EFFICIENCY OF *A. ARGUTA* (SIEBOLD ET ZUCC.) PLANCH. EX. MIQ. POLLINATION USING *A. DELICIOSA* POLLEN

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Abstract

Actinidia arguta is a dioecious vine commercially cultivated in many countries. Effective pollination is essential for good quality yield. Possibility of supporting the A. arguta natural pollination with commercially available A. deliciosa pollen was studied. The research was conducted on commercial plantation in central Poland. Regardless of the many troubles and threats associated with this procedure the effectiveness is insufficient. More appropriate solution seems to be optimizing natural pollination using A. arguta pollen.

Key words: *Hardy kiwifruit, Bower Actinidia, Kiwiberry, fruit quality, pollination, pollen*

1. INTRODUCTION

Actinidia arguta (Siebold et Zucc.) Planch. ex. Miq. is functionally dioecious woody vine, cultivated in North America, New Zealand, China and Chile. Recently it is also becoming more popular in some European countries, such as France, Belgium, Netherlands, Austria, Switzerland and Poland. The fruits - called hardy kiwifruits, kiwiberry or minikiwi, are grape-sized with green edible skin. High diversity of intensive aroma and flavour among the cultivars is well perceived by consumers. The fruits are rich in vitamin C and antioxidants, as well as mineral compounds, which makes them attractive and healthy accent in peoples' diet (Latocha et al. 2011, Latocha and Jankowski 2011). As all dioecious or self-incompatible plants it faces a problem of effective pollination. *Actinidia* species are thought to be adapted to both: wind and insects methods of pollination. Producing big amounts of pollen, which is observed in *A. arguta,* is characteristic to dioecious wind pollinated plants, but secretion of volatile compounds in attractive flowers with perianth, strongly suggest the role of pollinators, especially bees (Fraser and McNeilage 2016). Moreover *Actinida* are cryptic dioecious – female flowers with developed stamens are producing sterile pollen. According to the study on *A. polygama* made in Japan, pollinators did show significantly lower interest in female flowers after artificial stamens removal, comparing to observations of non-damaged flowers (Kawagoe and Suzuki 2004). Pollen quality plays the most important role in all methods of pollination. Viability and germination ability are basic parameters used to check the pollen quality. Previous research on *A. deliciosa* 'Tomuri' shows that both parameters may differ a lot even within cultivar. The viability of fresh pollen ranged between 97.9 % (north-west Portugal) and 70% (Santa Catarina, Brazil). Also different best conditions for pollen storage were observed. Generally long-term preservation is more effective in low temperatures, in pollen banks it is usually -196ºC, but every species can react in a different way and the best temperature should be selected individually. It is still not clear what temperature allows keeping good quality of *Actinidia* pollen. The results of the previous research do not correspond to each other, being -20ºC for Portuguese 'Tomuri' and -196ºC for Brazilian one (Abreu and Oliveira 2004, Borghezan et al. 2011, Gill 2014).

The pollen limitation, may also be caused by too large distance between male and female plants, as well as smaller pollinators interest on one sex, resulting in reduced seed set (de Jong et al., 2005). If it is only partially reduced, it is not a problem in natural environment, but is becoming a difficulty in commercial fruit production. As it was shown in research on *A. deliciosa*, increasing distance between female and male plants, causes reduction in seed number, and, consequently, fruit mass (Testolin

1991, Costa et al. 1993). The linear relationship between seed number and fruit weight was also observed in *A. arguta* 'Ananasnaya' (Tiyayon and Strik 2003). Another problem in *Actinidia* pollination seems to be short effective pollination period, about four days after anthesis in kiwifruit, after which decrease of stigmas receptivity and lower fruit set is observed (Gonzales, Coque & Herrero 1997). Comparison of different methods of pollination proved that natural pollination or with bee assistance is not as effective as hand pollination (Costa et al., 1993). On the other hand, it is still much more efficient than wind pollination (89%, 24% fruit set, respectively) (Howpage et al. 2001). In recent years self-fruiting *A. arguta* 'Issai' selection has been presented, but the fruit set (around 30%, comparing to the cross-pollination 'Issai' × male clones – 65%) has not been satisfactory (Mizugami et al. 2007), or like it had been tested in previous research in Polish conditions, the 'Issai' pollen had been sterile (Olszewska-Kaczyńska and Latocha 2004).

