

Selection of pollinators for particular pear cultivars (*Pyrus communis* L.) based on the observation of the pollen tubes

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Summary

Most fruit-tree species, including pear (*Pyrus*) have genetically controlled ability to outcrossing (self-incompatibility). From the orchard practice point of view outcrossing is desired for obtaining acceptable yield level. So, the aim of the present study was to learn problem of self-incompatibility or cross-compatibility of six pear (*P. communis*) cultivars (Amfora, Radana, Red Williams, Carola, Conference, Dicolor) and wild ecotype of *P. pyraster* and the evaluation of investigated cultivars as good pollinators for each other. The mode of pollination consists of intracultivar self- and cross-pollination and intercultivar diallel cross-pollination. The pollination was done in three different environmental conditions i.e. orchard, glasshouse and laboratory. Self-incompatibility or cross-compatibility of investigated cultivars was evaluated on the base of the pollen germination index (PGI). Six cultivars of pear and one ecotype of wild pear which were under investigation showed full self-incompatibility. In some inter-cultivar and interspecific pollinations full cross-incompatibility or unilateral incompatibility was observed.

Key words: *Pyrus*, self-incompatibility, pollen tubes, pear

INTRODUCTION

Pear is the third most important temperate fruit species after grape and apple. In the world two main species of the genus *Pyrus* are cultivated i.e. *Pyrus communis* L., the European pear, is grown in Europe and America, *P. pyrifolia* (Burm. f.) Nakai (= *P. serotina* Rehd.), the Assian pear or Nashi, is grown in Asia. According to calculation by the US Department of Agriculture (USDA), the total pear production in 2006/2007 season amounted to a bit over 17 million metric tons (MT) and reached 20 million MT in the 2007/2008 season. Within the European Union the economic situation for pear growing is substantially different from the situation of apple growing. While there is a worldwide crisis in apple production and prices, the economics of pear production are much better and pears have become an interesting way of economic diversification within modern fruit orchards. The total European pear production is rather stable and fluctuated during the last few years between 2.28 and 2.69 million MT (Poland: 0.03–0.09 million MT). Although there are about two thousand *P. communis* cultivars, the worldwide production of this species is based on a few cultivars, like ‘Williams’, ‘Conference’, ‘Passe Crassane’, ‘D. Jules Guyot’, ‘BeurréAnjou’ and ‘Beurré Bosc’ [1, 2]. Considering the cultivars, ‘Conference’ is the most cultivated pear in EU with production fluctuated during the last few years between 0.52 to 0.83 million MT (Poland: 0.02–0.04 million MT) and ‘Conference’ pears produced in the southern production areas are totally different in fruit shape from the ‘Conference’ pears produced in the northern production areas.

The genus *Pyrus* possesses many characteristics which can be used in breeding programs to modify pear production. The role of breeding in the improvement of pear was determined by summarizing opinions of 54 scientists associated with pear production in the world. Among the tree characteristics the components contributing to yield, compatibility, precocity and parthenocarpy were the most important, whereas, utilization of nutrients was considered least important [3]. Most fruit species exhibit self-incompatibility (SI) or cross-incompatibility (CI) which is a genetically controlled mechanism that prevents self- or cross-fertilization in flowering plants. In pear and other fruit-tree species there exists so called gametophytic self incompatibility (GSI) usually controlled by the S-locus. The S-allele products of the style are basic glycoproteins with ribonuclease activity (S-Nrases) that block incompatible pollen tube growth through the style [4, 5]. Determination of self-incompatibility (S-genotypes) are desirable in choosing compatible pollen donors, which are necessary for stable fruit production of SI fruit trees. However, the determination of S-genotype is difficult in tree plants because it is time-consuming to conduct genetic analyses. According to Hołubowicz [6] among 31 investigated old cultivars of *P. communis* exists both cross-fertility and cross-sterility. Cross-sterility e.g. was determined between such cultivars as ‘Williams Bon Chrétien’ and ‘Dobra Ludwika’ with ‘Seckel’; and ‘Dobra Ludwika’ with ‘Williams Bon Chrétien’. Traditionally, characterisation and identification of pear cultivars have been performed according to morphological and physiological

traits, which are sometimes ambiguous because of environmental and physiological effects [7].

