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ANALYSIS OF FERTILIZATION PROCESS IN RYE (*SECALE CEREALE* L. SSP. *CEREALE*) UNDER DIFFERENT GREENHOUSE CONDITIONS

Summary

The purpose of the work was to assess the germination process and the growth of pollen tubes in rye Secale cereale L. ssp. cereale) growing in greenhouse conditions taking into account different temperature conditions and exposures to light. The rye cultivar Dańkowskie Złote, was selected for the experiment and cultivar Dańkowskie Nowe as a control. Microscopic analysis were carried out using fluorescent techniques staining the preparations with aniline blue. Analysis were performed after 10 min, 1 h, 2 h and 3 h from the pollination. The results indicate a negative effect of greenhouse conditions on pollen germination, pollen tube growth and the fertilization process in rye Secale cereale (L.). The optimal greenhouse conditions for plants flowering in winter time are: exposure to the artificial lighting over plants in single day-phase 16 hours long and temperatures around 25°C for day. Temperatures lower than 20°C clearly interfere with the process of pollen grain germination, the growth of pollen tubes and the fertilization process, which in turn leads to the lower fertility and yielding of plants. In the process of producing new hybrid varieties and seed production in open-pollinated plants, plastic tunnels and greenhouses are used as isolators. In case of reproduction of the single plants or lines, selecting the optimal conditions for the fertilization process is of key importance.
***Key words**: Secale cereale ssp. cereale, rye, fertilization, pollen tube growth, temperature*

ANALIZA PROCESU ZAPŁODNIENIA U ŻYTA (*SECALE CEREALE* L. SSP. *CEREALE*) W RÓŻNYCH WARUNKACH SZKLARNIOWYCH

Streszczenie

Celem pracy była ocena procesu kiełkowania i wzrostu łagiewek pyłkowych u żyta Secale cereale L. ssp. cereale rosnącego warunkach szklarniowych, z uwzględnieniem różnych warunków temperaturowych i naświetlenia. Do doświadczeń wybrano odmianę uprawną żyta: Dańkowskie Złote a kontrolą była odmiana Dańkowskie Nowe. Przeprowadzono analizy mikroskopowe z zastosowaniem techniki fluorescencyjnej barwiąc preparaty błękitem anilinowym. Analizowano słupki utrwalone po: 10 min, 1 h, 2 h, 3 h od zapylenia. Wyniki wskazują na negatywny wpływ warunków szklarniowych na proces kiełkowania ziaren pyłku, wzrost łagiewek pyłkowych i zapłodnienie u żyta Secale cereale (L.). Optymalne warunki w szklarni dla roślin kwitnących w okresie zimy, to doświetlanie jednofazowe przez 16 godzin i temperatura ok. 25ºC w fazie dnia. Temperatury niższe od 20ºC i wyższe od 30ºC wyraźnie zakłócają proces kiełkowania ziaren pyłku, wzrost łagiewek pyłkowych i zapłodnienie, co w konsekwencji prowadzi do obniżenia płodności roślin i plonowania. W procesach wytwarzania nowych odmian heterozyjnych i produkcji nasiennej u roślin obcopylnych, stosuje się izolatory w postaci tuneli foliowych i szklarni. W przypadku rozmnażania pojedynków czy linii w procesie hodowli, dobranie optymalnych warunków do zapłodnienia to jedno z najistotniejszych zagadnień.
***Słowa kluczowe**: Secale cereale* ssp. *cereale, żyto, zapłodnienie, wzrost łagiewek pyłkowych, temperatura*

1. Introduction

For many years researchers specializing in many disciplines have pointed out the problem of global warming. It has been observed an increase in mean annual air and ocean temperatures, melting of ice caps and glaciers as well as an increase in the global average sea levels [1]. Comparable changes are also observed in Poland [2, 3]. When Michalska [4] was analyzed the course of weather conditions in a multi-annual period recorded in the 24 weather stations in Poland, she stated, that trends for mean annual temperature in the last two decades show a marked, significant increase over most of Poland's territory, particularly northern and western parts of the country. According to Michalska [4], in March, May and August the upward trend for temperatures in most weather stations of the Institute of Meteorology and Water Management is statistically highly significant and ranged from 0.2 to 0.6°C for the period of 10 years, while in the north of Poland also in April the average increase is 0.3°C. The observations indicate that the greatest increase in temperatures is found in early spring and spring. Such changes are also reported by farmers and crop breeders (unpublished information). The adequate growth and development conditions in the spring are necessary for the appropriate course of flowering, pollination and seed setting. Days in May with temperatures over 25°C contribute to the development of adverse conditions for the development of viable pollen grain and fertilization in greenhouses and plastic tunnels, where temperatures exceed 35°C. Authors faced a similar problem when conducting researches on rye [5]. Also Kolasińska [6, 7] reported that temperatures of approx. 35°C found in plastic tunnels had an adverse effect on the formation of fertile pollen grain and yield.

