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Impact of pollination services to apples goes beyond increased fruit set

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Introduction

Pollination services increase fruit and seed set of many crops, in particular that of vegetables, fruits, berries and nuts [1]. For this reason, it is well-recognized that animal-mediated pollination is an important service for the global food production in general and human nutrition in particular [1-3]. However, accumulating evidence shows that the positive effect of pollination services goes beyond increasing fruit and seed set, and may also increase quality [rapeseed, strawberries; 4, 5], affect nutritional composition [almonds, apples; 6, 7], decrease malformations [Fuji apples, 8] and increase shelf life-time [strawberries; 4] of crops. Still, the linkages between pollination services and fruit quality have only just started to be recognized and there is a need for research that evaluate the full economic and nutritional effects of pollination services.

Apple is a fruit crop with a strong dependence on animal-mediated pollination, in which all cultivars to some extent are self-incompatible and therefore require pollen transfer from another pollinizer cultivar to set commercially acceptable fruit levels [9]. It is the most geographically wide-spread temperate fruit and nowadays occur in Siberia as well as in high elevation areas in Colombia and Indonesia [10]. In Europe, apple is the most common pollinator-dependent crop and the economic gain from pollination-induced increases in fruit set is higher than that of any other crop [11]. However, for commercially produced fruits it is not only the quantity of fruit that matters, since marketable fruits also need to have an adequate quality for good storability and for attracting consumers. Even though higher insect pollination levels are known to improve fruit yields of apples [12, 13], the influence of insect pollination on quality aspects are more equivocal [7].

There are several quality aspects that have large economic impact on the apple production. Important quality attributes for consumers include flavour and flesh firmness, where firmer fruits are considered to have higher quality [14, 15]. Flavour is a complex attribute that is related to the dry matter content in fruits, where higher dry matter content often is preferred in consumer studies [15, 16]. The dry matter content (DMC) also influences the at-harvest and post-harvest fruit firmness, and is a good estimate of total soluble solids after storage [15, 17].

DMC has also been suggested to be a quality predictor for other climacteric fruits including avocado, mango and kiwifruit [16, 18, 19]. Another important quality aspect for producers and wholesalers is storability. Apples can be stored for protracted periods of time, which allows for longer market availability. However, even though the storage facilities have developed substantially, which has prolonged the storability of fruits, a lot of fruit is still discarded when taken out from the storage. For example, 20% of organic apples were disregarded during an experiment in Sweden 2010 and 9 – 27% of conventionally produced apples of different varieties from seven orchards were disregarded after storage during the period 2010-2015 (Ibrahim Tahir, SLU, pers. comm.). Many aspects influence the storability of apples, where harvest time and mineral concentrations are important modifiers. For example, low calcium content, and the ratio between magnesium or potassium and calcium, is connected to postharvest disorders including bitterpit, lenticel breakdown and Jonathan spot [20-24]. Calcium and its ratio with other elements (eg. K:Ca and N:Ca) is also connected to the softening of apples and resistance to diseases [25-27]. Consequently, calcium application both before and after harvest to increase fruit Ca-content is a common management action in modern orchards [22, 26].

A few studies have suggested that well-pollinated fruits differ in their mineral content from less pollinated ones, suggesting that the mechanisms influencing mineral allocation into fruits is related to pollination services. Bramlage, Weis [28] found a positive relationship between seed number and calcium concentrations in Richared Delicious apples, Volz *et al.* [29] found supplementary pollination to positively affected final fruit calcium concentrations of Braeburn apples. On the other hand, Bucceri and Di Vaio [30] found a positive relationship between higher seed set and calcium concentration in fruits from the cultivars Red and Golden Delicious, but not from the cultivars Annurca Rossa del Sud and Annurca Tradizionale, and Garratt *et al.* [7] found that supplementary hand-pollination decreased calcium concentrations in Gala apples, indicating that the effect may be cultivar specific. Since the mineral content in turn may be related to other quality aspects including firmness, postharvest disorders and storability, pollination service may have a more far-reaching role in the economy of apple production than what has earlier been estimated.

The aim of this study was to evaluate the effects from different levels of pollination on the mineral and dry matter concentration in apples and how this in turn affects the storability of the fruits. We hypothesized that higher pollination services will lead to generally higher element concentrations in fruits and that fruits that are well-pollinated will have a better storability.

Methods

Sites

Two orchards where selected in the apple growing area of Kivik, southern Sweden, which is the main apple growing region in Sweden. The orchards were separated by 8 km and were both certified with the Swedish organic KRAV-certification, which does not allow applications of synthetic fertilizers or pesticides. The orchards were irrigated and honeybee hives and commercial bumblebees were present during apple bloom. Several different varieties were planted in the orchards in separate rows, including compatible varieties, hence no specific pollinizer varieties were present. The apple harvests from the orchards are sold in the market or are used for production of apple juice and cider.