The existence of a large number of old and new commercial pear cultivars brings a need for their accurate identification, which requires the development of reliable cultivar identification techniques. These techniques should allow the identification of cultivars when fruits or other major organs are absent. Recently, DNA-markers like Randomly Amplified Polymorphic DNA (RAPD) [8] have been used for cultivar discrimination and to study taxonomic relationships within the genus *Pyrus* [9-12], but their utilization is limited by the small number of markers that can be used for analysis.

The aims of the present study are to assess the compatibility relationships among several *P. communis* new cultivars (including old traditional 'Conference' cultivar) and *P. pyraster* (wild ecotype existing in Poland) based on the observations of the pollen tube growth. Determination of compatibility relationships among pear cultivars can be useful in selecting both progenitors in breeding programs and, when planning an orchard, at least two intercompatible cultivars must be used to ensure successful pollination and consistently acceptable yield levels. The self- or cross-compatibility (incompatibility) of each cultivar thus needs to be determined in order to establish the group of its best pollinators.

MATERIAL AND METHODS

Plant material

Adult trees of *P. communis* cultivars 'Amfora', 'Radana', 'Red Williams', 'Carola', 'Conference' and 'Dicolor' were grown at Przybroda orchard of Poznań University of Natural Sciences, Poland and wild ecotype of *P. pyraster* came from a location in the city of Poznań area.

Pollination and pollen tube growth observation

The pollen of particular pollinators was placed on stigmas 1–2 days after emasculation which was done at the flower bud stage. The mode of pollination consists of intracultivar self- and cross-pollination and intercultivar diallel cross-pollination as it is presented in table 1. The pollination was done in three different environmental conditions i.e. orchard, glasshouse and laboratory conditions. Pollination under glasshouse (at a temperature of 20°C) and laboratory (at a temperature of 28°C) conditions was performed on shoots collected on field trials and placed in clean water. In order to monitor pollen tube growth in the pistils, samples were taken at 48–54 hours after pollination and fixed in Carnoy's solution [13] and stained with aniline blue according to Martin [14]. The observation of pollen tubes was done by means of fluorescence microscope. Self-incompatibility or cross-compatibility of the cultivars

under investigation was evaluated on the base of the pollen germination index (PGI) according to Matsuzawa [15]. These combinations of pollination in which PGI was equal to or higher than 2 were regarded as compatible.

RESULTS

Self-pollinations

Trees of all *P. communis* cultivars as well as ecotype of *P. pyraister* were self-incompatible and in all self-pollinations the pollen germination index (PGI) was below 2 and varied from 0.8 (cv. ‘Dicolor’ at glasshouse) to 1.7 (cv. ‘Red Williams’ at all three environmental conditions) (tab. 1). After selfing at three environmental conditions many pollen grains did not germinate on the stigma. Any way, there was still a lot of pollen grains which germinate and form pollen tubes which mostly reached 1/3 length maximum 1/2 length of the style and stop to grow (fig. 1).

Table 1.

Pollen germination index (PGI) after self- and cross-pollination in a diallel mode of 6 cultivated *P. communis* cultivars and one ecotype of wild *P. pyraister* in three environmental conditions (I, II and III)

