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EFFECT OF BIOPREPARATIONS ON THE HEALTH OF GRAIN OF SPRING BARLEY (*HORDEUM VULGARE*) IN ORGANIC SYSTEM

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Abstract

The effect of grain treatment and plant spraying with Biochikol 020 PC and Biosept 33 SL on harvested grain health was tested. Both preparations decreased grain infestation with spring barley pathogens *Bipolaris sorokiniana*, *Fusarium* spp. and *Drechslera teres*, however, the effectiveness of Biosept 33 SL was much higher. It was particularly evident in 2006, when the preparation significantly reduced *D. teres*, present in large numbers that year. The positive effect of Biosept 33 SL was also noted in the case of *B. sorokiniana*, which was the prevailing pathogen in 2007. Due to poor occurrence of *Fusarium* spp. on harvested grain, the effect of biopreparations on pathogens from this genus is difficult to determine. Molecular analysis, carried out besides of conventional mycological ones, confirmed the occurrence of *D. teres* in the material studied. Application of biopreparations, particularly Biosept 33 SL, seems an effective tool in barley protection against fungal disease factors in organic system.

Key words: barley, fungi, Biochikol 020 PC, Biosept 33 SL, organic farming, *Bipolaris sorokiniana, Fusarium*, *Drechslera teres,* SCAR, PCR

Introduction

Proceeding in accordance with the principles of organic farming as compared to conventional systems based on intensive technologies of production, is associated with yield reduction (Tamis and Van den Brink 1999). One of major factors limiting yield in organic farms are diseases caused by fungi. Previous studies of plant health and analysis of fungi occurrence in spring barley showed, that the serious threat in organic system is *Bipolaris sorokiniana* that infects roots, stem bases, leaves

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and grain, *Drechslera teres* – causal agent of leaf diseases and *Fusarium* spp. that infect roots, stem bases, heads and grain (Baturo and Łukanowski 2001, Baturo 2002, 2007, 2008). These pathogens are transmitted mainly with grain, and their use as sowing material is a serious problem (Healthy...). The possibility of preventing the occurrence of pathogens and the damage that they cause in the organic system is limited because traditional grain and chemical control during cropping season are banned. Therefore, natural substances are applied, which, as it turns out, have different efficiency, and they are one of the few possibilities of plant protection in organic farming.

The aim of the study was to determine the effect of grain treatment and plant spraying with biopreparations Biosept 33 SL and Biochikol 020 PC on the health of the harvested grain.

Materials and methods

The effect of cv. 'Damazy' grain treatment and plant spraying with Biochikol 020 PC (chitosan) 2.0%, Biosept 33 SL (grapefruit extract) 0.1% on grain health in comparison with control was tested in 2006–2008. Spring barley was grown in the five-year-rotation: potato, spring barley, pea, winter wheat, winter rye. Plot experiment was carried out in four replications, in organic farm in Kiełpin near Tuchola in Kuiavia-Pomerania province, north-western Poland. The organic farm has belonged to the EKOLAND association (Polish National Organic Organization) since 1991 year.

The plants in the plots where the sown grain was treated, were sprayed (with the exception of one combination for each biopreparation) during the cropping season one, two or three times with biopreparations, in the same concentrations, which were used for treatment, and the amount recommended by the producer. Spraying was performed every two weeks, starting from the beginning of shooting into stalk (first node), when disease symptoms showed very low intensity.

After harvesting, the effect of applied treatments on the grain health was analysed. For mycological analysis, carried out according to the method described by Baturo (2002), 4 × 100-grain samples were taken from each combination. Furthermore, the weight of 1000 grains and the mass of 100 heads was determined.

In order to complete the analysis, grain from all three years of the study was examined with molecular SCAR-PCR for the presence of *D. teres*. Reactions were carried out with two pairs of primers: DTT471h (F 5'-CCTGAGTAACTTGCCCCACC-3', R 5'-GAAAAGAGATGATGCGGACAC-3') and DTM494d (F 5'-TATTCTGCTAAGAGCTAGCATCCTA-3', R 5'-ACTGCGTACCAATTCTCTACAACTA-3'). They enabled verification of the pathogen presence in the samples and determination of the form: *teres* and *maculata* (Leisova et al. 2005).