Although there are some reports that *A. deliciosa* pollen can fertilize *A. arguta* (Strick 2005), successful pollination in kiwi fruit production should also be considered on genetic level. The ploidy among *Actinidia* taxes varies between diploides to hexaploids or, rarely, even to decaploid. Tetraploidy was the most common in previously studied genotypes of *A. arguta* and hexaploidy in *A. deliciosa.* High ploidy diversity in *Actinidia* may result in bigger crossing difficulties within than between taxa (Ferguson and Huang 2016).

Another problem in *Actinidia* pollination using foreign pollen is a risk of introducing bacterial pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa). The infection symptoms are leaf spots and necrosis, that usually leads to leaf loosing. Also flowers wilting, fruit scabs and specks or branch canker are observed. It causes economical losses in kiwifruit (*A. deliciosa* and *A. chinensis*) production all over the world (Marcelletti et al. 2011). Psa was also isolated from *A. arguta* wild plants in Japan (Ushiyama et al. 1992). Italian observation suggests that rapid spread of the pathogen may be caused by distribution of latently infected plant material, also, there is an evidence that pollen can be source of infection (Scortichini et al. 2012, Tontou, Giovanardi & Stefani 2014). High virulence and damage to fruit production are the reasons why European Union government decided to limit trade and transport of *Actinidia* plant material.

It is clear that successful pollination is crucial in fruit production. Lots of natural mechanisms, including huge amounts of pollen grains production, cryptic dioecy manifested by perfect flowers with sterile pollen on female plants, pistil secretions that enable pollen tube growth and fertilization, are observed *in vivo,* but still in some cases pollen quantity and quality limitation reduces the fruit set (Kawagoe and Suzuki 2004).

In present work, efficiency of pollination using *A. deliciosa* pollen as a support of natural pollination in comparison to only natural pollination was studied. Also controlled hand pollination, using *A. deliciosa* and *A. arguta* 'Weiki' pollen separately, was made to examine if *A. deliciosa* pollen is able to fertilize *A. arguta* flowers*.* Two female cultivars: Geneva and Weiki, the most commonly grown *A. arguta* cultivars in Europe, were chosen for this research.

2. MATERIALS AND METHODS

The experiments were carried out at the commercial plantation in Bodzew, Grójec region, Poland, in the years 2015-2016. Two female *A. arguta* cultivars 'Weiki' and 'Geneva' were studied. The pollinator in the orchard was male cv *A. arguta* 'Weiki'. Natural pollination at the vineyard includes wind and insects such as honey bees and bumblebees pollinating. Plants were grown in rows, 4,5 meters apart and were trained on 2 m high T-shaped support. Plants were 4 year old at the beginning of the experiment and plants were planted male to female ratio 1 to 6, shifted by one in each row. Experiment was designed in blocks. For each female cv and treatment 3 blocks containing four plants were selected. For each block the distance between female and male plants was always the same. The boxes with bumblebee families were put in rows, immediately before flowering. Two experiments were made. First – controlled pollination was provided on isolated shoots using 'Weiki' and *A. deliciosa* pollen separately. Verification if the hexaploid *A. deliciosa* is able to fertilize tetraploid *A. arguta*, was the purpose of the study. Three shoots, with buds number above 30, were isolated for each pollen on both female cultivars, a week before flowering. The isolators were taken off a week after flowers lost their petals. The 'Weiki' pollen, which was used in the experiment, had been previously extracted from male flowers in the „balloon stage", kept few hours in 25°C and then storaged in the fridge in 4°C. The *A. deliciosa* pollen, commonly used on commercial plantations as a supportive pollination, was bought in Argentina in 2014. It was transported frozen (about -12°C) and put in deep freezer (-80°C) immediately after arriving to Poland. The pollination method in this experiment was handmade, using little brushes.

The aim of the second experiment was comparing efficiency of natural pollination with supported one. The supportive pollination method used in the experiment was developed by BNS Micro-Nutrients NV (Belgium). The procedure included making dilution of suspension medium for wet application of kiwi fruit (*A. deliciosa*) pollen (250 ml) in deionised water (15L). After intimately mixing, adding 50g of pollen, previously progressively warmed during two days from -80°C, through -20°C and 4°C, to 17°C and mixing again, using wooden stick. The pollen solution was sprayed within one hour after preparation, using sprayer Stihl SR 200 with non-pressurized spraying turbine. The female plants were supportively pollinated at the full flowering stage (when all flowers were open). *A. deliciosa* pollen solution was prepared and sprayed on the half of the experimental blocks of *A. arguta* female plants.

