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Influence of microelements and attractants on the elements of yield structure and yield of white clover (*Trifolium repens* L.) seeds

Wpływ mikroelementów i atraktanta na elementy struktury plonu oraz plon nasion koniczyny białej (*Trifolium repens* L.)

Summary. A field experiment with the cultivation of white clover for seeds (Barda cv.) was carried out in 2009–2012, in the Experimental Farm in Parczew (22°53'60"E, 51°37'59"N), on typical brown soil (bonitation class IVb), by the split-plot method, in four replicates. The studies included: foliar fertilization with microelements (B + Mo) and attractant – Pollinus. The number of heads per 1 m^2, number of pods and seeds in the head as well as the seed yield were determined.
Weather conditions had the decisive influence on the yield of seeds and main elements of its structure. The highest seed yield (305.4 $kg \cdot ha^{-1}$) was obtained in 2010 characterized by more favorable thermal conditions and rainfall in the generative clover sub-period, slightly lower in 2012 (287.7 $kg \cdot ha^{-1}$), and the lowest in 2011 (49.3 $kg \cdot ha^{-1}$). Fertilization with microelements (B + Mo) significantly increased the number of heads per 1 m^2, the number of seeds in the head and the seed yield. The attractant used at the beginning and full flowering of clover significantly increased the yield of seeds by 35.2 and 78.0 $kg \cdot ha^{-1}$, respectively. The highest yield of white clover seeds (482.6 $kg \cdot ha^{-1}$) was obtained due to foliar fertilization with boron and molybdenum and using the attractant – Pollinus to fully flowering plants.

Key words: attractant, boron, white clover, molybdenum, seeds

INTRODUCTION

There has been observed an increase in the demand for white clover seeds, that are used as seeding material in the production of feed for farm animals, as well as in organic and recultivation crops, in recent years [Antonkiewicz and Radkowski 2006, Bojarczuk et al. 2011, Harkot and Gawryluk 2011, Klimont et al. 2013]. White clover is particularly important as a component of pasture mixtures for various soil habitats, for renovation of permanent grasslands and in the field cultivation as a component of clover-grass mixtures as well as undersown crops [Baryła and Kulik 2005, Prusiński and Kotecki 2006, Harasim 2008, Płaza et al. 2009, Bohra 2013]. It is also an important species in sustainable agriculture, because it does not require nitrogen fertilization, and furthermore, it supplies it to the soil as a consequence of symbiosis with *Rhizobium* bacteria. In domestic conditions on permanent pastures, it is about 3 kg $N \cdot ha^{-1}$ annually, for every 1% share in the sward [Kumar and Goh 2000, Prusiński and Kotecki 2006, Olszewska et al. 2008, Bohra 2013]. The market demand for white clover seeds is, to a small extent, covered by domestic production. The seeding material originates mainly from imports from New Zealand that is the world leader in the production of this species seeds [Chakwizira et al. 2011, Bohra 2013]. The domestic production potential of white clover seeds is small and highly dependent on habitat conditions and agro-technical factors.

Positive effects of certain micronutrients use and stimulating preparations were observed during the foliar fertilization of small-seeded bean plants [Goliński 2005, Twardowski and Hurej 2006, Ćwintal and Wilczek 2008, Wilczek and Ćwintal 2008a]. However, research in this area is relatively poor in relation to white clover, the cultivation of which for seeds requires an update of fundamental agrotechnical recommendations [Goliński 2005, Broniarz 2007].

A wide range of possibilities for using the white clover, both in productive and ecological terms in various natural conditions, and the need to guarantee the appropriate amount of seed material were the inspiration to carry out the present research. Its aim was to demonstrate the effect of foliar fertilization applying micronutrients (B + Mo) and the use of an attractant in various developmental stages of plants on the components of yield structure and yield of white clover of ‘Barda’ cv. grown on light soils.