Pollinator treatment

Within both orchards, rows with the apple cultivar Amorosa, a red sub-cultivar of Aroma, were identified. Aroma/Amorosa are among the most popular apple cultivars in Swedish orchards and were the most commonly planted cultivars in new plantations in 2012 [31, 32]. Distributed over two rows, 40 and 30 trees were marked in the two orchards, respectively. On each tree, three branches at similar height were marked and randomly assigned to one of the following treatments: pollinator exclusion, supplementary hand-pollination and control. When the flower buds were approaching balloon stage, the *pollinator exclusion* branches were bagged with 255 x 610 mm perforated Crispac-bags, plastic bags permeable to air through small holes (Ø = 0.5 mm), to exclude pollinators. All flowers on the branch were inside the bag. In the few cases when the bag did not cover all flower clusters, clusters outside the bag were removed. Bags were removed when all flowers had withered. At peak flowering in late May 2016, flowers on the *supplementary hand-pollination* branch, in addition to natural pollination from the present pollinator community, received supplemented hand-pollination using a cotton swab with fresh pollen collected from at least three different trees of a compatible variety (Holsteiner-Cox). Flowers that had received supplementary pollen were marked. The flowers on the *control* branches received only natural pollination from the present pollinator community. Number of flowers on the branches assigned to the different treatments as well as the total number of flowers on the whole tree were noted.

Fruit set

Approximately one month after peak flowering, the developing apples on the marked branches were counted as a measure of the initial fruit set. Some days before commercial harvest, 3.5 months after peak flowering, fruits were again counted on the marked branches. All developed fruits attached to the branches were counted, except for fully rotten fruit. The finest apples, without any major visible damage, were harvested from the branches. On the supplementary hand-pollinated branch only marked fruits were harvested. To compensate for the lack of fruits on some branches, up to four apples were taken from branches of the same treatment with more fruits. In one of the orchards, fruit set was extremely low (presumably affected by the high infestation of the rosy apple aphid, *Dysaphis plantaginea*), and extra control apples were picked from unmarked trees, hereafter called “extras”. In total 248 apples were picked.

Quality measurements

All collected apples were taken to the lab for measurements. All apples were weighed and their length, maximum and minimum diameter were measured using a digital caliper. Ground colour was estimated using a chartreuse colour chart where “1” was darkish green and “8” was yellow colouration of the skin, percent cover colour with red pigmentation was noted and if there were any pest damages or visible diseases on the apples. If these measurements were not taken on the same day as the harvesting date, the fruits were put in the refrigerator for a maximum of three days before measurements.

Following these initial measurements, apples were divided into two groups, where one group was selected for additional destructive measurements and the other used for measuring the storability and the quality of stored apples. The apples that were first stored were subjected to the same destructive measurements either when they were experiencing postharvest disorders or after a maximum of 162 days when the experiment ended. The stored fruits were checked every second week and the fruits that could no longer be regarded as first class fruit, due to postharvest disorders, were taken out from the storage and measured. Fruits that started to shrivel were kept in the storage if no other disorders were seen. During storage, the fruit was wrapped individually with paper, placed in perforated plastic backs commonly used for fruit packing, and placed in a 6°C refrigerator.

The destructive measures taken on all apples included firmness using a penetrometer (Model FT-327; Effegi, Italy; plunger diameter 8 mm), % sucrose (°Brix/soluble solids content) using an eclipse handheld refractometer (Bellingham + Stanley Ltd.), and counts of developed seeds. Following these measurements, fruits were frozen in a - 20 °C freezer for later analyses of acidity, dry matter and mineral content. The mineral content analyses was carried out with an ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry), Optima 8300, Perkin Elmer. Seven elements were analysed: Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Boron (B), Iron (Fe) and Zinc (Zn). Both dry and fresh weight concentrations were measured, but only fresh weight concentrations were used for statistical analyses. The concentration of the elements were adjusted according to the weight loss of each apple in storage (adjusted concentration = weight after storage / initial weight * element concentration). Titratable acidity (TA) in the apples was measured by extracting 5 ml of apple juice, diluting the juice with 15 ml ddH_2O and titrating as malic acid with 0.05N NaOH until a pH of 8.1 was reached. For the apples were extraction of flowing juice was not possible, 5.0 g apple sauce was used instead and the acidity was corrected accordingly (5 ml juice = 5.25 g).

Statistical analyses

Before any statistical analyses, all apples were checked if they meet the European Union marketing standards for apple. Fruits that were too small (lighter than 70 g) or had too low Brix value (if lighter than 90 g the Brix should be above 10.5) were disregarded, resulting in 218 apples.