Ten flowering long shoots were marked on each block before flowering and cut above the last inflorescense. The flower buds were counted on every shoot. Also 30 short shoots, were marked on each block. Before harvest fruit on each marked shoot were counted again. Then, fruits were collected at the commercial maturity stage. Kiwiberry yield, percentage of fruit set, fruit weight, were calculated or measured. Average yield per wine was determined too. The temperature during flowering period was also recorded. Two-way ANOVA and Tukey HSD test to compare the differences between means at significant level =0.05 were used, all statistical analysis were made using Statistica 13 software (USA).

Pollen viability and germination ability were measured every year just before pollination. The viability was checked using acetocarmine, which stains viable pollen grains in pink/red colour. The germination ability was provided on 10% sucrose agar medium with addition of boric acid (Borghezan et al. 2011). The pollen tubes were counted after 5 hours. The measurements were taken on 300 pollen grains for each species.

3. RESULTS AND DISCUSSION

3.1. Weather conditions

The weather conditions varied between years 2015 and 2016. The first year of experiment was rather dry: the average precipitation (Figure 1a) was more than 100 mm lower than long-term average: 425.4 mm, 531 mm respectively. Whereas in 2016 the precipitation was about 140 mm higher than long-term average, reaching value 671.2 mm. The average temperature (Figure 1b) was higher than long-term average for both years. It was 2.3°C higher in 2015 and 1.6°C in 2016. During the first year of the experiment, the average temperature was much higher than the long term one in the first half of the year, while in months July – November it was lower than the long term average. December 2015 was observed as warmer than usually. For a change in 2016 the difference was rather equal in the whole year, higher temperature was only noticed in winter months.

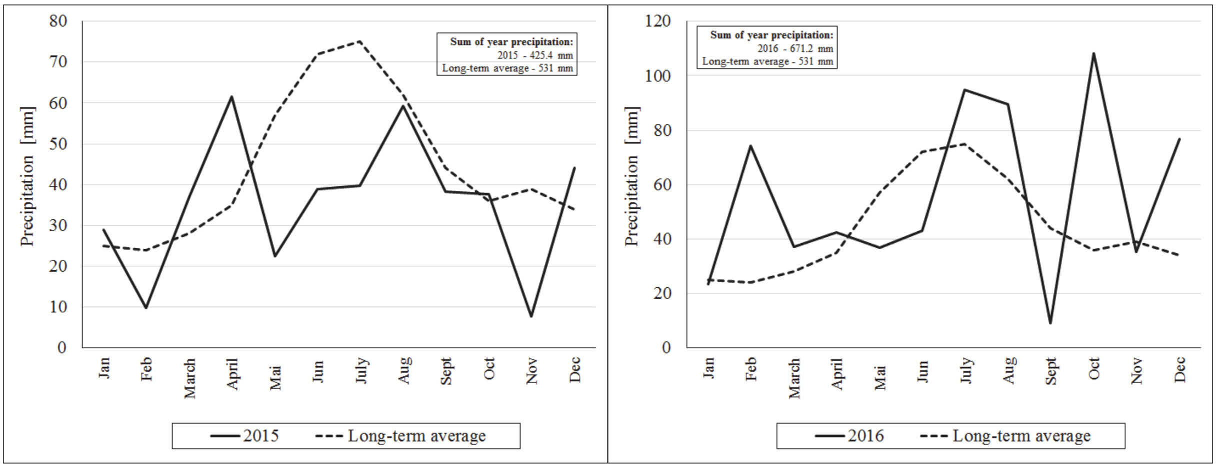

Figure 1a. Average precipitation in years 2015 and 2016 comparing to long term average.

