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Intra- and interspecific pollination between *Lupinus albus* L., *Lupinus mutabilis* Sweet. and *Lupinus angustifolius* L.

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Abstract. The pollen grain germination and the growth of pollen tubes after intra- and interspecific reciprocal pollination of *Lupinus albus* L., *L. mutabilis* Sweet. and *L. angustifolius* L. were examined. Results of microscopic observations of pollen grain germination and pollen tube growth enable to deny the existence of certain barriers that prevent fertilization after pollination of *L. albus* flowers with pollen of *L. angustifolius* and vice versa, as well as after pollination of *L. mutabilis* flowers with pollen of *L. angustifolius* and vice versa. Some delay in pollen grain germination and a slower growth of pollen tubes were observed in all combinations, as related to intraspecific ones, though in the case of each interspecific combination pollen tubes reached the ovary. Moreover, a different level of pollination efficiency (the number of growing pollen tubes) and various rates of pollen grain germination and pollen tube growth, depending on the direction of pollination, were found. The noted pollination efficiency may generally be considered to be low.

Key words: *Lupinus albus*, *Lupinus angustifolius*, *Lupinus mutabilis*, interspecific pollination, intraspecific pollination, pollen germination, tube growth.

Introduction

There are over 300 lupin species, some of which are of European origin, whereas others come from the American continents (DUNN 1984, GLADSTONES 1984). A few of them belong to the group of cultivated species, but a vast majority are free-living. Lupin species originating from the New World, characterized by a high natural variability, prevail among those described so far.

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In spite of such a great diversity, only one species of this group, i.e. *Lupinus mutabilis* Sweet. became domesticated (SAWICKA 1993). A high content of both protein with a good amino acid composition and high-quality fat (GROSS 1988) places this species among valuable breeding materials. Late ripening is its disadvantage, as it makes it impossible to obtain satisfactory seed yields.

White lupin (*L. albus* L.) is one of cultivated lupin species that ripes late in Polish conditions. It is of high-quality like *L. mutabilis*, but causes certain problems, especially in the north-eastern part of Poland.

One of the ways that may improve the feature in question is gene introgression from early ripening species. Both Andean and white lupins were crossed to different Mediterranean and American species (KAZIMIERSKI 1960, NOWACKI 1961, WILLIAMS et al. 1980, VUILLAUME, HOFF 1986, SCHÄFER-MENUHR et al. 1988). However, probably due to numerous hybridization barriers, the results of those crossings were not satisfactory. Prezygotic barriers that appear after pollination with pollen of an alien species may prevent the formation of hybrid embryos.

In view of the possibility of avoiding prezygotic barriers or diminishing their effects during wide crossing, observations should be conducted on the course of pollination and fertilization. So, the aim of this paper was to study the pollen grain germination and the process of pollen tube growth after reciprocal intra- and interspecific pollination of *L. albus* L., *L. angustifolius* L. and *L. mutabilis* Sweet .

Material and methods

Three lupin species were used in the course of investigations: white lupine, *Lupinus albus* L., cv. Wat, blue lupin, *L. angustifolius* L., cv. Polonez (seed samples were obtained from the Plant Breeding Station Wiatrowo) and Andean lupin, *L. mutabilis* Sweet., population No. 098903 (seed sample – from the Gene Bank in the Institute of Plant Breeding and Acclimatization). The experiment was carried out in a greenhouse; the seeds were sown into pots in five rounds from the first decade of April to the first decade of May. At flowering, intra- and interspecific pollinations were performed, according to the following scheme:

Intraspecific (control):

L. albus × *L. albus*

L. angustifolius. × *L. angustifolius*

L. mutabilis × *L. mutabilis.*

Interspecific:
L. albus. × *L. angustifolius*
L. angustifolius. × *L. albus*
L. angustifolius . × *L. mutabilis*
L. mutabilis × *L. angustifolius.*

The pistils were collected 6, 12, 24, 36 and 48 hours after the moment of pollination; after being checked for the presence of pollen grains on their stigmas, they were fixed in Carnoy solution and then stored in 70% ethyl alcohol. Pistils, together with the ovary, were macerated in 1N NaOH and dyed with aniline blue in 0.1N K_3PO_4 according to the method of ADACHI et al. (1983). After dyeing, the material was placed on a slide in a drop of glycerine and examined under a fluorescent microscope. On the basis of microscopic observations pollination efficiency (the number of growing pollen tubes), pollen grain germination on the stigmas and pollen tube growth in style and in ovary were estimated.

