

## Vigour evaluation of cocksfoot (*Dactylis glomerata* L.) seeds of different age

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### Abstract

Cocksfoot seeds cv. Baza and Bepro, harvested in 1986, 1985, 1984, and 1983 and stored in a store-house were studied. Seed lots did not differ greatly in their viability. However, significant reduction in seed vigour due to natural ageing as measured by soil emergence, seedling growth rate, and ethanol and osmotic stress tests was noted. A conductivity test did not indicate vigour changes of ageing seeds, and its correlation with field emergence was poor. The methodical aspects of vigour evaluation of cocksfoot seeds are discussed.

### INTRODUCTION

Stored seeds undergo ageing at a rate depending on the genetic properties of seeds, preharvest effects, seed maturity, and the influence of external factors, mainly moisture, temperature, and oxygen pressure (Grzesiuk and Kulka, 1981; Justice and Bass, 1978; Roberts, 1981). Ageing in seeds involves their deterioration due to structural, compositional, and functional changes within the tissues (Abdul-Baki, 1980; Bewley and Black, 1982; Górecki et al., 1985; Powell and Matthews, 1977; Roberts and Ellis, 1982; Ross, 1980). Vigour is the best indicator of the degree of seed deterioration because it decreases earlier and faster than viability (Copeland, 1976; Górecki, 1986).

Much work in the past has been done on the vigour examination of both naturally and artificially aged seeds of cereals and legumes (Ching, 1982; Górecki, 1982; Górecki and Harman, 1987; Grzesiuk and Tłucz-kiewicz, 1982). In this work we evaluated the vigour of naturally aged cocksfoot seeds. Different methods of vigour evaluation are also discussed.

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## MATERIALS AND METHODS

Cocksfoot (*Dactylis glomerata* L.) cv. Baza and Bepro seeds harvested in 1983, 1984, 1985, and 1986 at the Plant Breeding Station near Olsztyn were studied. After harvest seeds were dried at room temperature and stored in linen bags in a store-house under ambient conditions. Before starting experiments, seed lots were transferred into the laboratory and stored at 2-3°C and 60% relative humidity. Such conditions protected seeds from appreciable deterioration during the course of this study. All experiments were performed in the spring of 1987, when seed dormancy was over. One seed lot (collected from 2-3 sublots) represented each year and cultivar.

## LABORATORY GERMINATION AND SOIL EMERGENCE

Germination was assayed by placing 100 seeds on filter paper (Whatman No. 3) in a Petri dish moistened with 4 ml deionized water at 20°C. After 7 and 21 days, germination rate and germination capacity were recorded, respectively (International Seed Testing Association, 1976).

The soil emergence test was performed in the greenhouse (on 4 May) and in the field (on 25 May). Each time 100 seeds were sown by hand 5 mm deep in rows 1 m long in a medium loam soil. There were eight replicates and emergence was counted twice during a period of four weeks.

## VIGOUR DETERMINATION

Seedling growth rate analysis. 50 seeds were placed between moistened rolled blotters at 20°C. After 5 and 10 days the length of the coleoptile and radicle and the fresh weight of seedlings were determined.

Conductivity of leachates. For the analysis of leachates, 1 g of seeds was rinsed with deionized water, then soaked in 100 ml deionized water at 20°C for 24 hours. The electrical conductivity of the water was measured using a Radelkis OK-102/1 conductivity meter. The reading was expressed in  $\mu\text{S}$  per g seed air dry mass.

Osmotic stress. One hundred seeds of each lot were germinated on filter paper (Whatman No. 3) in a Petri dish in the presence of 4 ml 5, 10, 11, 12, 13, 14, 17.5 or 20% (w/v) polyethylene glycol (PEG) 6000 (Carbowax) water solution. Seed samples germinated in the presence of water were controls. Osmotic equivalents of PEG solutions were calculated according to Michel and Kaufmann (1973) and were: -57KPa, -163KPa, -190KPa, -219KPa, -250KPa, -283KPa, -318KPa, -524KPa, respectively. After 7 days of germination at 20°C (germination rate), counts were made of viable seeds.

Ethanol stress. Samples of 100 seeds were placed in a 125 ml Erlenmeyer flasks containing about 20 ml of 5, 10, 15 and 20% (v/v) ethanol. Flasks were stoppered with rubber stoppers and left for 0, 2, 4, 6, or 24 hours at 20°C; then the alcohol was poured off, and the seeds were washed with deionized water and tested for germination as above. Non-treated seeds were used as controls.