Rye (*Secale cereale* L. *ssp. cereale*) is an allogamous, wind pollinated and highly self-incompatible species [8]. The structure of the floret and reproductive organs is typical

of the family *Poaceae*. The pistil has no style and the stigma divided into two parts is pinnate. The role of numerous, multi-cellular papillae and hairs surrounding the ovary is to capture and hold pollen grain carried by the wind. Three anthers produce huge amounts of the threenucleate pollen grain. Under natural conditions the rye is flowering at a temperature of approx. 12°C [9] and pollen grain retains germinability for 5-7 days [10]. At the moment when pollen grain falls on the stigma, an interaction takes place between tissues of the female gametophyte (pistil, embryo sac) and pollen grain – the male gametophyte. The first stage in this interaction consists in grain hydratation, which is followed by the transfer of enzymes and glycoproteins responsible for the recognition of pollen by the gametophyte [10, 11, and 12]. Many authors indicate that ambient temperatures have a significant effect on the entire fertilization process [6, 7, and 8]. In order to accelerate the vegetation cycle, breeders frequently sow seeds in greenhouses at dates differing considerably from the recommended sowing dates. The target of presented study was to investigate reactions occurring on the stigma in rye, when flowering takes place under greenhouse conditions deviating greatly from those at natural sowing dates and pollination occurs at various temperature conditions. Insight into the reaction between the stigma and pollen grain will facilitate selection of the optimal conditions, particularly in research and breeding work conducted on rye.

2. Material and methods

Experiments were conducted on the rye cultivar: *Secale cereale* ssp. *cereale*: Dańkowskie Złote. Sowing material (elite) came from Danko Breeding Company Ltd. in Choryń in Poland. Experiments were carried out under artificial - greenhouse conditions, in 3 variants, including: a) temperature during plant growth and flowering, b) lighting, c) the period, in which plants were growing and pollination and fixation of materials for analyses were performed. The control, cultivar Dańkowskie Nowe was conducted in the greenhouse in May and under natural conditions in the field at dates recommended for cultivation operations. The experiments were conducted in greenhouses and in the experimental garden of the Department of Genetics and Plant Breeding, PULS, in 2009.

In order to conduct greenhouse experiments at dates differing from natural dates, it was necessary to perform artificial vernalization. Thus kernels were germinated and 1-week old seedlings were placed in a vegetation chamber at temperature of +1 to +4^{o}C for 8 weeks. After vernalization, plants were grown in a greenhouse until flowering, when material was collected for microscopic analyses. Germination and planting dates were selected so that the period of flowering came in April and March. In the control experiment sowing was performed at the end of September 2008 and plants underwent natural vernalization during the winter.

The experiments were conducted under greenhouse conditions in winter time and plants were grown under artificial light of lamps with Son-T Agro 400 light bulbs by Philips. In all the greenhouse experiments the length of the "daylight" phase was 16 h, which is consistent with the day length in Poland during rye flowering, i.e. in May. Temperature in the greenhouse was regulated by changing the location of lamps over plants. In three greenhouse experiments different temperature and lighting conditions were applied during the period of flowering:

Experiment 1 was conducted in January at a temperature of 8°C (night) and 12°C (day). In order to maintain the required temperature artificial single-phase lighting was used at the level of ears from 5 a.m. to 9 p.m. in the vicinity of plants, but not directly above them.

Experiment 2 was conducted in January at a temperature of 10°C (night) and 14°C (day). Artificial single-phase lighting was used from 5 a.m. to 9 p.m. directly onto plants.

Experiment 3 was conducted in January in the greenhouse at a temperature of 14°C (night) and up to 25°C (day). Artificial single-phase lighting was used from 5 a.m. to 9 p.m. directly over plants.