MATERIAL AND METHODS

The field experiment was carried out in the Experimental Farm in Parczew (22°53'60"E, 51°37'59"N) on typical podzolic soil with granulometric composition of dusty sandy loam (bonitation class IVb), by means of split-plot method, in four replicates. The soil’s abundance in nutrients was (in $mg \cdot 100\ g^{-1}$ soil): K_2O 19.3, P_2O_5 19.0, Mg 6.4 and in $mg \cdot 1\ kg^{-1}$ soil: B 0.61, Mn 198.1, Cu 1.51, Zn 12.58, Fe 925, Mo 0.022. The plot area was 15 m^2 (1.5 m × 10 m). Medium-leafed white clover of ‘Barda’ cv. was the subject of the study [Lista Opisowa Odmian 2007].

Following factors were taken into account in the studies after plant trimming:

1. foliar fertilization with B + Mo microelements – full budding,

2. application of the attractant – Pollinus – at the beginning of flowering and full flowering.

Boron and molybdenum were used in the forms of Borvit and Molibdenit. The dose of boron and molybdenum in the pure component was 0.3 and 0.01 $kg \cdot ha^{-1}$, respectively, while the Pollinus attractant was applied at the dose of 1.0 $dm^3 \cdot ha^{-1}$, as an aqueous solution at 300 $dm^3 \cdot ha^{-1}$. The control object consisted of plants not sprayed with the above preparations.

Winter triticale was the forecrop for the clover. Mineral fertilization was 35 kg P and 66.5 kg K·ha^{-1} used in spring before sowing and before vegetation start in the subsequent years of cultivation. Clover seeds of 'Barda' cv. originated from 2008; the 1000-kernel weight of seeds was 0.64 g; germination capacity was 92%. Seed sowing was done using a seed drill in row spacing every 20 cm to a depth of 0.5–1.0 cm on April 20–21, 2009. The sowing rate, calculated per 100% germination capacity, was 4 kg·ha^{-1} (620 seeds per 1 m^2). The clover was sown in pure sowing (without any protective plant).

In the year of sowing (2009), the plant density per 1 m^2 was determined and the field's emergence ability was calculated. Due to weed infestation and large diversity of the clover plant development stage making the use of herbicides against dicotyledonous weeds impossible, weeds-control trimming was performed twice in the sowing year. The first – on June 2nd to the height of 5–6 cm, while the second – on June 18th to a height of 10–12 cm. In addition, spraying with Fusilade Forte 150 EC herbicide at the dose of 2 dm^3·ha^{-1} against monocotyledonous weeds was carried out on July 1st.

In years of the full use (2010–2012), when plants reached a height of about 20 cm, the clover leaves were cut using a strip mower at the height of 8–10 cm revealing the buds of inflorescences. Then, after reaching the proper development stage of the clover, the aforementioned preparations were used.

Before seed harvest, at ripeness of about 70% of heads, their number per 1 m^2 was determined and samples from 0.25 m^2 were taken for further analyses. In 10 heads randomly collected from each sample, the number of pods with the appropriate number of seeds was determined (0, 1, 2, 3, 4, 5, 6 seeds). On this basis, the average number of pods and seeds in the head, the structure of seed placement in pods, 1000-kernel mass and seed yield were calculated.

The weather conditions during white clover growing season were determined based on data from the Automatic Weather Station ("Klimax" type) in Sosnowica about 10 km away from the experiment location.

After samples collection, the clover was harvested in two stages. Ripening plants were mowed with a strip mower for cuts, and after drying they were threshed using a plot combine-harvester.

The results were statistically processed applying variance analysis and Tukey's test at the significance level of $\alpha = 0.05$.

RESULTS

The length of vegetative and generative sub-periods for clover through years (2010–2012) as well as thermal and moisture conditions in these sub-periods are presented in Table 1.

In years of the full use, the vegetative period was from 63 to 65 days, while generative from 56 to 61 days. Similar air temperature (20.2°C and 20.0°C) occurred in 2010 and 2012 with lower rainfall in 2012. Lower air temperature (19.1°C) and very large amount of precipitation (327.6 mm) were recorded in 2011. On the basis of the Selyaninov's coefficient, it was shown that the generative sub-period in 2012 was dry, in 2010 quite dry, and in 2011 extremely humid.

