ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 10(3) 2011, 123-131*

CAPABILITY OF PRIMORDIA FORMATION AS A TRAIT DIFFERENTIATING WILD STRAINS OF *Agaricus bisporus* (Lange) Imbach AND *Agaricus arvensis* Schaeff. DERIVED FROM THE AREA OF WESTERN POLAND

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Abstract. The capability for the formation of primordia is one of the factors preconditioning their correct growth and development as well as high yields. The aim of the performed investigations was to evaluate the capability of primordia formation by several wild *Agaricus bisporus* and *Agaricus arvensis* strains derived from natural environment as well as cultivated strains of the above-mentioned species. It was demonstrated that the greatest numbers of primordia per 1 cm^2 were formed by the cultivated strain *A. bisporus* F59 (2.4 primordia/cm^2) and by the Ab/24/7 wild strain (2.2 primordia/cm^2). Similar numbers of primordia (2.1 primordia/cm^2) were developed by the Ar/21/I strain of the *A. arvensis* species. The cultivated strain of this species, i.e. KW7/35, formed only 0.9 primordium/cm^2.

Key words: button mushroom, horse mushroom, pinhead formation

INTRODUCTION

Agaricus bisporus (Lange) Imbach is one of the best known and common species of cultivated mushrooms in the world. In Poland, it has been widely cultivated since the 19th century. The *Agaricus arvensis* (Schaeff.) is a less known species used in commercial cultivations but it occurs very commonly in the natural environments of Europe, North America as well as Asia. Moreover, sporadically, it can also be found in Australia and New Zealand. These mushroom grow from May to October in well-spaced groups outside forests, most frequently on meadows, pastures, in scrubs and along forest edges.

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Fruiting bodies of this species are characterised by pleasant taste and smell of the anise [Michell and Walter 1999; Wojewoda 2003; Miller et al. 2006].

Proper growth and development of carpophores as well as high carpophore yields depends on many factors, among others, on the kind and quality of the applied substrate and cover [Visscher 1979; Noble et al. 2003], temperature and air humidity as well as on the amount of carbon dioxide present in the atmosphere in the cultivation facility. The level of yielding depends, to a considerable extent, on the capability of primordial development which is a species trait [Diamantopoulou and Philippoussis 2001; Vekiari et al. 2002, Pal et al. 2006]. The setting of carpophores is triggered off when the mycelium accumulates sufficient quantities of nutrients and overgrows the cultivation substrates completely. Mycelium hyphae begin to merge into thick threads or strings and create ball-like structures of diameters exceeding 1 mm which are referred to as carpophore primordia [Wood 1976; Umar and van Griensven 1997]. The formation of primordia is preconditioned by several important factors such as: substrate temperature, covering soil, humidity and concentration of carbon dioxide. The development of fruiting bodies is initiated by declining temperature and CO_2 concentration [Flegg and Wood 1985].

The covering soil characterised by special physical, chemical and microbiological properties is essential for the proper formation of carpophores of many mushroom species, among others: *Agaricus bisporus*, *A. arvensis*, *A. bitorquis*, *A. brasiliensis*, *Corpinus comatus* or *Stropharia rugoso-annulata* [Eger 1961; Flegg and Wood 1985]. This soil must also contain appropriate bacterial microflora in the form of such bacteria as: *Pseudomonas putida* [Hayes et al. 1969; Park and Agnihotri 1969] or bacteria from the *Bacillus* or *Alcaligenes* families [Fermor et al. 2000]. Wange et al. [2008] reported that they observed acceleration of primordia formation by 1 day following the application of a bio-fertiliser containing *Pseudomonas striata* bacteria, whereas Gitay and Wange [2007] obtained reduced dying of primordia of carpophores after the addition to the substrate, in the course of inoculation with mycelium, of a bio-fertiliser which contained bacteria from the *Azotobacter* genus.

The aim of the performed investigations was to ascertain the capability of primordia development of *Agaricus bisporus* and *Agaricus arvensis* wild strains derived from natural sites and to compare the obtained results with cultivated strains.

MATERIAL AND METHODS

Agaricus bisporus and *Agaricus arvensis* strains derived from natural sites were used in the described experiment. Their characteristics are presented in Table 1 and 2. The cultivated *A. bisporus* strain characterised by a very good yield-forming value Italspawn F59 and *A. arvensis* strain KW7/35 were used as control.

The trial was carried out in the biological laboratory of the Department of Vegetable Crops of Poznań University of Life Sciences in 2009. Spores were collected from carpophores and then mycelium was prepared using the method commonly employed in these kinds of investigations. The method was described by Sobieralski [1998].