Control 1 was established in the greenhouse in May with no temperature regulation. Plants wintered in the field and in March they were transplanted to the greenhouse. In May when the plants flowered, in the greenhouse the diurnal maximum temperature ranged from 20 to 37°C and pollination and sample collection were conducted at a temperature of 29 - 37°C. The temperature in the greenhouse depends of daily weather.

Control 2 was established in the field in the autumn of the previous year. Plants grew and flowered at natural dates and conditions. During flowering (in May) controlled pollination was performed and material for microscopic analyses was fixed. During flowering the diurnal maximum temperature ranged from 16 to 21°C and pollination and sample collection were conducted at a temperature of 17-18°C. The control cultivar was Dańkowskie Nowe.

After heading from leaf sheaths the androecium was removed from florets and bag isolators were placed on castrated ears. Controlled pollination was performed at 6 to 12 days after castration, when stigmas emerged from glumelles. Pollen grain viability was assessed for each cultivar in all the experiments, based on the analysis of preparations stained with the Belling solution (acetocarmine 2% + glycerin at 1:1) [13].

Within each experiment a total of 10-15 stigmas were collected at 10min, 1h, 2h, 3h and 4h after pollination and they were fixed in the Carnoy fixing solution (95% EOH + CH_3 COOH at 3:1) [13]. Aniline blue was applied in staining of microscopic slides [14]. Observations were conducted using a fluorescence microscope. The following parameters were recorded: a) the number of pollen grains on the stigma, b) the number of germinating pollen grains on the stigma, c) the number of pollen tubes reaching the ovary (base of stigma), d) the number of pollen tubes reaching the micropyle of ovule (ovary), e) the length of pollen tubes, f) atypical phenomena.

A 6–point scale was used to describe the observations:

- 0 – no pollen grain/pollen tubes on the stigma
- 1 – 1-5 pollen grain/pollen tubes on the stigma,
- 2 – 6-15 pollen grain/pollen tubes on the stigma,
- 3 – 16-30 pollen grain/pollen tubes on the stigma,
- 4 – 31-50 pollen grain/pollen tubes on the stigma,
- 5 – > 51 pollen grain/pollen tubes on the stigma.

The distance covered by pollen tubes was measured with the stage micrometer and was counted to µm. The mean distance from a) the end of the stigma to the ovary and b) from the end of the stigma to the micropyle was calculated based on measurements from 5 pistils of each cultivar. A total of 5 short pollen tubes and 5 long pollen tubes

were measured from each time point and the results were averaged.

3. Results

3.1. Assessment of pollen grain viability

Irrespective of the conditions, under which plants were growing and flowering, viability of pollen was similar and ranged from 82,9% to 92,3% (Tab. 1). The lowest viability was observed in plants flowering in May in the greenhouse, i.e. at the highest temperatures. Detailed results were given in Table 1.

3.2. Assessment of pollen germination and pollen tubes penetration

Average distance towards defeating by pollen tubes for two cultivars was calculated. From the end of the stigma to the base of stigma it was 3147 µm and from the end of the stigma to the micropyle it was 4031 µm . Detailed results of microscopic analysis were given in Table 2.

In experiments 1, 2, and 3, conducted in the greenhouse at dates different from natural, numerous irregularities were observed. In experiments 1, and 2 pollen grain abundance was poor or medium (except for one case with up to 30 grains). Germination of pollen tubes on the stigma was very weak (up to 5); the tubes were short and after 3 h did not reach the style (Fig. 1). No pollen tubes were observed in the ovary. Numerous irregularities were observed: the callose reaction manifested in pollen grains and pollen tubes, pollen tubes entwined or twisted around the grain, pollen tubes branched and broken. The recorded diminishing number of pollen grains on stigmas with the passing time from pollination indicates that grains were not anchored and were washed out in the course of slide preparation.

Table 1. Pollen viability of three cultivars of rye (*Secale cereale* L ssp. *cereale.)* determined in the different temperature conditions.
Tab. 1. Żywotność ziaren pyłku u trzech odmian żyta (Secale cereale L ssp. cereale.) określona w różnych warunkach temperaturowych.

No of experiment	Cultivar	Month	Place of the experiment	Mean temperature night/day [ºC]	Maximal day temperature [ºC]	Mean pollen viability [%]
Experiment 1.	Dańkowskie Złote	January	greenhouse	8/12	12	84,3
Experiment 2.	Dańkowskie Złote	January	greenhouse	10/14	14	85,1
Experiment 3.	Dańkowskie Złote	January	greenhouse	14/25	25	86,4
Control 1.	Dańkowskie Nowe	May	greenhouse	18/27	37	82,9
Control 2.	Dańkowskie Nowe	May	field	4,5/18,5	23	92,3

The temperatures were measured in 2009 year in greenhouse and garden of Dep. Gen. and Pl. Breed. PULS
Pomiary temperatury wykonano w maju 2009 r w szklarni i ogrodzie Kat. G i HR UP w Poznaniu.

