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Morphological variation of male *A. arguta* plants affects their flowering potential and pollen efficiency

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Abstract: *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. is functionally a cryptic dioecious plant and successful pollination is crucial for achieving high quality fruit. The extent and bases of morphological variability in female genotypes have been well studied, but here we focus on the males. Mature plants of seven male *A. arguta* genotypes were studied in 2016 and 2017 during which a suite of phenological and morphological features was measured on ten randomly chosen one-year-old canes on each plant. This analysis was complemented by two other, specialized measures potentially related to pollination efficiency, namely pollen quality, and quantity. The time of bud break was similar each year across all genotypes and the pollen quality was uniformly very high (viability – 95.0–99.9% and germination – 89.0–97.3%). However, the ten genotypes exhibited high variability in both the number of flowers per inflorescence and pollen quantity, indicating marked differences in their flowering potential and efficiency as pollinizers. The male kiwiberry indicators developed in this study – flowering potential and pollen efficiency, appear to be effective tools for the comparative evaluation of male *A. arguta* genotypes.

Keywords: kiwiberry; pollination; hardy kiwifruit; male genotype indicators; pollen quantity

Actinidia arguta (Siebold et Zucc.) Planch. ex Miq., is becoming an increasingly popular plant in fruit production. Commercial plantations are mainly located in North America, New Zealand and Europe, but the species is also cultivated in some countries in South America. The fruit called hardy kiwifruit, mini kiwi or kiwiberry, is grape-sized and covered by a smooth, edible skin usually green in colour. Flavour and aroma profiles differ greatly among current cultivars, and consumers find the small fruits attractive. Kiwiberries are a rich source of vitamins, minerals and antioxidants and provide a wide range of health benefits (Latocha 2017b). Because *Actinidia* is functionally a cryptic dioecious plant, effective cross-pollination is crucial to achieving large yields of high quality fruits (Costa et al. 1993; Tiyayon, Strik 2003b). Flowers on female plants are seemingly perfect, with pistils that produce pollen, although generally, the pollen is sterile. Even in the relatively rare cases of self-fruiting cultivars like 'Issai' fruit set after self-pollination is very low (Mizugami et al. 2007). In another departure from the strict dioecy typically associated with the genus, McNeilage (1991) examined the so-called 'fruiting male' form of *A. chinensis* var. *deliciosa*. In the case, pollen viability was found to be similar to typical male plants; but ovules were also produced, albeit in a lower number than functionally female plants, and the resulting fruit were small. Research on *A. polygama* revealed that the

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only function of stamens on female flowers appear to be attracting insects, thereby encouraging visitation to the flowers (Kawagoe, Suzuki 2004). Other studies indicate that the amount of produced pollen may also be determined by the male plant genotype (Kim et al. 2016).

Several studies have shown that *Actinidia* can be pollinated by the wind as well as insects, primarily bees and bumblebees (Fraser, McNeilage 2016). According to Howpage et al. (2001), open pollination (by wind and bees) generates 89–91% of the fruit set, a very high percentage in comparison to as authors declare, no-bee pollination, with some exposure to wind, which was only 24%. However, Costa et al. (1993) observed much higher fruit set while wind pollination (~81–98%), but fruit weight was lower and above 50% was unmarketable. Then it appears, that the most effective method of pollination tested on *A. chinensis* var. *deliciosa* is that of hand pollination (Costa et al. 1993). Also, some methods of artificial pollination using a water solution of previously harvested and preserved pollen have been examined as means of supplementing the natural process and making it more effective. Depending on the source of information, recommendations for long-term pollen storage conditions vary from –20 °C to –196 °C for the *A. chinensis* var. *deliciosa* 'Tomuri' (Abreu, Oliveira 2004; Borghezan et al. 2011; Gill 2014). More recent studies comparing bee and artificial (spraying pollen using machinery) kiwifruit pollinations indicate that the former is much more effective, producing 40% more fruit with higher seed number and weight (Sáez et al. 2019). Related research carried out on the female *A. arguta* cvs. 'Geneva' and 'Weiki' showed that supplemental pollination using pollen from *A. chinensis* var. *deliciosa* 'Tomuri' , did not have any significant effect on final fruit set or weight (Stasiak et al. 2017). In light of these findings, and given the fact that *A. arguta* pollen is not yet commercially available, it seems that natural pollination with *A. arguta* pollen may be the most appropriate and safest method [*A. chinensis* var. *deliciosa* pollen can be serve as a vector for *Pseudomonas syringae* pv. *actinidiae* (Psa), a devastating bacterial pathogen]. Although damages caused by Psa mainly impact the commercial production of *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa*, the pathogen has also been isolated from wild forms of *A. arguta* (Ushiyama et al. 1992; Marcelletti et al. 2011; Tontou et al. 2014). It was due to its virulence and damage to fruit production that in 2012 the European Union restricted the trade and transport of *Actinidia* plant material, including pollen.