Differences between pollination treatments for both initial and final fruit set were analysed by fitting generalized least squares (GLS) models, in the nlme package [33], using R [34]. Percent initial and final fruit set was calculated by dividing the number of fruitlets and ripe fruits respectively with the number of initial flowers on each branch and multiply it with 100. Percent *initial* and *final fruit set* were included as response variables and *treatment* and *site* as fixed factors. Final fruit set was square root transformed to meet the assumption of normally distributed model residuals. For the initial fruit set, the model allowing for unequal variance between pollination treatments had better fit (ΔAIC = 27.7), hence we modelled the variance for this factor with the *VarIdent* option. If a significant treatment effect was found with likelihood-ratio tests between models with and without the treatment variable, we continued with a posthoc tests using the glht-function from the “multcom package” in R [35] with the predefined contrasts control – supplementary hand-pollination treatment and control – pollinator exclusion treatment.

Seed set was analysed by fitting a generalized linear mixed-effects model, in the lme4 package [36], with the binomial response variable seed set (maximum 10 developed seeds per fruit), treatment as fixed factor and apple treeID as random effect. An observation-level random effect was added to the model to account for the overdispersion in the data [37]. If there was a significant treatment effect, we continued with a posthoc test using the glht-function with the same two predefined contrasts as for the fruit set analyses. Extra apples were excluded from the analysis.

The effects of the pollination treatment on the element concentrations (µg/g fresh weight) and the ratio between K and Ca (K:Ca) were analysed with separate linear mixed effect models for each element. Fixed factors in all models were *pollination treatment*, *initial weight* (possible dilution effect), and their interaction. *Total buds per tree* (initial investment by the tree), *final fruit set per branch* (possible dilution effect), *colour cover* and *site* were included as covariates and *tree ID* as a random effect. The continuous predictors were centred, using the scale-function. Response variables were transformed if needed to meet the assumption of normally distributed model residuals and the interaction term was removed if not significant. The interaction term was deleted if insignificant. The p-values where obtained using likelihood-ratio tests between full models and models with one of all the possible terms dropped. Extra apples were excluded from the analysis and two extreme outliers were removed from the Ca analyses (n = 197).

To analyse the DMC of fruits in relation to treatment and storage time, the data were divided into two datasets. One dataset (n = 159) included fruits that were measured initially, directly after harvest, and fruits that persisted in the storage until the end of the experiment, the final apples. The second dataset (n = 37) included apples that suffered from postharvest disorders during the storage time. Linear mixed effect models were fitted for both datasets, with percent *dry matter* as the response variable, *pollination treatment* and *storage category* (initial and final) or *days in storage*, and their interactions, as the explanatory variables, and *final fruit set per branch*, *initial fruit weight* and *total number of buds per tree* as covariates. The factor *site* was included to account for differences between sites in soil and management, and *tree ID* as a random effect. The interaction term was deleted if insignificant. If a treatment effect was found, we did a posthoc tests using the glht-function with the predefined contrasts control – supplementary hand-pollination treatment and control – pollinator exclusion treatment. Continuous predictors were centred, using the scale-function and p-values where obtained using likelihood-ratio tests. The other quality variables, sugar content, firmness, titrated acidity and weight loss during storage, which are all highly inter-correlated, were analysed with similar models as the DMC, but simplified to only include treatment, storage category/storage time and their interaction, fruit weight and site as predictor variables.

To assess direct and indirect effects of the pollination treatment on mineral content and the storability of apples, we developed a structural equation model (SEM). We used individual linear mixed-effects models (lme) from the nlme-package to be included in the initial piecewise SEM, were all models had treeID as a random effect. Each model was first individually evaluated by plotting standardized residual against fitted values and each predictor variable [38]. When necessary, we used the variance function varPower to model heteroscedasticity and log transformation to achieve normality of residuals. To assess the model fit of the SEM, we used directional separation test (D-separation test) which yields Fisher's C statistic that is chi-square distributed. If a missing path was detected with the D-separation test, the path was added. To simplify the model, insignificant paths were deleted from the model until the SEM's AIC did no longer decline or no other paths could be deleted. The relative importance of predictor variables was compared using standardised path coefficients.

Results

Fruit and seed set

The pollination treatment had an effect on both initial (L.Ratio=60.8, Δdf = 2, $p < 0.001$) and final fruit set (L.Ratio=61.1, Δdf = 2, $p < 0.001$) (Fig. 1). The control branches had 452% higher initial ($p < 0.001$) and 799% higher final ($p < 0.001$) fruit set than the branches in the pollinator exclusion treatment, which confirms that Amorosa apples are highly dependent on animal-mediated pollination. The supplementary hand-pollinated branches had 44% higher initial ($p < 0.03$) and 56% higher final ($p < 0.03$) fruit set compared to the control branches, indicating that there is a pollination deficit in the system. The seed set was higher in control fruits (mean = 2.3) compared to fruits in the pollinator exclusion treatment (mean = 0.4, glmer: $p < 0.001$), but there was no difference in seed set between control (mean = 2.3) and supplementary hand-pollinated fruits (mean = 2.4, $p > 0.8$).

