LETTER

Fathers, fruits and photosynthesis: pollen donor effects on fruit photosynthesis in wild parsnip

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Abstract

Chlorophyll is frequently present in plant reproductive tissues and indicates that photosynthesis is occurring in these parts. Photosynthesis by a reproductive organ can contribute as much as 65% to its own growth. Given the advantages that increased photosynthetic rates might have on development of individual seeds competing for resources, selection can be expected to favour the ability of offspring to influence photosynthetic rates of the tissues surrounding them. We report in this study the first evidence that the pollen genotype can influence the rate of photosynthesis in the fruit tissues surrounding the developing offspring. Using a novel chlorophyll fluorescence imaging instrument to quantify quantum efficiency of photosystem II, we found significant differences in photosynthetic rates among fruits in wild parsnip, *Pastinaca sativa* L, associated with different pollen genotypes.

INTRODUCTION

Flowers and fruits contribute to their own growth by in situ photosynthesis in grains (Singh 1993), tomatoes (Smillie *et al.* 1999), blueberries (Birkhold *et al.* 1992), grapefruits (Yen & Koch 1990), avocados (Whiley *et al.* 1992) and other species (Bazzaz *et al.* 1979). In large fruits such as avocados, mass-based photosynthetic rates are only 0.4–2.5% of those of avocado leaves (Whiley *et al.* 1992) and thus contribute little to fruit growth. Small-seeded rape fruits, by contrast, produce nearly all of their own carbon through their own photosynthesis (Singh 1993).

Insofar, as 'reproductive photosynthesis' can contribute substantially to the carbon gain of fruits, natural selection might favour offspring that enhance photosynthetic rates in the fruit tissues in which they are enclosed. Evolutionary models of investment in angiosperm seeds are complicated by the fact that seeds consist of three genotypes – maternal tissues (ovule wall), triploid endosperm and embryo. The 'interests' of these genotypes are not necessarily the same (Queller 1984; Haig 1990). For example, maternal plant fitness is enhanced by producing the greatest number of successful offspring, even if this goal necessitates abortion of some offspring. On the other hand, each offspring's fitness is enhanced by its ability to garner resources, sometimes at the expense of a sibling's fitness. These interests may not be compatible and may lead to parent–offspring or kin conflict.

The means by which offspring genotypes might influence maternal resource investment are not well understood. In this context, the ability of offspring to manipulate fruit photosynthesis may be advantageous as a means to garner more resources. For a number of reasons, an individual offspring's ability to influence fruit photosynthesis could benefit it. Faster rates might be associated with larger stored reserves, and hence larger, more competitive, seedlings (Zhang & Jiang 2000). Enhanced carbon gain might be beneficial in avoiding herbivore damage, either by hastening development and concomitantly reducing the window of opportunity for herbivores to consume them, or by bolstering chemical or physical defenses (Zangerl & Nitao 1998). As photosynthesis of fruits involves maternal tissues, offspring can affect carbon gain only by influencing maternal tissues. These so-called metaxenic effects may spur competition among offspring for limited maternal resources and may not be in the best fitness interests of the maternal plant, setting the stage for potential parent–offspring conflicts (Raju *et al.* 2001).

Until recently, technical barriers have prevented investigation of the role of offspring on fruit carbon gain; large

fruits suitable for measurement of photosynthesis by gas exchange often contain many seeds derived from multiple pollen donors. In fruits such as these, any benefit of manipulating photosynthesis by one offspring would be shared by all others, irrespective of their genetic relatedness. Manipulation in fruits that contain only one or two seeds is more likely to be favoured by selection, but these fruits are usually too small for conventional gas exchange, and require measurement of fruits one at a time. Recent advances in chlorophyll fluorescence imaging (Hunt 2003) allow spatial mapping of the flow of electrons through photosystem II, which is directly related to the rate of gross photosynthesis (Genty *et al.* 1989). Its value in the context of measuring fruit photosynthesis is that multiple fruits can be measured simultaneously, and fruit size is often not limiting because of the high resolution of the instrument.