paternal form ♂	maternal form ♀																				
	cultivars of <i>P. communis</i>															<i>P. pyraister</i>					
	‘Amfora’			‘Radana’			‘Red Williams’			‘Carola’			‘Conference’			‘Dicolor’			I*	II*	III*
	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*
‘Amfora’	1.0	0.9	1.5	2.1	2.0	1.7 ^b	2.3	2.6	2.4	2.1	2.4	1.1 ^a	2.3	2.1	1.4 ^b	2.2	2.2	2.2	2.4	2.2	2.4
‘Radana’	2.3	2.0	2.1	1.5	1.7	1.7	2.4	2.4	2.5	2.2	2.7	2.3	2.3	2.5	2.5	2.3	2.6	2.4	2.0	2.3	0.8 ^b
‘Red Williams’	2.5	2.3	2.3	2.4	2.6	2.4	1.7	1.7	1.7	2.4	2.5	2.5	1.0	1.4 ^b	2.1	2.1	2.3	2.1	2.1	2.2	1.4 ^b
‘Carola’	1.5 ^b	1.8 ^b	1.7 ^a	2.3	2.2	2.6	2.0	2.1	2.4	1.4	1.6	1.0	2.3	2.2	2.3	2.0	2.3	1.0 ^a	2.1	2.3	2.3
‘Conference’	2.2	2.1	2.2	2.1	2.1	1.3 ^b	2.0	2.1	2.1	2.1	2.0	1.2 ^b	1.4	1.7	1.7	2.2	2.3	2.3	2.3	2.3	2.5
‘Dicolor’	2.4	2.3	2.5	2.2	2.2	2.1	2.3	2.1	2.3	2.2	2.2	1.7 ^a	2.3	2.3	1.3 ^b	1.4	0.8	1.4	2.0	2.0	1.2 ^a
<i>P. pyraister</i>	2.2	2.3	2.3	1.4 ^b	1.0 ^b	2.1	2.1	2.4	2.3	2.2	2.1	2.2	2.1	2.4	2.3	2.3	2.1	1.0 ^a	1.7	1.4	1.2

* I – glasshouse conditions. II – culture room conditions. III – open field conditions. a – full cross incompatibility; b – unilateral cross incompatibility

Intercultivar cross-pollinations

Most cultivars of *P. communis* were reciprocally cross compatible (CC) with each other in three environmental conditions. In this case PGI ranged between 2.0 and 2.7 (tab. 1), but in some intercultivar crosses full- or unilateral cross-incompatibility was observed. Full cross-incompatibility (FCI) appeared in the crosses between pear cultivars: ‘Amfora’ × ‘Carola’ and ‘Carola’ × ‘Dicolor’, but only at the field

conditions (PGI from 1.0 to 1.7). At glasshouse and laboratory conditions full cross compatibility (FCC) between above mentioned cross combinations was observed. In the field conditions few cross combinations showed unilateral cross-incompatibility (UCI). Such UCI was observed in the crosses: 'Radana' × 'Amfora' and 'Conference' and 'Conference' × 'Amfora' and 'Dicolor' (PGI from 1.2 to 1.7). What is interesting, almost in all these cross combinations where both FCI or UCI exists in the field conditions, there was FCC at the glasshouse and laboratory conditions. The exception is 'Amfora' × 'Carola' cross where FCI was observed in the field but in the glasshouse and laboratory it was UCI. In general, there was better cross-compatibility under glasshouse and laboratory conditions as compared to the field conditions. In the glasshouse and laboratory only 'Amfora' and 'Conference' cultivars showed UCI with 'Carola' or 'Red Williams', respectively.

Interspecific cross-pollinations

In interspecific crosses of six *P. communis* cultivars with wild ecotype of *P. pyra-ster* there was only one cross combination: cv. 'Radana' × *P. pyra-ster* in which UCI exists, and one cross cv. 'Dicolor' × *P. pyra-ster* where FCI was observed. Interspecific cross combinations in which *P. pyra-ster* was used as a maternal form were FCC in four of six cross combinations. In crosses of *P. pyra-ster* with 'Radana' and 'Red Williams' cultivars the UCI was observed.

In all intercultivar and interspecific compatible combinations germinated pollen grains on the stigma and pollen tubes penetrating particular parts of the pistil were visible (fig. 2-4). The cross incompatible combinations, both FCI or UCI have some disturbances in pollen germination and pollen tubes growth. Usually in such combinations pollen tubes stop growing at 1/3 length of the style and occasionally germinated pollen grains with highly swollen short pollen tubes were present (fig. 5-7). Sometimes swollen pollen tubes formed calli plugs or cracked and occasionally two pollen tubes emerging from one pollen grain were observed (fig. 5).