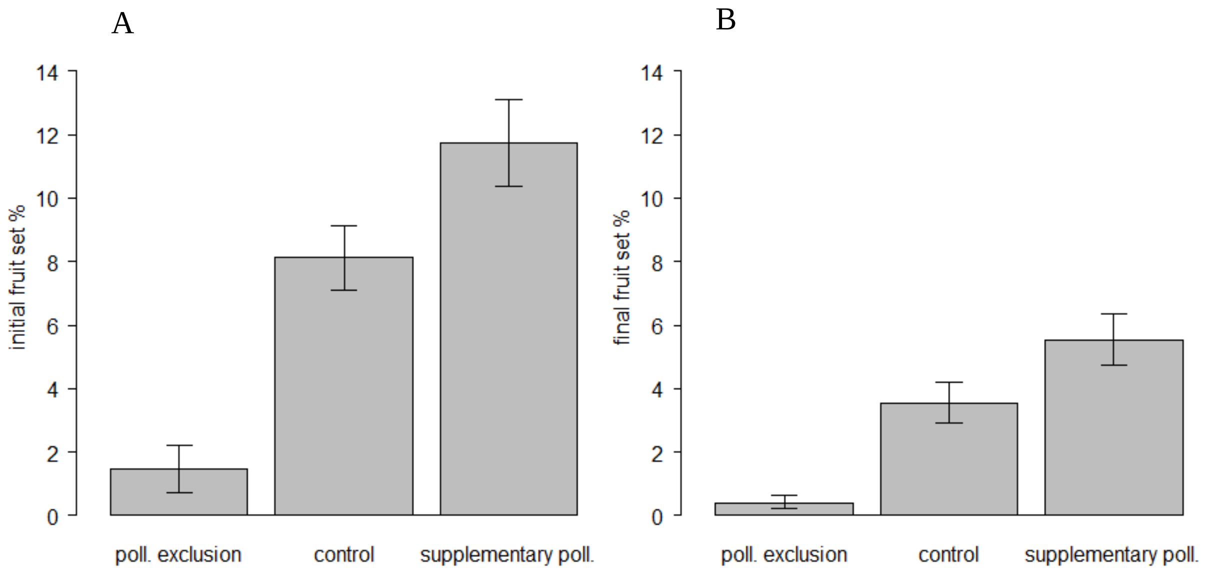

Fig 1. *The effect of the pollination treatments "pollinator exclusion", "control" and "supplementary hand-pollination" on A) the initial fruit set (number of initial fruits divided by number of flowers per branch) and B) final fruit set (number of ripe fruits divided by number of flowers per branch), using predicted values from the GLS-models. Bars represent model-estimated standard errors. The initial fruit set was analysed with a generalized least square model (GLS) to allow for different variances between pollination treatments. Both the model for the initial and final fruit set included Site as an additional predictor variable. In the supplementary hand-pollination treatment the fruit set was calculated for the whole branch but only 5-10 flowers on the branch was supplementary hand-pollinated. Estimated means per treatment and the standard errors in b) are back-transformed from squared rooted values.*

Mineral concentrations in fruit

The concentrations of the elements K, Zn, P and Mg in fresh weight apples were affected by the interaction between pollination treatment and initial apple weight (Table 1). The pattern underlying the interactions differed between elements, but indicated that the effect of pollination on mineral content depended on the weight of the apples. The pattern was similar for K and Mg, where the interaction effect arose because the supplementary hand-pollinated apples increased in K and Mg concentrations with apple weight, whereas the control (K: $t = -2.8$, $p = 0.007$, Mg: $t = -4.6$, $p <0.001$) and pollinator excluded fruits (K: $t = -2.3$, $p = 0.02$, Mg: $t = -3.5$, $p <0.001$) decreased in element concentrations with apple weight (Table 1). The variation in the concentration of P arose because the supplementary hand-pollinated apples increased in P concentration with increased weight, whereas the controls decreased ($t= -2.3$, $p =0.02$). The variation in concentration of Zn among treatments arose because control ($t = -3.0$, $p = 0.004$) and supplementary hand pollinated fruits ($t = -2.2$, $p = 0.03$) had a steeper decline in Zn concentration with increased weight compared to pollinator excluded fruits. We found a marginally significant effect of pollinator treatment on the K:Ca ratio in fruits, where the supplementary hand pollinated fruits tended to have lower K:Ca ratio compared to the other treatments (Table 1). For the remaining elements (Ca, Fe and B), the concentrations did not vary between pollination treatments or as a consequence of an interaction between pollination treatment and apple weight (Table 1). However, Ca concentration was negatively related to the initial weight of the apples (Table 1).