The ability of pollen parents to influence fruit photosynthesis via their genetic contributions to offspring and endosperm was investigated in wild parsnip, *Pastinaca sativa* L. An earlier study of wild parsnip reported that maternal seed investments in chemical defense but not endosperm were influenced by pollen parent, suggesting offspring manipulation of defense and a potential conflict between parent and offspring (Zangerl & Nitao 1998). Each fruit of this plant consists of two ovules. The primary umbel, which is the first to flower, may contain several hundred fruits. After successful fertilization the ovules expand and remain green until the embryo and endosperm are fully developed.
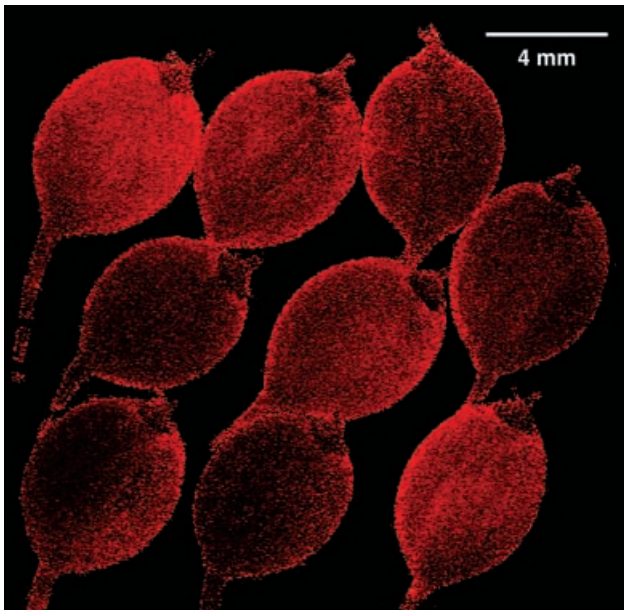

Figure 1 False-colour fluorescence imaging of photosystem II quantum efficiency (F_q'/F_m') showing variation among wild parsnip fruits in gross photosynthesis (rates of photosynthesis are directly proportional to redness).

At the same time, the levels of furanocoumarins, genotoxic defense compounds, also increase dramatically (Zangerl & Nitao 1998). We performed controlled pollinations with pollen from several different plants, and the resulting developing fruits were analysed with a specially designed chlorophyll fluorescence imaging instrument that yields images of apparent photosynthesis (Fig. 1).

MATERIAL AND METHODS

To effect controlled field pollinations, on 11 June 2002, we bagged the primary umbels (at the apex of the shoot) of five plants with flowers in the late male stage of development (flowers are protandrous, Cruden & Hermann-Parker 1977). The bags were constructed of fine nylon mesh (mesh openings of 0.3 mm) and were securely affixed with 10.2 cm cable ties to exclude insect pollinators. Three days later the flowers were receptive females (the bifurcated stigmas were exserted and curved). On that day, pollen was collected separately from each of several plants in the same field by tapping the male flowers of several secondary umbels on a plastic Petri dish. The bags were removed from the plants designated to be pollen recipients, and pollinations were performed according to Zangerl & Nitao (1998). For each pollen donor, all flowers on each of two umbellets were pollinated. The use of two umbellets on opposite sides of the primary umbel eliminated the potential for sectoriality to produce false pollen donor effects (unpublished dye-feeding experiments revealed that umbellets in close proximity to one another share vascular traces). The umbellet stalks were then marked with bands of indelible black ink to identify pollen donor. The umbel was subsequently enclosed in the same bag. The umbel of one plant (plant A) was collected on 20th June for analysis. The bag was removed from the umbel and the nearly complete absence of fertilized ovules on umbellets, not manually pollinated, confirmed the success of the pollinator exclosure. The few fertilizations that did occur on these umbellets was likely caused by smearing of pollen that we applied when the umbel was reinserted into the bag. Plants B and C were harvested and analysed 2 days after plant A. Plants A and B were successfully pollinated by five shared fathers. Plant C was successfully pollinated by four fathers, two of which were shared with plants A and B.

