowering (c6) and physiological seed maturity (c7). Dry weight of nodules was determined after drying at 60^{o} C for 24 hours.

The occurrence and the count of symbiotic bacteria in soil after lupin harvest were defined by planting out the seedlings of serradella (the test plant) in two reps into plastic bags filled with sterile river sand moistened with the Jensen medium. Three days after planting, the seedlings were inoculated with an aqueous suspension of the substrata sampled from the field under intercrops (factor A) and the methods of seed preparation for sowing (factor B), applying the technique of multiple dilutions (dilutions from 10^{-1} to 10^{-6}). After four weeks the test plants roots were evaluated in terms of the number of nodules and the count of symbiotic bacteria was estimated based on the total number of plants derived from all the dilutions on the roots of which nodules appeared (Martyniuk et al. 2000).

To determine the Nmin content at the Chemical and Agricultural Station in Bydgoszcz (Polska Norma PN-R-04028), soil was sampled 3 times with the use of KRET-type soil sampler: prior to intercrops sowing (August over 2008-2010); from each field from the depth of 0-30 and 30-60 cm, prior to yellow lupin sowing; from split-plots A after each intercrop as well as after yellow lupin harvest (after each treatment for factor A and B). Determination of Nmin is based on the spectrophotometric measurement of the concentration of ammonium and nitrate ions in the extract of the soil 1% (m/m) sodium sulfate (VI) solution. The mean results from 4 reps are given in kg $N{\cdot}ha^{-1}$ ($Nmin = N\text{-}NO_3 + N\text{-}NH_4$).

The Minolta SPAD 502 meter was used to determine greenness of leaves, e.g. relative amount of chlorophyll present at full plant flowering by measuring the transmittance of the leaf in two wave bands (600-700 and 400-500 nm); it is an indirect indicator of plant N status – the chlorophyll content increases in proportion to the amount of nitrogen present in leaves. Each result is a mean of 30 leaf-records from each plot. The mean value of $N_{iż}$ (*nitrogen harvest index*) is expressed in % as the ratio of the amount of N in lupin seeds to the total N content in the plants at the stage of maturity. The efficiency of the utilization of soil nitrogen and N from symbiosis W_{gn} was calculated from the quotient of the seed yield and the total nitrogen content in the plant. The total N content in the under and aboveground parts of lupine plans as well as in dry matter of leaves were determined with Kjeldahl method.

The results obtained in the experiment were verified with the analysis of variance (STATISTICA®) for the split-plot design and the significance of differences was verified with the Tukey test at the level of $\alpha = 0.05$.

The precipitation and temperature conditions most favourable and evenness for the growth and development of plants were recorded in 2011. The April drought over

2009 and 2011 delayed the field emergence and the size of nodulation at the flowering stage, however, further plant vegetation coincided with favourable temperature and precipitation conditions and very high precipitation in July delayed ripening (Table 1).

Table 1. Mean air temperature and rainfall according to the Mochełek Experiment Station
Tabela 1. Średnia temperatura powietrza i sumy opadów według notowań Stacji Badawczej w Mochełku

Specification Wyszczególnienie	Year Rok	Month – Miesiąc					
		IV	V	VI	VII	VIII	IX
Mean air temperature, °C Średnia temperatura powietrza	2009	9.8	13.2	14.5	18.6	18.2	13.7
	2010	7.8	11.5	16.7	21.6	18.4	12.2
	2011	10.5	13.5	17.7	17.5	17.7	14.3
Mean air temperature over 1949-2010 Średnia temperatura powietrza,°C w latach 1949-2011		7.4	12.7	16.2	18.0	17.5	13.2
Precipitations, mm – Opady	2009	0.40	85.3	57.4	118.0	17.6	34.4
	2010	33.8	92.6	18.1	107.4	150.7	74.7
	2011	13.5	38.4	100.8	132.5	67.7	37.0
Precipitations over 1949-2009, mm Opady w latach 1949-2010		27.7	43.2	52.9	72.2	53.0	41.4