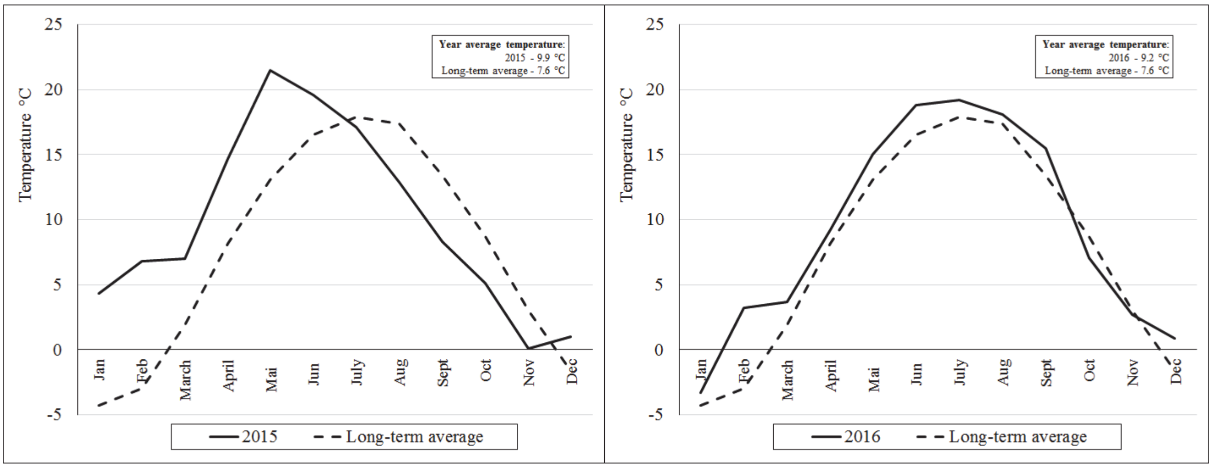

Figure 1b. Average temperature in years 2015 and 2016 comparing to long term average.

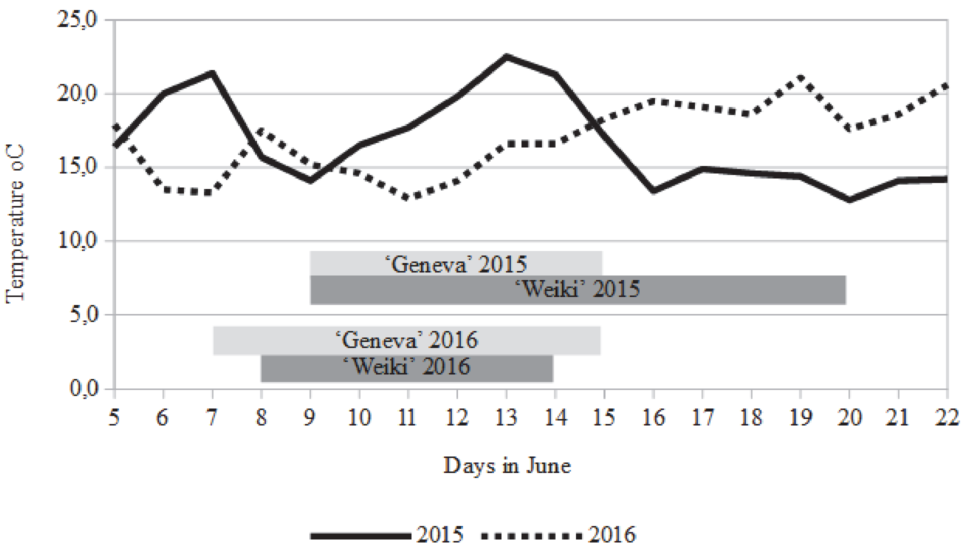

Figure 2. Average daily temperature during flowering period in the years 2015 and 2016, with marked term of flowering for 'Geneva' and 'Weiki' for each year.

The average temperature was 16.6°C in 2015 and 15.5°C in 2016 during the flowering time of 'Geneva' and 'Weiki' (Figure 2). 3-4°C higher temperature was noticed few days before the beginning of flowering and was about 15°C when flowers started to open.

In 2015 'Weiki' flowering period was almost two times longer than 'Geneva', whereas in 2016 it was two days shorter for 'Weiki'. Lower temperature together with, more equal flowering time for both cultivars in 2016, may suggest higher pollinators activity in such conditions.