A strong correlation has been observed between fruit weight and seed number in *A. chinensis* var. *deliciosa,* suggesting that effective pollination has a high impact on traits of primary economic importance, namely fruit size and total yield (Costa et al. 1993). A linear relationship between the fruit weight and seed number has also been reported for *A. arguta* 'Ananasnaya' (Tiyayon, Strik 2003b). Less-than-optimal pollination among dioecious plants, may be due to excessive distance between male and female plants, and pollinator bias, which can result in lower frequency of visits to female plants (Costa et al. 1993; de Jong et al. 2005). Seal et al. (2013a,b) showed that the genotype of the pollen source can not only affect final fruit weight, but also influence the synthesis of some bioactive compounds (e.g. anthocyanin) and the dry matter content, characteristics thought until then to be determined by the interaction of the female plant with the environment (Howpage et al. 2001).

The most reasonable and effective way of pollinating *A. arguta* plants is likely using insect-vectored *A. arguta* pollen, and studies like the ones mentioned above underscore the importance of the pollen source (i.e. the male genotype).

It comes as no surprise that female cultivars of *A. chinensis* var. *deliciosa*, *A. kolomikta* and *A. arguta* have been evaluated more frequently than their male forms (Jie, Thorp 1986; Snowball 1997; Tiyayon, Strik 2003a, 2004; Česonienė, Viškelis 2007; Kim et al. 2014). Evaluation of *A. kolomikta* fruiting potential indicated significant differences among genotypes due to both genetic and environmental reasons (Česonienė, Viškelis 2007). So far, very little research has been carried out on the morphological differences among male *A. arguta* cultivars (Kim et al. 2014, 2016).

There are certain limitations on breeding *Actinidia* due to ploidy difference within and among species. Some interspecific crosses have been shown to be more successful than crosses within a species, particularly when the intraspecific crosses are between plants of different ploidy (Ferguson, Huang 2016). However, there are reports that the female diploid *A. chinensis* 'Hort16A' pollinated with pollen from tetraploid *A. chinensis* or hexaploid *A. deliciosa* bears fruit with higher fresh weight compared to that resulting from pollination with pollen from a male diploid *A. chinensis* line (Seal et al. 2013b).

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Similarly, the tetraploid *A. arguta* 'Weiki' and 'Geneva' were successfully hand-pollinated with hexaploid *A. deliciosa* pollen in controlled conditions (Stasiak et al. 2017; 2019). In this case, although fruit set and weight was similar to that achieved via pollination using pollen from male *A. arguta* tetraploids, some biochemical differences were observed (Stasiak et al. 2019).

There is an impressive level of diversity in ploidy among and within *A. arguta* species. Research on wild genotypes of *A. arguta* in Japan revealed that most (87 out of 127) were tetraploids (Asakura, Hoshino 2017), but geographically separated and relatively localized populations of diploids and hexaploids were also found (Katakoa et al. 2010). Further studies showed that naturally occurring in the area of Hokkaido (Japan) are diploid accessions of both *A. kolomikta* and *A. polygama*, along with tetraploid *A. arguta* (Asakura, Hoshino 2016). In contrast, Bogačioviene et al. (2019) examined 6 genotypes of *A. arguta* that are grown in Europe and found that one male was diploid, four females were tetraploids, and one female was hexaploid.

In view of the growing interest and investment in the commercial cultivation of *A. arguta* and the lack of systematic evaluation of male cultivars, despite the clear importance of pollen source to fruit production and quality, we have decided to examine in detail pollinizing selections used in European conditions. Specifically, the aim of this work is to evaluate the morphology, flowering potential and pollen efficiency of seven *A. arguta* male genotypes.