Table 1. *The effect of the interaction between pollination treatment and the apple weight at harvest on the element concentrations, analysed with linear mixed effect models (lme, nlme-package in R) with the random effect treeID. When interactions were non-significant, the effect of treatment and apple weight is presented separately. P-values were obtained using log-likelihood tests. Df = 2 for interaction and treatment effects and df =1 for apple weight. N = 188.*

Response variable	Treatment * apple weight	Slope estimates ± std. error			Treatment	Apple weight
		pollinator exclusion	*control*	*supplementary hand-pollination*		
Log(K_Ca)	p = 0.33				p = 0.051 poll ex: 3.5 ± 0.11 con:3.5 ± 0.09 supp: 3.3 ± 0,12	p = 0.073
Ca*	p = 0.22				p = 0.091	**p = 0.0023**
K	**p = 0.018**	-0.89 ± 0.41	-1.08 ± 0.36	0.75 ± 0.58		
Sqrt(Zn)	**p = 0.012**	-0.000046 ± 0.00029	-0.0011 ± 0.00025	-0.0011 ± 0.00042		
Sqrt(P)	**p = 0.044**	-0.00068 ± 0.0023	-0.0050 ± 0.0019	0.0033 ± 0.0032		
Log(Fe)	p = 0.32				p = 0.14	p = 0.99
Mg	**p < 0.001**	-0.071 ± 0.021	-0.10 ± 0.018	0.055 ± 0.030		
Log(B)	p = 0.35				p = 0.30	p = 0.20

*two outliers were removed

The effect of storage time on fruit dry matter and mineral content

DMC in fruits was affected by pollination treatment (L.Ratio = 28.5, Δdf = 2, $p < 0.001$ (initial and final fruit data); L.Ratio = 8.6, Δdf = 2, p =0.014 (apples that suffered from postharvest disorders), where fruits from the supplementary hand-pollination treatment had the highest DMC (pairwise comparison with control: z = 3.8, p <0.001 (initial and final fruit data); z = 2.48, p = 0.026 (apples that suffered from postharvest disorders)) (raw data showed in Fig. 2). Furthermore, for the healthy fruits included in the analysis of the initial and final fruits the control fruits had higher DMC compared to the pollinator excluded fruits (z = - 3.0, p = 0.005).

The fruits that suffered from postharvest disorders during storage showed a similar tendency with higher DMC in control compared to pollinator excluded fruits, but the effect was only marginally significant ($z = -2.1$, $p = 0.063$). DMC decreased with days in storage in fruits that were removed from storage when we found them to have postharvest disorders (L.Ratio = 20.2, $\Delta df = 1$, $p < 0.001$). DMC also had a tendency to be higher in apples that were measured directly after harvest compared to apples that had remained in the storage for the entire storage period (maximum 162 days) (L.Ratio = 3.7, $p = 0.056$). We found no interaction effect between pollination treatment and storage time in these analyses, showing that fruits from the supplementary hand-pollination treatment had consistently higher DMC throughout the storage time.

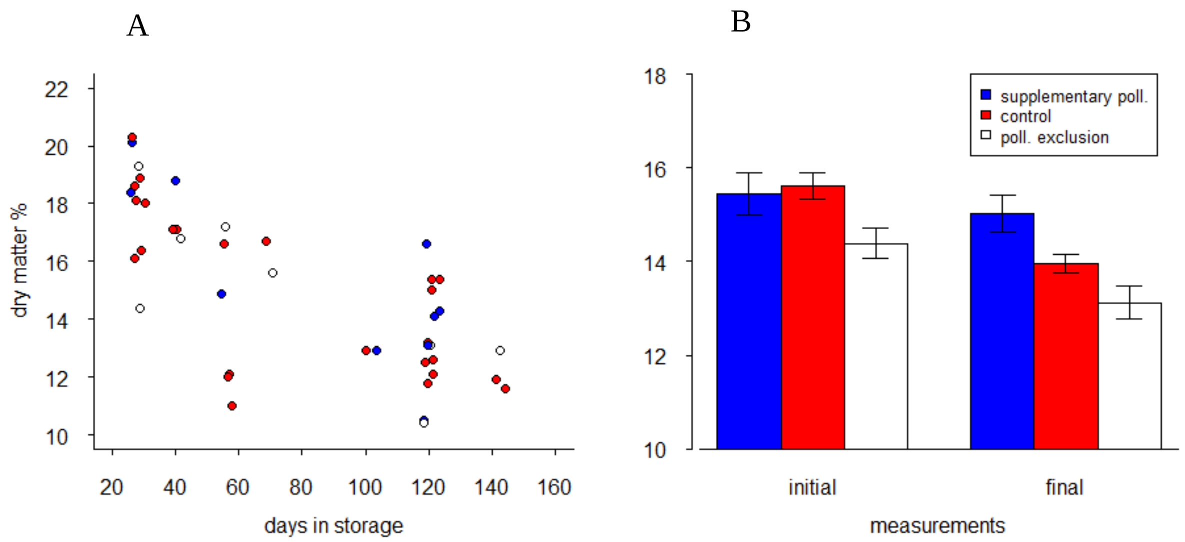

Fig 2. *Dry matter content (%) in relation to (A) the number of days the fruits were in storage, i.e. until they could no longer be considered to be first class fruit, and (B) against the categories initial (directly after harvest) and final measured fruits (after 161-162 days in storage). The maximum storage time was 162 days. Apples that had started to shrivel were considered as first class fruit if no other damages were detected on the skin. Supplemented pollinated fruits are represented by blue, controls by red and pollinator exclusion by white. In A) dots are jittered to make all dots visible.*