Results

Pollination efficiency

In spite of the fact that each pistil subject to fixation was checked for the presence of pollen, in none of examined combinations the number of pistils with a trace of pollination was equal to 100%, but varied from 19.9% in the case of *L. mutabilis* × *L. mutabilis* combination to 65.9% in that of *L. augustifolius* × *L. augustifolius* (Table 1). The pollination efficiency amounted to 30% in *L. albus* × *L. albus* combination, while among interspecific cross combinations the number of efficiently pollinated pistils was found to be the greatest in the cases where *L. angustifolius* was a female form (34.4% with *L. albus*, 47.2% with *L. mutabilis*). In the combination where white lupin was pollinated with pollen of blue lupine, the pollination efficiency was slighly higher than in the control (*L. albus* × *L. albus*), and *L. mutabilis* was pollinated better with pollen of *L. angustifolius* (26.2%) than with its own pollen (19.9%).

Pollen grain germination and pollen tube growth

Lupinus albus × *Lupinus albus*

First pollen tubes that reached the ovary were observed 36 hours after pollination; they constituted only 2.4% of all examined pistils. Much more such pistils (14.3%) were noted 48 hours after pollination. The same number of pollen tubes in pistil canals was observed after equal periods of time.

Table 1. The course of intra- and interspecific pollination of *L. albus*, *L. angustifolius* and *L. mutabilis*

Pollination	Total number of pollinations	Efficient pollinations*		Number of hours after pollination	Pistils with non-germinating pollen grains		Pistils with pollen tubes					
							on stigma		in style		in ovary	
		No.	%		No.	%	No.	%	No.	%	No.	%
1	2	3	4	5	6	7	8	9	10	11	12	13
Intraspecific												
L. albus × *L. albus*	140	42	30.0	6	0	0	3	7.1	2	4.8	0	0
				12	0	0	2	4.8	2	4.8	0	0
				24	0	0	3	7.1	3	7.1	0	0
				36	3	7.1	1	2.4	0	0	1	2.4
				48	2	4.8	0	0	0	4.3	6	14.3
L. angustifolius × *L. angustifolius*	91	60	65.9	6	1	1.7	5	8.3	3	5.0	0	0
				12	2	3.3	1	1.7	0	0	3	5.0
				24	0	0	0	0	11	18.3	3	5.0
				36	0	0	0	0	5	8.3	11	18.3
				48	0	0	0	0	4	6.7	11	18.3
L. mutabilis × *L. mutabilis*	156	31	19.9	6	1	3.2	2	6.5	2	0	0	0
				12	1	3.2	3	9.7	2	16.1	0	0
				24	0	0	0	0	3	22.6	1	3.2
				36	0	0	1	3.2	8	16.1	0	0
				48	0	0	0	0	6	12.9	1	3.2
Interspecific												
L. albus × *L. angustifolius*	422	136	32.2	6	14	10.3	11	8.1	3	2.2	0	0
				12	9	6.6	2	1.5	5	3.7	0	0
				24	12	8.8	6	4.4	6	4.4	0	0
				36	2	1.5	21	15.4	16	11.8	4	2.9
				48	0	0	5	3.7	11	8.1	9	6.6

1	2	3	4	5	6	7	8	9	10	11	12	13
L. angustifolius × *L. albus*	430	148	34.4	6	3	2.0	1	0.7	6	4.1	0	0
				12	5	3.4	5	3.4	21	14.2	0	0
				24	0	0	7	4.7	17	11.5	3	2.0
				36	0	0	3	2.0	13	8.8	8	5.4
				48	0	0	7	4.7	31	20.9	18	12.2
L. angustifolius × *L. mutabilis*	322	152	47.2	6	0	0	5	3.3	3	2.0	0	0
				12	1	0.7	7	4.6	12	7.9	1	0.7
				24	0	0	3	2.0	22	14.5	4	2.6
				36	9	5.9	1	0.7	8	5.2	18	11.8
				48	4	2.6	0	0	17	11.2	37	24.3
L. mutabilis × *L. angustifolius*	298	78	26.2	6	4	5.1	8	10.3	3	3.8	0	0
				12	2	2.6	6	7.7	2	2.6	0	0
				24	0	0.	7	8.9	8	10.3	0	0
				36	1	1.3	4	5.1	14	17.9	0	0
				48	0	0	1	1.3	8	10.3	10	12.8

* efficient pollinations acc. to the authors mean the number of growing pollen tubes.

Lupinus angustifolius × *Lupinus angustifolius*

Penetration of pollen tubes into the ovary (Fig. 1) was observed in 5% of the studied pistils as quickly as 12 hours after pollination. The same result was obtained 24 hours after pollination, and the presence of pollen tubes in pistils was noted when the number of pistils was over three times greater (18.3%). After 36 and 48 hours, the percentage of pistils with pollen tubes in the ovary increased over threefold.

Lupinus mutabilis × *Lupinus mutabilis*

16.1% of pistils with pollen tubes in canals were observed 12 hours after pollination. Their number increased even more after the next 12 hours. Pollen tubes entered the ovary for the first time (Fig. 2) in 3.2% of the examined pistils also after the same period of time. The same result was achieved 48 hours after pollination.