Table 1. Strains of *Agaricus bisporus* used in the experiment
Tabela 1. Rasy *Agaricus bisporus* użyte w doświadczeniu

Strain Rasa	Date of harvest Data zbioru	Place of harvest Miejsce zbioru	Site Stanowisko
Ab/11/3	X 2007	Regional Directorate of State Forest (RDLP) Piła, Krzyż Forest District, Gieczynek Forest-range	brushes zarośla
Ab/2/14	X 2007	RDLP Piła, Krzyż Forest District, Wizany Forest-range	meadow łąka
Ab/17/8	X 2007	RDLP Szczecin, Bolewice Forest District, Lewice Forest-range	glade polana
Ab/1/14	IX 2008	RDLP Szczecinek, Miastko Forest District, Biały Bór Forest-range	glade polana
Ab/23/7	IX 2007	RDLP Szczecin, Dobrzany Forest District, Błotno Forest-range	Meadow łąka
Ab/28/2	IX 2009	RDLP Piła, Trzcianka Forest District, Wrząca Forest-range	meadow łąka
Ab/47/3	VIII 2009	RDLP Piła, Krzyż Forest District, Chojno Forest-range	glade polana
Ab/70/1	VIII 2007	RDLP Poznań, Konstantynowo Forest District, Krajkowy Forest	wasteland nieużytek
Ab/64/8	VIII 2008	RDLP Zielona Góra, Zielona Góra Forest District, Wilkanowo Forest-range	meadow łąka
Ab/53/2	IX 2008	RDLP Piła, Wronki Forest District, Pustelnia Forest-range	meadow łąka
Ab/19/13	VIII 2008	RDLP Szczecin, Dobrzany Forest District, Karkowo Forest-range	glade polana
Ab/11/5	IX 2007	RDLP Poznań, Piaski Forest District, Mchy Forest	meadow łąka
Ab/24/7	IX 2008	RDLP Szczecinek, Miastko Forest District, Sępólno Forest-range	meadow łąka
Ab/47/2	IX 2009	RDLP Szczecin, Kłodawa Forest District, Płomykowo Forest-range	meadow łąka
Ab/83/11	VIII 2007	RDLP Szczecinek, Miastko Forest District, Biały Bór Forest-range	meadow łąka
Ab/40/28	VIII 2008	RDLP Szczecin, Karwin Forest District, Gościnowo Forest-range	brushes zarośla
Ab/15/27	VIII 2008	RDLP Piła, Wronki Forest District, Lubowo Forest-range	wasteland nieużytek
Ab/93/6	IX 2009	RDLP Piła, Trzcianka Forest District, Jędrzejewo Forest-range	meadow łąka
F59	-	strain from the mushroom collection of the Department of Vegetable Crops of Poznań University of Life Sciences odmiana z kolekcji grzybów Katedry Warzywnictwa Uniwersytetu Przyrodniczego w Poznaniu	-

*Regional Directorate of State Forest – Regionalna Dyrekcja Lasów Państwowych;
Forest District – Nadleśnictwo,
Forest-range – Leśnictwo

The assessment of the primordia formation was carried out in glass cylinders of 600 cm^3 volume and 10 cm diameter. The experimental cylinders were filled to 2/3 of their height with wheat grain mycelium of the examined *A. bisporus* and *A. arvensis* strains. A 6-centimeter layer of peat cover was placed on the top mycelium surface and

then incubation was carried out at the temperature of 25°C and at 90–95% relative air humidity until the mycelium grew into the cover to the height of 5.5 cm. After incubation, the temperature was decreased to 15°C. Primordia which were formed on the surface of the cover until day 16 (*A. bisporus*) and day 20 (*A. arvensis*) from the moment of the temperature decrease were counted. The number of primordia was converted into 1 cm^2 of area. Detailed description of the method as well as the method of cover preparation were given by Sobieralski [1998].