Source: own work / Źródło: opracowanie własne

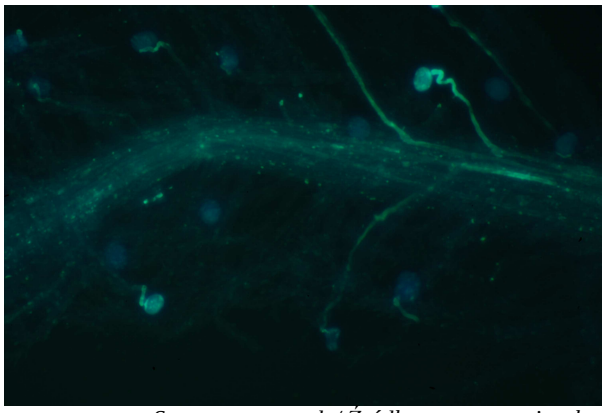
Table 2. Germination of pollen grain and penetration of pollen tubes into the ovary in (*Secale cereale* ssp. *cereale* L.) in the different condition of greenhouse and in the field
Tab. 2. Kiełkowanie ziaren pyłku i wnikanie łagiewek pyłkowych do zalążni żyta (Secale cereale ssp. cereale L.) określona w różnych warunkach szklarniowych i w polu.

Place	Time from pollination	Pollen grain abundance*	The number of pollen tubes			The length of pollen tubes [µm]	
			stigma	base of stigma	ovary	minimal	maximal
Experiment 1.							
greenhouse	10”	2	1	0	0	10	40
	1h	3	1	0	0	10	100
	2h	2	1	0	0	100	150
	3h	2	1	0	0	100	250
Experiment 2.							
greenhouse	10”	4	1	0	0	10	30
	1h	2	1	0	0	70	200
	2h	3	1	0	0	100	250
	3h	2	1	0	0	100	500
Experiment 3.							
greenhouse	10”	5	1	0	0	10	400
	1h	5	4	0	0	20	550
	2h	5	3	1	0	20	2200
	3h	5	3	1	1	100	3900
Control 1.							
greenhouse	10”	4	1	0	0	10	200
	1h	3	2	0	0	10	1200
	2h	3	3	1	0	20	2400
	3h	4	3	1	1	20	3100
Control 2.							
field	10”	3	3	0	0	10	400
	1h	3	4	0	0	20	2500
	2h	4	5	2	1	40	3500
	3h	5	5	3	2	100	4000

*a 6-point scale was applied (as described in the text)
**Zastosowano skalę 6-stopniową (opis w tekście)*

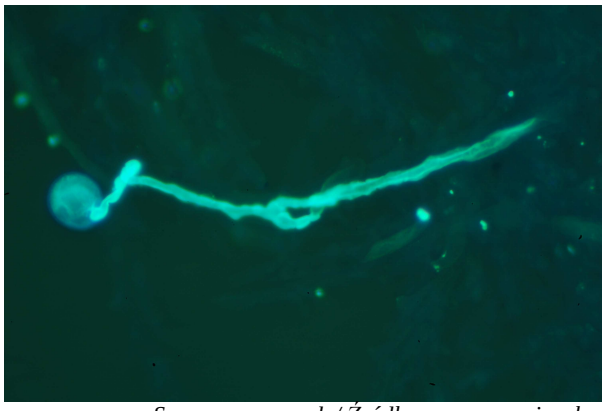
Source: own work / Źródło: opracowanie własne

Results of experiment 1 and 2 showed that at a temperature 12°C and at poor lighting during flowering, and 14°C (day) with the single-phase lighting from 5 a.m. to 9 p.m. directly onto plants, the process of germination and penetration of pollen tubes was completely inhibited (Fig. 1 and 2). Stigmas in experiment 1 were curved and papillae were clustered providing no conditions for pollen grain settlement. Such a structure of pistils shows that they were not prepared to accept pollen grain and to fertilization.