Table 1. Characteristics of meteorological conditions in the white clover vegetation sub-periods

Specification	Year	Vegetative sub-period	Generative sub-period	Vegetation period
Date of occurrence	2010 2011 2012	29.03–02.06 02.04–04.06 27.03–28.05	03.06–02.08 05.06–30.07 29.05–28.07	29.03–02.08 02.04–30.07 27.03–28.07
Length of the sub-period in days	2010 2011 2012	65 64 63	61 56 59	126 120 122
Average daily air temperature in the sub-period (^{o}C)	2010 2011 2012	13.2 12.6 13.0	20.2 19.1 20.0	16.7 15.6 16.5
Total precipitation in the sub-period (mm)	2010 2011 2012	189.5 93.1 98.8	150.7 327.6 104.8	340.2 420.7 203.6
Hydrothermal coefficient *k* by Selyaninov	2010 2011 2012	2.21 1.15 1.21	1.22 3.06 0.89	– – –

Value *k* – sub-period: ≤ 0.40 – extreme dry (ss); 0.41–0.70 – very dry (bs); 0.71–1.00 – dry (s); 1.01–1.30 – quite dry (ds); 1.31–1.60 – optimum (o); 1.61–2.00 – quite humid (dw); 2.01–2.50 – humid (w); 2.51–3.0 – very humid (bw); > 3.00 – extremely humid (sw).

Plant density after emergence did not significantly differ depending on the factors studied and it was on average 233 plants per 1 m^2. In relation to the number of seeds sown, the field emergence ability of white clover ranged from 36.3% to 38.7% and it also did not show any significant variation. Low percentage of the field emergence ability was caused by drying of some seedlings after germinating as a result of unfavorable moisture conditions that occurred immediately after sowing at the end of April.

The diversification of developmental stage of seedlings after emergence was prevented by the use of herbicides against dicotyledonous weeds, therefore trimming was performed. This treatment modified the course of plant growth and development. Under such conditions, in the sowing year, white clover produced few inflorescences, hence no seeds were harvested. Proper yielding of white clover for seeds can be obtained in years of the full use.

The number of white clover heads per 1 m^2 was significantly different depending on the weather conditions in years of research and micronutrients, as well as their interaction with years (Table 2). Remarkably the highest density of inflorescences (528 pieces) was recorded in 2010, and the smallest in 2011 (320 pieces). Foliar fertilization with boron and molybdenum significantly increased the average head density per 1 m^2 compared to the object without micronutrients. There were no differences in the density of white clover inflorescences per 1 m^2 depending on the attractant. However, the synergistic effect between microelements and weather conditions in particular years in relation to the discussed feature was visible. Significantly higher number of heads was recorded in 2010 in an object with microelements, while in 2011 and 2012, the number of heads was also higher in objects with microelements, but the differences were irrelevant.

Table 2. White clover heads (pcs · m^{-2})

Year	Attractant	Without microelements	Microelements (B + Mo)	$\overline{X}$
2010	control	435	591	513
	beginning of flowering	500	562	531
	full flowering	461	620	540
	$\overline{X}$	**465**	**591**	**528**
2011	control	293	328	310
	beginning of flowering	316	343	329
	full flowering	320	320	320
	$\overline{X}$	**310**	**330**	**320**
2012	control	443	486	464
	beginning of flowering	421	491	456
	full flowering	452	489	470
	$\overline{X}$	**439**	**489**	**464**
$\overline{X}$	control	390	468	429
	beginning of flowering	412	465	439
	full flowering	411	476	443
$\overline{X}$		**404**	**470**	–
$LSD_{0.05}$		24.8		37.2
in interaction: years × microelements = 74.3				

The number of pods in a head was determined significantly by weather conditions and microelements. Value of this feature ranged from 48 (2011) to 71 pieces (2012) (Table 3). Foliar fertilization with micronutrients increased the number of pods in the head by an average of 6 pieces, while the attractant did not significantly affect the size of this feature.

There was large diversity in the number of seeds in a head – from 22 to 139 seeds against the background of the factors studied (Table 4). The weather conditions in the years of research, fertilization with microelements, attractant as well as interaction of years × microelements and microelements × attractant, had significant influence on its variability. The highest variability in the number of seeds in the head occurred between years. The highest number of seeds in the inflorescence (108 seeds) was recorded in 2010, and the lowest (30 seeds) in 2011. Such result was determined by the weather course, that was more favorable for the plant flowering and the pollinating insects in 2010 (Table 1).