3.2. Pollen quality

The viability of *A. arguta* and *A. deliciosa* pollen was high in both years (Figure 3), reaching 100% and 99.7% for *A. arguta* and 94.1% and 89.3% for *A.deliciosa* pollen in 2015 and 2016 respectively. Lower viability of *A. deliciosa* pollen was a result of more than two-year-long storage, *A. arguta* pollen was freshly collected in both seasons. The pollen germination was also high for both species in every year of the experiment, being 97.1% for *A. arguta* and 90.9% for *A. deliciosa* in 2015, and 97.5% and 86.4% in 2016, respectively. The obtained result for *A. deliciosa* pollen viability (94.1% in 2015 and 89.3% in 2016) were similar to previous Abreu and Oliveira (2004) observations for 'Tomuri' cultivar (97.9%), but different from Borghezan et al. (2011) – 70%. The storage temperature analysis showed that temperature -80°C is suitable for long-term preservation of *A. deliciosa* pollen. This significantly differs from conclusions of previous research made by the same authors,(-20°C and -196°C respectively) where analyzed storage period was maximum one year. The temperature -80°C in Abreau and Oliveira research (2004) was also checked, but the decrease in viability and germination ability of stored pollen was higher than at -20°C. Also temperature -196°C with the viability around 60% and germination ability under 20%, was even less satisfying than when preserved at -80°C. In our conditions storage at -80°C gave very good results. After two and half years of storage *A. deliciosa* pollen viability and germination ability of were 89.3% and 86.4%, respectively. Comparing so different results of optimal conditions suggests, that beside preservation temperature, pollen preparation for storage can play very important role. Length of keeping the anthers in room or above room temperature to dehiscence and pollen collection, precooling the pollen before freezing to -80°C or cryopreservation and keeping optimal humidity may have high impact on storage success.

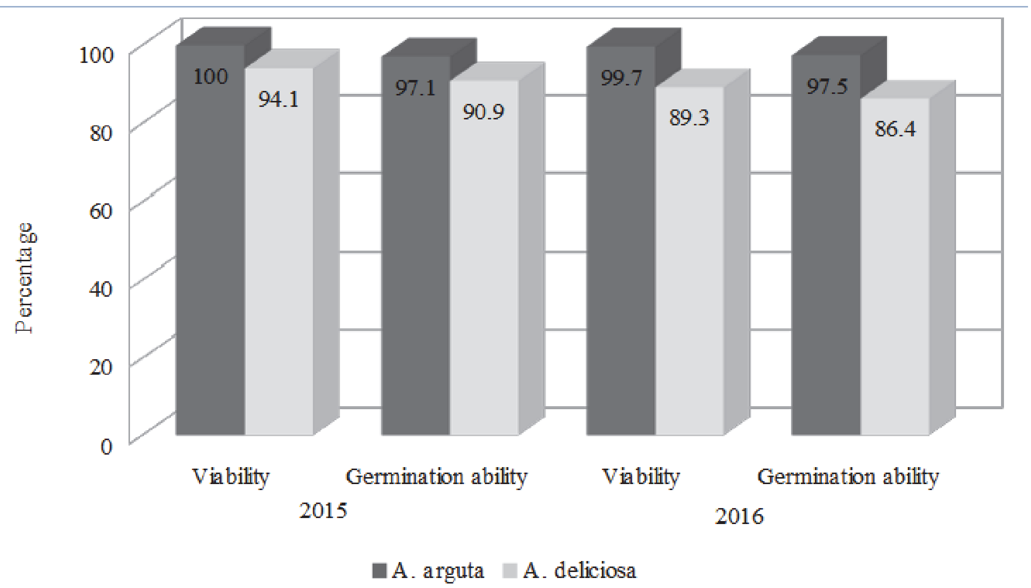

Figure 3. Pollen viability and germination ability of A. arguta 'Weiki' and A. deliciosa in 2015 and 2016