In 2010 extremely dry conditions were reported in June and the first two decades of July. Their unfavourable effect on a fast completion of vegetation and the disappearance of nodule bacteria were intensified by high air temperature at that time. The precipitation exceeding 100 mm in the third decade of July and 81 mm in the first decade of August that year did not, in fact, affect lupin yielding. The differentiation in water conditions over the vegetation period was mostly recorded in June; from 18.1 mm in 2010 to 100.8 mm in 2011. Mean air temperature from April to August over 2010-2011 was similar (15.2-15.4^{o}C) and higher than in 2009 (14.7^{o}C). The highest mean temperature was reported in July (21.6^{o}C, in the second decade 24.7^{o}C) and August (18.4^{o}C) 2010.

RESULTS

In successive research years, prior to intercrops sowing (2008-2010) and then prior to sowing and after the harvest of yellow lupin (2009 – 2011) soil was sampled from two layers of the soil profile: 0-30 cm and 30-60 cm to assay the Nmin content. Prior to sowing intercrops, the mean Nmin content in the 0-30 cm layer was 33.4 kg·ha^{-1} and in the 30-60 cm layer – 23.9 kg Nmin·ha^{-1} (Fig. 1), while in spring, prior to yellow lupin sowing, the lowest Nmin content in the 0-30 and 30-60 cm layer was found in the control (24.8 + 14.0 kg Nmin·ha^{-1}). Growing intercrops in poor rye complex soil slightly increased, as compared with the control, the Nmin content in the soil profile 0-30 cm deep and decreased (only after mustard harvested before frosts) deeper in the profile layer. Whereas after lupin harvest, slightly more Nmin was found for the treatments with intercrops, especially rye in the 0-30 cm layer (Fig. 2). The method of seed preparation for sowing played a greater role in developing the Nmin content in none of the two soil profile layers analyzed after lupin harvest (Fig. 3).

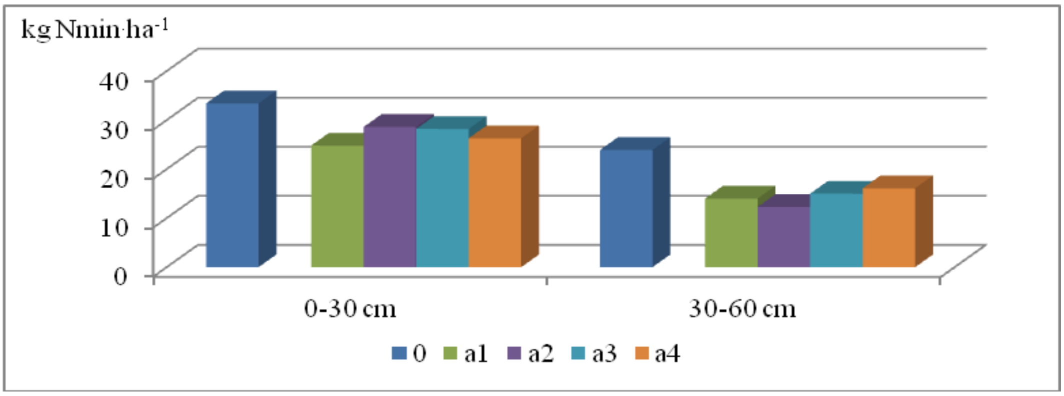

Fig. 1. Mean content of Nmin in soil before sowing of intercrop (0) and yellow lupin depending on intercrops (a1-a4)
Rys. 1. Średnia zawartość Nmin w glebie przed siewem międzyplonów (0) oraz łubinu żółtego w zależności od zastosowanego międzyplonu (a1-a4)

