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Pollen viability of 'Besztercei plum' clones depending on the effect of the year*

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Key words: plum, pollen viability, microclimatic effects, effects of the year

Summary: The percentage of dark staining pollen grains was higher in spring of 1996 than in the previous year. Data in 1998 resemble those of 1995, concerning the large amount of medium staining pollen grains in the majority of clones. Some clones produced excellent quality pollen also in the third year, whereas there were significant differences in other clones in various years.

The warmer February-March period in 1995 induced an early blooming, and frost affected the orchard not only in winter months, but also immediately before and during blooming. Thus, frost was the possible cause of weaker quality pollen this year. In 1996 warming began a bit late, but it was not broken by drastic falls in temperature, except for the middle of April, when a smaller frost affected the orchard. It is likely that this frost did not influence pollen quality of 'Besztercei' and 'Early Besztercei' plum clones significantly. In 1998 warming was continuous and steady, the orchard was not affected by frost immediately before blooming. In March, however, there was frost almost every day, according to daily minimum temperatures.

Introduction

In floral biological studies of plum cultivars, pollen viability is of major significance, since it influences fruit set and affects the value of the bee pasture, as well (*Halmágyi & Keresztesi,* 1975). If pollen is of poor quality, i.e. few pollen grains develop a suitable pollen tube, or the pollen tube is short and not functioning, fertilisation of flowers may not happen due to the pollen population effect (*Lee*, cit. *Surányi,* 1985). The role of pollen cannot be neglected either in self-sterile or in self-fertile cultivars. In pollen viability studies, it is recommended to take into consideration the effect of the year, as well, since pollen viability is influenced also by weather conditions, e.g. air temperature, relative humidity, light and wind etc. and their daily course cannot be neglected either (*Pálfi & Gulyás,* 1985).

In the average of several years 'Early Besztercei' blooms between 17–27 of April, while 'Besztercei plum' blooms a bit later, between 18–29 of April. The flowers of both are self-fertile and they set fruit well. Despite of these, there can be differences in fertility, although the ability for self-fertility and pollen viability are inherited features (*Rémy,* 1954, *Linskens,* 1974, *Gulyás & Pálfi,* 1987). According to the quality of the spur, even in the same year and under the same conditions, topological effects are not insignificant for flowers and the viability of pollen, either (see *Tóth & Surányi,* 1980, *Surányi,* 1991).

The clones in the two 'Besztercei' cultivar groups are microtaxa whose floral biology differs only slightly in a certain sense (*Surányi & Orosz-Kovács,* 1992). Pollen viability has hardly been investigated in recent years, especially not for several years, including its dependence on environmental factors (*Surányi,* 1985, 1996a & 1996b).

Pollen viability can be studied in a number of ways. Determining of pollen viability by pollen germination has been known for long (*Hámori-Szabó,* 1959). Duration of bloom is short, pollen enclosed within anthers in the flower bud cannot be stored, and consequently researchers have searched for quick staining methods. Histochemical staining reactions were based on the activity of a certain enzymatic system, e.g. TTC worked well with several species, although this method also has some uncertainty (*Szalai & Sárkány,* 1966). At the same time, analysis based on the study of the

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pollen tube is slow and only a limited number of samples can be studied, although this is the best model for natural conditions. The quick proline-staining procedure developed by *Gulyás* & *Pálfi* (1985), based on the statement of *Stanley* & *Linskens* (1974) that proline content of pollen grains correlates with its fertility, has been found suitable for determining pollen viability. When applying the isatine staining procedure, in the so-called "proline-type" plants proline content reaches 1–2% or even more of pollen dry matter. The other group includes the "non-proline-type" species. In these the proline concentration of pollen is lower than 0,2%, thus their viability cannot be determined by this method. *Gulyás* & *Pálfi* (1986) found that the 18 studied species of the Rosaceae family belong to the "proline-type" plants. Each plant species within a genus belongs either to "proline-type" or "non-proline-type", with no exception (*Pálfi* & *Gulyás,* 1985, *Gulyás* & *Pálfi,* 1986, *Pálfi* et al., 1987). Thence it follows that the presence or absence of proline is a hereditary characteristic (*Gulyás* & *Pálfi*, 1989).