DNA from grain was isolated according to a modified method of Edwards et al. (2001). Coffee grinder was used to crush 14 g of grain into very small parts and total DNA was extracted using CTAB buffer (5.0% CTAB, 5.0M NaCl, 0.5M EDTA, 1.0M Tris-HCl (pH 8.0), PVP, β-mercaptoethanol, and H_2O). To flasks containing

10-g samples of crushed grain, 30 ml of buffer were added, mixed and incubated at 65°C for 16 h. The tube contents were mixed and centrifuged (3000 rpg, 15 min). DNA was extracted from a 1.2-ml aliquot of supernatant in chloroform: isoamyl alcohol (24:1) mixed with phenol (1:1), precipitated with 95% ethanol, and washed twice with 70% ethanol. RNA was eliminated by addition of RNAase.

Reactions were carried out in a 12.5 μl volume of PCR Core Kit (Qiagen, USA) with 20 ng of each primer and 100 ng of DNA. Amplifications were performed in a thermocycler (Biometra) according to the following protocol: 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 62°C (annealing temperature for primer pair DTT471h) or 30 s at 55°C (annealing temperature for primer pair DTM494d), 40 s at 72°C, and a final extension 5 min at 72°C. Amplification products were separated by electrophoresis on 1.6% agarose gels with TBE running buffer and stained with ethidium bromide. A molecular marker of 100 bp (EURx, Poland) was used. The results were scanned into a computer imaging file with a gel documentation system (Vilber Lourmat) equipped with a digital camera.

Results

The large number of *Alternaria alternata* and *Epicoccum nigrum* were found on the grains in all the years of the study, while the presence of major pathogens of barley in different years was differentiated.

Mycological analysis carried out in 2006 showed that the highest number of grains from the control was most strongly infected by *D. teres*. Grain treatment and plant spraying with both biopreparations reduced the pathogen, however advantageous effect of Biosept 33 SL was much stronger. The effect of the Biochikol 020 PC was observed after three spraying and Biosept 33 SL after a single spraying or even in combination without spraying, where only the grains were treated. Biosept 33 SL, depending on the number of sprayings, reduced the occurrence of the pathogen from 44% (for treated grains and no spraying) to 72% (in the case of grain treatment and spraying applied twice). Advantageous effect of Biochikol 020 PC, with efficacy similar to Biosept 33 SL, was noticeable only after three treatments. This year, the smallest number of fungi was isolated in comparison to 2007 and 2008, but the occurrence of *D. teres* was the highest. *Bipolaris sorokiniana* and fungi from *Fusarium* genus were isolated sporadically (Table 1).

In 2007 *B. sorokiniana* was prevalent among pathogenic fungi. Biosept 33 SL explicitly limited its presence, particularly in the plots where spraying was applied. Positive impact of Biochikol 020 PC was not clear and, like in the previous year in the case of *D. teres*, only after three sprayings. Furthermore, in the material harvested from the plot sprayed once, the presence of the pathogen in the greatest intensity was found, higher even than in the control. *Drechslera teres* was noted very rarely, probably because of weather conditions during the growing season, which was not conducive to its development. It was noted occasionally, only on the sprayed combinations (Table 2).

Table 1

Fungi isolated from harvested grain, weight of 100 heads and 1000 grains in 2006

Fungus species	Control	Grain treated with Biosept 33 SL				Grain treated with Biochikol 020 PC			
		no plant spraying	1 × plant spraying	2 × plant spraying	3 × plant spraying	no plant spraying	1 × plant spraying	2 × plant spraying	3 × plant spraying
Grain infection with fungi (%)									
Alternaria alternata	51	65	76	81	79	58	68	62	81
Alternaria consortiale	–	–	–	–	1	–	–	1	–
Aspergillus fumigatus	–	–	–	–	–	–	2	1	–
Aspergillus niger	–	–	1	2	1	–	–	–	–
Aureobasidium bolleyi	4	3	2	4	2	5	7	7	2
Bipolaris sorokiniana	1	0	1	–	–	1	1	1	–
Botrytis cinerea	–	–	–	1	–	–	–	–	1
Cladosporium herbarum	1	1	3	–	–	–	2	–	1
Drechslera teres	33	22	15	11	14	28	22	25	14
Epicoccum nigrum	17	16	18	13	16	16	15	19	13
Fusarium avenaceum	–	–	–	–	1	1	1	–	1
Fusarium langsethiae	–	–	–	1	–	–	–	–	–
Fusarium poae	–	–	–	–	–	–	–	–	1
Penicillium spp.	–	–	–	1	–	1	–	1	–
Non-sporulating fungi	–	1	–	1	2	–	1	–	–
Total number of colonies isolated from each 400-grain sample	428	436	464	460	464	440	476	468	456
Weight of 100 heads (g)	85.7	86.0	85.8	84.4	83.2	85.3	85.8	85.8	88.2
Weight of 1000 grains (g)	45.6	45.2	45.1	44.1	45.7	45.3	45.4	46.8	44.7