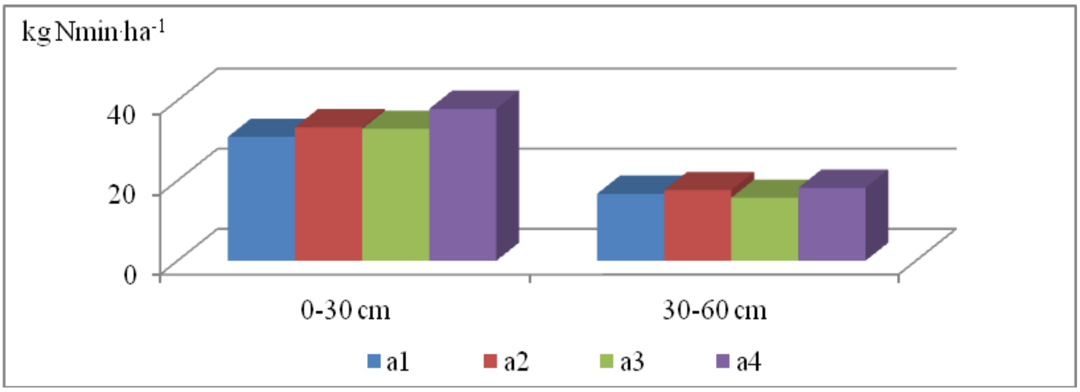

Fig. 2. Mean content of Nmin in soil after yellow lupin harvest depending on the intercrops used (a1-a4)
Rys. 2. Średnia zawartość Nmin w glebie po zbiorze łubinu żółtego w zależności od zastosowanych międzyplonów (a1-a4)

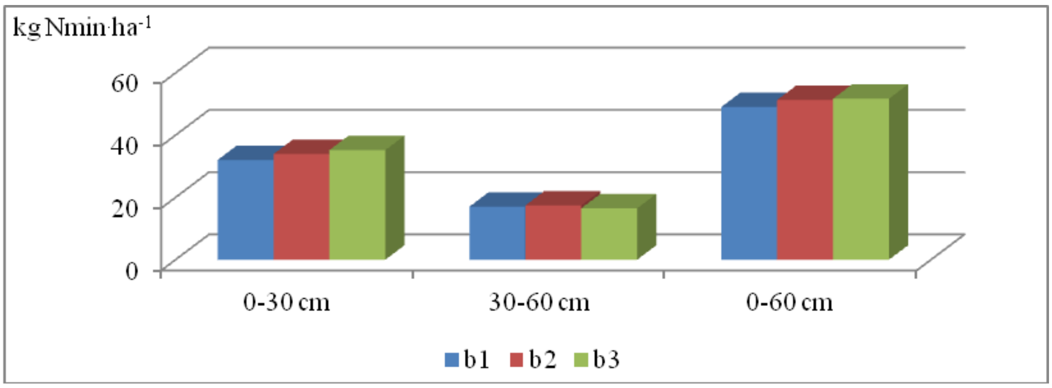

Fig. 3. Mean content of Nmin after yellow lupin harvest depending on the methods of seeds preparing for sowing (b1-b3)
Rys. 3. Średnia zwartość Nmin glebie po zbiorze łubinu w zależności od zastosowanych sposobów przygotowania nasion do siewu (b1-b3)

The factors applied in the experiment did not have a significant effect on the mean number of nodules per yellow lupin plant, which for the many-year period was 13.7, from 10.5 in 2009 to14.5-15.5 in the other research years (Table 2). The number of nodules which appeared right after emergence was growing regularly up to the physiological maturity of seeds over 2009-2010 and up to the flowering stage in 2011 (Fig. 4).

Table 2. Mean number of nodules per yellow lupin plant depending on the factors used
Tabela 2. Liczba brodawek na jednej roślinie łubinu żółtego w zależności od zastosowanych czynników

Typ of intercrop Rodzaj międzyplonu	Method of seed preparation for sowing Sposób przygotowania nasion do siewu			Mean Średnia
	Control Sarfun 50WP Kontrola Sarfun 50WP	Sarfun 50WP + *Bradyrhizobium lupini*	Sarfun 50WP + *Bradyrhizobium lupini* + genistein (genisteina)	
Control – Kontrola	14.5	13.9	13.7	14.1a
White mustard harvested before frosts – Gorczyca biała zebrana z pola przed nastaniem mrozów	13.9	13.6	13.8	13.8a
White mustard left for winter in a form of mulch – Gorczyca biała pozostawiona na zimę w formie mulczu	12.9	13.1	13.9	13.3a
Winter rye ploughed-in in spring – Żyto ozime zaorane wiosną –	13.8	14.2	13.3	13.8a
Mean – Średnia	13.8 A	13.7 A	13.7 A	13.7