According to *Gulyás* & *Pálfi* (1989) the proline concentration of plum (*Prunus domestica* L.) reaches 1,66%, thus it can be classified into the "proline-type" group, so the isatine staining reaction can be applied with success in determining the pollen viability of plum cultivars. Successful pollen viability studies using the proline test have been carried out on other fruit species belonging to the *Rosaceae* family, for example on apple and pear, including several cultivars of these (*Nagy-Tóth* & *Farkas,* 1996, *Farkas* et al., 1996, *Szabó* et al., 1997). The degree of quality of pollen grains in cultivars belonging to the same species is indicated sensitively by the percentage results of positive isatine staining (*Pálfi* & *Köves,* 1984, *Gulyás* & *Pálfi,* 1986).

The aim of this study was to select clones with a stable pollen quality, i.e. good pollen viability, which does not strongly depend on microclimatic conditions. For this reason the chosen 16 clones have been studied for several years.

Material and methods

Pollen was collected in the cultivar collection of the Research Station for Fruitgrowing, Cegléd (from 1-3 trees, at least 20–50 flowers). Samples were collected on 10-13 April 1995, 29-30 April 1996 and 16–17 April 1998 from the flowers of the following clones of 'Besztercei plum' and 'Early Besztercei':

Besztercei szilva C. 1	Besztercei szilva Slapanicka
Besztercei szilva C. 35	Besztercei szilva Wurzelechte
Besztercei szilva C. 224	Besztercei szilva 10/b-64
Besztercei szilva C. 970	Besztercei szilva 105/58
Besztercei szilva KD. 10	Besztercei szilva 142/59
Besztercei szilva Kruft	Korai Besztercei Tv. 46
Besztercei szilva Magyarnándor	Korai Besztercei Tv. 52
Besztercei szilva Prettini	Korai Besztercei Tv. 56

According to *Stanley* & *Linskens* (1974) proline concentration does not change significantly until at least 4 days after pollen shed. Pollen samples were fixed within 2–3 days after pollen shed by the method of *Gulyás* & *Pálfi* (1986): pollen was heated at 90 °C for 10 minutes, then stored in airtight containers until studied. Pollen viability was determined by the isatine staining procedure described by *Pálfi* & *Gulyás* (1985). Viable pollen grains are stained blue, dark blue or black by the isatine reagent, non-viable grains show brown, light brown or yellow colour (*Pálfi* & *Köves,* 1984). The treated, variously stained pollen grains were counted in the divided field of sight of a light microscope.

If the proline concentration of pollen grains is between 1.0–2.0%, the quality of the grains is good, thus they are blue, dark blue or black. The staining of these pollen grains is called "positive isatine staining". These species belong to the "proline-type" on the basis of their pollen. Below 1.0% proline content the pollen grain gives yellow, brown, red or greenish colour with the isatine reagent. The pollen of these species is "non-proline-type", so it gives a "negative isatine staining" (*Gulyás* & *Pálfi,* 1989). We have worked out a qualification system, based on the percentage values of staining and have applied this concept to cultivars:

97–100%	excellent
70–89%	good
50–69%	medium
31–49%	weak
1–30%	poor
0%	sterile

For the analysis of the effects of the year, meteorological data about the periods between 1 February–13 April 1995, 1 February–30 April 1996 and 1 February–17 April 1998, provided by the Meteorological Station of the Research Station for Fruitgrowing, Cegléd, were used. Out of the data daily maximum and minimum temperatures and daily mean temperature have been used.

Results and conclusions

The 16 'Besztercei plum' and 'Early Besztercei' clones studied for three years were classified as good or bad pollen donors, according to whether the pollen produced by a given clone is suitable or not to develop a pollen tube in one or more years. Viability of pollen grains, concerning dark staining, varied to a great extent *(Table 1)*. The least pollen grains with intensive staining were produced by 'Besztercei' Prettini, e.g. 19% in 1995 (*Figure 1*). Apart from this, the pollen viability of the studied clones proved to be suitable. The ones reacting to the effects of the year only to a small extent stain in high percentage, these can be considered ideally staining cultivars and clones, i.e. they are excellent pollen donors.