All germination and vigour tests were conducted at least four times in two series.

## RESULTS

### 1. GERMINATION AND SEEDLING EMERGENCE

Cocksfoot seeds lost their viability rather quickly as measured in terms of speed of germination (Fig. 1). Germination capacity showed smaller differences in seed quality and ranged between 93% (1-year-old seeds) and 75% (4-year-old seeds). Both indicators pointed, however, to more rapid ageing of seeds of cv. Baza than Bepro.

Soil emergences also depended on the seed age; three- and four-year-old seeds always emerged weaker than one- and two-year-old ones (Fig. 2). Field emergences were 20 to 40% lower than germination. The correlation coefficients for both cultivars between laboratory germination and field emergence were highly significant (Table 2). However, the fact is that field emergence corresponded the best to greenhouse emergence ( $r = 0.79, 0.81$ ).

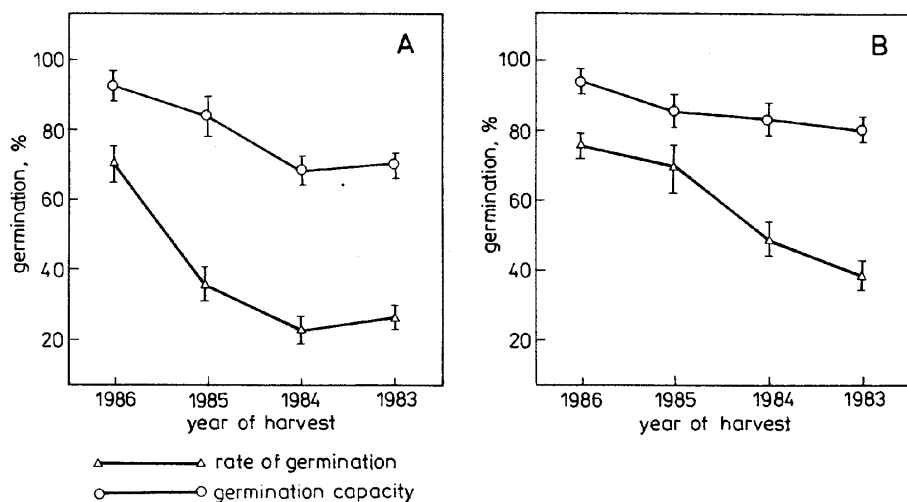


Fig. 1. Germination of cocksfoot seeds cv. Baza (A) and Bepro (B) of different ages

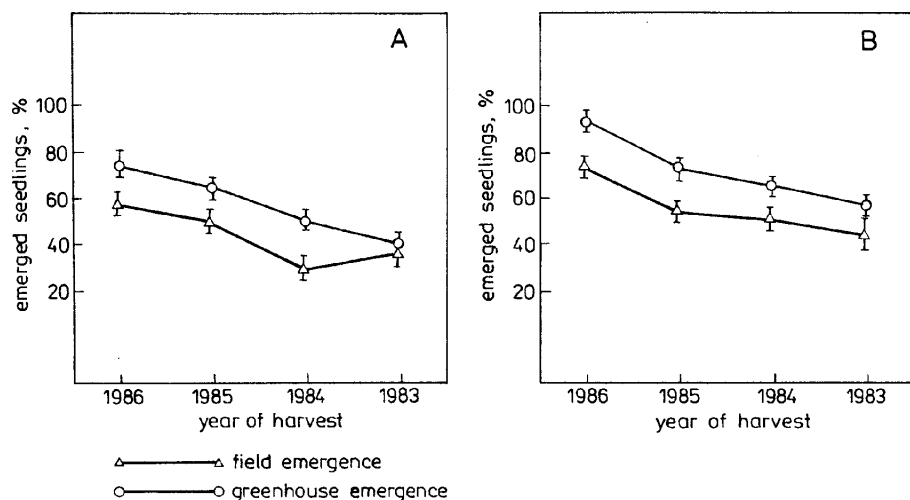


Fig. 2. Field and greenhouse emergence of cocksfoot seeds cv. Baza (A) and Bepro (B) of different ages

## 2. VIGOUR

A gradual decrease of seed vigour during their ageing was observed when a method of seedling growth rate was used (Table 1). The oldest seeds produced the weakest seedlings, whereas the best seedlings were produced by the youngest ones. Generally, radicle length and seedling fresh weight showed vigour reduction more than coleoptile length. The differences between years were most pronounced when measurements were made after 5 days of germination. It can be noticed that cocksfoot seeds of both cultivars lose their vigour much earlier and faster than viability. For example the germination capacity of seeds of cv. Bepro decreased after 4-year-ageing about 10%, but seedling fresh weight (determined after 5 days) was lowered over 60% (Fig. 1B, Table 1).