Mean values followed by the same capital letters in rows and lower-case letters in columns did not differ significantly at α = 0.05
Średnie zaznaczone tymi samymi dużymi literami w wierszach i małymi w kolumnach nie różniły się istotnie przy α = 0,05

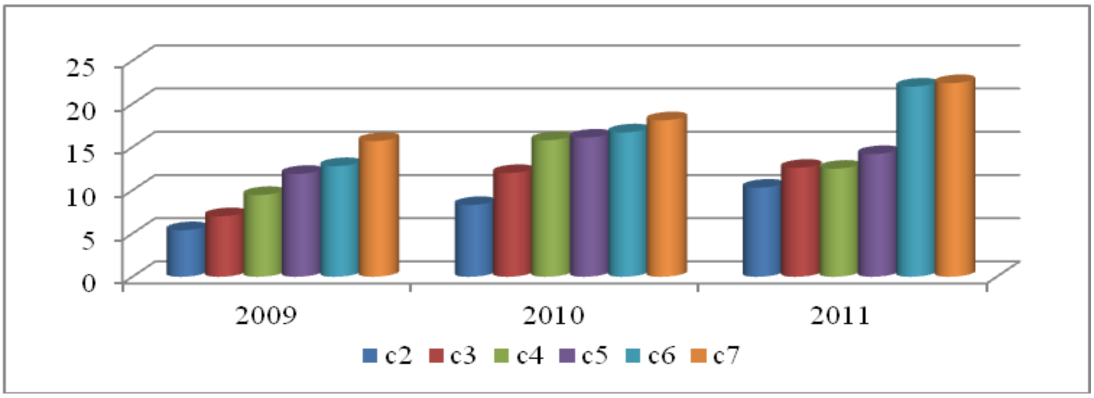

Fig. 4. Dynamics of yellow lupin nodulation (LSD 2009 – 1.16; LSD 2010 – 1.65; LSD 2011 – 3.61) (no of days after plant emergence: c2 – 14, c3 – 21, c4 – 28, c5 – 35; c6 – full of plant flowering, c7 – physiological seed maturity)
Rys. 4. Dynamika brodawkowania łubinu żółtego w latach badań (NIR 2009 – 1,16; NIR 2010 – 1,65; NIR 2011 – 3,61) (liczba dni po wschodach: c2 – 14, c3 – 21, c4 – 28, c5 – 35; c6 – pełnia kwitnienia, c7 – dojrzałość fizjologiczna nasion)

The factors affected the dry weight of yellow lupin nodules significantly (Table 3). The intercrop made up from white mustard left for winter in a form of mulch significantly decreased, as compared with the control, the mean dry weight of nodules per yellow lupin plant. The inoculation with rhizobia, on the other hand, with genistein added, significantly increased the dry weight of yellow lupin nodules. In 2009 with the development of plants and seeds, the dry weight of root nodules got significantly lower, however in 2011 the opposite tendency was reported (Fig. 5).

Table 3. Dry weight of nodules per yellow lupin plant depending on the factors used
Tabela 3. Sucha masa brodawek na jednej roślinie łubinu żółtego w g w zależności od zastosowanych czynników doświadczenia

Typ of intercrop Rodzaj międzyplonu	Methods of seed preparation for sowing Sposób przygotowania nasion do siewu			Mean Średnia
	Control Sarfun 50WP Kontrola Sarfun 50WP	Sarfun 50WP + *Bradyrhizobium lupini*	Sarfun 50WP + *Bradyrhizobium lupini* + genistein (genisteina)	
Control – Kontrola	0.508	0.462	0.558	0.509 a
White mustard harvested before frosts – Gorczyca biała zebrana z pola przed nastaniem mrozów	0.453	0.440	0.473	0.456 ab
White mustard left for winter in a form	0.430	0.421	0.429	0.427 b

of mulch – Gorczyca biała pozostawiona na zimę w formie mulczu				
Winter rye ploughed-in in spring – Żyto ozime zaorane wiosną	0.466	0.480	0.522	0.489 ab
Mean – Średnia	0.464 B	0.451 B	0.496 A	0.470