Lupinus albus × *Lupinus angustifolius*

Pollen tubes of *L. angustifolius* reached the ovary of *L. albus* 36 hours after pollination (Fig. 3). The presence of pollen tubes inside the pistil was noted in a 4-fold larger number pistils. After 48 hours, the number of pistils with pollen tubes in the canal decreased while the number of pistils with pollen tubes reaching the ovary grew to 6.6%.

Lupinus angustifolius × *Lupinus albus*

Pollen tubes of *L. albus* were observed in a few ovaries of *L. angustifolius* as quickly as 24 hours after the moment of pollination (Fig. 4). The number of pistils with pollen tubes in the ovary was growing within the next hours, and after 48 hours it constituted 12.2% of all examined pistils. Besides, tubes penetrating the pistil canal were also relatively frequent (in 20.9% of the examined pistils) after the same period of time.

Lupinus angustifolius × *Lupinus mutabilis*

Single pistils with pollen tubes in the ovary were observed after 12 hours (Fig. 5). Their percent was 2.6% after the next hours increasing to 11.8% after 36 hours and reached 24.3% after 48 hours following pollination. The percentage of pistils penetrated by pollen tubes 24 hours after pollination amounted to 14.5%, whereas 48 hours after pollination constituted 10.3%.

Lupinus mutabilis × *Lupinus angustifolius*

With the passage of time the number of pistils with pollen tubes growing inside the pistil increased (by ca. 3% after 6 and 12 hours, ca. 10% after

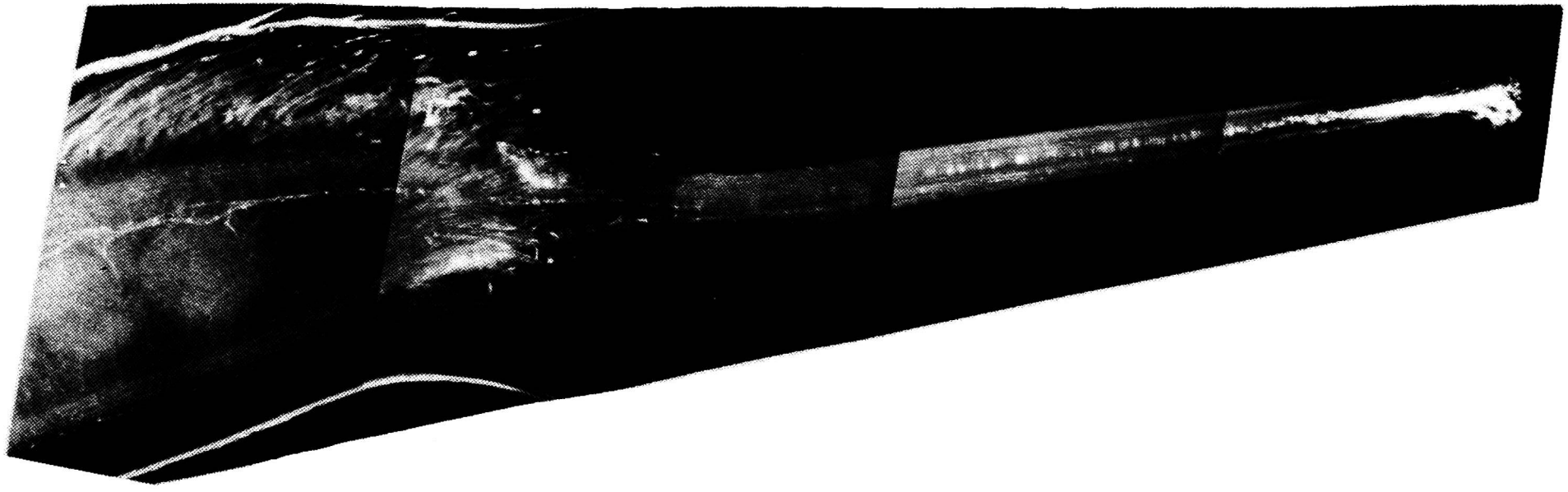

Fig. 1. *L. angustifolius* pollen tubes in *L. angustifolius* ovary 24 hours after pollination

Fig. 2. *L. mutabilis* pollen tubes in *L. mutabilis* ovary 48 hours after pollination

Fig. 3. *L. angustifolius* pollen tubes in *L. albus* ovary 36 hours after pollination