Table 2. Strains of *Agaricus arvensis* used in the experiment
Tabela 2. Rasy *Agaricus arvensis* użyte w doświadczeniu

Strain Rasa	Date of harvest Data zbioru	Place of harvest Miejsce zbioru	Site Stanowisko
Ar/11/B	IX 2008	RDLP Szczecinek, Drawsko Forest District, Łowicz Forest-range*	brushes zarośla
Ar/7/2	X 2008	RDLP Szczecinek, Świerczyna Forest District, Buczyna Forest-range	brushes zarośla
Ar/7/11	IX 2008	RDLP Szczecinek, Manowo Forest District, Osetno Forest-range	brushes zarośla
Ar/75	IX 2007	RDLP Szczecinek, Ustka Forest District, Peplino Forest-range	wasteland nieużytek
Ar/7/N	VIII 2009	RDLP Piła, Krzyż Forest District, Żelichowo Forest-range	meadow łąka
Ar/23/A	VIII 2008	RDLP Szczecinek, Bytów Forest District,	wasteland nieużytek
Ar/21/I	VIII 2008	RDLP Szczecinek, Drawsko Forest District, Czertyń Forest-range	brushes zarośla
Ar/12/7	X 2007	RDLP Piła, Krzyż Forest District, Wizany Forest-range	brushes zarośla
Ar/12/S	VIII 2009	RDLP Szczecinek, Borne Sulinowo Forest District	brushes zarośla
Ar/11/9	IX 2009	RDLP Szczecin, Dobrzany Forest District, Karkowo Forest-range	brushes zarośla
Ar/34/5	VIII 2009	RDLP Szczecin, Kłodawa Forest District, Płomykowo Forest-range	wasteland nieużytek
Ar/34/9	IX 2008	RDLP Poznań, Piaski Forest District, Mchy Forest-range	wasteland nieużytek
Ar/35/1	IX 2008	RDLP Szczecin, Łobez Forest District, Zagorzyce Forest-range	wasteland nieużytek
Ar/36/3	X 2008	RDLP Piła, Trzcianka Forest District, Jędrzejewo Forest-range	brushes zarośla
Ar/39/4	IX 2008	RDLP Szczecinek, Miastko Forest District, Wołcza Forest-range	wasteland nieużytek
Ar/48/5	VIII 2007	RDLP Piła, Wronki Forest District, Chojno Forest-range	wasteland nieużytek
KW7/35	-	strain from the mushroom collection of the Department of Vegetable Crops of Poznań University of Life Sciences odmiana z kolekcji grzybów Katedry Warzywnictwa Uniwersytetu Przyrodniczego w Poznaniu	-

*Regional Directorate of State Forest – Regionalna Dyrekcja Lasów Państwowych;
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The experiment was established in a completely randomized design in 4 replications and 2 series. The results were analyzed using variance for two-factorial experiments at the level of significance of $\alpha = 0.05$. The results were discussed on the basis of mean values obtained from two series of experiments.

RESULTS AND DISCUSSION

The evaluation of carpophore setting requires a number of operations similar to complete cultivation of the common mushroom and is a fairly troublesome process. The selected assessment method – in glass cylinders of 600 cm^3 volume – is effective in the case of investigations carried out on a large number of strains due to limited cultivation area as well as to the amount of the applied substrate. At the same time, it is a fully reliable method and can be used in breeding work quite extensively [Sobieralski 1998].

The formation of primordia by *A. bisporus* and *A. arvensis* strains derived from natural sites varied considerably. In the case of *A. bisporus*, majority of the examined wild strains developed primordia significantly less readily than the cultivated strain. The number of formed primordia ranged from 0.4 to 2.4 primordia per 1 cm^2. On the other hand, majority of wild *A. arvensis* strains was characterised by a considerably higher number of set carpophores in comparison with the control. The number of formed fruiting bodies in the examined *A. arvensis* strains ranged from 0.3 to 2.1 primordia per 1 cm^2 (fig. 1).