Table 1 Pollen viability of studied clones between 1995–1998, Cegléd

Clones	1995			1996			1998		
	Dark st	Med. st	Non st	Dark st	Med. st	Non st	Dark st	Med. st	Non st
Besztercei Kruft	87	8	9	78	21	1	51	28	21
Besztercei C. 970	93	4	3	99	1	0	42	52	6
Besztercei Wurzelechte	70	23	7	93	6	1	87	12	1
Besztercei KD. 10	93	6	1	56	44	0	58	36	6
Besztercei Magyarnándor	16	31	53	97	2	1	69	26	5
Besztercei C. 1	89	10	1	96	4	0	46	52	2
Besztercei 10/B-64	89	8	3	89	11	0	99	1	0
Besztercei Prettini	19	46	35	83	14	3	89	10	1
Besztercei 142/59	96	3	1	94	4	2	23	71	6
Besztercei 105/58	75	18	7	98	0	2	52	46	2
Besztercei C. 35	38	41	21	99	1	0	97	3	0
Besztercei Slapanicka	20	26	54	97	1	2	45	51	4
Besztercei C. 224	83	14	3	95	4	1	81	17	2
Korai Besztercei Tv. 56	96	3	1	96	2	2	97	2	1
Korai Besztercei Tv. 46	64	22	14	93	5	2	89	9	2
Korai Besztercei Tv. 52	88	9	3	49	50	1	45	47	8

Abbreviations:

Dark st. = Dark staining
Med. st. = Medium staining
Non st. = Non staining

In the average of three years the following clones had the best pollen viability: 'Besztercei plum' C. 224, 10/b-64 and Wurzelechte clones, furthermore the 'Early Besztercei' Tv. 56 clones, because their dark staining pollen could be classified into the excellent or good categories in all three years and the percentage of their vital pollen grains was above 70%. The clones with the poorest staining were 'Besztercei plum' Kruft and Prettini, and 'Early Besztercei' Tv. 52, in these the amount of dark staining pollen grains did not reach the excellent category on any occasion.

Viable pollen grains of the 'Besztercei' C. 224 clone were present in smaller amount in the samples in 1995 (83%) than in the following year (95%), i.e. they were of good and excellent quality. Data in 1998 resembled those in 1995 to a great extent; pollen had good quality also in this year (81%). The amount of medium staining pollen grains was similar in 1995 and 1998 (14 and 17%, respectively), whereas it was only 4% in 1996. This cultivar, giving uniformly good quality pollen, can be recommended as pollinizer *(Figure 1–3)*.

'Besztercei plum' 10/b-64 also gave pollen with strikingly good viability in all three years, 89% of its pollen showed dark staining in two years, and 99% in 1998, the amount of non-viable pollen grains was insignificantly small accordingly. Consequently, this clone is also an excellent pollen donor *(Figure 1–3.)*.

In the 'Besztercei plum' Wurzelechte clone there is a considerably great difference in the ratio of viable and medium staining pollen grains in the various years. While in 1995, 70% of the pollen grains belonged to the dark staining category, in 1996 this value reached 90%, thus this clone could be classified into the good and excellent pollen viability category in1995 and 1996, respectively. In 1998, however, pollen could be qualified "only" as good (the ratio of dark staining pollen grains was 87%). The clone having good or excellent viability pollen is suitable for pollen giving by all means *(Figure 1–3)*.

The pollen of the 'Early Besztercei' Tv. 56 clone is of excellent quality, the amount of dark staining pollen grains was almost the same (96, 96, 97%) in all three years. Accordingly, the number of medium staining (3, 2 and 2%) and sterile (1, 2 and 1%) pollen grains can be neglected (Fig. 1–3.).

According to our assessment, clones reacting slightly to the effects of the year can be considered the best pollen donors.

On the basis of the three years the following clones were classified as having weak pollen viability.