The other quality measurements - acidity, sugar, firmness and weight loss - did not have common responses to pollination treatment and storage time (Fig. 3). Sugar concentrations in apples from the control and the pollinator excluded treatment declined between fruits measured initially and fruits that had been in storage during the whole experiment, while fruits from the supplementary hand-pollination treatment had stable sugar levels across time (Fig. 3). The weight loss in fruits was highest for apples in the supplementary hand-pollination treatment, both for fruits that were removed due to post-harvest disorders and especially for healthy fruits at the end of the experiment (Fig. 3). We found no relationship between firmness and titratable acidity and pollination treatment. However, longer storage time had a general negative impact on titratable acidity, sugar and firmness and site had an impact on all quality variables (Fig. 3).

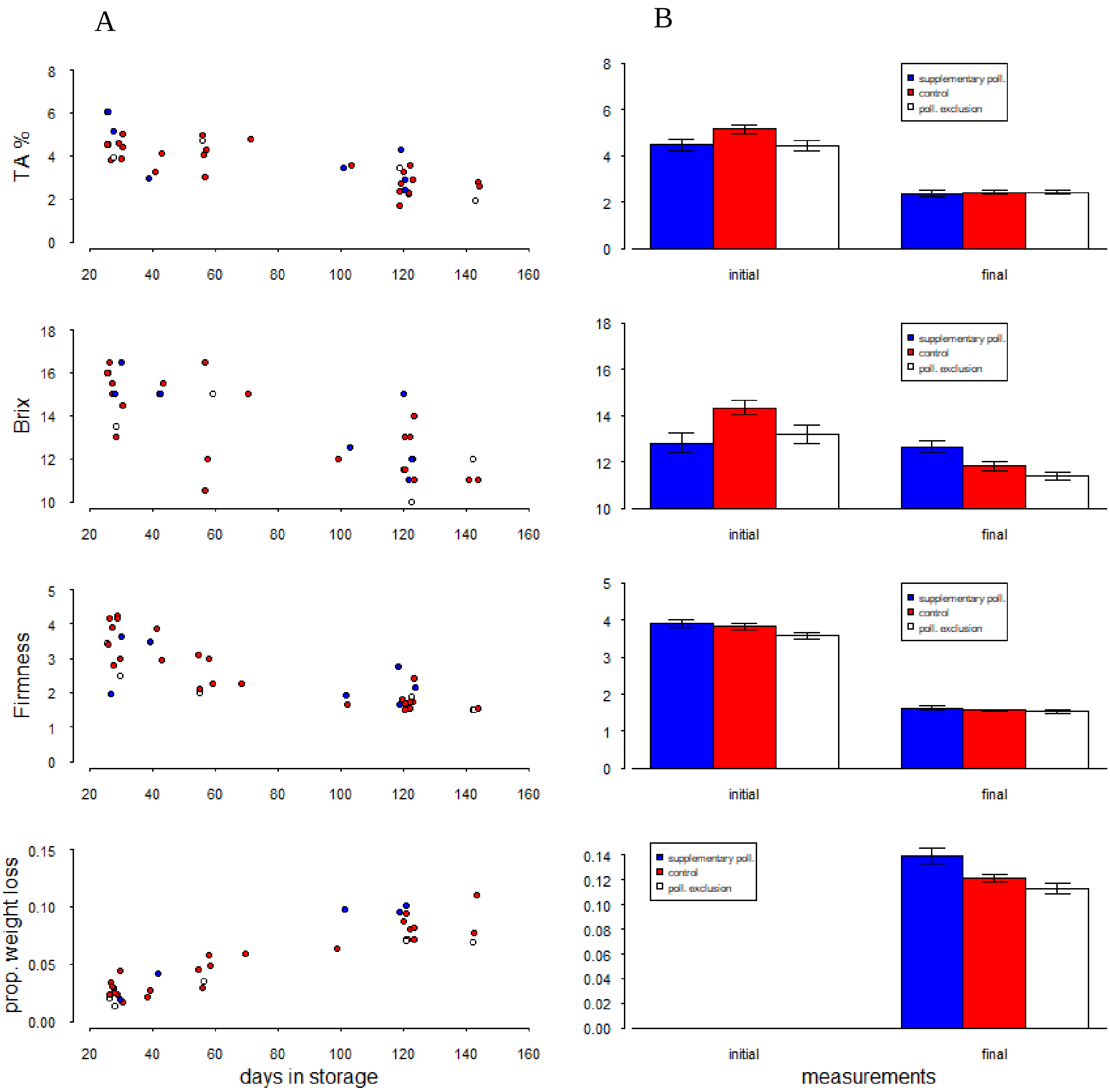

Fig. 3. The quality measurements titratable acidity %, brix/sugar, firmness and weight loss in relation to pollination treatment and storage time of apples that suffered from postharvest disorders during the storage time (A) or storage category of fine fruits that were measured initially before storage (initial) and apples that stayed in the storage the entire experiment (final) (B).