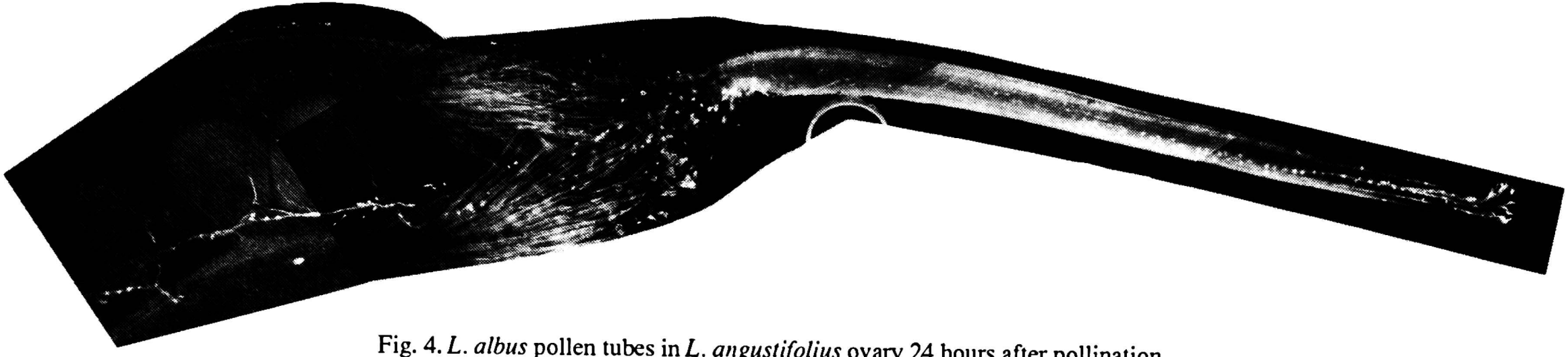

Fig. 4. *L. albus* pollen tubes in *L. angustifolius* ovary 24 hours after pollination

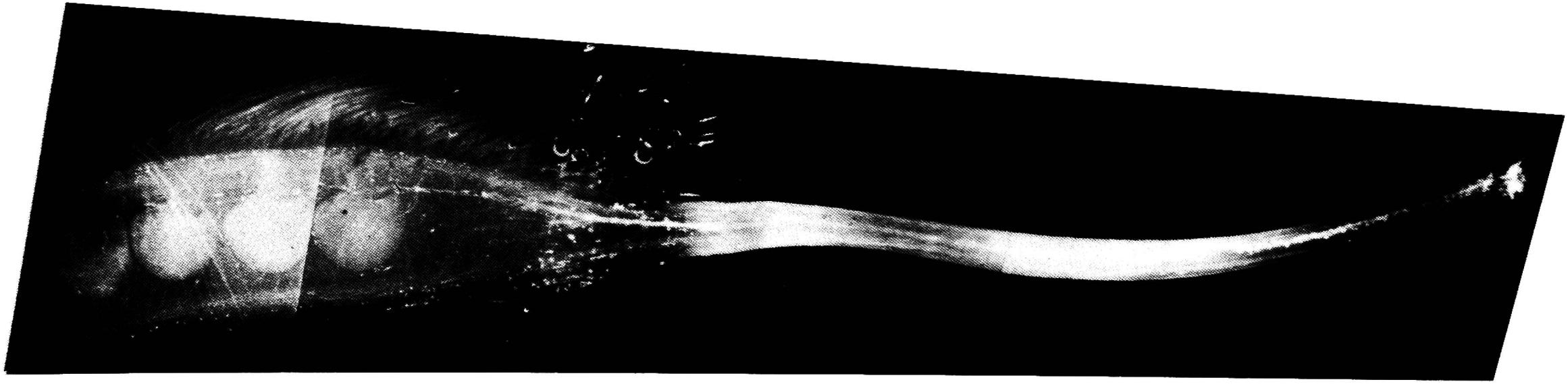

Fig. 5. *L. mutabilis* pollen tubes in *L. angustifolius* ovary 24 hours after pollination

Table 2. Pollen tube growth after intra- and interspecific pollination of *L. albus*, *L. angustifolius* and *L. mutabilis*

Pollination	Number of efficient pollinations*	Non-germinating pollen grains		Pollen tubes							
				on stigma		in style		in ovary		total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Intraspecific											
L. albus × *L. albus*	27	5	18.5	1	3.7	14	51.9	7	25.9	22	81.5
L. angustifolius × *L. angustifolius*	51	2	3.9	1	2.0	20	39.2	28	54.9	49	96.1
L. mutabilis × *L. mutabilis*	20	0	0	1	5.0	17	85.0	2	10.0	20	100.0
Interspecific											
L. albus × *L. angustifolius*	68	2	2.9	26	38.3	27	39.7	13	19.1	66	97.1
L. angustifolius × *L. albus*	83	0	0	10	12.1	44	53.0	29	34.9	83	100.0
L. angustifolius × *L. mutabilis*	144	14	9.7	11	7.6	59	41.0	60	41.7	130	90.3
L. mutabilis × *L. angustifolius*	19	0	1	1	5.3	8	42.1	10	52.6	19	100.0

* efficient pollinations acc. to the authors mean the number of growing pollen tubes.