MATERIAL AND METHODS

The plant material and growing conditions. The work was dedicated to four *A. arguta* male cultivars ('Weiki', 'Nostino', 'Joker' and 'Rubi'), one *A. arguta* var. *purpurea* cultivar ('Rot'), and two hybrids between *A. arguta* and *A. arguta* var. *purpurea* (F7 and F21), all tetraploids (Table 1). All plants of were grown at the Experimental Garden of the Environmental Protection Department at the Warsaw University of Life Sciences in central Poland and each genotype was represented in the study by three mature plants, each at least five-years-old. All plants were grown in similar soil and water conditions and were maintained and fertilized according to standard recommendations (Strik 2005, Latocha 2017a). The study was conducted in 2016 and 2017. Both seasons were warmer and wetter than the long-term average, but average temperature was similar across the two study years. Total annual precipitation was about 100 mm lower in 2016 than in 2017, and differences in precipitation were very high during the spring time when flowering occurred (May-June) (Figure 1). In both growing seasons, plants were watered during dry weather to avoid water stress.

Morphological measurements and observations. Ten one-year-old canes, each at least 50 cm long, were randomly chosen on each plant in early spring; and suite of the measurements was made in late spring, including cane length, number of buds per cane, average internode length, percentage bud break, number of flowering summer shoots, and number of flowers per flowering shoot. Afterwards, the flowering potential (FP), defined as the number of flowers per winter bud or per unit cane length, was calculated for each genotype.

Pollen quality and quantity. To compare pollen efficiency per flower, anthers were counted in thirty randomly chosen flowers per vine and pollen quantity per anther was measured. To accomplish this, anthers were isolated from flowers just before anthesis and pollen grains were collected and evaluated according to Bieniasz et al. (2017). For each genotype, four groups of ten anthers each were placed in 1 mL Eppendorf microcentrifuge tubes and dried at 30 °C for 12 hours. A 2% eosin solution (1 mL) was then added to the tubes and mixed using a Vortex shaker for 30 seconds. Then, 0.2 mL of the homogeneous suspension was load-

Table 1. Information about the male genotypes used in this study

Clone	Species	Place of origin
'Weiki'	*A. arguta*	Clematis Nursery, Poland
'Nostino'	*A. arguta*	Häberli Nursery, Switzerland
'Joker'	*A. arguta*	own selection
'Rot'	*A. arguta* var. *purpurea*	Werner Merkel, Germany
'Rubi'	*A. arguta*	own selection
F7	*A. arguta* × *A. arguta* var. *purpurea*	own selection
F21	*A. arguta* × *A. arguta* var. *purpurea*	own selection

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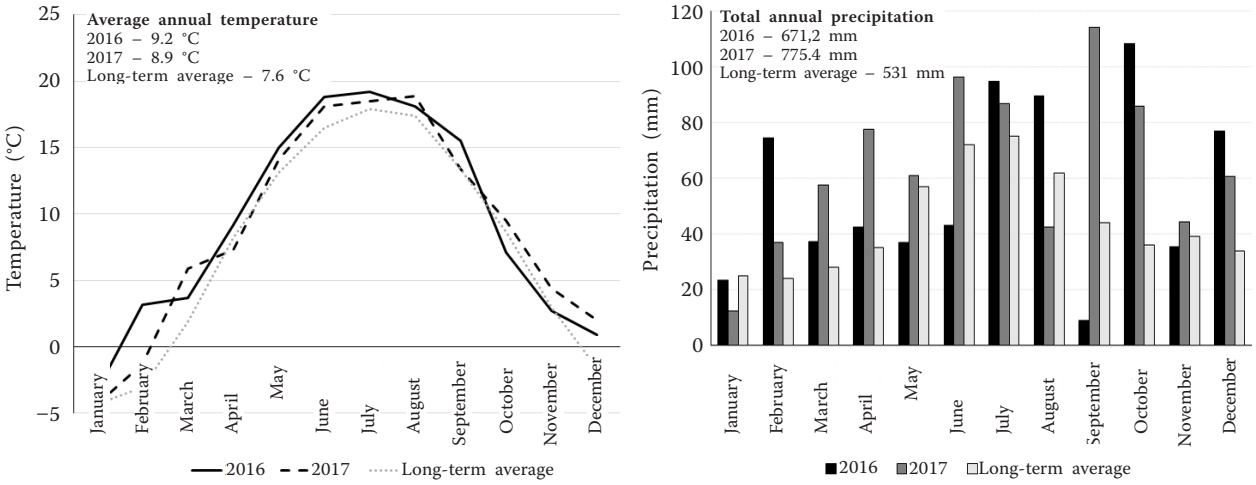

Figure 1. Weather conditions recorded in 2016 and 2017

ed into a Bürker hemacytometer (Merck, Poland). and pollen counts were conducted under an AXIO Imager M2 microscope (from Carl Zeiss, Germany) at 100× magnification, For each vine, pollen quantity was calculated by multiplying the number of anthers per unit cane length by the pollen grain count, standardized to pollen grains per anther.