Mean values followed by the same capital letters in rows and lower-case letters in columns did not differ significantly at α = 0.05
Średnie zaznaczone tymi samymi dużymi literami w wierszach i małymi w kolumnach nie różniły się istotnie przy α = 0,05

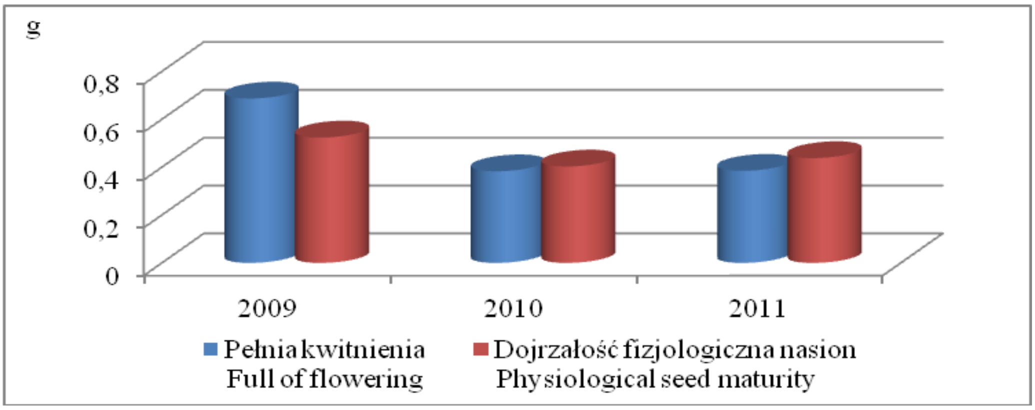

Fig. 5. Dynamics of dry weight of yellow lupin nodules over the research years (LSD 2009 = 0.022; LSD 2010 = ns; LSD 2011 = 0.011)
Rys. 5. Dynamika suchej masy brodawek łubinu żółtego (NIR 2009 =0,022; NIR 2010 = ni; NIR 2011 = 0,011)

In the soil from the control (without the intercrop), the application of rhizobia and genistein for seed inoculation increased the presence of symbiotic bacteria with a nodulation potential (Table 4). As for the mustard left for winter in a form of mulch in the control and where rhizobia were applied only, no symbiotic bacteria were identified at all. Interestingly, irrespective of the intercrop, the highest count of symbiotic bacteria in soil after lupin harvest was observed when genistein was used.

Table 4. Mean number of symbiotic bacteria (in g^{-1} dry weight of soil) after harvest of yellow lupin seeds depending on the factors used
Tabela 4. Liczebność bakterii symbiotycznych (w g^{-1} sm gleby) po zbiorze nasion łubinu żółtego w zależności od zastosowanych czynników

Rodzaj międzyplonu Type of intercrop	Method for seed preparation for sowing Sposób przygotowania nasion do siewu

	Control Sarfun 50WP Kontrola Sarfun 50WP	Sarfun 50WP + *Bradyrhizobium lupini*	Sarfun 50WP + *Bradyrhizobium lupini* + genistein (genisteina)
Control – Kontrola	0	2.0	2 x10^3
White mustard harvested before frosts – Gorczyca biała zebrana przed nastaniem mrozów	2.0 x 10^2	6.9 x 10^2	2.0 x 10^2
White mustard left for winter in a form of mulch – Gorczyca biała pozostawiona na zimę w formie mulczu	0	0	2.0 x 10
Winter rye ploughed-in in spring Żyto ozime zaorane wiosną	2.0	0	2 x10^3