Table 2

Fungi isolated from harvested grain, weight of 100 heads and 1000 grains in 2007

Fungus species	Control	Grain treated with Biosept 33 SL				Grain treated with Biochikol 020 PC			
		no plant spraying	1 × plant spraying	2 × plant spraying	3 × plant spraying	no plant spraying	1 × plant spraying	2 × plant spraying	3 × plant spraying
Grain infection with fungi (%)									
Alternaria alternata	83	79	76	75	79	88	74	75	79
Alternaria consortiale	–	–	–	1	–	–	–	–	–
Arthrinium phaeospermum	1	–	–	–	1	–	–	–	–
Bipolaris sorokiniana	17	12	12	13	14	8	27	15	12
Botrytis cinerea	2	–	–	–	–	1	–	1	1
Cladosporium herbarum	–	–	–	1	–	–	–	–	–
Drechslera teres	–	–	3	1	–	–	3	2	1
Epicoccum nigrum	26	20	34	30	20	17	18	16	22
Fusarium avenaceum	2	1	2	3	–	1	2	1	–
Fusarium langsethiae	–	1	–	1	–	–	–	–	–
Fusarium poae	3	4	2	–	–	1	–	1	2
Fusarium tricinctum	–	3	3	–	2	7	3	5	3
Gelasinospora cerealis	–	2	–	3	–	1	1	1	1
Gonatobotrys simplex	3	2	1	1	–	–	4	5	4
Nigrospora oryzae	2	1	–	–	4	–	1	5	–
Penicillium spp.	–	–	–	1	1	–	–	–	–
Stemphylium botryosum	–	1	–	–	–	–	–	–	–
Total number of colonies isolated from each 400-grain sample	556	504	532	520	484	496	532	508	501
Weight of 100 heads (g)	86.5	88.0	85.0	87.0	84.0	86.0	86.6	87.0	89.5
Weight of 1000 grains (g)	48.1	48.2	46.3	45.0	45.6	46.8	46.9	45.8	47.3