Umbels were cut with a razor blade, the base immediately submerged in degassed water and cut again. The material was brought to the laboratory and all fruits with developing endosperms were cut at the bases of their stalks. The stalks were inserted into window screening that was glued to a glass Petri dish filled with water. This design permitted the fruits to have access to water during the measurements and also permitted the fruits to lie flat on the surface. For plant A, four sets of 12 fruits,

including fruits from each of five pollen donors, were imaged. Samples sizes per pollen donor ranged from 7–14. Quantum efficiency through photosystem II (F_q'/F_m'), which is linearly related to net photosynthesis in wild parsnips (Zangerl *et al.* 2002), was then measured for each set using a chlorophyll fluorescence imaging instrument (Zangerl *et al.* 2002). The resulting images were analysed with SCION for Windows 4.02 software (Scion, Frederick, MD, USA). Because of slight variations in the topography of fruits, we selected a 5-mm^2 circle at the center of each fruit for measurement of average F_q'/F_m' values. The fruits of umbels from plants B and C were imaged the following day. The fruits from five pollen donors were imaged in the second plant (four sets of 15 fruits, 9–13 fruits per pollen parent) and from four pollen donors for the third plant (five sets of 15, 16–20 fruits per pollen parent). The data for each maternal plant were analysed by two-way analysis of variance with pollen donor and measurement set as fixed main effects.

Additional fruits from all the plants and representing all pollen donors were dissected, removing the non-commissural (outside) walls of the fruits for furanocoumarin analysis (Zangerl & Nitao 1998). Prior study had documented the influence of pollen donor on the furanocoumarin content of this tissue (Zangerl & Nitao 1998). Xanthotoxin, being both abundant in parsnip fruits (Zangerl & Nitao 1998), and highly phototoxic (Berenbaum & Zangerl 1996), was quantified by high-pressure liquid chromatography (Zangerl & Nitao 1998). Pollen donor effects on xanthotoxin content were analysed by one-way analysis of variance, if the data were normal and variances homogeneous, or, if not, by Kruskal–Wallace tests.

RESULTS

Pollen donors were indeed found to influence quantum efficiency of photosystem II in the outer layers of the fruits. Of the three maternal plants in which fruits were measured, all three exhibited significant pollen donor effects (Table 1). Variation among pollen donors ranged as high as 14.9, 17.8 and 8.9% in three maternal plants (Table 2). We also found significant differences in the xanthotoxin content of the non-commissural faces of fruits of maternal plants A and C that were attributable to pollen parent (Kruskal–Wallace chi-square values were 10.1 and 10.8, respectively, with *P*-values of 0.039 and 0.013, respectively), but not in the other maternal plant (ANOVA $F = 0.595$ and $P = 0.668$).

DISCUSSION

One potential benefit to an offspring of enhanced fruit photosynthesis is increased defense. Wild parsnip fruits contain high concentrations of furanocoumarins, and this

Table 1 Effects of pollen donor on photosynthesis measured as the photosystem II quantum efficiency (F_q'/F_m') of developing fruits in three wild parsnip plants. 'Pollen' refers to the effect of pollen donor. Photosynthesis measurements were performed on groups of fruits ('set' effect)

Source	d.f.	Mean square	%	F	P
Plant A					
Pollen	4	0.00288	28.1	2.742	0.048
Set	3	0.00874	47.1	8.301	<0.001
Pollen × set	12	0.00053	17.9	0.508	0.892
Error	28	0.00105			
Plant B					
Pollen	4	0.00333	26.2	3.283	0.021
Set	3	0.00264	17.4	2.596	0.067
Pollen × set	12	0.00089	22.3	0.884	0.570
Error	37	0.00101			
Plant C					
Pollen	3	0.00188	14.2	2.823	0.048
Set	4	0.00200	19.1	3.004	0.027
Pollen × set	12	0.00023	7.5	0.347	0.975
Error	51	0.00066		2.838	