3.3. Controlled pollination

First experiment included hand pollination in controlled conditions using *A. arguta* 'Weiki' and *A. deliciosa* pollen separately. The aim was to check if pollen of different species can pollinate *A. arguta*, as effectively as the same species. Declared ploidy of all experimental *A. arguta* genotypes was tetraploidy and *A. deliciosa* – hexploidy. According to Ferguson and Huang (2016) the effects could not be positive. However, the results were satisfying – in all combinations the fruit set was about 90% (Table 1), which confirmed Strick (2005) observations. The lowest fruit set was observed for combination 'Geneva' × *A. arguta* male (87.9%) and the highest one – for 'Weiki' × *A. arguta* male (96.7%), but these differences were not statistically proved. Similarly, the fruit number per shoot varied between 24.7 and 28.5 and the differences were also not significant. The lowest percentage of marketable fruit (84.4%) and average fruit weight (5.8 g) were observed in the same combination 'Weiki' × *A. deliciosa* male. On the other hand, the highest weight of average fruit (6.2 g) was observed in combination 'Weiki' × *A. arguta* male, but the highest percentage of marketable fruit was noticed when 'Geneva' was pollinated with *A. arguta* pollen (90.3%). The reasons could be visible fruit skin damages (rosting), even if the fruit size was high enough to qualify it as a commercial quality.

Table 1. Comparison of basic yield characteristics for A. arguta Geneva and Weiki cultivars

	Pollinator	Percentage of fruit set [%]	Fruit no/shoot	Percentage of marketable fruit [%]	Average fruit weight [g]
Geneva	A. arguta	87.9	28.5	90.3	6.0
	A. deliciosa	92.4	24.7	85.8	6.0
Weiki	A. arguta	96.7	27.2	87.8	6.2
	A. deliciosa	91.1	26.5	84.4	5.8

3.4. Supportive pollination impact on selected morphological yield parameters

Despite different weather conditions in both years, the results were similar and statistical analysis did not show significant differences between seasons. Therefore, the results were shown as two-year average. The sprinkling irrigation system used on plantation, might be the reason why no relevant differences were observed.

Some differences in cultivar yield characteristics were noticed between female *A. arguta* 'Weiki' and 'Geneva'. Fruit number per long and short shoots was higher for 'Weiki', and fruits are bigger than at 'Geneva' (Table 2). 'Weiki' fruits were lengthened and laterally flattened, usually covered with red blush, but the flesh was green, whereas 'Geneva' fruits were rather round, rarely having blush, but often with reddish flesh inside, when fully riped. 'Weiki' fruits ripens about two weeks later than 'Geneva'.

Hardly any research about efficiency of supportive pollination were published. The only female cultivar that was checked in different pollination combinations was parthenocarpic *A. arguta* 'Issai', where addition of male *A. arguta* pollen showed significant impact on fruit set and weight.

In this research the two-way ANOVA showed no significant impact on average fruit weight for long shoots comparing natural or supportive pollination, for both cultivars. Tough 'Geneva' fruits were smaller and weighed 7.4 g and 7.5 g on average for plants pollinated with *A. arguta* male and supported by *A. deliciosa* pollen, respectively. 'Weiki' fruits reached 7.8 g and 8.1 g in the same combinations (Table 2). On the other hand average fruit weight on short shoots of 'Geneva' significantly increased after supported pollination with *A. deliciosa* pollen, 4.8 g for natural and 5.3 g

– for supported pollination. Although for 'Weiki' the fruit mass difference was similar as for 'Geneva' (0.5 g), it was not statistically proved.

Table 2. Comparison of basic yield characteristics of cultivars 'Geneva' and 'Weiki' using natural or supportive pollination.

Cultivar	Pollen source	Short shoots			Long shoots		
		Fruit no/shoot	Perc. marketable fruit [%]	Average fruit weight [g]	Fruit no/shoot	Perc. marketable fruit [%]	Average fruit weight [g]
'Geneva'	A. arguta	14.6 b	75.2	4.8 a	22.2	92.3	7.4
	A. arguta+A. deliciosa	13.1 a	76.4	5.3 b	20.3	95.7	7.5
'Weiki'	A. arguta	22.0	76.8	5.7	27.4	92.9	7.8
	A. arguta+A. deliciosa	20.2	79.8	6.2	28.0	95.3	8.1