In addition to pollen quantity, viability and germability were also measured as indicators of pollen quality. Pollen from 50 flowers in balloon stage was collected for this analysis. Anthers cut out of flowers were put on Petri dishes and left for 24 h to reveal pollen, For analysing pollen viability, the Alexander method was used, in which viable pollen grains appear red and the non-viable green (Alexander 1969). Pollen germability was assessed directly on a 15% sucrose agar medium poured into Petri dishes and allowed to cool to room temperature. Using the same microscope as above, observations were taken after a 12 hour incubation period at room temperature. The base for calculating both parameters consisted of 50 pollen grains, and observation were repeated four times at 100× magnification. In analysing germability, a pollen grain was considered germinated only if the length of its pollen tube exceeded twice its grain diameter.

Statistical analysis. The normality of the data was verified using the Shapiro-Wilk test. When the assumption of normality was met, an ANOVA analysis was performed. Otherwise, the non-parametric equivalent, a Kruskal-Wallis H test was carried out at a 5% significant level. In both instances, pairwise comparisons were made to identify homogeneous groups, withthe Tukey test and the Wilcoxon signed-rank test performed for the normal and non-normal datasets, respectively. All statistical analyses were made using the Statistica software, version 13.0 [TIBCO Software Inc. (2017), http://statistica.io, USA].

RESULTS AND DISCUSSION

Pollination is a crucial process in fruit production and one in particular need of strategic management in the cultivation of dioecious plants such as *A. arguta*. As discussed in detail in the introduction, several studies have established a positive correlation between pollination and fruit production in several *Actinidia* spp. (Costa et al. 1993; Howpage et al. 2001; Tiyayon, Strik 2003b; Sáez et al. 2019). There are some reports that efficient pollination in kiwifruit and kiwiberry may be achieved using pollen of a higher ploidy level than that of the receiving female genotype (Seal et al. 2013b; Stasiak et al. 2017, 2019). Other studies suggest that the genotypes and ploidies of both parents may have an impact on some fruit quality parameters (Seal et al. 2013b, Stasiak et al. 2019). Data collected during this two-year study lays the foundation for a method of selecting male genotypes which may serve as effective commercial pollinizers for female plants.

Plant and flowering morphology. Analysis of basic morphological parameters indicated that the average internode length did not vary between seasons but did vary significantly among tested genotypes (Table 2). The shortest internodes were recorded for 'Nostino' (2.5 cm) and the longest for 'Rubi' and F7 (4.8 and 4.2 cm, respectively). Internode length

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Table 2. Characteristics of one-year-old canes of four *A. arguta* ('Weiki', 'Nostino', 'Joker', 'Rubi', one *A. arguta* var. *purpurea* ('Rot') and two *A. arguta* × *A. arguta* var. *purpurea* (F7 and F21) male genotypes. Data, representing a two-year average, were analysed using the Kruskal-Wallis and Wilcoxon signed-rank test with a significance level of 0.05

Genotype	Average one-year-old cane internode length (cm)	Percentage of terminating summer shoots
'Weiki'	3.6[bc1]	91.8
'Nostino'	2.5[d]	94.7
'Joker'	2.7[cd]	88.3
'Rot'	3.6[bc]	87.2
'Rubi'	4.8[a]	83.9
F7	4.2[ab]	74.7
F21	2.9[cd]	87.3
Mean	3.5	86.8
Median	3.5	87.3
Significance[2]	***	NS

[1]Values in each column marked with the same letter do not differ significantly; [2]significance level designations: *** for $P \leq 0.001$, ** for $0.001 < P \leq 0.01$, * for $0.01 < P \leq 0.05$ and *NS* for $P > 0.05$

may differ depending on shoot origin on the vine (Tiyayon, Strik 2003a). Comparing shoots of similar origin, namely one-year-old canes growing directly from the cordon, the internode length for 'Ananasnaya' was found to be 2.5 cm in one research location and 3.2 cm in another. Therefore, local conditions and plant treatment may play an important role in shaping plant morphological features (Tiyayon, Strik 2003a).

