Insect pollination as a key factor for strawberry physiology and marketable fruit quality

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A B S T R A C T

About 35% of global crop production arises from crop species that benefit from animal pollination, especially by insects. Animal pollination can enhance yields and increase fruit quality, but the effects of insect pollination on pre- and post-harvest fruit physiology and quality are largely unknown. For the first time, we analysed in much detail the physiological responses of fruit development and marketable quality improvements to different pollination treatments such as self-pollination, open-pollination and hand-pollination. In strawberries, self-pollination led to reduced seed set (fertilized achenes), reduced concentration of the phytohormone auxin, highest share of deformed fruits (> 90%), smallest and lightest fruits, considerably lower commercial value (8% of the value of open- or hand-pollinated fruits), and reduced shelf life of fruits. Overall, insect pollination increased the average commercial value of marketable fruits by 92%. The commercial value of hand-pollinated and open-pollinated strawberries did not differ. We conclude that pollination services are not merely important for yield, but also vital for physiological processes that result in better marketable quality (e.g. fruit appearance, flavour-enhancing constituents, prolonged shelf life) and commercial value of many pollinator-dependent crops.

1. Introduction

Fruit and seed set of many crops depend on pollination through insects, typically wild and honey bees, and about 35% of global crop production arises from crop species that benefit from animal pollination (Klein et al., 2007). Exponential world population growth and the related increase of global food demand has led to an intensification of agricultural production, which carries the risk of pollinator losses (Garibaldi et al., 2011). Insufficient insect pollination leads to reduced seed set and crop yield. These yield losses emerge mostly from non-developing fruits and fruit deformations (Svensson, 1990; Żebrowska, 1998), which undermine the marketability of fruits. Only few studies report pollination effects improving quality parameters, such as: increased flavour-enhancing constituents like sugars and acids in oriental melon (Shin et al., 2007), higher oil and less chlorophyll contents in oilseed rape (Bartomeus et al., 2014; Bommarco et al., 2012), reduction of empty seeds in buckwheat (Bartomeus et al., 2014), improved commercial grades of strawberries (Bartomeus et al., 2014), and

improved size, shape and commercial grade of apples (Garratt et al., 2014).

The garden strawberry (*Fragaria x ananassa* DUCH.) has previously been used as a model crop for tests of pollination effects on crop quantity and quality (Klatt et al., 2014a). Strawberries are commercially cultivated around the world, with Europe exhibiting the globally largest cultivated area with strawberries (FAO, 2017). Klatt et al. (2014a) calculated that improvement of strawberry quality and storability by bee pollination contributes 1.44 billion US$ to the total turnover of 2.90 billion US$ of the European market. This effect is caused by improved shape and size of insect-pollinated fruits (Klatt et al., 2014a; Żebrowska, 1998). Insect pollination leads to improved fertilization of strawberry flowers and thereby increases the number of fertilized achenes (Klatt et al., 2014a; Svensson, 1990). In addition to size and shape, insect pollination can also enhance colour, firmness and acidity of fruits (Klatt et al., 2014a). These quality aspects are subject to significant changes during fruit development and ripening and assumed to be driven by phytohormones.

Growth and ripening processes of non-climacteric strawberry fruits seem to be controlled mainly by auxin (indole-3-acetic acid, IAA) (Nitsch, 1950; Given et al., 1988), certain gibberellic acids (GA), abscisic acid (ABA), brassinosteroids and ethylene (Cherian et al., 2014). It is assumed that fertilized achenes release IAA into the receptacle, where it promotes fruit enlargement (Nitsch, 1950; Symons et al., 2012, Dreher and Poovaiah, 1982). Immediately after anthesis, IAA synthesis increases, reaching a maximum in small green fruits (Symons et al., 2012). When IAA concentration drops during later fruit development, ripening processes are initiated (Given et al., 1988; Symons et al., 2012; Manning, 1998). ABA and ethylene synthesis is then accelerated during late fruit development and ripening (Cherian et al., 2014; Jia et al., 2011; Jiang and Joyce, 2003). However, the timing and mechanisms by which pollination determines fruit development, phytohormone expression and finally fruit growth and quality are unknown to date.

Ripening of strawberry fruits is characterized by red colouring (anthocyan formation), softening (Given et al., 1988; Culpepper et al., 1935) and accumulation of soluble sugars (sucrose, fructose and glucose) and organic acids (citrate, malate and ascorbic acid) (Woodward, 1972; Pérez et al., 1997). Beside aroma compounds, the ratio between sugars and acids is an important quality parameter that influences the flavour of strawberry fruits (Hancock, 1999; Wozniak et al., 1997). Fruit firmness contributes to improved shelf life, decreases during ripening and is primarily influenced by the cell wall composition and by the water content and turgor (Jackman and Stanley, 1995). Strawberry fruits are classified as highly perishable goods as their storability is very limited (Caner et al., 2008). Based on fruit firmness Klatt et al. (2014a) calculated reduced losses of at least 11% during storage in case of firmer bee-pollinated fruits if compared with wind- and self-pollinated fruits. These predictions have been tested by a storage experiment with fruits differing in their degree of deformations, which is considered as an indirect indicator of pollination limitations (Klatt et al., 2014b). Yet, the effects of pollination limitation on shelf life have not been tested directly in a storage trial with fruits from different pollination treatments.