Table 3

Fungi isolated from harvested grain, weight of 100 heads and 1000 grains in 2008

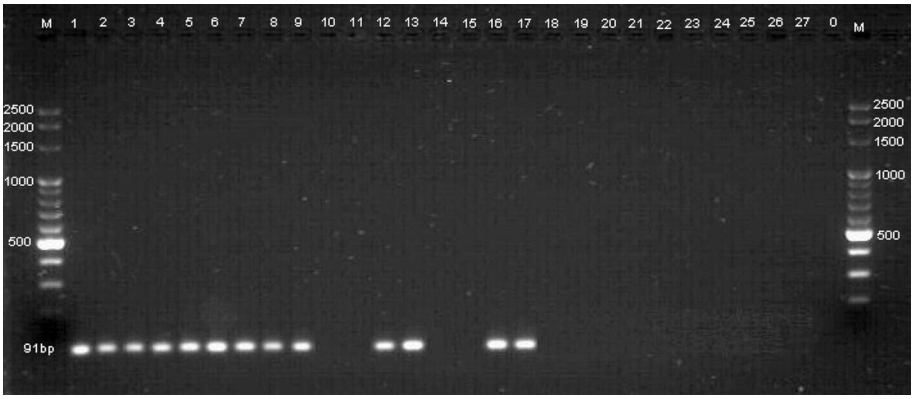
Fungus species	Control	Grain treated with Biosept 33 SL				Grain treated with Biochikol 020 PC			
		no plant spraying	1 × plant spraying	2 × plant spraying	3 × plant spraying	no plant spraying	1 × plant spraying	2 × plant spraying	3 × plant spraying
Grain infection with fungi (%)									
Alternaria alternata	86	81	76	81	89	89	77	84	80
Ascochyta sp.	–	–	–	–	–	–	1	–	–
Aspergillus niger	–	–	1	–	–	–	–	1	–
Aureobasidium bolleyi	5	–	–	3	2	3	1	2	2
Bipolaris sorokiniana	5	7	5	2	–	6	6	3	6
Botrytis cinerea	–	1	3	1	1	–	–	1	4
Cladosporium herbarum	2	1	1	–	–	–	–	–	–
Epicoccum nigrum	28	30	36	37	34	39	39	33	35
Fusarium avenaceum	1	–	3	–	1	–	–	3	–
Fusarium equiseti	–	1	–	–	–	–	–	–	–
Fusarium poae	–	–	2	1	1	1	1	–	–
Fusarium tricinctum	1	–	–	3	–	–	5	–	–
Gonatobotrys simplex	–	1	3	2	1	2	–	2	–
Mucor mucedo	–	–	1	2	1	–	–	–	–
Penicillium spp.	–	2	–	–	–	–	–	–	–
Non-sporulating fungi	–	–	–	–	1	–	1	–	–
Total number of colonies isolated from each 400-grain sample	513	496	524	527	525	559	524	516	508
Weight of 100 heads (g)	124.5	125.8	120.8	129.9	119.0	129.4	121.3	124.2	132.0
Weight of 1000 grains (g)	56.0	56.2	55.2	56.0	55.7	55.5	56.2	56.0	55.0

The results obtained in 2008 indicated that Biosept 33 SL to some extent limited *B. sorokiniana*, which occurred less numerously than in the previous year. The effectiveness of the biopreparation increased with the number of sprayings. There was no positive effect of Biochikol 020 PC spraying: the same frequency of the pathogen occurred in plots that were not sprayed, sprayed once or three times. No effect of biopreparations on the occasional occurrence of *Fusarium* spp. was observed. *Drechslera teres* was not noted (Table 3).

Among the rarely isolated species of *Fusarium*, such as pathogenic *F. avenaceum* and nonpathogenic *F. equiseti*, *F. poae* or *F. tricinctum*, single isolates of *F. langsethiae* were noted (Tables 1 and 2).

Weight of heads was slightly higher after three sprayings with Biochikol 020 PC and smaller after three applications of Biosept 33 SL. However, it did not affect the weight of thousand grains, which was similar in all samples (Tables 1, 2, 3).

Molecular analysis revealed the presence of the *D. teres* in the material tested. The results largely confirmed the conventional mycological analysis results. In all samples in 2006 the pathogen occurrence was found. In 2007, when the fungus occurred sporadically, molecular method did not detect it even in those samples, where its presence was observed in studies on PDA medium. In the grain samples in 2008 year, using as well traditional as molecular method, the presence of *D. teres* was not identified. Product of 161 bp was obtained for none of the samples in the case of reaction with primers DTM494d. The obtained reaction products of a size 91 bp with a pair of primers DTT471h indicated the occurrence of *teres* form in the test material. Results of SCAR-PCR analysis are shown in Photograph 1.



Phot. 1. A gel showing amplification products generated with primer DTT471h.
Lanes 1–9 refer to grain samples harvested in 2006, 10–18 in 2007, 19–27 in 2008. In each year lanes refer as follows: control, grain treated with Biosept 33 SL with no spraying, and 1–3 × spraying with Biosept 33 SL and adequately for combinations with Biochikol 020 PC, M – molecular weight marker, 0 – negative control (photo by A. Baturo)