Shoot type analysis revealed a strong disproportion between terminating and non-terminating shoots growing from one-year-old canes. Terminating shoots dominated regardless of the genotype, accounting for 83.9–94.7% of all the examined summer shoots in the cases of 'Rubi' and 'Nostino', respectively (Table 2). Certain other parameters exhibited seasonal variation. The percentage of spring bud break, for instance, was similar for all genotypes (Table 3) but on average higher in 2016 (ranging from 46.7–62.7%) than in 2017 (37.2–56.8%). Similar results have been recorded by Snowball (1997) as well as by Tiyayon, Strik (2003) for female

Table 3. Characteristics of canes and summer shoots of four *A. arguta* ('Weiki', 'Nostino', 'Joker', 'Rubi', one *A. arguta* var. *purpurea* ('Rot') and two *A. arguta* × *A. arguta* var. *purpurea* (F7 and F21) male genotypes

Genotype	Percentage of spring bud break (A)		Percentage of flowering summer shoots (A)		Number of flowers per 10 cm of flowering shoot	
	2016	2017	2016	2017	2016 (A)	2017 (B)
'Weiki'	49.3	44.9	43.8[1]	92.3	19.3[ab]	25.4[c]
'Nostino'	50.6	40.7	100.0[a]	96.2	52.8[a]	48.9[a]
'Joker'	48.1	37.6	99.2[ab]	92.5	28.3[ab]	25.7[c]
'Rot'	46.9	37.2	81.3[ab]	73.9	26.3[ab]	26.9[c]
'Rubi'	46.7	46.3	90.4[ab]	100.0	25.7[ab]	30.7[bc]
F7	62.4	56.8	87.6[ab]	100.0	27.1[ab]	43.3[ab]
F21	55.4	42.7	95.6[ab]	83.8	16.1[b]	8.7[d]
Mean	50.0	43.2	85.2	89.2	35.3	35.0
Median	48.7	43.3	93.9	92.3	34.9	37.5
Significance[2]	NS	NS	**	NS	*	***

Data were analysed using a two-year average with the Kruskal-Wallis and Wilcoxon signed-rank test (A), one-way ANOVA and Tukey's test (B), both with a significance level of 0.05

[1]Values in each column marked with the same letter do not differ significantly; [2]significance level designations: *** for $P \leq 0.001$, ** for $0.001 < P \leq 0.01$, * for $0.01 < P \leq 0.05$ and *NS* for $P > 0.05$

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A. arguta cultivars (44.3–49.8% and 34–57%, respectively), suggesting that this feature may be typical for the species as a whole, rather than varying greatly among genotypes.

In this study, the percentage of floral summer shoots differed between 'Weiki' and 'Nostino' in 2016 (43.8% vs. 100%, respectively); but no differences were observed in 2017 (Table 3). This result suggests that the 'Weiki' cultivar may exhibit greater sensitivity to ambient weather conditions. In general, the percentage of floral summer shoots observed in this study, was much higher than that observed by Snowball (1997) on the female selections of *A. arguta* var. *arguta* and *A. arguta* var. *cordifolia* (13.0–33.1%). In comparison much higher (above 70%) percentage of fertile shoots was observed on the commercial female 'Ananasnaya' (Tyiayon, Strik 2003b). No differences in the percentages of fertile shoots originating from different wood types was noticed in 'Ananasnaya' (Tyiayon, Strik 2003a). This may suggest a greater variation in the density of fertile shoots among female genotypes and indicate that well-selected female commercial cultivars could have more fertile shoots. However there are reports of some environmental factors, such as plant insolation, suggesting that overhead shading for 2 month before harvest may lower the floral shoots number in subsequent year (Tyiayon, Strik 2004). Flower density, calculated as the number of flowers per 10 cm of summer shoots is shown in Table 3. The lowest flower density was exhibited by F21 (16.1 and 8.7 flowers per 10 cm of shoots in 2016 and 2017, respectively) and the highest by 'Nostino' (around 50 per 10 cm of summer shoots). In 2016, the flower densities of the other genotypes ranged between those recorded for F21 and 'Nostino' and, according to the Kruskal-Wallis test, were statistically similar to each. The situation differed in 2017, however. Data analysed using the ANOVA and Tukey tests indicated a normal flower distribution and F7 (43.3 flowers per 10 cm of summer shoots) grouping with both 'Nostino' (48.9) and 'Rubi' (30.7). The largest differences across the two years is seen in the cases of the F7 and F21 (16.2 and 7.4 flowers per 10 cm of summer shoots, respectively).