24 hours and 17.9% after 36 hours). Their number 48 hours after the moment of pollination dropped to the level of 24 hours, and flowers with pollen tubes in the ovary constituted ca. 13%.

To compare individual combinations to the control, only the results concerning time period when pollen tubes reached the ovary were taken into account (Table 2). The percentage of pistils with pollen tubes reaching the ovary and ovules was lower in the case of all interspecific combinations than in the control, with only one exception – *L. mutabilis* × *L. angustifolius* where pistils with tubes in the ovary were over two times more frequent than in *L. mutabilis* × *L. mutabilis*. The number of pistils with pollen germinating on the stigmas was higher in all examined interspecific combinations than in the control; such pistils were more frequent in *L. albus* × *L. angustifolius* and in *L. mutabilis* × *L. angustifolius* than in the control. In the case of combinations involving *L. angustifolius* as a female form, the number of pistils with tubes on the stigmas was comparable to that of the control.

A higher number of pistils with non-germinating pollen on the stigmas than in intraspecific pollinations, was observed in all studied interspecific combinations; ca. 30-60% of flowers with tubes in the pistil canal were observed in all combinations, both intra- and interspecific. Only in the case of *L. mutabilis* × *L. mutabilis* the number of flowers with pollen tubes penetrating the pistil constituted more than 60%.

Pollen grain germination and pollen tube growth depending on the direction of pollination

When *Lupinus albus* was used as a female form in interspecific pollination with *L. angustifolius*, the pollination efficiency was lower than that in the intraspecies (*L. albus*) combination. However, it was a relatively good pollinator, as 34.4 % out of its 148 pollinated flowers in the interspecific combination with *L. angustifolius* appeared to be pollinated efficiently (Table 3, Fig. 6). Pollen of *L. albus* germinated on the stigmas of its own species (in 21.4% of the examined pistils) to a higher degree than on the stigmas of *L. angustifolius* (in 15.51% of examined pistils), while *L. albus* stigmas proved to be a relatively good environment for *L. angustifolius* pollen, which germinated on 33.1% of the pistils. Pollen tubes of *L. albus* were found in the pistil canal (59.5%) and in the ovary (19.6%) of *L. angustifolius* pistils. The percentage of *L. angustifolius* pistils with *L. albus* pollen tubes in the pistil canal and in ovary was comparable to the control. Pollen tubes of *L. angustifolius* were found in *L. albus* pistil canal in ca. 30% of pistils (in comparison to

Table 3. Pollen grain germination and pollen tube growth depending on the direction of pollination of *L. albus*, *L. angustifolius* and *L. mutabilis*

Direction of pollination	Total number of polli-nations	Efficient pollinations*		Non-germinating pollen		Pollen tubes							
						on stigma		in style		in ovary		total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
L. albus × *L. albus*	140	42	30.0	5	11.9	9	21.4	21	50.0	7	16.7	37	88.1
L. albus × *L. angustifolius*	422	136	32.2	37	27.2	45	33.1	41	30.1	13	9.6	99	72.8
L. angustifolius × *L. angustifolius*	91	60	65.9	3	5.0	6	10.0	23	38.3	28	46.7	57	95.0
L. angustifolius × *L. albus*	430	148	34.4	8	5.4	23	15.5	88	59.5	29	19.6	140	94.6
L. angustifolius × *L. mutabilis*	322	152	47.2	14	9.2	16	10.5	62	40.8	60	39.5	138	90.8
L. mutabilis × *L. mutabilis*	156	31	19.9	2	6.5	6	19.3	21	67.7	2	6.5	29	93.5
L. mutabilis × *L. angustifolius*	298	78	26.2	7	9.0	26	33.3	35	44.9	10	12.8	71	91.0

* efficient pollinations acc. to the authors mean the number of growing pollen tubes.