Discussion

Reduction of the major pathogens' number by application of biopreparations suggests that they could be an effective tool in barley protection. Good results were obtained with Biosept 33 SL. Grain treatment and plant spraying resulted in lower infection of harvested grain with *D. teres* and *B. sorokiniana*. Previous studies (Baturo 2006) showed also a good effect of Biosept 33 SL on the health of spring barley seedlings in organic system. Grain treatment with Biosept 33 SL resulted in the lowest plant infection with *B. sorokiniana* in comparison to thermotherapy, Biochikol 020 PC and highly antagonistic strain of *Trichoderma viride*. Similarly, Mazur and Nawrocki (2007) in the studies on carrot infection with *Alternaria* spp. observed advantageous effect of plant spraying with Biosept 33 SL on the health of carrot roots in contrast to Biochikol 020 PC. Applying grapefruit extract for seed treatment was also highly effective in protection of bean against soil-borne pathogens (Patkowska and Pięta 2004). Plant spraying resulted in reducing *Colletotrichum gloeosporioides* causing lupine anthracnose (Jeske 2006) and *C. lindemuthianum*, causal agent of anthracnose of kidney bean, under field and laboratory conditions (Stompor-Chrzan 2007) and alternariosis (*Alternaria* spp.) of organically grown potato (Lenc 2007). Studies of Orlikowski and Skrzypczak (2003) showed a wide biological activity of grapefruit extract in the control of leaf and soil-borne pathogens like: *Fusarium* spp., *Botrytis* spp. and *Phytophthora cryptogea*.

Biosept 33 SL, containing 33% grapefruit extract, directly affects pathogenic factors and induces plant resistance to certain pathogens (Orlikowski et al. 2002). It is possible that the mechanism of direct effect on pathogens caused its higher efficacy in comparison to Biochikol 020 PC. Chitosan, its main ingredient, is known primarily as a potential elicitor of plant defense reactions and also an active inhibitor of fungal growth (Wojdyła 2004). Many reports inform about the inhibiting impact of chitosan on growth and development of fungi and bacteria, but it is not always observed, for instance: chitosan did not limit, neither *in vitro* nor after introduction into the soil, some forms of *F. oxysporum* (Skrzypczak and Orlikowski 1998). Studies carried out by Roller and Covill (1999) showed antifungal properties of chitosan *in vitro* against a few filamentous fungi. They also noted that some fungal strains could be resistant to that chitin derivative which showed that not all fungi were susceptible to chitosan. Varied reaction of pathogens to chitosan was observed also by Wojdyła (2003): *C. gloeosporioides* and *P. cryptogea* strongly reacted to the substance as opposed to *Cylindrocarpon scoparium*, *Myrothecium roridum* and *Diplocarpon rosea*, which were less sensitive. However, in many studies clearly positive effect of Biochikol 020 PC was observed. Pięta et al. (2000) noted that treatment with chitosan satisfactorily protected germinated seeds of bean and then roots and stem bases against soil-borne pathogens. Patkowska (2005) observed advantageous effect of Biochikol 020 PC and Biosept 33 SL on soybean health. Biochikol 020 PC was beneficial to the health of roots and leaf sheaths of spring barley seedlings (Baturo 2003) and in the case of older plants with disease symptoms, after applying of the biopreparation, *B. sorokiniana* was isolated less fre-

quently, although the effect was weaker than after the application of Biosept 33 SL (Baturo 2006). In this study, the effect of Biochikol 020 PC on reduction of *B. sorokiniana* was not satisfactory, while in the *in vitro* studies (Baturo 2003, Łukanowski 2003) its limiting effect on this severe pathogen of barley and *Fusarium* spp. was clear.

In the tested samples, beside the *Fusarium* spp. common on cereal grain, *F. langsethiae* was isolated. This species, that is morphologically similar to *F. poae* and toxicologically to *F. sporotrichioides*, was described relatively recently (Schmidt et al. 2004, Torp and Nirenberg 2004). In Poland it was found in wheat grain (Łukanowski et al. 2008). Due to the small number of *Fusarium* spp. isolates from the test material, it is difficult to determine the impact of biopreparations on their occurrence. Reports on this problem are varied. Research carried out by Horoszkiewicz-Janka and Jajor (2006) showed that Biosept 33 SL used for grain treatment of barley significantly limited the seedling blights. Furthermore, compared with Biochikol 020 PC as well as Bioczos BR, Biosept 33 SL was more effective in protection of older plants, despite the fact that *Fusarium* spp. were isolated from their stem bases. Patkowska (2006) noted considerable protective effect of Biosept 33 SL against soil-borne pathogenic fungi, e.g. *Fusarium* spp. in germinating seeds, roots and stem bases of common bean, runner bean and pea. Research shows that the reaction of the species may be differentiated. Tests carried out by Łukanowski (2003) showed that the biopreparation limited the growth of *Fusarium* spp., but towards one species, *F. solani*, its effect was weak. Solarska and Jończyk (2003) showed no effective reduction of *Fusarium* spp. by wheat spraying with Biosept 33 SL in organic system. Studies carried out by Orlikowski and Skrzypczak (2001, 2003) revealed another aspect: probably grapefruit extract protects plants against pathogens of the genus *Fusarium* for a short time and may stimulate plant defense response to the pathogen.