At full flowering lupin plant phase there was determined the state of nutrition and the use of N by yellow lupin plants (Table 5). The mean SPAD value was 38.0 units, from 35.2 in 2010 to 38.7-38.9 over 2009 and 2011. The method of seed preparation for sowing did not differentiate the state of nitrogen nutrition of the plants, however lupin grown after mustard left for winter revealed a significantly higher SPAD value than in the control field (b1). The mean value of $N_{iż}$ (*nitrogen harvest index*) at the stage of maturity was high and it accounted for 71.8%. In the control where no intercrops were applied its value was relatively highest, however neither the kind of the forecrop nor the method of seed preparation for sowing differentiated its value significantly. The efficiency of the utilization of soil nitrogen and N from symbiosis (W_{gn}) was on average, 11.4 kg of seeds per 1 kg N, yet under the present conditions none of the factors differentiated its value. The mean total N content in the underground parts of yellow lupin plants accounted for 2.44% and it did not vary in the successive research years. The application of rhizobia with genistein in the control decreased the total N content in the roots significantly, as compared with the other treatments of factor B. The mean N content in aboveground parts of lupin, on the other hand, accounted for 3.30%; from 3.06% in 2010 to 3.53% in 2009. In the synthesis for the research years there was reported a significant effect of the intercrop on the N content in the aboveground parts; in the control plants it was significantly lower than in the other treatments of factor B. The mean N content in lupin seeds was almost 6.32%; from 6.22% in 2009 to 6.41% in 2010 and it did not depend on the experimental factors significantly. In the seeds of lupin grown after mustard left in the field for winter there was found a non-significantly lower N content in the seeds than for the other treatments of factor A.

Table 5. Mean values of nitrogen nutritional status of yellow lupin plants
Tabela 5. Średnie wartości wskaźników stanu odżywienia azotem roślin łubinu żółtego

Specification Wyszczególnienie	Years – Lata			Mean Średnia
	2009	2010	2011	

SPAD units – jednostki SPAD	38.9	35.2	38.7	37.9
$N_{iż}$ – N harvest index – Indeks żniwny N, %	71.7	71.5	72.4	71.8
W_{gn} – N utilization efficiency, kg seeds per kg N – Efektywność wykorzystania N pobranego z gleby oraz z symbiozy	10.3	11.4	11.5	11.4
N content in the dry weight in underground parts of plant, % Zawartość N ogółem w suchej części podziemnej jednej rośliny	2.43	2.44	2.47	2.44
N content in the dry weight in aboveground parts of plant, % Zawartość N ogółem w suchej części nadziemnej jednej rośliny	3.53	3.06	3.32	3.30
N content in the dry weight of seeds, % Zawartość N w suchej masie nasion	6.22	6.41	6.32	6.32

DISCUSSION

The population of nodule bacteria in the soils of Poland is getting smaller and smaller and their variation and adaptation to symbiosis after many years of its absence can pose potential difficulties in the construction of an efficient symbiotic system (Martyniuk et al., 2003). According to Pudełko (2010), a high variation in the population and a wide range of occurrence of Bradyrhizobium lupini in our country point to their excellent adaptation to the local environmental conditions. At the Experiment Station of the Faculty of Agriculture and Biotechnology at Mochełek for more than 60 years lupin experiments have been performed at various intensity and the N rates applied in the reference fields under cereals do not exceed 100-120 $kg \cdot ha^{-1}$, which must have resulted in an average Nmin content in both soil profile layers analysed (Fotyma and Pietruch 2000) and a lack of a considerable effect of the intercrops implementation to lower its content in spring, at least in the nodulation zone. In general, the Nmin content is higher in autumn than in early spring (prior to the use of fertilisers), which demonstrates that it depends strongly on the uptake by the plants and the organic matter mineralization intensity as well as the transformations of nitrogen itself in soil [22]. According to Płaza et al. (2009) growing intercrops enhances the mineral nitrogen content in spring. Undoubtedly, however, the intercrops take out considerable amounts N min from the soil profile, mostly in aboveground parts (Duer 1996, Dzienia and Boligłowa 1992; Jaskulski and Jaskulska 2004; Płaza et al. 2009; Sosulski and Mercik 2009; Wilczewski 2009).