DISCUSSION

In this study, self-incompatibility (SI) and cross-incompatibility (CI) among six cultivars of *P. communis* and one wild ecotype of *P. pyra-ster* were analyzed. Knowledge on self-incompatibility of pear cultivars may be useful in selecting compatible parents in pear breeding programs aiming the creation of new cultivars in the future. Field data cannot distinguish between fully compatible and half-compatible crosses because in some cases fruit set can be affected by parthenocarp [7, 16]. Better understanding of SI problem could be particularly useful under severe conditions, where semi-compatibility is a disadvantage and can lead to yield reduction, since half of the pollinator's pollen is rejected [17]. Most pear cultivars have been traditionally considered as completely or almost completely

self-incompatible, while some of them can be partially self-compatible, depending on the environment [18]. In our work for pear incompatibility testing the reliable method of pollen tube growth along the pistil was used. The data obtained after self- and cross-pollination at three different temperature conditions show that incompatibility in pear can be affected by the environment as in some cross-combinations in the field there existed full cross-incompatibility but in the glasshouse and laboratory full cross-compatibility was observed and vice versa. Moreover, the reaction on the environment conditions was different and there was no regularity in the reaction of particular cultivars. In some combination after pollination in the field full or unilateral cross-incompatibility existed. Meanwhile, in other two tested environments crossed cultivars were cross-compatible. Other combinations were cross-incompatible both in the laboratory and glasshouse but cross-compatible in the field. Such different reaction of particular cultivars on the environment factor can be caused by different alleles of incompatibility (S-allele) which are present in their genotypes. This idea is partially supported by the report of Hawlader and Mian [19] who found several cultivars with moderately strong SI in *Raphanus sativus* then Lee et al. [20] and McCubbin et al. [21], who observed intermediate reaction in SI expression in *Petunia inflanta*. According to some scientists dealing with *Brassica* (e.g. Thompson and Taylor [22], Zuberi et al. [23]) there are a lot of dominant S-alleles which are classified both to low or high series and the expression of SI depends on to which series belongs S-allele responsible for incompatibility. Different expression of SI was also observed in *B. campestris* ssp. *chinensis*, which is described as very strong SI species, by Wojciechowski (unpublished date) who observed completely break down of incompatibility in the glasshouse when temperature was higher than 28°C. Reduction of SI expression by high temperature is also described in pear by Hiratsuka and Tomita [24] who also noticed different expression of incompatibility at different stages of flower bud development. In younger buds expression of SI was weaker as compared to older ones. Therefore, the expression of incompatibility in pear can vary not only with the plant physiological state but also with environmental factors, which could take place in our experiment.

CONCLUSIONS

The obtained results suggest that *P. communis* six cultivars as well as wild ecotype of *P. pyraster* which were under investigations may have different S-genotype which causes different expression of incompatibility under different environmental factors.

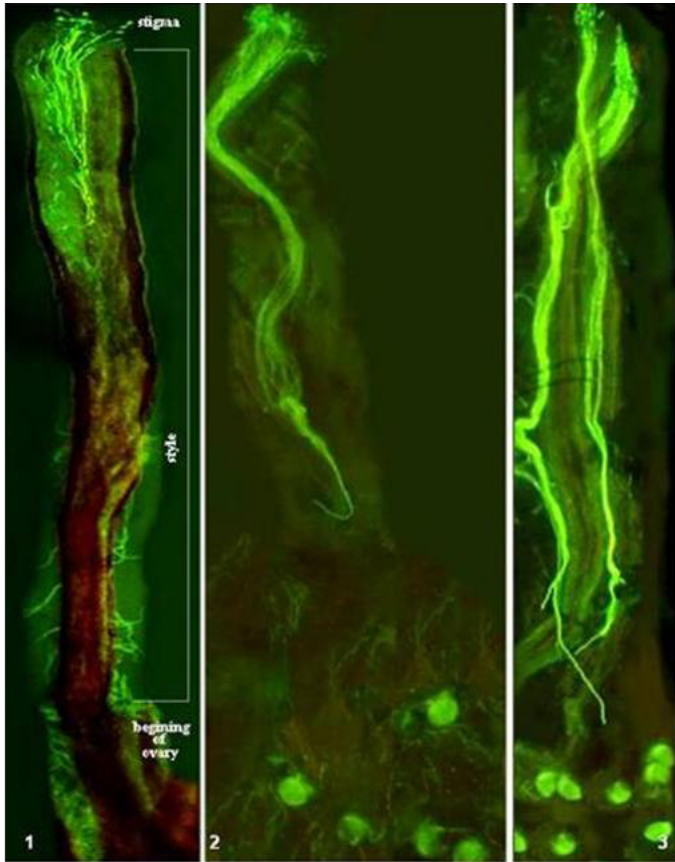


Figure 1. The pistil of 'Red Williams' cultivar with pollen tubes stopping their growth at 1/3 length of the style, 48 h after self-pollination