Source: own work / Źródło: opracowanie własne

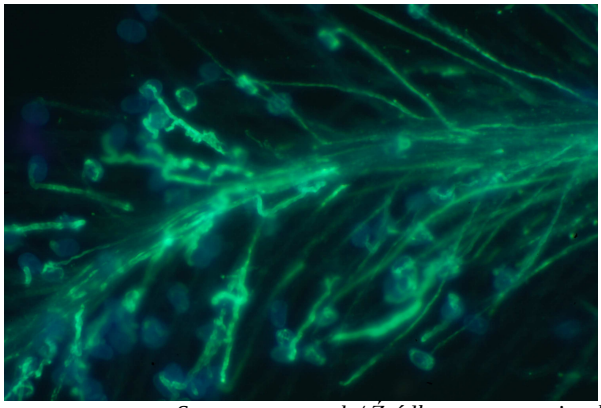
Fig. 1. Very weak pollen grain abundance and pollen tubes germination on the stigma of rye *(Secale cereale* L. ssp. *cereale*) 2 h after pollination in a greenhouse at a temperature 14°C. (Experiment 2)
Rys. 1 *Słabe opylenie, kiełkowanie ziaren pyłku i wzrastanie łagiewek pyłkowych na znamieniu. żyta (Secale cereale ssp. cereale L.) po 2 h od zapylenia w szklarni w temperaturze 14°C. (Doświadczenie 2)*



Source: own work / Źródło: opracowanie własne

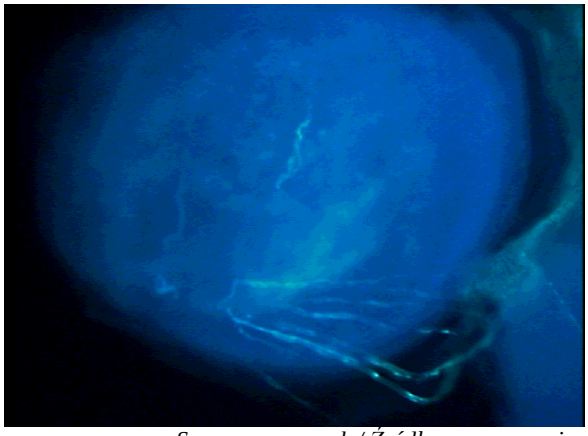
Fig. 2. The branched and broken pollen tube with callose reaction in rye *(Secale cereale* L. ssp. *cereale*) 2 h after pollination in a greenhouse at a temperature 16°C. (Experiment 4)
Rys. 2. *Rozwidlona łagiewka pyłkowych z reakcją kalozową na znamieniu żyta (Secale cereale L. ssp. cereale) po 2 h od zapylenia w szklarni w temperaturze 16°C. (Doświadczenie 4)*

In experiment 3, conducted in the greenhouse at a temperature of 25°C, pollen grain abundance was very high and numerous pollen grains germinated at all the analyzed time points (considerably exceeding 50). Pollen tubes already after 2 h reached the base of stigma and after 3 h single pollen tubes were observed in the ovary. In 5 cases out of the 15 tested penetration of pollen tubes to the micropyle was recorded (Fig. 4). Moreover, reactions were also observed indicating an inappropriate pistil/male gametophyte interaction. They were similar irregularities to those described above.



Source: own work / Źródło: opracowanie własne

Fig. 3. Intensive pollen grain abundance and pollen tubes germination on the stigma in rye *(Secale cereale* L. ssp. *cereale*) 3 h after pollination in a greenhouse at a temperature *25°C*. (Experiment 3)
Rys. 3. Intensywne opylenie, kiełkowanie ziaren pyłku i wzrastanie łagiewek pyłkowych na znamieniu żyta (Secale cereale L. ssp. cereale) po 3 h od zapylenia w szklarni w temperaturze 25°C. (Doświadczenie 3)



Source: own work / Źródło: opracowanie własne

Fig. 4. Ovule and pollen tubes penetration into the micropyle in rye *(Secale cereale* L. ssp. *cereale)* 3 h after pollination in a field condition. (Experiment 6)
Rys. 4. Zalążek i łagiewki podążające do milkopyle u żyta (Secale cereale L. ssp. cereale) po 3 h od zapylenia w warunkach polowych. (Doświadczenie 6)

The control no 1, conducted in the greenhouse in May, with oscilation of the temperature inside couses the temperature outside, pollen grain abundance was medium (up to 50 grains) and germination intensity reached about 30 pollen tubes on the stigma after 1 h. After 2 h single pollen tubes were observed in the style and after 3 h - in the ovary. Pollen tubes reached maximum length of 3050 µm, they did not reach the micropyle. At all the time points large numbers of very short pollen tubes were recorded (20-50 µm). Numerous irregularities were observed, e.g. the callose reaction manifested in pollen grains and pollen tubes, breaking of pollen tubes, strongly twisted pollen tubes and penetration in a wrong direction. No pollen tubes at the micropyle were observed after 3 h.