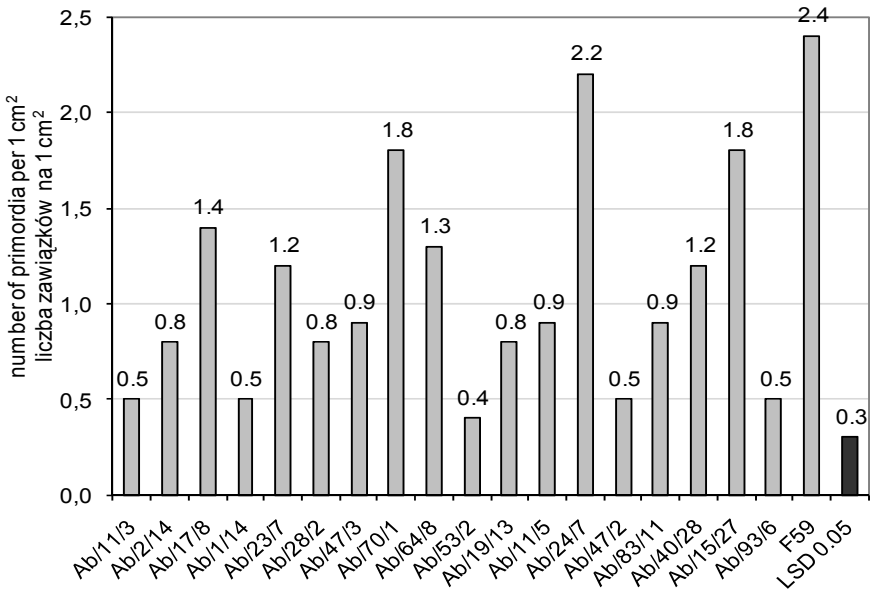

Fig. 1. Primordia formation of wild and cultivated strains of *A. bisporus*
Ryc. 1. Tworzenie zawiązków przez dzikie rasy oraz rasę uprawną *A. bisporus*

It was found that the greatest number of properly developed primordia per 1 cm^2 was formed in the case of the cultivated F59 strain of *A. bisporus* and as well as its wild strain Ab/24/7. The remaining examined *A. bisporus* strains exhibited a significantly worse capability for the formation of primordia in relation to the strains mentioned above. It was observed that among the examined strains, a high – albeit significantly lower – number of primordia was developed by strains Ab70/1 and Ab/15/27 (1.8). Even smaller quantities of primordia were found to be formed by the following strains: Ab/17/8, Ab/64/8, Ab40/28 and Ab/23/7 (respectively: 1.4, 1.3, 1.2 and 1.2 primordia per 1 cm^2). The remaining strains of *A. bisporus*, namely: Ab/11/5, Ab/47/3, Ab/83/11, Ab/19/13, Ab/2/14 and Ab/28/2 formed distinctly smaller numbers of primordia amounting to fewer than 1 properly developed primordium per 1 cm^2. The smallest number of primordia (from 0.4 to 0.5 primordia per 1 cm^2) was recorded in the case of the following strains: Ab53/2, Ab/11/4, Ab/11/3, Ab/47/2 and Ab/93/6.

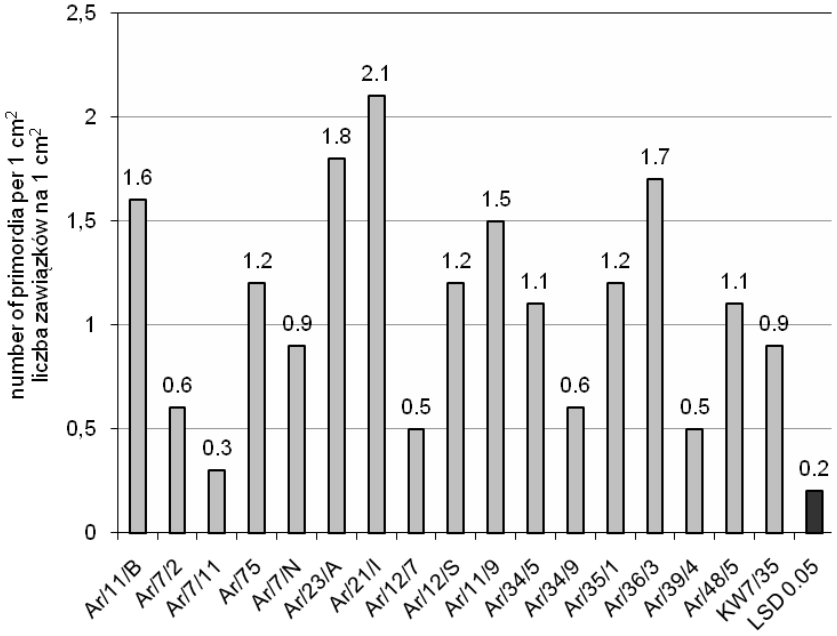

Fig. 2. Primordia formation of wild and cultivated strains of *A. arvensis*
Ryc. 2. Tworzenie zawiązków przez dzikie rasy oraz rasę uprawną *A. arvensis*

In the case of the *A. arvensis*, the highest number of properly formed primordia (2.1 primordia per 1 cm^2) was recorded in the case of the wild Ar/21/1 strain, while the cultivated KW/7/35 strain developed only 0.9 primordium calculated per 1 cm^2. High, albeit statistically significantly lower, numbers of primordia were formed by Ar/23/A (1.8), Ar/36/3 (1.7), Ar/11/B (1.6) and Ar11/9 (1.5) strains. Ar/75, Ar/12/S and Ar/35/1 strains formed 1.2 primordia per 1 cm^2 each, whereas Ar/34/5 and Ar/48/5

strains – only 1.1 primordia per 1 cm^2 each. The remaining strains, i.e. Ar/7/N, Ar/7/2, Ar/34/9, Ar/12/7 and Ar/39/4 formed fewer than 1 primordium per 1 cm^2 (0.5 to 0.9). The smallest number of primordia per 1 cm^2 (only 0.3 primordium per 1 cm^2) was developed by strain Ar/7/11 (fig. 2).