The pollen viability of the 'Besztercei plum' Kruft clone proved to be better in 1995 than in 1996 (87 and 78%, respectively), thus the clone can be characterised by good pollen viability in these two years. In 1998 the produced pollen was only of medium quality (51%). In 1995 the amount of medium staining pollen grains was 8%, in 1996 21% and in 1998 it was 28%. It has to be noted that in 1998 the frequency of sterile pollen grains increased (9, 1 and 21% in 1995, 1996 and 1998, respectively). This clone is variable, thus it cannot be recommended as a pollen donor because of its pollen viability varying with the years *(Figure 1–3)*.

In the dark staining category of 'Besztercei plum' Prettini clone there was a significant difference in the first two years: in 1995 it was 19%, the next year the value was 83%, i.e. in 1995 the clone had a very poor pollen viability, whereas in 1996 pollen quality was good. The 1998 results resembled the latter; pollen could be qualified as good (89%).

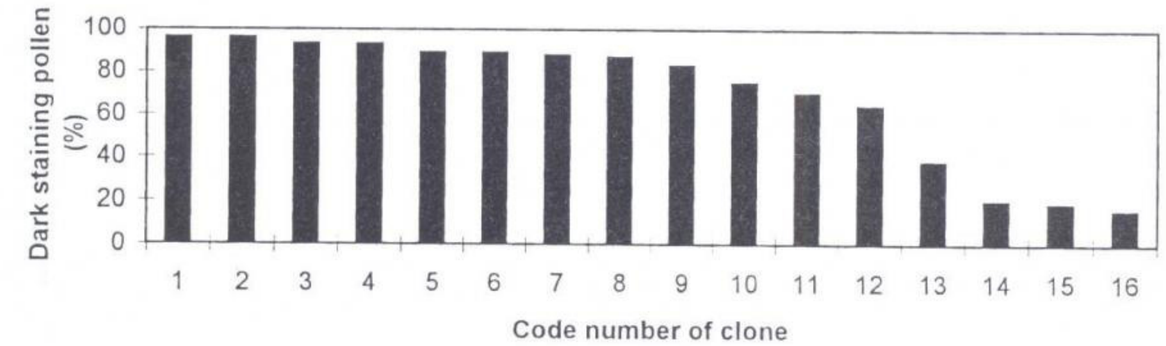

Figure 1 Pollen viability of 'Besztercei plum' clones, Cegléd, 1995.

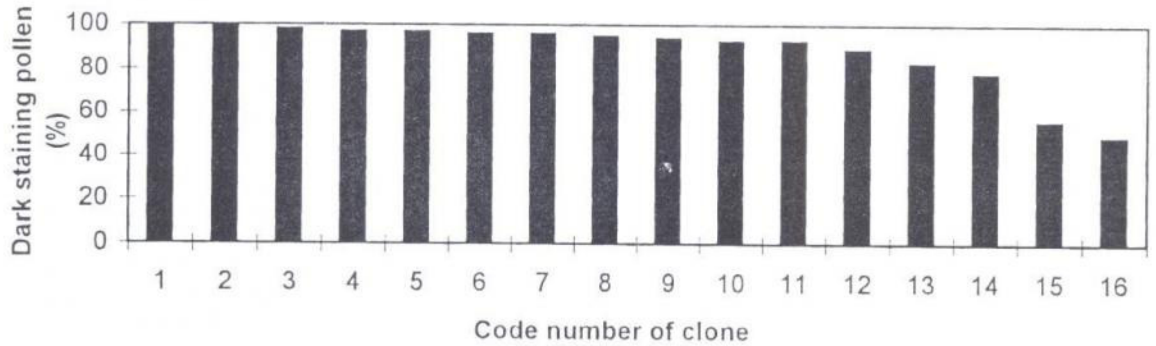

Figure 2 Pollen viability of 'Besztercei plum' clones, Cegléd, 1996.