General trend observed in fruit weight from natural and supportive pollination, was higher fruit mass when using the second method. It may suggest that, even if the differences were not significant on the level of single fruit weight, the average yield mass might be significantly higher. But as the results are presented on figure 4, the yield for both cultivars was higher when flowers were naturally pollinated (*A. arguta* pollen: 'Weiki' - 33.1 kg, 'Geneva' 26.6 kg., comparing to mixed pollen: 'Weiki' - 29.7 kg and 'Geneva' - 23.6 kg). This may be related to higher fruit set while natural pollination (Figure 5). The parameter was above 95% in all combinations, but the highest was noticed at naturally pollinated cultivars: 'Weiki' (98.7%) and 'Geneva' - 98.4%. At supportive method of pollination the fruit set was lower - 98.5% for 'Weiki' and 96.6% for 'Geneva' (Figure 5) and resulted in smaller number of fruits per shoot observed in all short shoots combinations in both cultivars. Similarly, lower fruit number per long shoot was observed in 'Geneva', when using supportive method of pollination. Whereas, 'Weiki' result was slightly higher in such combination. Another parameter was percentage of marketable fruit, which was higher among the fruits originating from long shoots of both cultivars. The average was between 92.3% (combination 'Geneva' × *A. arguta* pollen) and 95.7% ('Geneva' × mixed pollen). The same parameter was lower on short shoots, varying from 75.2 % for naturally pollinated 'Geneva' to 79.8 % for supportively pollinated 'Weiki'. Generally the commercial fruit percentage was higher, when supportive method of pollination was used. This may be related to lower number of fruit per shoot, which, as we can conclude from the results, had positive impact of the single fruit weight – parameter that mostly determines marketable fruits.

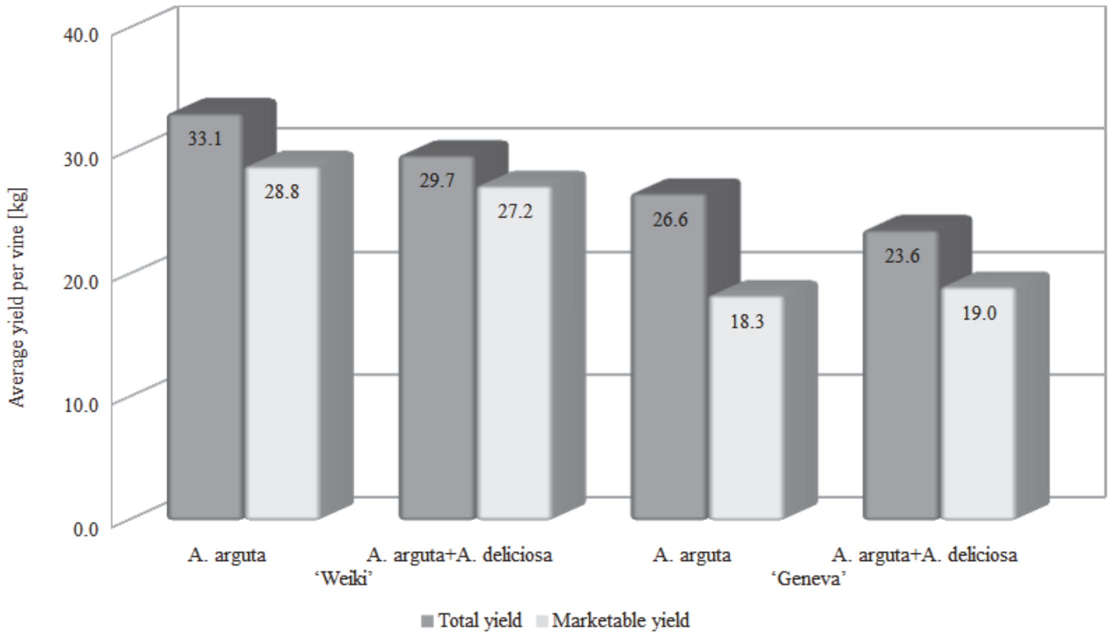

Figure 4. Comparison of average total yield per vine of cvs 'Weiki' and 'Geneva' using different methods of pollination.