Hence, this study analyses for the first time the direct effects of insect pollination on phytohormone-driven processes of fruit quality formation during fruit growth and ripening using strawberry as a model plant. Furthermore, storability, marketability and commercial value of ripe fruits were examined. To manipulate pollination success, three types of pollination treatments were performed: autonomous self-pollination, open-pollination and hand-pollination (with genetically identical pollen). Additionally, we inoculated ripe fruits from self-, open- and hand-pollinated flowers with grey mould (*Botrytis cinerea)* to analyse the direct effects of pollination success on shelf live under standardised conditions.

2. Materials and methods

2.1. Experimental set-up

The study was carried out on an open strawberry field (without polytunnel, ca. 1.5 ha) near Holtensen (Fig. A1), close to the city of Göttingen (southern Lower Saxony, Germany). In 2011, the field was planted with the garden strawberry cultivar *Fragaria* x *ananassa* 'Honeoye' (50 rows of about 90 cm row spacing) and not fertilized in 2014. In addition, nesting aids for wild bees and managed honey bee hives could be found in the vicinity of the strawberry field (for detailed information see Appendix File A1). Field work (fungicide application, pollination treatment, pollinator assessment, fruit sampling) was carried out from April until June 2014 (see Fig. A2 for climatic details).

Three pollination treatments were applied to all first order flowers of experimental strawberry plants: autonomous self-pollination, hand-pollination and open-pollination. To enable self- and hand-pollination, buds were covered with Osmolux bags (Pantek, Montesson, France), which are permeable for water vapour and thus allow microclimatic

exchange but prevent wind and cross-pollination. Furthermore, hand-pollinated flowers were manually pollinated (using brushes) with pollen of the same flower during anthesis. Plastic bags were removed after all stigmas became unreceptively for pollen (normally associated with fruit set; see detailed description of pollination treatments in the Appendix File A1). Open-pollinated plants remained uncovered to allow cross-pollination through insects and wind. In all treatments, we focused on first order flowers to standardise the effects of the number of stigmas and fruit weight, as higher order flowers generally have fewer stigmas and hence produce smaller fruits (Galletta and Himelrick, 1990). Pollination treatments were replicated in four plots, which were located in one central row with a distance of 20 m to each other and a minimum distance of 15 m from field margin. In addition, plots had a distance of at least 15 m from field margins to avoid edge effects. Each plot consisted of the three pollination treatments randomly arranged per each plot. The treatment areas had a length of 6 m, respectively, and were separated from each other by one meter in every plot. Each pollination treatment was applied to 64 plants (Fig. A3). A total of 768 first order flowers were labelled and their date of blossom was noted. During blossom of first order flowers, standardised transect walks were performed to record the pollinators (four transects of 100 m length, located on the left and right of the treatment plots with randomised starting points of transect walks; Westphal et al., 2008; see Appendix File A1). Out of the 768 experimental first order flowers, a total of 689 strawberry fruits were sampled (76 fruits had disorders like fungal infections). Each treatment * replication combination composed of several fruits (between 55 and 60 fruits; see Table A1 for details) that were harvested in three development stages (green, white and red fruits).

2.2. Fruit quality and physiological parameters

2.2.1. Size, weight, colour and firmness

Size (horizontal diameter), weight and colour of 689 fruits were measured during harvest (size) or within eight hours after harvest (weight and colour). Fruit size was determined by a digital sliding calliper. Fresh weight was measured without pedicels and sepals. For fruit colour the a-value, which indicates the green-red composition (the more negative, the greener and the more positive, the redder) was recorded with a portable colorimeter (CR-410 Chromameter, Konica Minolta, Badhoevedorp, The Netherlands) at two opposite sides of the fruit in the L*a*b*-colour space. Firmness was measured on 562 fruits with the texture analyser TxT2 (Stable Micro System, Surrey, UK) which was equipped with a 25 kg compression cell and a 5 mm diameter probe and configured with a 4 mm fruit penetration depth. Firmness measurements of red strawberries were applied on bisected fruits (Klatt et al., 2014a).

2.2.2. Total soluble solids, titratable acids, pH-value and fertilized achenes

Total soluble solids (sugars), titratable acids, pH-value and number of fertilized achenes (seed set) were determined in 10 ripe fruits per replication × treatment combination (in total 120 fruits) and homogenized with an immersion blender (4179 300 W, Braun, Spain). Subsequently, a subsample (between 54 and 77 g) was centrifuged (Centrifuge 5416, Eppendorf, Wesseling-Berzdorf, Germany) at 7000 rpm for 10 min. The supernatant was used to determine pH-value, sugar and acid concentration. Sugar concentration (%) was quantified through a handheld refractometer (HRO 32, Krüss, Hamburg, Germany). The pH-value of the supernatant was analysed through a pH-meter (inoLAB pH Level 1, WTW, Weilheim, Germany). Acids were determined according to Caner et al. (Caner et al., 2008): 3 ml of the supernatant was diluted with 20 ml of distilled water. Acid concentration (%) was calculated on the basis of the milliequivalent factor (meq) of citric acid (0.064 meq) after titration to the end point of pH 8.1 with 0.1 N NaOH by an automated titrator (titration unit TitroLine 96, Schott, Hofheim, Germany). Sugar-acid-ratio was calculated by dividing the sugar by the acid concentration. Finally, the used subsamples