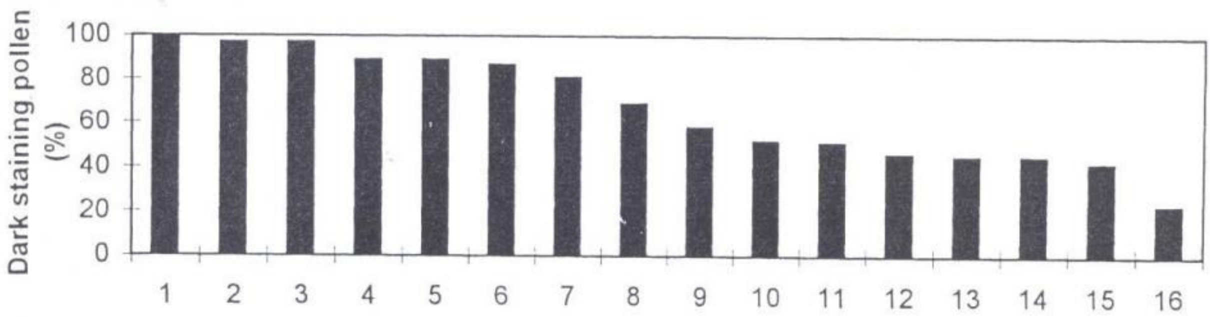

Figure 3 Pollen viability of 'Besztercei plum' clones, Cegléd, 1998.

Captions to Figure 1:

Code number of clones:

1: Besztercei 142/59
2: Korai Besztercei Tv. 56
3: Besztercei C. 970
4: Besztercei KD. 10
5: Besztercei C. 1
6: Besztercei 10/B-64
7: Korai Besztercei Tv. 52
8: Besztercei Kruft
9: Besztercei C. 224
10: Besztercei 105/58
11: Besztercei Wurzelechte
12: Korai Besztercei Tv. 46
13: Besztercei C. 35
14: Besztercei Slapanicka
15: Besztercei Prettini
16: Besztercei Magyarnándor

Captions to Figure 2:

Code number of clones:

1: Besztercei C. 970
2: Besztercei C. 35
3: Besztercei 105/58
4: Besztercei Magyarnándor
5: Besztercei Slapanicka
6: Besztercei C. 1
7: Korai Besztercei Tv. 56
8: Besztercei C. 224
9: Besztercei 142/59
10: Besztercei Wurzelechte
11: Korai Besztercei Tv. 46
12: Besztercei 10/B-64
13: Besztercei Prettini
14: Besztercei Kruft
15: Besztercei KD. 10
16: Korai Besztercei Tv. 52

Captions to figure 3:

Code number of clones:

1: Besztercei 10/B-64
2: Besztercei C. 35
3: Korai Besztercei Tv. 56
4: Besztercei Prettini
5: Korai Besztercei Tv. 46
6: Besztercei Wurzelechte
7: Besztercei C. 224
8: Besztercei Magyarnándor
9: Besztercei KD. 10
10: Besztercei 105/58
11: Besztercei Kruft
12: Besztercei C. 1
13: Besztercei Slapanicka
14: Korai Besztercei Tv. 52
15: Besztercei C. 970
16: Besztercei 142/59

Accordingly, in 1995 the ratio of the medium staining pollen grains was the highest (46%), but also the value of the non-staining category was high (35%). Due to especially the latter data, this clone cannot be recommended as a pollen donor with certainty *(Figure 1–3)*.

The percentage of viable pollen grains in 'Early Besztercei' Tv. 52 clone reached 88% in 1995, i.e. pollen was of good quality, in 1996 and 1998, however, viability was poor (49 and 45%, respectively). Years 1996 and 1998 could be characterised mainly by the great amount of medium-staining pollen grains (50 and 47%, respectively). The ratio of non-staining, sterile pollen was below 10% each year. The clone producing pollen with poor viability cannot be recommended as a pollinizer *(Figure 1–3)*.

In the three years studied the following clones possessed varyingly good or bad pollen viability: 'Besztercei plum' C.1, C.35, C. 970, KD 10, Magyarnándor, Slapanicka, 105/58, 142/59 and 'Early Besztercei' Tv. 46 clones *(Figure 1–3)*. Consequently, these clones can be recommended as safe pollen donors only in certain years, because the success of their fertilisation is questionable. It can be supposed, however, that despite of this the pollen of these cultivars should be used, and in this case the yearly staining studies can help in clarifying the actual situation. As it could be seen, however, clones uniformly having a large amount of dark staining, viable pollen in the samples each year, meaning that they are less sensitive to effects of the year, are safe pollen donors. Self-fertile clones can be suitable for independent planting even if their pollen viability is medium or poor, since less pollen with good viability may also be sufficient for pollination within the flower.