The control no 2 conducted in the field, pollen grain abundance at all analyzed time points was very high. Pollen grains were found on stigmas in numbers considerably exceeding 100. Already after 10 min intensive germination of pollen tubes was observed and the number of germinated

grains increased with time from pollination, so that after 2 h it exceeded 100 pollen grains. After 2 h numerous pollen tubes were seen penetrating through stigma tissues towards the ovary. At the base of the stigma the number of pollen tubes was also very high; however, it dropped drastically within the ovary to several only. Single pollen tubes reached the micropyle. In 8 cases out of 15 analyzed penetration of pollen tubes to the embryo sac was recorded (Fig 4), i.e. after 3 h it was fertilized. The reported irregularities included single branching pollen tubes, the callose reaction and twisted pollen tubes.

4. Discussion

High viability of pollen grain observed in all cases (Tab. 1) indicates that variable plant growth and development conditions did not have a negative effect on the process of development of male gametophytes. The lowest mean viability was recorded for plants growing in the greenhouse and flowering in May, when maximum diurnal temperature ranged from 21 to 37°C. Similarly as Kolasińska [6, 7], the authors also stated a negative effect on viability of pollen grain in rye (*Secale cereale* L.) for conditions found in greenhouses or plastic tunnels, particularly high temperatures. The slight decrease in viability to 82.9%, observed in control 1 should not have a negative impact on the process of fertilization. Pollen grain coverage of stigmas was abundant and grain germination was intensive; however, in comparison to processes occurring under natural conditions (control no 2) penetration of pollen tubes was markedly slowed. After 3 h only single pollen tubes reached the base of stigmas, while under natural conditions after such a time pollen tubes were seen at the micropyle (Fig. 4). It may be assumed that after a longer time fertilization might have occurred. This is what actually happens, since crop is obtained when cultivating plants under identical artificial conditions, although it is lower than normally expected [5, 6, 7]. The slowed process of pollen tube penetration seems to be caused by the negative effect of elevated temperatures in the greenhouse, reaching 37°C on warm days. Such extreme conditions resulted in reduced turgor in plants, and thus also in stigmas, which in turn disturbed the process of pollen grain hydratation and nutrition of pollen tubes.

After a pollen grain fall on the stigma, the first stage consists on its hydratation followed by recognition. Within the first few minutes the pollen grain is either accepted or rejected [8, 11]. Studies on *Arabidopsis* [15] indicate that lipids play a key role in the initial process of pollen grain recognition and next its anchoring, germination and growth of the pollen tube. Grain hydratation takes place through tunnels in the plasma membranes of stigma cells [16-25]. Helsop-Harrison reported that in rye hydratation of pollen grain takes place 2 min 22 s after contact [26]. If the hydratation process is disturbed, pollen grain germination or penetration of pollen tubes into the stigma tissue is inhibited. Studies conducted by many authors confirmed the active role of pistils and the female gametophyte in the process of recognition and acceptance of the male gametophyte and the further process of pollen tube growth [25]. In these processes the key role is played by the proteins in the regulation of water flow between cells [24]. Water content in the grain settled on the stigma increases as a result of its release from the stigma. This drastic change in water content requires a cascade of a whole series of structural, mechanical and molecular mechanisms in pollen grain so that it may germinate into the pollen tube, penetrate into the pistil tissues and successfully deposit two spermatozoids to the embryo sac [23, 26, and 27]. Two major factors determine the process of male gametophyte/female gametophyte interactions, with the first factor being the competition between pollen tubes, while the second factor consists in the modulation of these differences by the gynoecium [20]. They cover physical and physiological barriers as well as the genetic pollen grain–pistil interaction. Physiological limitations may be determined by the availability of nutrients, since most substances comprising the pollen tube wall are collected from the stigma. Thus pistil tissues participate not only in the initial process of male gametophyte recognition and its anchoring, but also in further processes of growth, nourishment and targeting of pollen tubes [24, 25]. For the appropriate course of these processes environmental conditions have to be adequate for a given species, with ambient temperature being one of the major factors in this respect. Studies conducted on different plant species indicate a significant relationship of the volume of seed yield with temperature during flowering [11]. Species vary in terms of their temperature requirements and the differences result from evolutionary changes adapting plants to different habitat conditions.