The flowering potential and the pollen efficiency. The analysis of the number of anthers per flower revealed significant differences among genotypes. More anthers in one flower were recorded in case of F7, 'Nostino', 'Joker' and 'Rubi' (36.5, 37.0, 39.0 and 40.0, respectively) (Table 4). The F21 had the lowest number of anthers followed by 'Weiki' and 'Rot'.

As mentioned before, one of the basic parameters used to measure flowering potential is number of flowers per inflorescence, a parameter that is usually applied in selecting genotypes with high flowering potential. As shown in Figure 2, the most common number of flowers per inflorescence on terminating shoots for majority of the male genotypes

Table4. Flower morphology and flowering potential (FP) of four *A. arguta* ('Weiki', 'Nostino', 'Joker', 'Rubi', one *A. arguta* var. *purpurea* ('Rot') and two *A. arguta* × *A. arguta* var. *purpurea* (F7 and F21) male genotypes

Genotype	Number of anthers per flower (A)	FP_1 (A)	FP_2 (B)
'Weiki'	30.0b[c1]	7.6[bc]	276[bc]
'Nostino'	37.0[a]	16.5[ab]	550[a]
'Joker'	39.0[a]	15.0[ab]	325[b]
'Rot'	34.0[b]	7.9[bc]	271[bc]
'Rubi'	40.0[a]	21.1[a]	557[a]
F7	36.5[ab]	24.0[a]	223[bc]
F21	28.0[c]	3.9[c]	124[c]
Mean	35.0	14.1	332.9
Median	34.5	13.1	289.5
Significance[2]	***	***	***

Data were analysed using two-year average with the Kruskal-Wallis and Wilcoxon signed-rank test(A),one-wayANOVA and Tukey's test (B), both with asignificance level of 0.05;FP_1– number of flowers per winter bud; FP_2–number of flowers per 1 m of one-year-old cane

[1]Values in each column marked with the same letter do not differ significantly; [2]significance level designations: ***for $P \leq 0.001$,**for $0.001 < P \leq 0.01$, *for $0.01 < P \leq 0.05$ and *NS* for $P > 0.05$

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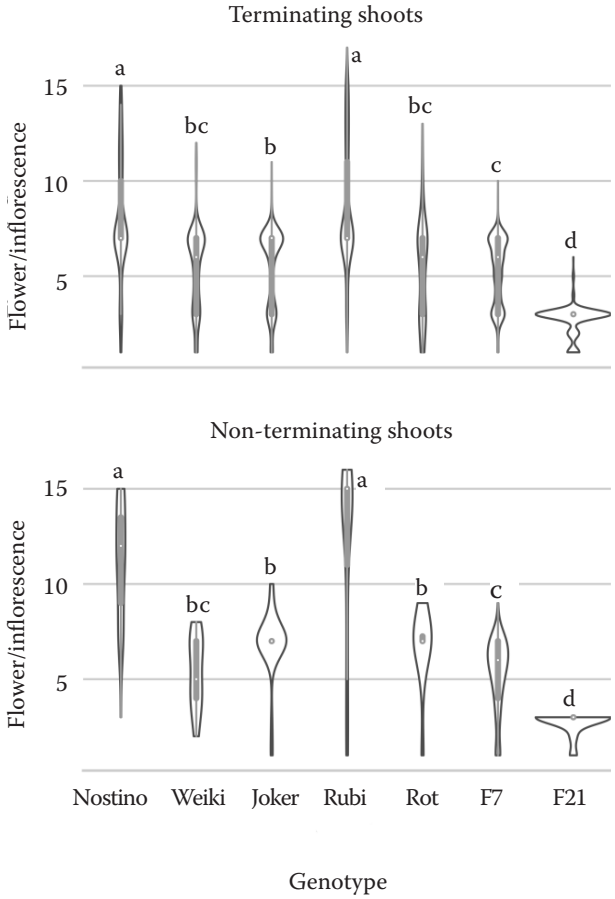