In objects with foliar fertilization with boron and molybdenum, the average number of seeds in the head was 88 pieces, which was significantly higher compared to the object without microelements (71 items). Similar dependencies occurred when using an attractant, with more beneficial effect achieved when applied at full than at the beginning of plant flowering.

Table 3. Number of pods in the head of white clover

Year	Attractant	Without microelements	Microelements (B + Mo)	$\overline{X}$
2010	control	64	66	65
	beginning of flowering	61	72	66
	full flowering	64	76	70
	$\overline{X}$	**63**	**71**	**67**
2011	control	45	48	47
	beginning of flowering	48	48	48
	full flowering	49	51	50
	$\overline{X}$	**47**	**49**	**48**
2012	control	68	74	71
	beginning of flowering	69	77	73
	full flowering	66	75	70
	$\overline{X}$	**68**	**75**	**71**
$\overline{X}$	control	59	63	61
	beginning of flowering	59	66	62
	full flowering	60	67	63
$\overline{X}$		**59**	**65**	–
$LSD_{0.05}$		3.59		5.17
in interaction: years × microelements = 7.91				

Table 4. Number of seeds in the head of white clover

Year	Attractant	Without microelements	Microelements (B + Mo)	$\overline{X}$
2010	control	83	106	94
	beginning of flowering	98	120	109
	full flowering	103	139	121
	$\overline{X}$	**95**	**122**	**108**
2011	control	22	27	25
	początek kwitnienia beginning of flowering	25	30	28
	full flowering	31	40	36
	$\overline{X}$	**26**	**33**	**30**
2012	control	79	93	86
	beginning of flowering	97	112	104
	full flowering	105	127	116
	$\overline{X}$	**94**	**111**	**102**
$\overline{X}$	control	61	75	68
	beginning of flowering	73	87	80
	full flowering	80	102	91
$\overline{X}$		**71**	**88**	–
$LSD_{0.05}$		4.83		7.14
in interaction: years × microelements = 14.2, attractant × microelements = 14.2				

The interaction of the attractant with weather conditions also influenced the discussed feature. In 2010 and 2012, significantly more seeds in the head were found in object with the use of this preparation in full flowering, while in 2011 with weather conditions unfavorable for the pollinators' flight in the generative sub-period of white clover, Pollinus preparation had no significant impact on the discussed feature.

Percentage of pods in the inflorescence with the number of seeds from 0 to 6 depending on the factors studied and years, is shown in Figures 1, 2, 3. Under the influence of micronutrients used (B + Mo), there was a reduction in the share of pods without seeds by 4.4% and a slight increase in the share of pods with the number of seeds from 1 to 6, compared to the control object. The most positive effect of micronutrients was the most evident in the case of 3-, 4- and 5-seed pods.

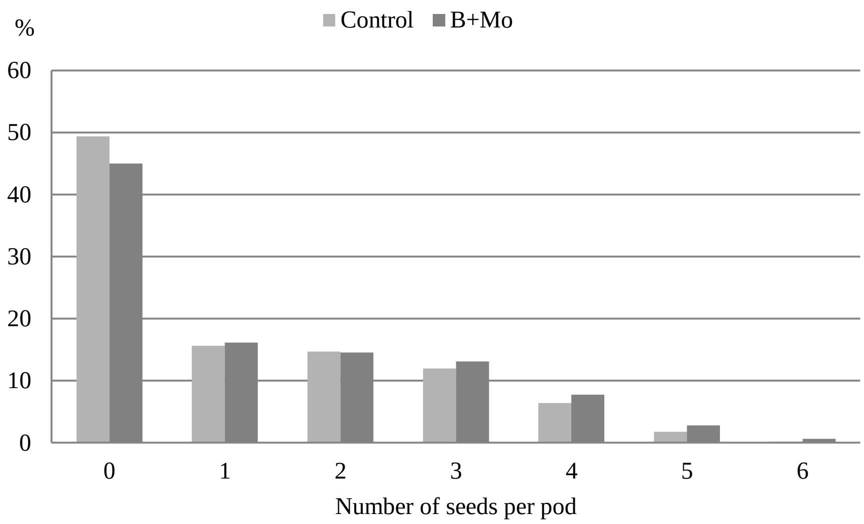

Fig. 1. Share of pods in the head with the number of seeds from 0 to 6 pcs depending on the micronutrients

The Pollinus attractant positively influenced the discussed feature. In both dates of application, it reduced the share of pods without seeds, however, the date of full plant flowering was more beneficial. The first date of application was more beneficial for the growth of pods with one and two seeds, while the second application time favored the increase in the share of the remaining pods, mainly 3-, 4- and 5-seed ones.