Cocksfoot seeds showed differentiated reactions to osmotic stress (Fig. 3). Moderate water stress (up to  $-160$  KPa) did not cause significant changes in seed germination. High concentrations of PEG ( $-300$  and more KPa) strongly limited seed germination. Seeds of cv. Baza were more sensitive to osmotic stress than those of cv. Bepro. For example, the stress required to lower the germination of seeds from 1986 was  $-205$  KPa for Baza and  $-310$  KPa for Bepro. Four-year-old seeds, i.e. with low vigour level, were more sensitive to the osmotic stress than one-year-old seeds with high vigour level.

Germination of seeds subjected to ethanol stress depended on alcohol concentration, time of stress duration, and seed age (Figs. 4 and 5). The highest deterioration was caused by 24 hours soaking in 15 and 20% ethanol. Seed

Table 1

Seedling growth rate of cocksfoot seeds, cv. Baza and Bepro of different ages

Year of harvest	Mean coleoptile length mm		Mean radicle length mm		Whole seedling fresh weight mg	
	after 5 days	after 10 days	after 5 days	after 10 days	after 5 days	after 10 days
cv. Baza						
1986	3.8±0.9	28.2±1.5	19.5±1.3	43.9±1.6	4.5±0.4	7.4±0.2
1985	2.8±0.9	27.4±1.7	15.2±2.5	43.4±1.6	4.2±0.3	7.8±0.7
1984	2.7±1.0	25.3±2.6	16.2±1.7	33.9±1.7	3.1±0.4	6.3±0.4
1983	0.3±0.3	20.4±2.4	4.9±3.5	32.9±2.5	1.9±0.7	6.3±0.2
cv. Bepro						
1986	8.8±0.9	39.0±1.4	29.2±0.5	53.4±1.2	5.2±0.3	8.0±0.1
1985	6.4±7.0	35.7±2.3	24.6±1.2	47.3±0.9	4.5±0.2	7.0±0.3
1984	4.1±0.7	32.3±2.1	20.1±1.4	46.7±1.8	3.9±0.2	7.2±0.2
1983	1.7±0.2	33.2±1.7	15.7±0.7	46.1±1.2	3.7±0.2	7.1±0.2

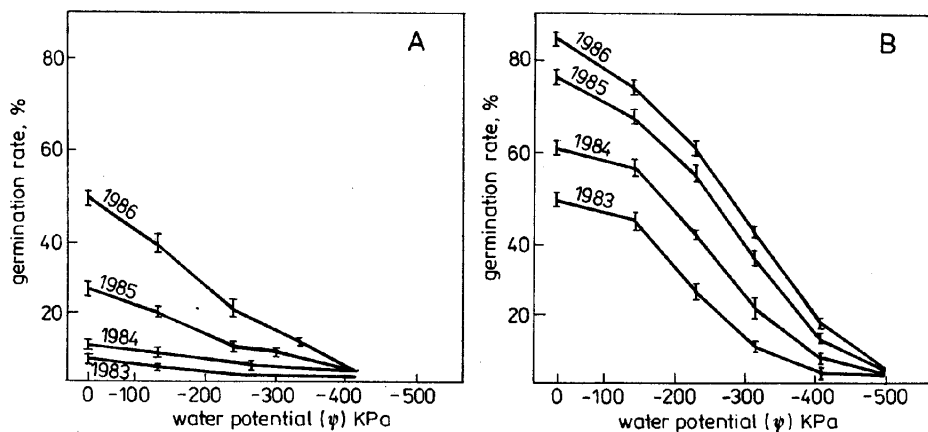


Fig. 3. Germination rate of cocksfoot seeds cv. Baza (A) and Bepro (B) of different ages in the solutions of PEG 600 with different water potentials.

treatment by 5 and 10% ethanol for 2 hours did not affect seed quality significantly. Surprising data were received while treating seeds of both cultivars with high (40-96%) alcohol concentrations, even during 24 hours (data not presented here). Such treatment resulted in small reduction of germination. Considering the resistance of seeds of different ages to alcohol stress the following dependence can be noticed: the older the seed, the more sensitive to stress.