The warmer February-March period in 1995 induced the beginning of bud development and an early blooming, since the necessary heat amount was reached sooner because of higher temperatures *(Figure 4)*. Consequently, in 1995 the blooming of 'Besztercei plum' can be considered as early, because the bloom of certain clones began in the first half of April. In February, immediately before and during bloom a significant frost affected the flowers, which could cause the weaker quality of pollen in 1995 *(Figure 5)*.

In 1996 the frosty period lasted longer than generally, spring warming started only in the middle of March, approximately from 13 of March *(Figure 6)*. For this reason in 1996, in contrast with the previous year, bloom began later than usually, but the difference was only two days, which can be considered as minimal. This year the warming period was not interrupted by frosty days, except for the middle of April. Temperature was below zero only for a few days, but not to a critical degree *(Figure 7)*. Such a weak frost could not possibly influence the pollen viability of

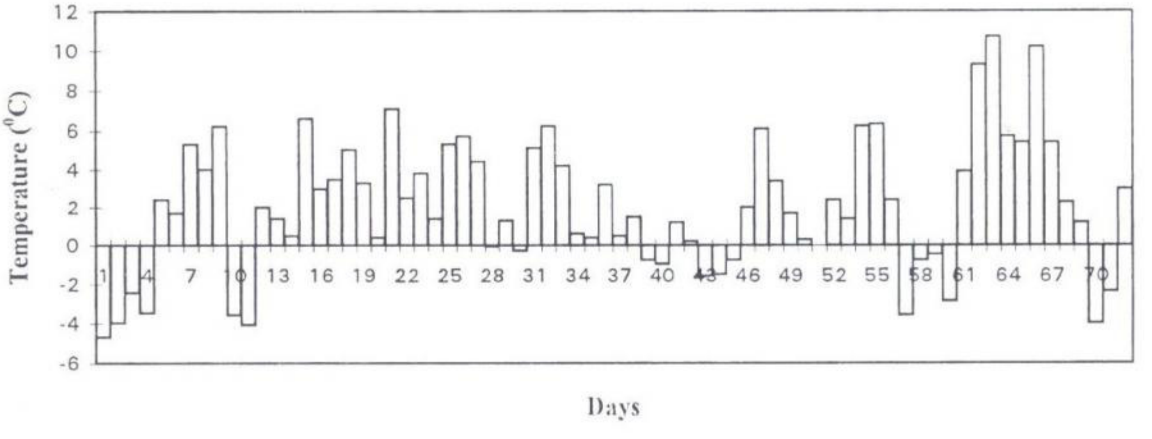

Figure 4 Daily minimum temperatures, 01. 02. 1995. – 13. 04. 1995., Cegléd

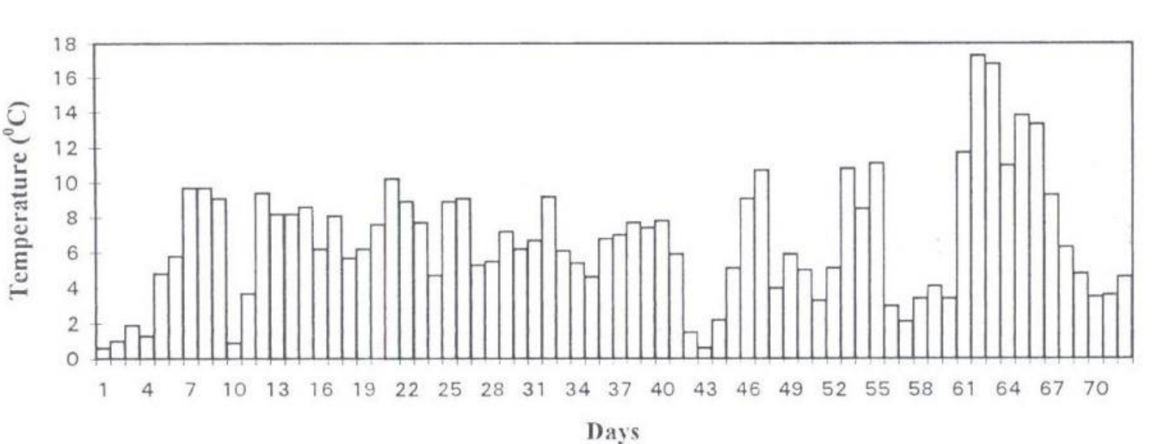