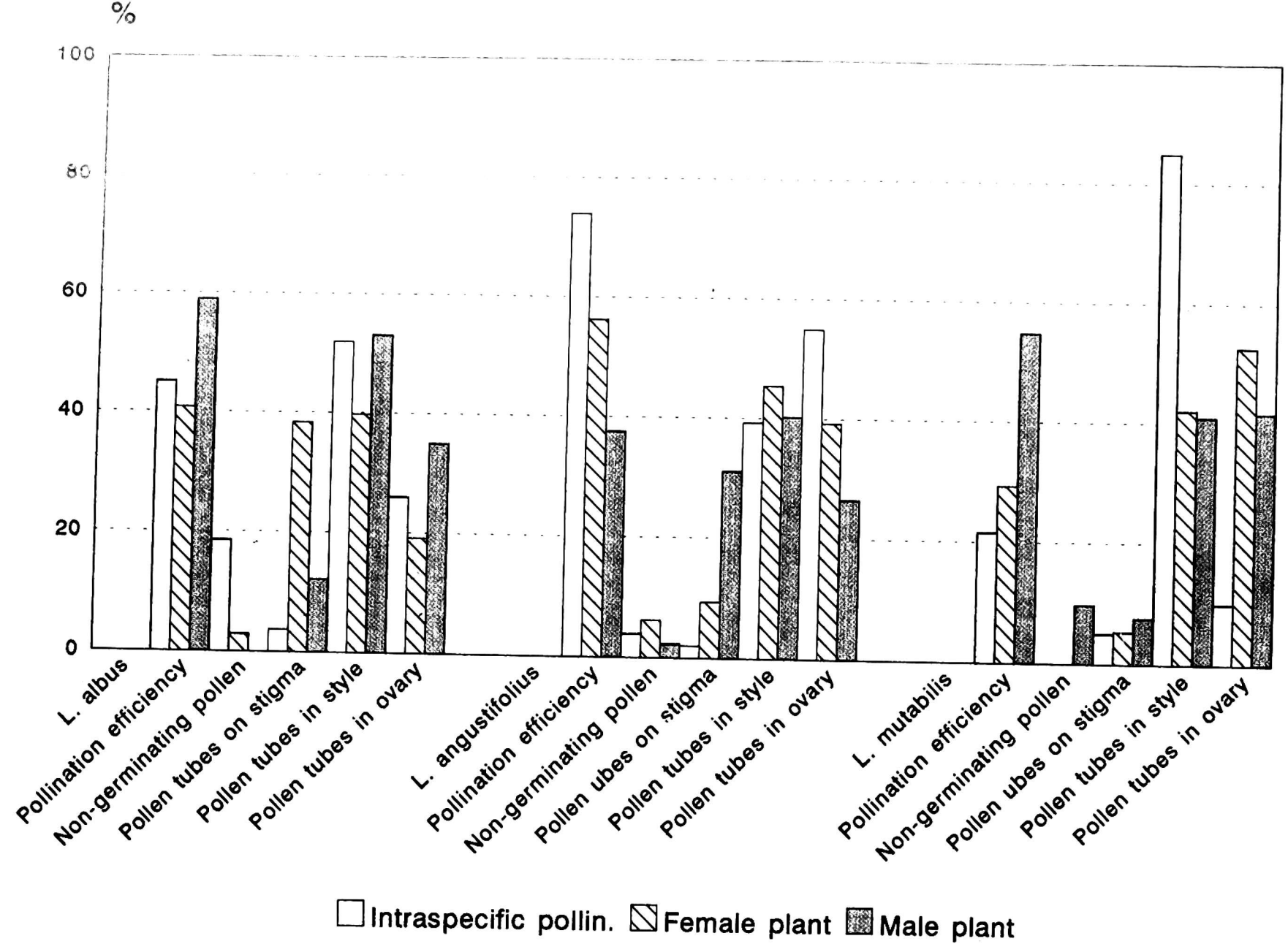

Fig. 6. Pollen tube growth depending on the direction of pollination (*L. albus* × *L. angustifolius*, *L. angustifolius* × *L. albus*, *L. angustifolius* × *L. mutabilis* and *L. mutabilis* × *L. angustifolius*)

50.0% within the species), and in the *L. albus* ovary – 9.6% (within the species – 16.7%).

Lupinus angustifolius was pollinated the best with its own pollen, and the pollination efficiency in that case was equal to 65.9%. The efficiency in question was lower than that of the control when this species was used as a female form. Stigmas of *L. albus* and *L. mutabilis* were not receptive enough to pollen of *L. angustifolius* (the pollination efficiency in that case amounted to on average 40.8%). Pollen tubes of alien species reached *L. angustifolius* ovary in 29.6% of the flowers. *L. angustifolius* pollen tubes reached alien ovaries in 11.2% of flowers. The presence of alien pollen tubes was observed in *L. angustifolius* styles in 50.2% of pistils, and in comparison with intraspecific pollination, they were 12% more numerous. Pollen tubes of *L. angustifolius* penetrated the stigmas and grew down through the styles with the frequency similar to that of the control combination. When *L. angustifolius* was a female form, both germinating and non-germinating pollen was found on the stigmas of its pistils. The both numbers were higher than those in the case of intraspecific pollinations. Among 214 *L. albus* and *L. mutabilis* flowers, efficiently pollinated with *L. angustifolius* pollen, ca. 30% had pollen germinating on the stigmas, while only 18.1% had non-germinating pollen.