SEM

When running the initial SEM to explore the causal relationships between pollination treatment, fruit weight, fruit set, K:Ca ratio and storage duration, the D-separation test revealed a missing link between fruit weight and storage duration (Fig. 4). This path was therefore added to the model. We decided to include the K:Ca ratio in the model, and not the other elements, since this ratio's effect on storability has strong support in the literature. In the final SEM, we found that pollination treatment had an indirect effect, mediated through fruit weight and K:Ca ratio, on storage time of fruits (Fig. 4). Fruit weight had both a direct negative effect and an indirect effect – where fruit weight had a positive effect on the K:Ca ratio which in turn had a negative effect – on storage time. Site had a strong direct effect on both K:Ca ratio and storage time. Final fruit set was affected by treatment, but did not in turn affect the K:Ca ratio.

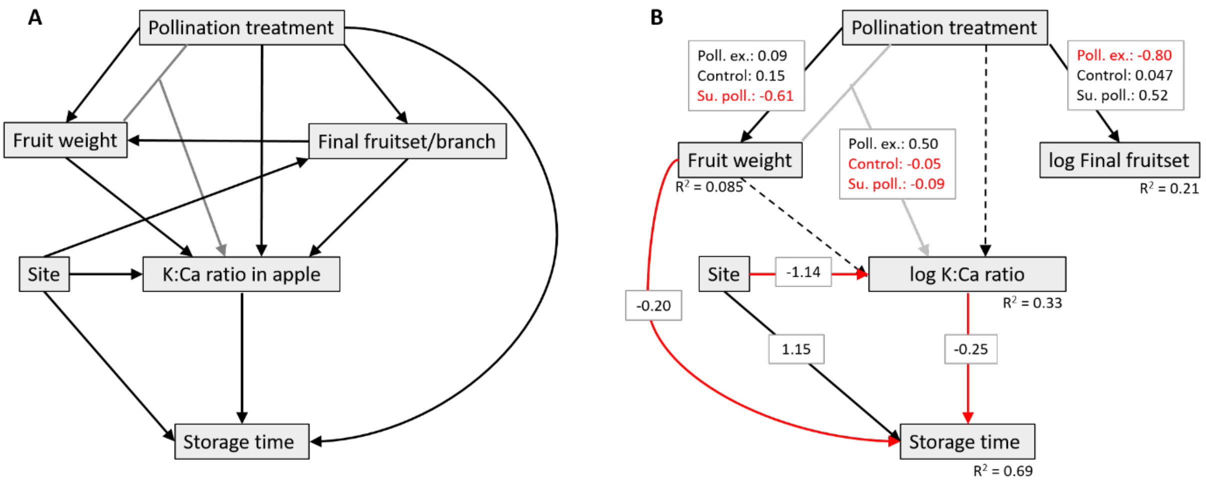

***Fig 4.** Piecewise structural equation model (SEM) exploring the relationship among pollination treatment, fruit weight, fruit set, fresh weight K:Ca ratio, site and storage time. A) Initial SEM showing all analysed paths, and B) is the final analysed SEM after variable transformations, addition of a missing path, and deletion of insignificant relationships. Arrows represent significant unidirectional relationships (p <0.05), where black arrows represent a positive relationship, red arrows a negative relationship, the grey arrow an interaction term and the dashed lines the variables included in the interaction term.*

Discussion

We found the level of pollination to not only affect the quantity of apples produced but also the DMC in apples, which in turn is connected to several fruit quality aspects such as flavour, sucrose, acidity and fruit texture [15-17]. Several elements (K, Zn, P, Mg) were also affected by the interaction between pollination treatment and fruit weight, and the K:Ca ratio tended to be related to pollination treatment, which indicated that pollination levels were involved in the accumulation of these substances into fruits. The supplementary hand-pollination treatment, which represent optimum pollination levels, resulted in both the highest fruit set and the highest levels of DMC in fruits. Furthermore, the SEM showed that the interaction between the pollination treatment and fruit weight directly affected the K:Ca ratio which then indirectly had a negative impact on the storability. Since high K:Ca ratios have been associated with several post-harvest disorders in other studies [23, 24], our results indicates that large poorly pollinated fruits suffer from higher risks of quality failure during storage compared to those that had been animal or hand pollinated. Hence, the results are partly in accordance with our hypothesis, that higher pollination services will lead to generally higher element concentrations in fruits, as the level of pollination service modified the concentration of several elements in fruits, but the pollination effect depended on the weight of the apples. Some of these elements (the ratio between K:Ca) were also related to storability of fruits.