Table 2 Least square means and standard errors (in parentheses) for photosystem II quantum efficiencies (F_q'/F_m') of wild parsnip fruits sired by different pollen donors

Pollen parent/ maternal parent	A	B	C
1	0.33 (0.010)	0.30 (0.013)	
2			0.26 (0.006)
3	0.29 (0.011)	0.28 (0.009)	
4	0.29 (0.009)	0.26 (0.009)	0.27 (0.008)
5	0.30 (0.013)	0.30 (0.010)	0.26 (0.006)
6	0.30 (0.011)	0.30 (0.009)	
7			0.28 (0.006)

investment is costly (Zangerl & Berenbaum 1997). With increased carbon, offspring might be better protected from herbivores via greater investment in these compounds. Previous studies revealed that furanocoumarin allocation to the non-commissural (outward) face of fruits is influenced by pollen parent (Zangerl & Nitao 1998). Xanthotoxin is the most abundant furanocoumarin in fruits (Zangerl & Nitao 1998) and, of the furanocoumarins found in wild parsnip, is the most UV-phototoxic (Berenbaum & Zangerl 1996). However, there was no clear relationship between xanthotoxin investment and apparent rates of photosynthesis, suggesting that defense investment is not a benefit to offspring of manipulating fruit photosynthesis.

Alternatively, higher rates of photosynthesis may enable earlier seed maturation or larger seed sizes. Because harvest of

the fruits for the photosynthesis measurements precluded measurement of their final size or maturation time, we cannot say for certain that this alternative scenario is operative in wild parsnip. However, because carbon from fruit photosynthesis appears to remain in the fruit (Bazzaz *et al.* 1979) and biomass consists of other elements in addition to carbon, fruits with enhanced carbon gain presumably compete with other fruits for limiting nutrients. It then seems reasonable to assume that parsnip fruits with higher rates of photosynthesis are not only gaining carbon more rapidly but also may be gaining limited nutrients more rapidly at the expense of siblings. If competition among siblings leads to reduced parental fitness, parent-offspring conflict may be possible. Indeed, seed set in wild parsnip appears to be resource-limited. Plants typically produce as many seeds in the primary umbel as there are successful pollinations. In contrast, fruits do not always develop in later-developing secondary and higher order umbels, unless flowers, buds, flowers, or fruits are lost from the primary umbel to herbivores (Hendrix & Trapp 1981) or mechanical removal (Nitao & Zangerl 1987). Absent competition for seed resources (i.e. no differences in seed size among fruits as a function of pollen genotype/photosynthesis), selection may nevertheless favour enhanced photosynthetic rates if they enable faster development. The major cause of fruit mortality in wild parsnips is the parsnip webworm, which feeds exclusively on reproductive parts up until fruits begin drying out. Often 80% or more of the plants in midwestern US populations are attacked by this insect (Zangerl & Rutledge 1996), and offspring that are able to make their fruits mature more quickly may be more likely to escape predation.

Having detected offspring-influenced photosynthesis in the first species we examined, it seems likely that the phenomenon occurs in other plants as well. The mere existence of the phenomenon can complicate models or studies of offspring conflict in plants. For example, theoretical models of parent–offspring conflict in plants (Zhang & Jiang 1998) suggest that the cost of soliciting maternal resources and the premium on doing so may actually reduce average seed size. If, however, individual offspring can secure additional carbon via enhanced fruit photosynthesis, this cost of solicitation may be minimized, thereby reducing parent–offspring conflict without diminishing conflict among siblings. Thus, the ability of offspring to affect photosynthetic rates of surrounding maternal tissues adds another dimension by which siblings compete with each other during development.

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