When comparing the influence of weather conditions during the years of research on the discussed feature, it should be noted that 2010 was the most favorable for seed setting. In that year, the lowest share of empty pods (28.4%) and those with one seed (14.9%) was recorded, while the highest number of multi-seeded pods compared to the remaining years. A very unfavorable seed-forming structure was found in 2011. At that time, empty pods made up 66.0%, and single-seeded ones 17.0%.

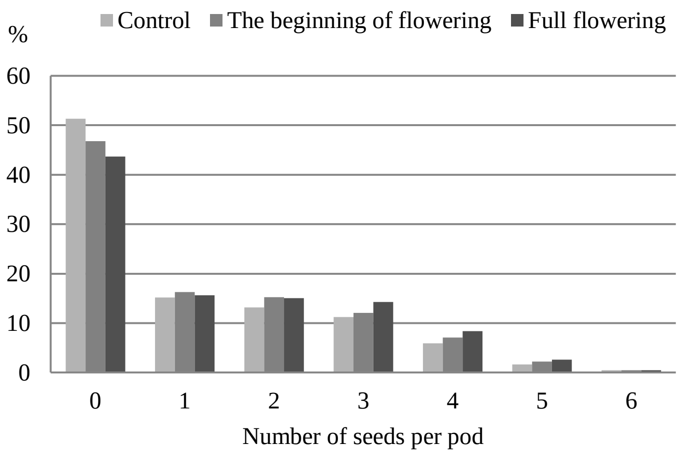

Fig. 2. Share of pods in the head with the number of seeds from 0 to 6 pieces depending on the attractant

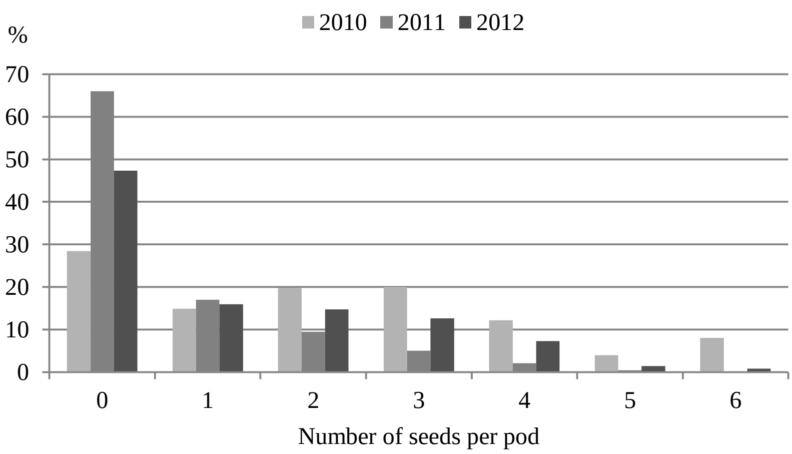

Fig. 3. Share of pods in the head with the number of seeds from 0 to 6 pcs depending on the years

The 1000-kernel mass of white clover seeds was significantly higher in object with micronutrient fertilization and it amounted to an average of 0.54 g (Table 5). Weather conditions and attractants did not differentiate the value of this feature. The yield of white clover seed varied from 33.0 to 482.6 kg·ha^{-1} and it depended on the weather course in individual years, micronutrients, attractant and the interaction of these factors with years (Table 6). Weather conditions were the most diversified. Significantly higher yield (305.4 kg·ha^{-1}) was obtained in 2010 and 2012 (287.7 kg·ha^{-1}), compared with 2011 (49.3 kg·ha^{-1}). Under the influence of fertilization with microelements, significant increase in seed yield was recorded, by 109.9 kg·ha^{-1} on average. Attractant used at the beginning and full flowering of white clover was also beneficial. The obtained yield was significantly higher by 35.2 and 78.0 kg·ha^{-1}, respectively, in reference to the control object.