Lupinus mutabilis was pollinated more effectively with pollen of *L. angustifolius* than with its own pollen (Table 3). Pollen tubes of *L. angustifolius* reached *L. mutabilis* ovary in 12.8% of pistils, i.e. two times more frequently than after intraspecific pollination. *L. mutabilis* pollen reached the ovaries of 39.5% of examined *L. angustifolius* pistils. Almost the same number of pollen tubes in pistil tissue was observed in *L. angustifolius* × *L. mutabilis* and *L. mutabilis* × *L. angustifolius* combinations. In the both cases, the number of pistils with tubes in the styles was lower than that in intraspecific pollination. However, germinating pollen was found on 33.3% of the examined pistils after pollination with *L. angustifolius* pollen, and on 19.3% after pollination with its own pollen. *L. mutabilis* pollen germinated on 10.5% of the stigmas of *L. angustifolius* pistils.

Discussion

Results of observations on pollen grain germination and pollen tube growth in pistils in vivo as a result of reciprocal pollination among *Lupinus* species were presented only by few researchers. JARANOWSKI (1962a, 1962b) conducted a cytoembryological research on *Lupinus* species concerning the process of fertilization and embryo development following autogamy and reciprocal

crossing between *L. albus*, *L. angustifolius* and *L. luteus*. Based on observations of pollen grain germination and pollen tube growth, WILLIAMS et al. (1980) evaluated cross-compatibility between European species (*L. albus*, *L. angustifolius*, *L. luteus*) as well as between European species and *L. mutabilis* from South America. Investigations on interspecific compatibility among *Lupinus* species carried out by FALUYI and WILLIAMS (1981) resulted in the discovery of self- and cross-incompatibility in intra- and interspecific crossings of *L. albus*. The course of pollen grain germination and pollen tube growth were observed by BUSMANN-LOOCK et al. (1992) to identify crossing barriers between *L. luteus* and *L. hartwegii* as well as between *L. mutabilis* and *L. hartwegii*.

Cross-incompatibility is connected with the lack of pollen grain germination on the stigma, with inhibition of pollen tube growth on the stigma or with its gradual reduction, leading to cessation of growth at the pistil base. The presented results of microscopic observations concerning pollen grain germination and pollen tube growth enable one us to deny the existence of certain barriers that prevent fertilization after pollination of *L. albus* flowers with pollen of *L. angustifolius* and vice versa, as well as after pollination of *L. mutabilis* flowers with pollen of *L. angustifolius* and vice versa. Some delay in pollen grain germination and a slower growth of pollen tube were observed in all studied combinations, in comparison with intraspecific pollination, but in the case of each interspecific combination pollen tubes reached the ovary. The same was found by WILLIAMS et al. (1980) who evaluated cross-compatibility among European and American lupin species. JARANOWSKI (1962b) carried out a cytological and embryological research concerning the possibility of obtaining hybrids between *L. albus* and *L. angustifolius*, which resulted in proposing his thesis that the above mentioned species are characterized by sexual compatibility enabling their reciprocal fertilization. Fertilization processes, however, were delayed to a considerable degree in comparison with fertilization accompanying self-pollination of parental forms; the barrier effect was revealed after joining parental gametes.

It should also be noted that the conducted microscopic observations showed that pollen tubes in the case of all cross-combinations reached the ovary within the period of time equal or similar to that described by JARANOWSKI (1962a, 1962b) and WILLIAMS et al. (1980). An exception were only two combinations *L. angustifolius* × *L. angustifolius* and *L. angustifolius* × *L. mutabilis*, where the presence of pollen tubes was noticed in some cases as early as after 12 hours. Due to self-pollination of *L. angustifolius* and due to the fact that pollination takes place at the bud stage, this result may be an artefact (pollination may have occurred before castration and isolation of flowers).

It is also worth emphasizing that the pollination efficiency and rate of pollen grain germination as well as the growth rate of pollen tube were different depending on the direction of pollinations. Such phenomenon was also observed by WILLIAMS et al. (1980) in the crossings of *L. mutabilis* with European species. BUSMANN-LOOCK et al. (1992) found that inhibition of pollen tube growth in the pistil was stronger in the cross *L. luteus* × *L. hartwegii* than in *L. hartwegii* × *L. luteus*. Unilateral incompatibility was also found in the cross *L. luteus* × *L. rothmaleri* (KAZIMIERSKI, KAZIMIERSKA 1965).

The noted pollination efficiency expressed in the rate of pollen tubes growth, may generally be considered as low. Blue lupin used to pollinate itself with its own pollen relatively easily, in contrast to other species examined in the experiments, which pollinated themselves to a slight degree with both their own and alien pollen. Differences in the pollination efficiency may be associated with the existence or non-existence of mechanisms and features that make self-pollination difficult or even impossible. Such mechanisms do not exist in blue lupine, which is an autogamic species. Its pistil stigma can receive pollen grains at the moment when anthers are capable of releasing fertile pollen, and pollination takes place before the flowers have opened. The mechanisms and features that make self-pollination difficult exist in the case of white lupin and *L. mutabilis*, which are allogamous species, and protandry is one of them. JUNCOSA and WEBSTER (after WOJCIECHOWSKA 1993) carrying out observations with the help of a scanning electron microscope found that there was no trace of elicitor indispensable for adhesion and germination of pollen grains on the surface of a one-day stigmas of *L. nanus* subsp. *latifolius*, while stigmas of two- and three-day flowers were covered with the elicitor and pollen grains. Moreover, the same author states that stigmas cut out of newly-opened *L. mutabilis* flowers were dry and clean, and pollen collected from them efficiently pollinated stigmas of older flowers. The above facts may help to explain why pollen was not found on the stigmas of most flowers after fixation of the material though it was present there before the treatment.