Karapanos et al. [28] investigated the effect of high temperature (30°C) on respiration of pollen grains in tomato in an in vitro experiment. Respiration is a key metabolic process in pollen grains and it affects hydratation and germination processes as well as growth of pollen tubes. Studies showed that ambient temperature has a significant effect on respiration intensity and thus affects the process of fertilization. The same authors stated that low light reduces the photosynthetic capability of the plant and therefore the availability of substrates (sugars) for pollen metabolism. In the presented study we observed phenomena, which have a negative effect on temperatures above 30°C. Despite high pollen grain abundance many grains did not germinate and growth of pollen tubes was markedly slowed. An intensive callose reaction was observed, manifested in pollen grains and in pollen tubes. This reaction was stronger in comparison to the control run under natural conditions. It may be assumed that this strengthening was caused by the death of poorly hydrated pollen tubes. The process of embryo targeting was also disturbed, as evidenced by the pollen tubes growing in the opposite direction. These observations indicate that under the conditions of contrel1 the gynoecium did not function properly. This may be attributed to the negative effect of high temperatures in the greenhouse, reaching 37°C on warm days. Such extreme conditions resulted in a decreased turgor in plants, thus also in stigmas, and this in turn disturbed the process of pollen grain hydratation and pollen tube nourishment.

The rejection reactions were observed in all experiments. To a considerable degree it was a normal reaction for rye as a self-incompatible plant. Under natural conditions many pollen grains fall on the stigma and germinate into pollen tubes; however, the number of pollen tubes growing towards the ovary successively decreases so that the ovary is reached by a greatly limited number of pollen tubes and only single tubes reach the micropyle [19]. In cereals the ovary contains only one embryo sac. Only one pollen tube transporting two spermatozoids is required for successful fertilization. The system of self-incompatibility found in rye eliminates many pollen tubes, which genes are identical to those of the plant accepting the pollen grain, i.e. incompatible [15, 16]. However, within the huge number of grains falling in the stigma there should be a sufficient number of these genetically compatible. It is only crucial for them to have optimal conditions to penetrate into pistil walls.

Temperature conditions below 12°C and low lighting of plants (experiment 1) turned out to be particularly adverse for fertilization. Despite the temperature during flowering adequate for rye [9], the process of pollen grain anchoring as well as germination and growth of pollen tubes was most considerably hindered among all the tested variants. Since grains

were viable (84.3%), causes may be attributed to the stigmas not being prepared to accept male gametophytes. An adequate amount of light is a key factor in the appropriate course of physiological process in plants. Conditions of experiment 2 proved to be equally disadvantageous. The process of pollen grain anchoring was very weak and a very low number of pollen grains germinated into pollen tubes. After 3 h pollen tubes were too short to reach the embryo.

Among the three greenhouse experiments, the conditions of experiment 3, i.e. at a temperature 25°C, proved to be the most advantageous for germination of pollen tubes. The number of anchored grains was very high and they germinated intensively. Initially they formed bundles composed of numerous pollen tubes. Similarly as Hormaza et al. [29], a successive elimination of pollen tubes was observed with the shortening distance to the ovary. Only a very limited number of several pollen tubes penetrated into the ovary. The rate at which pollen tube reached the embryo was similar as in the field experiment (control 2), as fertilization was observed after 3 h.

5. Conclusions

1. The significant and negative effect of greenhouse conditions was observed in the process of pollen grain germination, pollen tube growth and fertilization in rye *Secale cereale* (L.).
2. Optimal conditions in greenhouses for plants flowering during winter time are: lighting in a single-phase 16 hours long directly over plants and a temperature of approx. 25ºC during the day phase.
3. Temperatures below 20ºC and over 35ºC markedly disturb germination of pollen grains, growth of pollen tubes and fertilization.
4. Results indicate that studies on the process of pollination and fertilization should not be conducted under artificial conditions and in periods deviating from natural, due to the high probability of erroneous conclusions being drawn as a result of considerable disturbances in the germination of pollen grain and growth of pollen tubes and as a consequence – reduced fertility of plants.

6. References

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