Table 5. Mass of 1000 seeds

Year	Attractant	Without microelements	Microelements (B + Mo)	$\overline{X}$
2010	control	0.50	0.50	0.50
	beginning of flowering	0.52	0.54	0.53
	full flowering	0.50	0.56	0.53
	$\overline{X}$	**0.51**	**0.53**	**0.52**
2011	control	0.51	0.53	0.52
	beginning of flowering	0.50	0.54	0.52
	full flowering	0.51	0.53	0.52
	$\overline{X}$	**0.51**	**0.53**	**0.52**
2012	control	0.52	0.55	0.53
	beginning of flowering	0.51	0.55	0.53
	full flowering	0.52	0.56	0.54
	$\overline{X}$	**0.52**	**0.55**	**0.53**
$\overline{X}$	control	0.51	0.53	0.52
	beginning of flowering	0.51	0.54	0.52
	full flowering	0.51	0.55	0.53
$\overline{X}$		**0,51**	**0.54**	–
$LSD_{0.05}$		0.029		n.s.

Table 6. Seed yield (kg·ha^{-1})

Year	Attractant	Without microelements	Microelements (B + Mo)	$\overline{X}$
2010	control	180.5	313.2	246.8
	beginning of flowering	254.8	364.2	309.5
	full flowering	237.4	482.6	360.0
	$\overline{X}$	**224.2**	**386.7**	**305.4**
2011	control	33.0	47.8	40.4
	beginning of flowering	39.5	56.5	48.0
	full flowering	50.3	68.5	59.4
	$\overline{X}$	**40.9**	**57.6**	**49.3**
2012	control	182.0	302.3	242.1
	beginning of flowering	208.3	346.2	277.2
	full flowering	246.8	440.9	343.8
	$\overline{X}$	**212.4**	**363.1**	**287.7**
$\overline{X}$	control	131.8	221.1	176.4
	beginning of flowering	167.5	255.6	211.6
	full flowering	178.2	330.7	254.4
$\overline{X}$		**159,2**	**269.1**	–
$LSD_{0.05}$		14.7		21.4
in interaction: years × microelements = 40.7, attractant × microelements = 40.7, years × attractant = 66.2				

There was also significant interaction of micronutrients × years and attractant × years in influencing the yield of seeds. The highest yield of white clover seeds (482.6 $kg \cdot ha^{-1}$) was obtained after the use of micronutrients and the attractant in plant full flowering in 2010, when weather conditions favored the cultivation of clover.

DISCUSSION

White clover seed yield is determined mainly by the head density per 1 m^2, number of seeds in the head and 1000-kernel mass. These elements are modified by both habitat and agrotechnical factors. In the present study, immediately after sowing, adverse humidity conditions occurred, which affected the low value of field clover emergence and diversified the plant development phase. Similar observations were presented in their works by Olszewska [2004] and Grzesik et al. [2012], who underlined, that the effects of water shortage in the environment cause plant stress, delays in emergence, as well as their inhibition. According to Olszewska [2004], white clover requires 300–450 mm of rainfall during the growing season.

In agricultural practice, white clover seeds are harvested only in the second year of cultivation (Wilczek 2000). The results obtained in this experiment confirm this finding. With regard to perennial legumes grown for seeds, there is a consistent view that the size of the seed yield is determined to a greater extent by weather conditions during plant vegetation than agrotechnical factors [Wilczek 2000, Bodzon 2005, Ćwintal and Wilczek 20012]. They affect the formation of vegetative mass and generative organs by plants and affect the intensity of the pollinating insects flight, on which the number of seeds in the head depends [Wilczek 2000, Wilczek and Ćwintal 2008a]. Excessive precipitation during the generative sub-period of 2011 caused the rotting of the earliest set inflorescences, that fell completely or partly dropped their pods. It resulted in a lower head density per 1 m^2 and pods in the head. In addition, under high rainfall conditions, high humidity hindered the fertilization and seed formation, therefore their number in the head was low (30 on average). Up to 6 ovules can be found in embryo sac of white clover and it should be much more [Cebrat et al. 1982]. Unfavorable weather conditions in 2011 caused a large share of the number of pods without seeds and a small number of seeds in the fertile pods, resulting in a yield of 49.3 $kg \cdot ha^{-1}$.