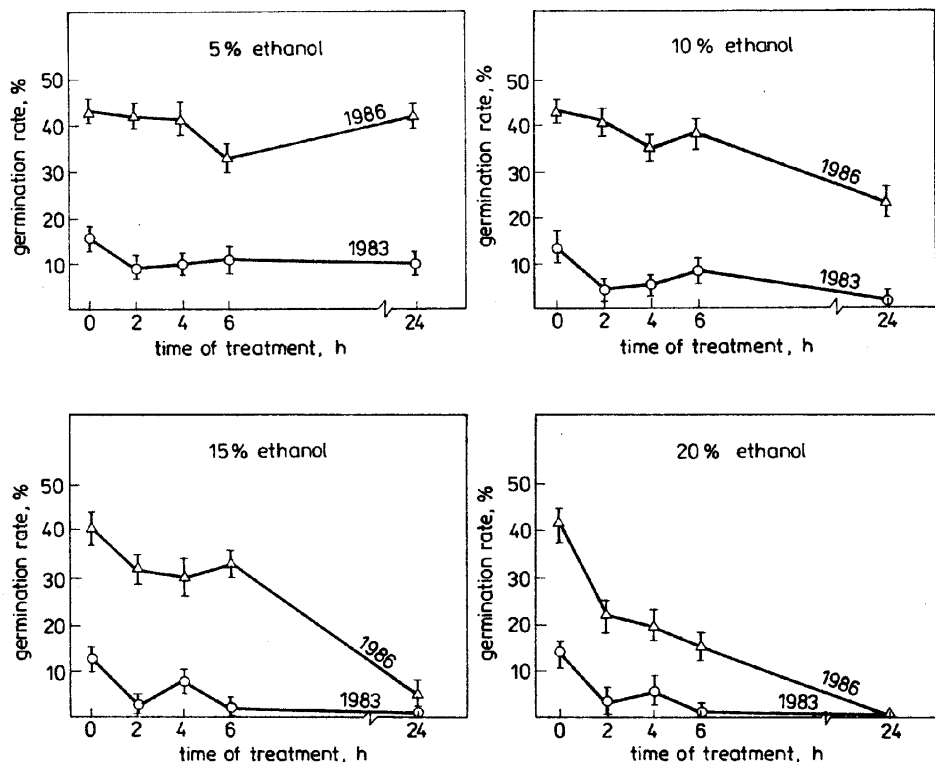


Fig. 4. Germination rate of cocksfoot seeds cv. Baza harvested in 1986 and 1983, treated with different concentrations of ethanol solutions during 2, 4, 6 and 24 hours

Electrical conductivity of steep water was different within the examined samples (Fig. 6). Increased conductivity was seen in cv. Baza from 1983 and 1984 in comparison to the seeds from 1985 and 1986. Such a dependence was not noticed in cv. Bepro, because the conductivity reading for the seeds from 1984 was lower than from 1985 and 1986. These data show some disagreement in vigour estimation of cocksfoot seeds by the conductivity method in comparison to other tests.

#### DISCUSSION

Several years seed storage of cocksfoot in a store-house did not affect greatly their viability, but it decreased their vigour significantly. Therefore, vigour examination is very important for a proper estimation of cocksfoot seed quality. Up till now there have not been worked out any single simple and uniform methods for vigour determination of different seed species. It is due to different understandings of vigour term and vigour complexity (Górecki,