Figure 2. Distributions of flower number per inflorescence of four *A. arguta* ('Weiki', 'Nostino', 'Joker', 'Rubi', one *A. arguta* var. *purpurea* ('Rot') and two *A. arguta* × *A. arguta* var. *purpurea* (F7 and F21) male genotypes. Medians are indicated with open white dot, and bold grey lines span the distance between the first and third quartile

Data were analysed using the Kruskall-Wallis and Wilcoxon range tests with a significance level of 0.05. Within each plot, genotypes marked with the same letter do not differ significantly

in this study was seven. One exception proved to be F21 typically with only three flowers per inflorescence. The second number is also typical for male *A. chinensis* var. *deliciosa* according to McNeilage (1991). On the other hand, most genotypes characterised with a wide distribution in the number of flower per inflorescence, ranging from 1 to 10 (e.g. in 'Weiki', 'Joker' and 'Rot') or even as high as 17 ('Nostino' and 'Rubi'). As the results were not distributed normally, non-parametric tests were used for statistical analysis, in which the genotypes were divided into four homogenous groups, as shown in Figure 2. The highest mean numbers of flowers were observed in 'Nostino' and 'Rubi' (>7), whereas F7, 'Weiki' and 'Rot' had 3–7 flowers. Non-terminating shoots, which accounted for 5.3–16.1% of all shoots, exhibited similar distributions as those found on terminating shoots (Figure 2). Generally, the number of flowers per inflorescence was higher compared to that reported previously in different *Actinidia* species (Snowball 1997). In the same study, only *A. guilinensis* and *A. latifolia* have been observed to have higher densities exceeding 50 flowers per inflorescence.

The flowering potential was defined as the number of flowers per unit length of cane, or the number of flowers per winter bud and was calculated for each male genotype. As shown in Table 4, FP_1 (the number of flowers per winter bud) was the highest in the case of F7 and 'Rubi' (24.0 and 21.1, respectively). The figure was slightly lower for 'Nostino' and 'Joker', and the lowest number was observed for F21. FP_2 (number of flowers per meter of cane) –was the highest for the 'Rubi' and 'Nostino' (557 and 550, respectively) and the lowest for F21 (124). Regardless of the assessment method used (FP_1 or FP_2), the essential ranking of genotypes was generally consistent; however, large discrepancies between these two parameters were noted in the cases of 'Rot' and F7. Such discrepancies may be accounted for by morphological differences (e.g. the length of internodes), and indeed one might posit that shorter internode lengths result in higher flower production. While such a relationship is evident in the cases of 'Nostino' and 'Joker', the internodes lengths of 'Rubi' and F7 are nearly twice as long (4.2–4.8 cm) (Table 2) and yet these cultivars produced a very large number of flowers. It would seem that the internode length is an independent factor which may or may not have an impact on flowering potential. Consequently, more attention should be paid to other features, such as, for example, the number of flowers per inflorescence.

The pollen efficiency (PE) of each genotype was calculated based on the number of pollen grains per anther multiplied by the mean number of anthers per flower, and the FP. Differences among the male selections were observed, based on the number of pollen grains per anther (Table 5). F21 was the genotype with the lowest number of grains per anther (5 573), roughly 9.5 times lower than 'Joker' (52 992) and almost 11.5 times lower than that of 'Weiki' (64,150). A higher number of pollen grains per anther was observed in 'Rot', 'Nostino' and F7 genotypes (ranging between 76 483–79 633 grains). The highest number of pollen grains was produced by anthers of 'Rubi'

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Table 5. Comparison of pollen quantity and quality of four *A. arguta* ('Weiki', 'Nostino', 'Joker', 'Rubi', one *A. arguta* var. *purpurea* ('Rot') and two *A. arguta* × *A. arguta* var. *purpurea* (F7 and F21) male genotypes