Figure 2, 3. Pollen tubes penetrating the style: 'Amfora' cultivar, 48 h after pollination with pollen of *P. pyraeaster* (fig. 2), two styles and ovary with ovules of cv. 'Radana', 54 h after pollination with 'Carola' pollen (fig. 3)

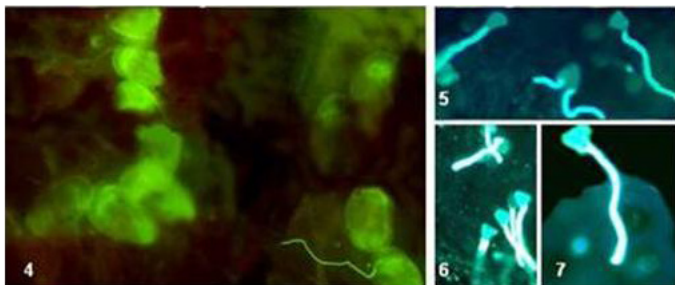


Figure 4. The ovary of cv. 'Dicolor' with the pollen tube entering the ovule, 48 h after pollination with cv. 'Radana' pollen

Figure 5–7. Pollen tubes on the stigma of cv. 'Amfora' with 2 pollen tubes appearing from 1 pollen grain (fig. 5) and swollen ones (fig. 6-7), cross cv. 'Amfora' × cv. 'Carola' 48 h after pollination

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REFERENCES

1. Bell RL. Pears. In: Moore JN, Ballington JR (eds.). Genetic Resources of Temperate Fruit and Nut Crops. Wageningen 1990:657-96.
2. Deckers T, Schoofs H. The pear production in EU. Proc. Xth IS on Pear Eds. A.D. Webster and C.M. Oliveira. Acta Hort 2008:95-105.
3. Van der Zwet T. Role of breeding in improving pear production. Acta Hort 1977; 69:87-96.
4. Kao TH, McCubbin A. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc Natl Acad Sci USA 1966; 93:12059-65.
5. Ushijima K, Sasa H, Tao R, Yamane H, Dandekar AM, Gradziel TM, Hirano H. Cloning and characterization of cDNAs encoding S-RNases in almond (*Prunus dulcis*): primary structural features and sequence diversity of the RNases in the (*Rosaceae*). Mol Gen Genet 1998; 260:261-8.
6. Hołubowicz T. Sadownictwo w Wielkopolsce. In: Rejman A (ed.) Pomologia. Odmianoznawstwo roślin sadowniczych. Warszawa 1994.
7. Ishimizu T, Inoue K, Shimonaka M, Saito T, Terai O, Norioka S. PCR-based method for identifying the S-genotypes of Japanese pear cultivars. Theor Appl Genet 1999; 91:691-8.
8. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990; 18:6531-5.
9. Botta R, Akkac A, Me G, Radicati L, Casavecchia V. Identification of pear cultivars by molecular markers. Acta Hort 1998; 457:63-70.
10. Monte-Corvo L, Cabrita L, Oliveira C, Leitao J. Assessment of genetic relationships among *Pyrus* species and cultivars using AFLP and RAPD markers. Gen Res Crop Evolution 2000; 47:257-65.
11. Oliveira C, Mota M, Monte-Corvo L, Goulão L, Silva D. Molecular typing of *Pyrus* based on RAPD markers. Sci Hortic 1999; 79:163-174.
12. Ye GN, Hemmat M, Lodhy MA, Weeden NF, Reisch BI. Primers longer than 16 bases are particularly useful for RAPD mapping and fingerprinting of grape and pear. Bio Techniques 1996; 20:368-71.
13. Wojciechowski A. Interspecific hybrids between *Brassica campestris* and *B. oleracea*. I. Effectiveness of crossing. Observations of pollen tube growth. The course of embryogenesis. Genetica Polonica 1985; 26/4:423-36.
14. Martin F. Staining and observing pollen tubes by means of fluorescence. Stain Technology 1959; 34:125-8.
15. Matsuzawa Y. Studies of the interspecific hybridisation in genus *Brassica*. II. Crossability in interspecific crosses, *B. oleracea* × *B. campestris*. Japan J Breed 1983; 33:321-30.
16. Broothaerts W, Verdoodt L, Keulemans J, Janssens GA, Broekaert WF. The self-incompatibility gene in apple and determination of the S-genotype of apple cultivars by PCR. Acta Hort 1996; 423:103-9.
17. Zisovich A, Stern R, Shafir S and Goldway M. Identification of seven S-alleles from the European pear (*Pyrus communis*) and determination of compatibility among cultivars. J Hort Sci Biotech 2004, 79:101-6.
18. Nyék J, Soltész M, Ivancsics J. Self-fertility of pear varieties conditioned by natural self-pollination (autogamy). Int. J Hort Sci 2000; 6:110-13.
19. Hawlader MSH, Mian MAK. Self-incompatibility studies in local cultivars of radish (*Raphanus sativus* L.) grown in Bangladesh. Euphytica 1997; 96:311-15.
20. Lee HS, Huang S, Kao TH. S-proteins control rejection of incompatible pollen in *Petunia inflanta*. Nature 1994; 367:560-3.
21. McCubbin AG, Chung YY, Kao TH. A mutant S₃ RNase of *Petunia inflanta* lacking RNase activity has allele-specific dominant negative effect on self-incompatibility reactions. Plant Cell 1997; 9:85-95.