One can assume that under field conditions the Nmin content in early spring in the 0-30 cm layer (where nodulation occurs) was not high enough to inhibit or to limit nodulation and to affect the number of nodules on the lupin roots significantly. With such inconsiderable changes in the presence of Nmin in early spring (mineral nitrogen fertilisation was not applied) there was reported no significant variation in the growth and development of yellow lupin plants. However, one shall stress that both in the control and whenever the sowing seeds were inoculated with genistein added, the count of symbiotic bacteria after plant harvest was highest, which can suggest a favourable effect of isofalvonoid on the survival rate of bacteria in soil (Rengel 2002).

The white mustard left in the field for winter in a form of mulch showed a negative effect on the dry weight of yellow lupin nodules and on the N content in the dry weight of plants and seeds, which could have been due to specific root secretions to the rhizosphere (Scott and Knudsen 1999) including glucosinolates (Sawicka and Kotiuk 2007) and phenolic compounds characteristic for many species representing family *Brassicaceae* (Cartea et al., 2011). Similarly, the stimulating effect of organic matter of white mustard on the growth and development of saprophytic microorganisms is common knowledge (Bełkot and Pięta 2005). According to Hoflich et al. (1999) the forecrop (cereals, sugar beet) can have a significant effect on the infestation of the rhizosphere of the aftercrop, including e.g. an increase in the nodulation of pea and the effectiveness of symbiosis considerably.

The method of seed preparation for sowing played a greater role in developing the Nmin content in none of the two soil profile layers analyzed. A poor reaction of *Fabaceae* plants to mineral nitrogen fertilisation means that they can use both forms of nitrogen: mineral and symbiotic, depending on their availability in soil. All that could account for the statements by some authors that the size of the nodulation is not a factor determining the seed yield size (Andrzejewska 2002). Beyond any doubt, the legumes must be provided with the conditions for possibly the best use of its production potential by a complete self-supply of nitrogen. To do so, it is indispensable to balance all the nutrients adequately (Tsai et al., 1993).

The weather pattern, especially precipitation conditions, pointed to a special susceptibility of nodules to water deficit (2010) or excess (2011) in the rhizosphere; in both cases there was noted a drastic decrease in the number and dry weight of nodules or even their disappearance already 28-35 days after emergence, similarly as in pea (Prusiński et al., 2012), which must have been due to a strong reaction of nitrogenase to such conditions resulting from the C deficit or a limited oxygen availability in the nodules (Serraj et al., 1999).

Growing intercrops to decrease the Nmin content did not result in the expected decrease in the Nmin content in soil, the content of which turns out to be not quite high enough for an effective symbiosis. Similarly, despite an inconsiderable share of *Fabaceae* plants in the crop structure in our country, and the fears that in the soils of Poland the nodule bacteria can be either incapable of the assimilation of N_2 or they can be absent at all, the method to increase the yellow lupin nodulation applied did not bring the expected results.

CONCLUSIONS

Growing intercrops in the soil of poor rye complex soil decreased neither the Nmin content in early spring nor the size of yellow lupin nodulation considerably. After lupin harvest slightly more Nmin was found in the treatments with intercrops, especially rye in the 0-30 cm layer. The weather pattern, precipitation mostly, pointed to a special susceptibility of nodules to the water deficit or excess in the rhizosphere; in both those cases there were noted a considerable decrease in the number and the dry weight of nodules or even their disappearance already 28-35 days after emergence. The method of preparing the seeds for sowing played a greater role in the developing of the Nmin content in neither of the two soil profile layers analysed after yellow lupin harvest. The white mustard left in the field in a form of mulch showed a negative effect on the dry weight of yellow lupin nodules and the total N content in the dry weight of the plants and seeds. Similarly, growing white mustard created least favourable conditions for the survival and the capacity for initiating symbiosis by symbiotic bacteria left in the soil after lupin harvest. Both in the control and when the sowing seeds were inoculated without or with genistein added, the count of symbiotic bacteria after plant harvest was highest. The state of yellow lupin nutrition with nitrogen was not affected by the experimental factors applied.

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