Conclusions

The noted pollination efficiency expressed in the rate of pollen tube growth in the pistil, may generally be considered to be low. Some delay in pollen grain germination and a slower growth of pollen tubes were observed in all combinations, although in each interspecific combination pollen tubes reached the ovary. This study showed different levels of pollination efficiency and various rates of pollen germination and pollen tube growth depending on the direction

of crossing. The results of microscopic observations concerning pollen germination and pollen tube growth enabled us to deny the existence of certain barriers that prevent fertilization after pollination of *L. albus* flowers with pollen of *L. angustifolius* and vice versa, as well as after pollination of *L. mutabilis* flowers with pollen of *L. angustifolius* and vice versa.

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REFERENCES

ADACHI T., KAWABATA K., MATSUZAKI N., YABUYA T. (1983). Observation of pollen tube elongation, fertilization and ovule development in autogamous autotetraploid buckwheat. Proc. 2 Int. Symp. Buckwheat, Miyazaki, Japan, 7-10 September 1983: 103-114.

BUSMANN-LOOCK A., DAMBROTH M., MENGE-HARTMANN U. (1992). Histological observations on interspecific crosses in the genus *Lupinus*. Plant Breeding 109: 82-85.

DUNN D.B. (1984). Cytotaxonomy and distribution of new world lupin species. Proc. 3th Int. Lupin Conf. La Rochelle, France, 4-8 Juni 1984: 68-85.

FALUYI M.A., WILLIAMS W. (1981). Studies on the breeding system in lupin species: a) self and cross compatibility in the three European lupin species, b) percentage of out-crossing in *Lupinus albus*. Z. Pflanzenzuchtung 87: 233-239.

GLADSTONES J.S. (1984). Present situation and potential of Mediterramean African lupins for crop production. Proc. 3th Int. Lupin Conf. La Rochelle, France 4-8 Juni 1984: 18-37.

GROSS R. (1988). Lupins in human nutrition. Proc. IV Int. Lupin Conf. Poznań, July 1988: 51-64.

JARANOWSKI J. (1962a). Fertilization and embryo development in the genus *Lupinus*. Part I. Seed development in cases of autogamy. Genet. Pol. 3: 210-246.

JARANOWSKI J. (1962b). Fertilization and embryo development in the genus *Lupinus*. Part II. Fertilization and embryo development following reciprocal species hybridization. Genet. Pol. 3: 333-368.

KAZIMIERSKI T. (1960). An interspecific hybrid in the genus *Lupinus* (*Lupinus albus* L. × *Lupinus jugoslavicus* Kazim. et Now.). Genet. Pol. 1: 3-60.

KAZIMIERSKI T., KAZIMIERSKA E. (1965). Studies on a species hybrid *Lupinus rothmaleri* Klink. × *Lupinus luteus* L. Genet. Pol. 6: 125-140.

NOWACKI E. (1961). An interspecific hybrid *Lupinus mutabilis* Sweet. × *Lupinus ornatus* Duogl. × *Lupinus douglasi* Lindl. Genet. Pol. 2: 1-17.

SAWICKA E.J. (1993). Indukowane mutacje u łubinu andyjskiego (*Lupinus mutabilis* Sweet.). Prace Ogrodu Botanicznego PAN Warszawa, 3: 3-13.

SCHÄFER-MENUHR A., CZERWIŃSKI T., BRUSMANN A. (1988). Der Einsatz von Embryokultur zur Gewinung von Artbastarden aus der Kreuzung *Lupinus mutabilis* × *Lupinus hartwegii*. Landbaufors. Völk. 38, 3: 173-177.

VUILLAUME E., HOFF T. (1986). Development in vitro d'embryons immatures de *Lupinus albus* L. et de *Lupinus mutabilis* Sweet. par culture de gousses, d'ovules ou d'embryons isoles. Agronomie 6(10): 925-930.

WILLIAMS W., AKHTAR M.A., FALUYI M. (1980). Cross compatibility between European and American *Lupinus* species. Bot. J. Linnean Soc. 81: 225-232.

WOJCIECHOWSKA W. (1993). Finezja zapylania łubinów. Wszechświat 11(94): 271-277.