The performed investigations demonstrated that the capability to form carpophore primordia is a species-dependent trait which was also corroborated by experiments conducted by Wannet et al. [1999], Vekiari et al. [2002], Noble et al. [2003] as well as Pal et al. [2006].

The capability to form high numbers of primordia is an advantageous trait and can affect subsequent numbers of mature carpophores and the level of yields. Noble et al. [2003] maintain that not all developed primordia grow into mature fruiting bodies. This thesis was also corroborated by Seaby [1999] who showed that the yield level depended not only on the strain, substrate moisture content of temperature of the cultivation but also on the number of primordia formed on the cover surface.

Investigations show that the presence of bacterial microflora is not essential for the proper formation and development of fruiting bodies and may depend on strain. In his own experiments Kerrigan [1995] demonstrated that several strains obtained from natural environment exhibited readiness for carpophore development also in the case when no bacterial microflora was present in the cover, including covers from organic and non-organic substrates. Strains investigated in Kerrigan's [1995] experiments were capable to further proper carpophore development with no visible deformations. It is possible that low numbers of the developed fruiting bodies in our own studies resulted from the lack of bacterial microflora appropriate for the examined strains.

Other experiments carried out on the composition and preparation of the cover soil revealed that carpophore formation as well as yields were delayed in the case of covers subjected to sterilisation, while in the cover mixed with charcoal the process followed its standard rate. This appears to suggest that the presence of microorganisms does not aid carpophore formation but rather causes inactivation of inhibitors of primordia formation by mycelium [Eger 1961; Long and Jacobs 1974; Wood 1976].

There is little information, both in Polish and world literature, about correlations between the capability of primordia formation and common mushroom strains. Moreover, this also refers to mechanisms (especially molecular) responsible for the formation and development of carpophores. Therefore, it seems necessary to carry out further investigations in this field.

CONCLUSIONS

1. The examined wild strains of *A. bisporus* and *A. arvensis* derived from natural sites were characterised by varying capabilities of primordia formation.

2. Only one of the examined wild *A. bisporus* strain, i.e. Ab/24/7, formed the number of primordia similar to that of the cultivated strain. The remaining strains were characterised by worse capability for primordia formation.

3. In comparison with the cultivated strain of *A. arvensis*, i.e. KW/7/35, the majority of the examined wild strains of this species was characterised by a higher or similar number of developed primordia.

4. The highest number of developed primordia was recorded in the wild strain of *A. arvensis* designated as Ar/21/1.

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ZDOLNOŚĆ WIĄZANIA OWOCNIKÓW JAKO CECHA RÓŻNICUJĄCA DZIKIE RASY *Agaricus bisporus* (Lange) imbach I *Agaricus arvensis* Schaeff. POCHODZĄCE Z TERENU POLSKI ZACHODNIEJ

Streszczenie. Zdolność do tworzenia zawiązków owocników jest jednym z czynników warunkujących ich prawidłowy wzrost i rozwój oraz wysoki plon. W badaniach dokonano oceny zdolności tworzenia zawiązków owocników przez kilkanaście dzikich ras *A. bisporus* oraz *A. arvensis* pozyskanych ze środowiska naturalnego, a także ras uprawnych wymienionych gatunków. Wykazano, że największą liczbę zawiązków na 1 cm^2 wytworzyła rasa uprawna *A. bisporus* F59 (2,4 szt./cm^2) oraz rasa dzika Ab/24/7 (2,2 szt./cm^2). Podobną liczbę zawiązków wytworzyła rasa Ar/21/I (2,1 szt./cm^2) gatunku *A. arvensis*. Rasa uprawna tego gatunku, tj. KW7/35 wytworzyła zawiązki tylko w liczbie 0,9 szt./cm^2.

Słowa kluczowe: pieczarka dwuzarodnikowa, pieczarka polowa, tworzenie zawiązków

Accepted for print – Zaakceptowano do druku: 1.06.2011