Figure 5 Daily mean temperatures, 01. 02. 1995. – 13. 04. 1995., Cegléd

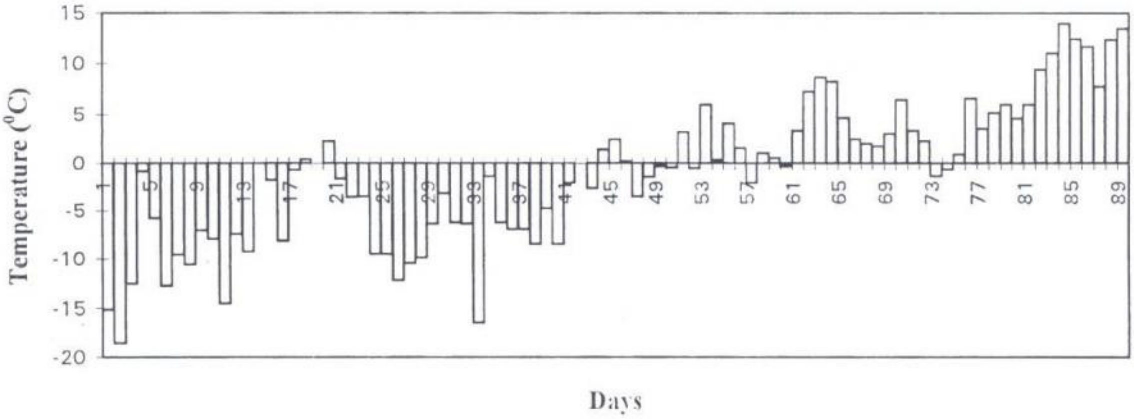

Figure 6 Daily minimum temperatures, 01. 02. 1996. – 30. 04. 1996., Cegléd

'Besztercei plum' and 'Early Besztercei' clones significantly. It is likely that better results are due to these facts.

In 1998 warming was gradual from the first week of February, and there was no significant fall in temperature (fig. 8.). However, daily minimum temperatures indicated weaker frosts even in March, especially in the second half of the month. In April warming was continuous, the two weeks immediately preceding bloom were completely frost-free *(Figure 9)*.

As a summary it can be stated that pollen viability can be determined both by isatine staining and in hanging drop culture in 10-15% sucrose (see *Surányi* 1985, 1996a and 1996b), and differences really exist between certain clones of 'Besztercei plum' and 'Early Besztercei' cultivar groups. Variations can be explained by genetic reasons, fluctuation can mainly be in relation with effects of the year or the varying plum pox infection of the trees, sample-taking topology (see *Surányi* 1997). The suitability of the staining procedure, which allows a faster viability-determination