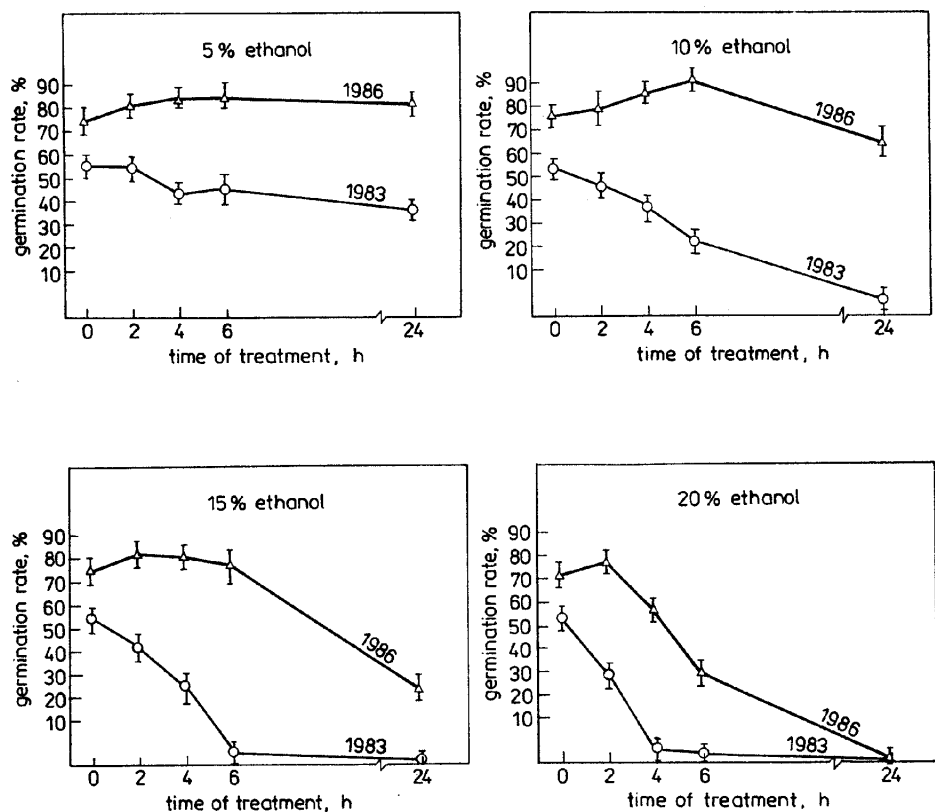


Fig. 5. Germination rate of cocksfoot seeds cv. Bepro harvested in 1986 and 1983, treated with different concentrations of ethanol solution during 2, 4, 6 and 24 hours

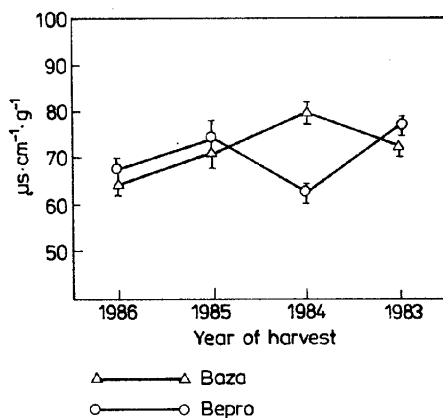


Fig. 6. Conductivity of cocksfoot seeds leachates

1986; Halmer and Bewley, 1984). Therefore, vigour should be examined by a few methods, adjusted to proper seed material (Grzesiuk and Górecki, 1981). The object of any vigour test must be to indicate or rank the ability of a seed lot to perform under stress, whether this is due to ageing during storage or other factors. The final test must be what happens in the field.

Germination rate (percentage of viable seeds after 7 days) can be a very simple and useful test for cocksfoot seed vigour determination. It showed differences in seed quality and correlated with field emergences relatively well ( $r = 0.7-0.6$ ) as compared to other indicators (Table 2), though no better than germination capacity.

Table 2

Correlations of field emergence with viability and vigour indicators of cocksfoot seeds

	Correlation coefficient			
	cv. Baza		cv. Bepro	
Germination capacity	0.60**		0.67**	
Rate of germination	0.69**		0.60**	
Greenhouse emergence	0.81**		0.79**	
Seedling growth rate after 5 and 10 days				
Coleoptile length	0.23	0.30	0.70**	0.33
Radicle lenght	0.46**	0.59**	0.61**	0.70**
Seedling fresh weight	0.54**	0.44**	0.72**	0.29
Osmotic stress				
10% PEG	0.74**		0.59**	
11% PEG	0.72**		0.61**	
12% PEG	0.70**		0.58*	
13% PEG	0.65**		0.66**	
14% PEG	0.57**		0.68**	
15% PEG	0.66**		0.54**	
17.5% PEG	0.00		0.27	
Ethanol stress, 4 h				
5%	0.72**		0.88**	
10%	0.83**		0.84**	
20%	0.59*		0.75**	
Ethanol stress, 24 h				
5%	0.76**		0.78**	
10%	0.63**		0.78**	
20%	0.00		0.00	
Conductivity of leachates	−0.68**		0.28	

\*Significant at  $P = 0.05$ ; \*\*Significant at  $P = 0.01$ .



Seedling growth rate is a commonly used method of seed vigour examination in many species (Górecki, 1986; Grzesiuk and Tłuczkiwicz, 1982; Perry, 1977). In our studies this test appeared to give rather low  $r$  values, especially for coleoptile length (Table 2).