22. Thompson KF, Taylor JP. Self-compatibility in kale. *Heredity* 1971; 27:459-71.
23. Zuberi MI, Zuberi S, Lewis D. The genetics of incompatibility in *Brassica*. I. Inheritance of self-compatibility in *Brassica campestris* L. var. *toria*. *Heredity* 1981; 46:175-90.
24. Hiratsuka S, Tomita A. Incompatible pollen tube growth and protein composition in styles of Japanese pear following high temperature treatments. *Euphytica* 1989; 43:191-6.

DOBÓR ZAPYLACZY DLA POSZCZEGÓLNYCH ODMIAN GRUSZY DOMOWEJ (*PYRUS COMMUNIS* L.) NA PODSTAWIE OBSERWACJI ŁAGIEWEK PYŁKOWYCH

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Streszczenie

Wiele gatunków drzew owocowych, w tym także grusza (*Pyrus*), posiada genetycznie determinowaną skłonność do obcozapyleń (samoniezgodność gametofitowa). Z punktu widzenia praktyki sadowniczej zapylenie krzyżowe jest niezbędne dla uzyskania wysokiego plonowania. Dlatego celem niniejszej pracy było poznanie zjawiska samoniezgodności oraz zgodności i niezgodności krzyżowej u sześciu odmian gruszy domowej *P. communis* (Konferencja, Bonkreta Williamsa, Dicolor, Amfora, Radana, Carola) i dzikiego ekotypu *P. pyraeaster* oraz ocena przydatności wybranych odmian w charakterze dobrych zapylaczy. Pyłek poszczególnych zapylaczy umieszczony został na znamieniu słupka w kwiatach kastrowanych w stadium zamkniętego pąka kwiatowego. Sposób zapyleń obejmował samo- i obcozapylenie w obrębie odmiany oraz zapylenie wszystkich badanych obiektów w układzie diallelicznym. Zapylanie wykonano w sadzie oraz w warunkach laboratoryjnych na odciętych pędach przy dwóch zakresach temperatury, tj. 20°C i 28°C. Obserwacje łagiewek wykonano przy użyciu mikroskopu fluorescencyjnego. Samoniezgodność, zgodność lub niezgodność krzyżową oceniono na podstawie indeksu kiełkowania pyłku (PGI). Badane sześć odmian gruszy uprawnej i jeden ekotyp dzikiej gruszy wykazały całkowitą samoniezgodność. W niektórych krzyżowaniach międzyodmianowych i międzygatunkowych obserwowano całkowitą lub jednostronną niezgodność.

Słowa kluczowe: *Pyrus*, samoniezgodność, łagiewki pyłkowe, grusza