The inflorescences density per 1 m^2 in the present experiment was smaller than that reported by Goliński [2005], which for the 'Romena' cv. was 596–738 pieces. In turn, the average number of pods and seeds in the head was similar.

Foliar fertilization with boron and molybdenum resulted in a significant increase in the density of heads per 1 m^2 and seeds in the head as well as seed yield. Observations confirming the beneficial effect of these micronutrients on the growth and generative development of plants are emphasized by other authors [Wilczek and Ćwintal 2008, Szewczuk and Sugier 2009, Sun et al. 2013]. In turn, studies by Stoltz and Wallenhammar [2014] with the use of boron alone towards white clover did not confirm significant differentiation of seed yields, although they showed a 10% upward trend.

Setting the seeds of perennial *Fabaceae* depends on the presence of pollinating insects, their activity and the length and course of flowering in various weather conditions [Wilczek and Ćwintal 2003, Warda and Kozłowski 2012]. Pollinus fragrance agent attracting bees, is expected to increase their number and activity on flowering plants (Grzesik et al. 2012). Its positive effects in the experiment consisted in a decrease in the number of pods without seeds and an increase in the share of multi-seeded pods, which affected a greater number of seeds in the head and seed yield. It should be emphasized that the greatest impact of this preparation was noted in favorable weather conditions in 2010 with the application during full flowering. These results are consistent with Majewski's [2010] statement that after applying Pollinus on blooming plants, more bees appear, they are more active and carry more pollen on flowers.

CONCLUSIONS

1. Weather conditions in particular cultivation years had the decisive influence on the elements of the yield structure (density of inflorescence per 1 m^2, number of pods and seeds in the head, structure of seed embedding in pods) and the yield of white clover seeds. Significantly higher values of these elements and yield of seeds were obtained in 2010 characterized by favorable thermal and moisture conditions during the clover's generative sub-period.

2. Foliar application of micronutrients (B + Mo) significantly increased the density of heads on the surface unit, number of seeds in the head and the seed yield.

3. Attractant used at the beginning and full flowering of the clover caused significant increase in the share of seeds in the head and increase in the seed yield.

4. In the cultivation of white clover for seeds, the highest yield (482.6 $kg \cdot ha^{-1}$) was obtained in 2010, when fertilizing with boron and molybdenum, and using the attractant – Pollinus at the stage of fully plant flowering.

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Streszczenie. Eksperyment polowy z uprawą koniczyny białej na nasiona (odmiana Barda) przeprowadzono w latach 2009–2012 w Gospodarstwie Doświadczalnym w Parczewie (22° 53′ 60″E, 51°37′59″N), na glebie płowej typowej, (klasa bonitacyjna IVb), metodą split-plot, w czterech powtórzeniach. W badaniach uwzględniono: dolistne nawożenie mikroelementami (B + Mo) i atraktant – Pollinus. Określono liczbę główek na 1 m^2, liczbę strąków i nasion w główce oraz plon nasion.

Decydujący wpływ na plon nasion oraz główne elementy jego struktury miały warunki pogodowe. Największy plon nasion (305,4 $kg \cdot ha^{-1}$) uzyskano w roku 2010, charakteryzującym się korzystniejszymi warunkami termicznymi i opadami w podokresie generatywnym koniczyny, nieco niższy w roku 2012 (287,7 $kg \cdot ha^{-1}$), a najniższy w 2011 (49,3 $kg \cdot ha^{-1}$). Nawożenie mikroelementami (B + Mo) istotnie zwiększało obsadę główek na 1 m^2, liczbę nasion w główce i plon nasion. Atraktant stosowany na początku i w pełni kwitnienia koniczyny istotnie zwiększał plon nasion odpowiednio o 35,2 i 78,0 $kg \cdot ha^{-1}$. Najwyższy plon nasion koniczyny białej (482,6 $kg \cdot ha^{-1}$) uzyskano, nawożąc dolistnie borem i molibdenem oraz stosując atraktant – Pollinus w pełni kwitnienia roślin.

Słowa kluczowe: atraktant, bor, koniczyna biała, molibden, nasiona

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