In Tables 1 and 2 data are shown for 5 days of germination. Analysis performed after 3 or 4 days did not provide any better information about cocksfoot seed quality (data not shown here). Thus seedling growth rate analysis, however, being a very simple method can be considered as a supplementary test for cocksfoot seed vigour studies.

Grass seeds are often subjected to drought stress which results in poor field emergence. Such drought conditions can be stimulated in the laboratory by germinating seeds in PEG 6000 solution with specific osmotic potential (Hadas, 1977). The rate of germination of cocksfoot seeds under water stress was markedly reduced. Taking into consideration our experimental data (Fig. 3, Table 2) we have come to the conclusion that PEG solution with osmotic potential ranging from  $-160$  KPa to  $-320$  KPa (10 to 15% PEG) may be used in vigour analysis of cocksfoot. However, it should be mentioned that this stress test was no better in predicting field emergence than germination capacity.

Seed treatment by proper concentration of methanol or ethanol causes seed deterioration, and the rate of it is suggested to be an indicator of soybean and sunflower seed quality (Fetch and Schneiter, 1983; Mugnisjah and Nakamura, 1986; Musgrave et al., 1980). Fetch and Schneiter (1983) found that the 20% methanol stress test during 2 hours gave the best separation into varying degrees of seed vigour in sunflower. The present report indicates a deteriorating effect of ethanol stress on cocksfoot seeds and its usefulness in a vigour examination of these seeds. Data presented in Table 2 show that only the stress test (at concentrations 5 and 10%, during 4 hours) appears to give better  $r$  values than germination. The other vigour tests correlated more poorly with field performance.

Controlled deterioration and AA-test are suggested as routine tests for small-seeded species (AOSA 1983; Perry, 1981). We found that the AA-test (seeds stored at  $40^{\circ}\text{C}$  and 100% relative humidity) was not effective in vigour determination of cocksfoot (unpublished data). In contrast to our findings, Marshall and Naylor (1985) presented a significant correlation between field emergence and a controlled deterioration test in Italian ryegrass.

The conductivity test is used successfully as an indicator of field emergence of several large-seeded legume species and cereals (Grzesiuk and Tłuczkiwicz, 1982; Powell, 1986; Powell and Matthews, 1981). The best correlation between conductivity and seed vigour was obtained when ageing seeds were studied (Górecki, 1982). Such a good relationship was not observed in cocksfoot seeds. Also Marshall and Naylor (1985) and Ching and Schoolcraft (1968) found poor correlation between conductivity and

germination or field emergence of Italian ryegrass. This may be due to leaching of electrolytes from the lemma and palea masking the differences between seed lots (Marshall and Naylor, 1985). Besides, cocksfoot seed density is rather small and differentiated within the seed lot; therefore seeds do not submerge uniformly in water, which in turn could make difficult leaching out electrolytes into the water. Additionally, different conditions at seed ripening and harvest between years could account for different accumulations of electrolytes in seeds, masking the effect of storage conditions.

### CONCLUSION

None of the vigour tests beyond the ethanol test was better than germination in the evaluation of ageing cocksfoot seed quality. Application of both the ethanol and osmotic stress test needs further standarization studies. Seedling growth rate analysis, however, showing a marked reduction of seed vigour due to storage, did not predict satisfactory seedling performance in the field. Surprisingly, the conductivity method did not indicate factual vigour changes of ageing seeds and consequently can not be used as a vigour test of cocksfoot seed.

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## Ocena wigoru nasion kupkówki (*Dactylis glomerata* L.) o różnym wieku

### Streszczenie

Badano nasiona dwu odmian kupkówki pospolitej (*Dactylis glomerata* L.) ze zbiorów w latach 1986, 1985, 1984 i 1983, przechowywanych w magazynie o nie kontrolowanej temperaturze i wilgotności względnej powietrza. Próbkę nasion nie różniły się istotnie pod względem żywotności. Znaczące zróżnicowanie stwierdzono w wigorze nasion, który oznaczano na podstawie wschodów polowych, szybkości wzrostu siewek, testu stresu osmotycznego i testu stresu etanolowego. Test konduktometryczny okazał się metodą nieprzydatną do określenia wigoru nasion kupkówki. W pracy przedyskutowano także metodyczne aspekty oznaczania wigoru nasion tego gatunku.