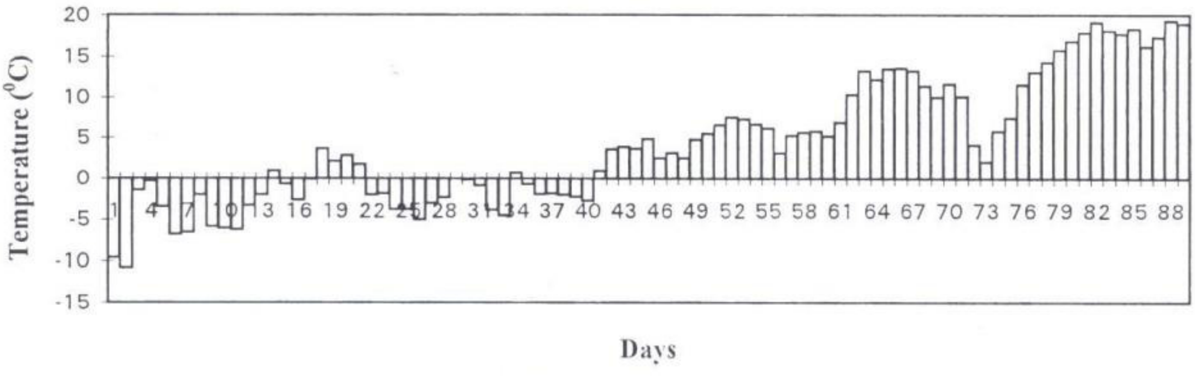

Figure 7 Daily mean temperatures, 01. 02. 1996. – 30. 04. 1996., Cegléd

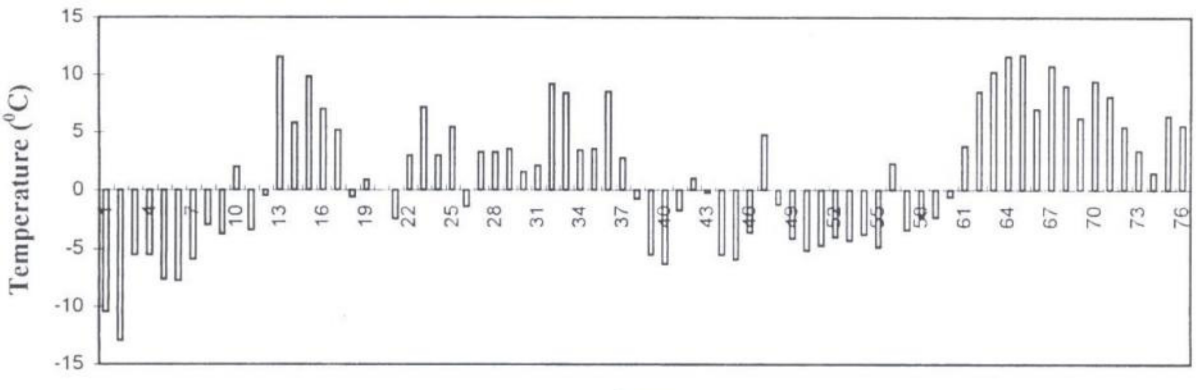

Figure 8 Daily minimum temperatures, 01. 02. 1998. – 17. 04. 1998., Cegléd

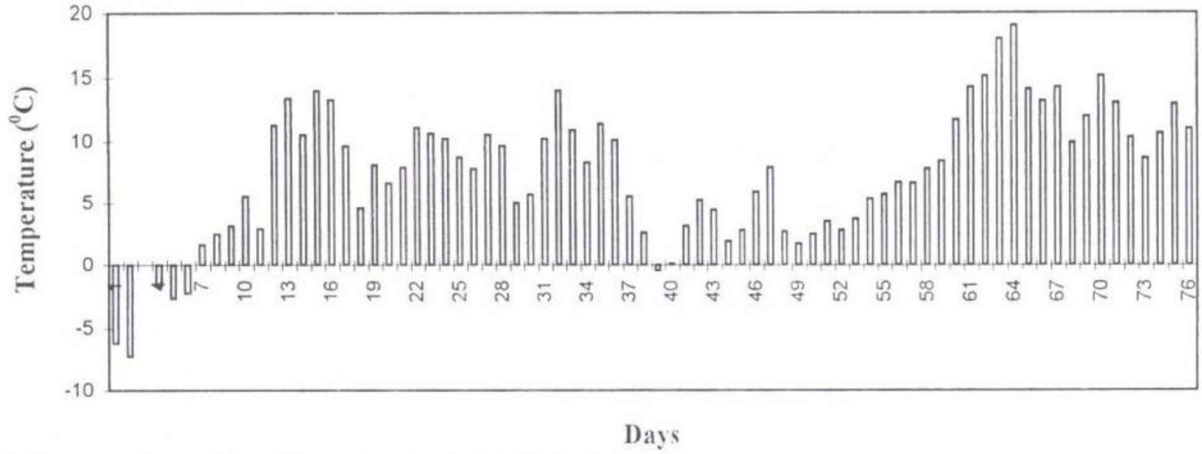

Figure 9 Daily mean temperatures, 01. 02. 1998. – 17. 04. 1998., Cegléd

than previous methods, has been verified in the present study. It can help to suffice the requirements of both breeders and practical producers.

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