Genotype	Number of pollen grains per anther (A)	Number of pollen grains per flower (B)	Pollen efficiency (A) (PE_1)	Pollen efficiency (A) (PE_2)	Pollen viability (A) (%)	Pollen *in vitro* germability (A) (%)
Weiki'	64 150^{b1}	2 189 793^{b}	16 576 360^{bc}	435 768 810^{f}	99.9	97.3
'Nostino'	77 717^{ab}	2 881 073^{b}	47 766 811^{abc}	1 457 822 940^{a}	99.0	95.5
'Joker'	52 992^{b}	2 152 058^{b}	32 192 640^{abc}	596 120 070^{e}	96.4	92.1
'Rot'	76 483^{ab}	2 706 896^{b}	22 647 351^{abc}	699 513 520^{d}	96.4	89.0
'Rubi'	94 817^{a}	4 063 152^{a}	85 529 350^{a}	1 027 977 460^{b}	97.7	92.4
F7	79 633^{ab}	2 821 116^{b}	67 565 728^{ab}	930 968 280^{c}	99.8	95.4
F21	5 573^{c}	153 976^{c}	866 535^{c}	22 057 250^{g}	95.0	90.5
Mean	64 481	2 424 009	38 840 754	738 604 047	97.7	93.2
Median	53 700	2 443 432	32 192 640	697 507 200	97.7	92.8
Significance^{2}	*	***	***	***	NS	NS

Data were analysed using the Kruskal-Wallis and Wilcoxon signed-rank test (A) and ANOVA with Tukey's test (B) with a significance level of 0.05; PE_1 – Pollen efficiency as number of pollen grains per winter bud; PE_2 – Pollen efficiency as number of pollen grains per 1 m of cane

[1]Values in each column marked with the same letter do not differ significantly; [2]significance level designations: *** for $P \leq 0.001$, ** for $0.001 < P \leq 0.01$, * for $0.01 < P \leq 0.05$ and *NS* for $P > 0.05$

(about 20% more than those of F7). When calculated per flower, the highest number of pollen grains was recorded for 'Rubi' (>4 million) and the lowest for 'F21' (~154 000). The number of pollen grains per flower in the other genotypes, comprising one significance group, were 30–50% lower than that for 'Rubi'. The remainder of genotypes tested did not differ significantly in the number of pollen grains per flower (Table 5). According to the data (Table 5), 'Rubi', F7 and 'Nostino' exhibited the highest PE_1 values, with ~85.5, ~67.6 and ~47.8 million of pollen grains per winter bud, respectively (Table 5). When calculated per meter of cane, PE_2 the highest performing cultivars were again 'Nostino', 'Rubi' and F7 and the lowest was F21 (Table 5). Taken all together, these results indicate that the pollen efficiency (PE) calculated per winter bud (PE_1) or per meter of cane (PE_2) appear equally informative when comparing pollen efficiency of male selections. These results confirm the findings of Kim et al. (2016) that in *Actinidia* there are tremendous differences, genotype-to-genotype, in the amount of pollen produced.

Of equal or arguably even more importance than pollen quantity is quality. As shown in Table 5, pollen viability and *in vitro* germability, were consistently, high across all tested genotypes. Such high quality pollen, obtained in this study, agrees with reports for *A. chinensis* (Seal et al. 2013a, b). That being said, lower pollen quality was observed in the cases of *A. chinensis* var. *deliciosa* 'Tomuri' and 'Matua' (Abreu, Oliveira 2004); Borghezan et al. 2011) as well as other recent research on *A. arguta* in which germability varied greatly among genotypes, ranging from 20–70% (Kim et al. 2016). The reason such variation was not seen in this study is not known.

There is no doubt that efficient pollination is crucial for the commercial production of high-quality *A. arguta* fruit. As the most effective sources of pollen, male genotypes within a commercial vineyard must produce large quantities of quality pollen grains. This study has shown that parameters such as the number of flowers per inflorescence, the percentage of fertile shoots, the number of flowers per shoot, the number of anthers per flower, and the number of pollen grains per anther, provide a useful bases for differentiating male selections in terms of the pollinating potential.

CONCLUSION

The FP and PE developed male kiwiberry indicators - proved to be effective tools for drawing a comparison between male *A. arguta* genotypes. Data regarding morphological features of tested male genotypes indicate a close relationship between flowering potential (FP) and pollen efficiency (PE), as well as the range of variability of these traits. It means is possible to evaluate adult male plants in field conditions

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using only FP method, which is simple to check and available without specialized equipment. However evaluation can be made only short time during vegetation, which is before and during flowering period. Then it is reasonable to look for vegetative morphological features correlated to flowering potential, which would allow to evaluate male plants regardless of the flowering period.

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