Beside the pathogens mentioned, other fungi common in barley grain were also isolated. The occurrence of *Alternaria alternata*, *Epicoccum nigrum*, *Aureobasidium bolleyi*, *Cladosporium herbarum* and *Botrytis cinerea* were noted in many studies (Knudsen et al. 1995, Baturo 2002).

Molecular analysis enabled the rapid identification of *D. teres* in the grain samples. However, in some cases, it proved unreliable and less efficient than the conventional mycological method. SCAR-PCR method is widely used for identification of cereal pathogens such as *Fusarium* spp., *Tapesia yallundae* and *T. acuformis* directly in the plant material (Turner et al. 1999, Fordoński et al. 2001) and for species identification of pure mycelium such as *Fusarium* spp. (Nicholson et al. 1998, Akinsanmi et al. 2004, Łukanowski et al. 2008). In this study, the method also was used to identify the forms of *D. teres*. The reaction with appropriate primers revealed that in the tested material only *D. teres* f. sp. *teres*, causing *net* symptoms of disease, was present. This is consistent with the literature data. Many authors report that this form prevails in the pathogen population (Jonsson and Anderberg 1997, Kosiada and Weber 2003, Serenius et al. 2005).

The results obtained here showed a significantly advantageous effect of Biosept 33 SL on the grain health. Toppe et al. (2007), examining the effect of grapefruit

extract on powdery mildew of cucumber, found that the substance could have been a supplement or even a replacement to some of the current fungicides. These results and data in literature concerning cereals are also promising, which is very important under organic and integrated farming conditions.

Conclusions

1. Applying biopreparations, especially Biosept 33 SL, can be effective in reducing fungal diseases in barley grown in the organic system.
2. The results obtained in different years are not clear, but they indicate that the effect of biopreparations, especially Biosept 33 SL, is noticeable in the case of some pathogen populations such as *D. teres* in 2006 and *B. sorokiniana* in 2007.
3. For complete results, in addition to molecular analysis, it is advisable to carry out the conventional mycological analysis.

Streszczenie

WPŁYW BIOPREPARATÓW NA ZDROWOTNOŚĆ ZIARNA JĘCZMIENIA JAREGO (*HORDEUM VULGARE*) W SYSTEMIE EKOLOGICZNYM

Badano wpływ zaprawiania ziarna i opryskiwania roślin Biochikolem 020 PC i Bioseptem 33 SL na zdrowotność ziarna. Celem było stwierdzenie, czy biopreparaty ograniczają rozwój grzybów patogenicznych dla jęczmienia jarego, takich jak: *Bipolaris sorokiniana*, *Fusarium* spp. i *Drechslera teres*. Obydwa preparaty ograniczały rozwój patogenów, jednak wyraźnie lepszy efekt wystąpił w przypadku Bioseptu 33 SL. Było to szczególnie zauważalne w 2006 roku, w którym preparat ten wyraźnie przyczynił się do zmniejszenia liczebności izolatów *D. teres*. Ograniczający efekt działania Bioseptu 33 SL odnotowano również w przypadku *B. sorokiniana*, przeważającego wśród patogenów wyizolowanych z ziarna w 2007 roku. Ze względu na małą liczbę wyosobnień *Fusarium* spp. trudno określić wpływ badanych preparatów na nasilenie występowania tych patogenów. Analizy molekularne SCAR-PCR, przeprowadzone oprócz tradycyjnych badań mikologicznych, potwierdziły obecność *D. teres* w badanym materiale. Wyniki trzyletnich badań wskazują, że stosowanie biopreparatów, szczególnie Bioseptu 33 SL, może być efektywnym narzędziem ograniczania chorób powodowanych przez grzyby na jęczmieniu uprawianym w systemie ekologicznym.

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