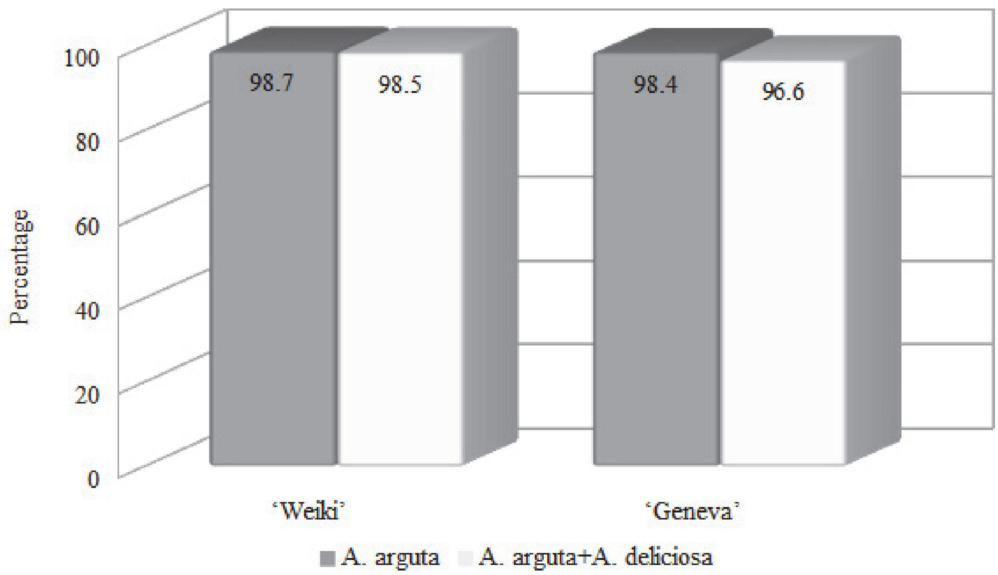

Figure 5. Comparison of percentage of fruit set of cvs 'Weiki' and 'Geneva' using different methods of pollination.

Comparing all experimental results suggests that supportive pollination using *A. deliciosa* pollen is not reasonable in Polish climatic conditions. Average yield per wine was higher, when flowers were pollinated naturally. 'Weiki' commercial yield was similarly higher, only 'Geneva' was slightly lower, when pollination was natural. Therefore, natural pollination in the research conditions seems to be effective enough to gain high, good quality yield. As there is no significant impact on commercial yield, it can be assumed that noncommercial fruits defects, such as rosting or small weight, depend more on weather conditions than on source of pollen.

Considering size of yield per hectare, where every vine yield is around 25 kg, all male plants generate lost of almost 2 tons per hectare. Then, the possibility of eliminating the male plants and replacing them with female vines (like on *A. deliciosa* plantations), seems to be economically reasonable. On the other hand, in the research the main source of pollen was male cultivar *A. arguta* 'Weiki', while *A. deliciosa* pollen was used only as a support. If it was the only source of pollen on plantation, the one-time pollination procedure may not be enough to fertilise all the flowers. According to Gonzales, Coque and Herrero (1997) *A. deliciosa* losses stigmas receptivity in four days after anthesis, then it can be presumed it is similarly in *A. arguta* and at least one-week-long flowering period requires repeating pollination, which increases the cost. Still, because of different ploidy of both species according to Ferguson and Huang (2016), there is no guarantee the yield would be satisfying. Then artificial pollination using *A. arguta* pollen should be taken into consideration, but the pollen is not commercially available. Also, as it was previously said the quality of *A. deliciosa* pollen may differ a lot between places of origin (Abreu and Oliveira 2004, Borghezan et al. 2011). Furthermore, the pollen storage procedure is not clearly developed and the quality, after long term preservation, may be significantly lower than in this research, making successful pollination impossible. Another problem of supportive pollination is spreading high virulent bacteria *Pseudomonas syringae* pv. *actinidiae*, which according to Scortichini et al. (2012) and Tontou, Giovanardi &Stefani (2014), may also be transmitted by pollen. The import of pollen was forbidden by the EU government of this reason. Moreover, there is no data about influence of solvents used in preparation of pollen solution on viability and germination ability of pollen grains. Producers claims it can be used only in one hour after preparation, otherwise the pollination will not be effective. Then covering big area requires several preparations of the pollen solution, or investing in bigger turbine. There is also no research on flowers reaction on the sprayed solvent and, consequently, on the impact on fertilisation.

4. CONCLUSIONS

Taking into consideration all the results, it can be concluded that the process of supportive pollination did not have an impact on measured parameters and is not economically reasonable in Polish conditions. The procedure generate costs of pollen, solvents, spraying turbine and increases the risk of Psa infection with imported pollen. The possible usage of *A. arguta* pollination with *A. deliciosa* pollen is replacement of male plants with female ones to increase the total yield per hectare, but it requires developing a pollen quality assurance procedure for transport and storage.

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