DMC has recently been proposed as a predictive tool for estimating fruit quality, where higher DMC gives better quality [15-17], due to its connection to firmness of apples at harvest, softening rates during storage [15, 17], better flavour [16] and increased consumer preference [15]. In earlier studies, soluble solids and firmness was considered to be the main quality measurements of apples, but since apples are living tissues the metabolism continues during storage and hence the composition of the substances change during maturation [15]. Thus, soluble solids and firmness as well as acidity will depend on the apple ripeness when measured and are not stable and reliable quality indicators [15]. DMC measured at harvest is a more reliable predictor of total soluble solids (mainly sugar) after 12 weeks in storage compared to measurements of soluble solids at harvest [15]. In our experiment, DMC was lower for fruits that remained longer in storage, which is not unexpected as a consequence of respiration during storage. However, since we did not measure respiration, we cannot distinguish this from fruits with high DMC suffering more from early post-harvest disorders. However, we found supplemented hand-pollinated fruits to retain higher DMC compared to the fruits from the other treatments throughout the storage time which indicate a persistently higher quality of well-pollinated fruits.

In agreement with earlier studies, we found that a lower K:Ca ratio in fruit resulted in better storability of fruits [23, 24]. Even when there is good availability of Ca in the soil solution, apples can still suffer from a low Ca level [39]. The uptake, translocation and redistribution of Ca within the plant is generally low in comparison to elements like Mg and especially K. Because the uptake of Mg and K are easier for the plant, these element tend to accumulate at a more uniform rate in the fruit than Ca [39]. Hence, the most likely cause for a lower higher K:Ca (and Mg:Ca) ratio may be a reduction in the accumulation of Ca in the fruit [39]. It also seems likely that the low levels of Ca rather than the high levels of K or Mg per se is the cause for post-harvest disorders, but the low Ca level is most obvious in contrast with K and Mg [39]. Since the K:Ca ratio were marginally affected by pollination treatment, as well as several single elements in interaction with apple weight, a reasonable conclusion is that the allocation of these elements into fruit is connected to the pollination levels. A possible mechanism was suggested by Bramlage, Weis [28], who argued that increased seed numbers are connected to increase translocation of Ca into fruits. Apple seeds produce auxins and the transport of Ca and auxins is closely linked. When there is greater auxin transport out from the fruit, due to high seed numbers, Ca transport into the fruit may be enhanced [28]. On the other hand, Bramlage et al. [28] did not find seed numbers to affect Mg and K in fruits, confirming the importance of Ca concentrations for the role of K:Ca ratios. Similar, Volz et al [29], found supplementary hand-pollination to increase both seed number and Ca concentration in early flowering Braeburn apples, while Mg and K were unaffected by pollination treatment. In our study, Mg and K levels in fruits changed with pollination treatment in interaction with fruit weight. While the seed number and auxin related mechanism seems plausible, additional mechanisms may be at play as we found differences in the K:Ca ratio in apples between control and supplementary hand-pollinated fruits while at the same time no effect on seed set.

As evidenced by the SEM analysis, pollination treatment is not the only factor affecting apple quality aspects (Fig. 4). Site had a strong influence on both fruit set and K:Ca ratio. Factors related to site could be differences in management, general apple tree health, the availability of elements and nutrients in the soil, presence of pests and pathogens in the orchard and tree related factors including fruiting position within the apple tree and density of leaves (eg. [29]). However, it is clear that pollination services in orchards needs to be considered in the management since it obviously affects fruit set and several quality aspects of apple. To increase or obtain high natural pollination services in orchards, high species richness and abundance of wild bees, seems to be the key [40-42]. Higher species richness is generally related to higher functional trait diversity and pollinators with different traits, including behaviour, can complement each other both spatially and temporally [40, 42, 43]. The effectiveness of specific pollinator species also depend on the interacting species [44]. For example, honeybees are more efficient as almond pollinators if wild bees are present, since wild bees disturb the honeybees and makes them switch flower more frequently [6]. Even though honeybees are commonly used as apple pollinators, their effectiveness as crop pollinators have recently been questioned [40, 45], and some authors actually suggest that orchard owners should focus un supporting wild communities of pollinators rather than investing in honeybee hive rental [41].

In order to increase sustainability of apple production, it is necessary to better understand the relationships between pollination services, fruit set, fruit quality and storability. Increased understanding can guide management decisions, and one key aspect is to understand when increased pollination services is needed. We have found that high pollination services are generally beneficial, since pollination increases both fruit set and dry matter content in fruit. Experimental set-ups including more cultivars and orchards, different thinning-regimes, control of inter-annual effects and pollination treatment application to whole trees (to account for resource allocation within trees) are needed to evaluate how general the results for this study are and the over-all economic impact of pollination services on apple fruit quality.

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Planned scientific publications:

Samnegård, U., Hambäck P.A. and Smith, H.G., Impact of pollination services to apples goes beyond increased fruit set.