EXPERIMENTALLY INCREASED SOURCE–SINK RATIO: A METHOD TO SCREEN YIELD POTENTIAL IN SUNFLOWER

Eckhard Grimm, Martin-Luther-Universität Halle-Wittenberg, Institut für Acker- und Pflanzenbau, Ludwig-Wucherer-Str. 2, D-06099 Halle (Saale), Germany
E-mail: grimm@landw.uni-halle.de

Merianne Alkio, Martin-Luther-Universität Halle-Wittenberg, Institut für Acker- und Pflanzenbau, Ludwig-Wucherer-Str. 2, D-06099 Halle (Saale), Germany; present address: Department of Biology, University of Massachusetts-Boston, 100 Morrissey Blvd., Boston, MA 02125, USA
E-mail: alkio@landw.uni-halle.de

Wulf Diepenbrock, Martin-Luther-Universität Halle-Wittenberg, Institut für Acker- und Pflanzenbau, Ludwig-Wucherer-Str. 2, D-06099 Halle (Saale), Germany
E-mail: diepenbrock@landw.uni-halle.de

Abstract

Seed set and seed development as affected by the source–sink ratio was described using two sunflower (*Helianthus annuus* L.), hybrids cvs. Rigasol and Kintasol. The source–sink ratio was defined as the upper green leaf area per floret. It was altered by shading sunflowers during floret initiation. Compared with the control, the upper green leaf area and the floret number were smaller in the shaded plants. However, the source–sink ratio was enlarged in shaded plants because the floret number was more reduced relative to the leaf area. The increased source–sink ratio corresponded with a lower percentage of unfilled achenes (seed set) and a higher dry mass per filled achene (seed filling). Data suggest that achene development in sunflower is source limited. A significantly improved seed set and seed filling was achieved when green leaf area per floret amounted to 10 cm sq. The shading method is proposed as a practical means for testing yield potential of sunflower crosses.

Introduction

One of the most striking problems in sunflower cultivation is the failure of seed development, especially in the centre of the capitulum. However, empty grains (achenes) also occur, although less apparently, in the outer whorls (Alkio et al., 2003). The empty achenes consist of a pericarp (fruit hull) enclosing the reduced seed with the rudimentary embryo. Even though the phenomenon is well known, the physiological background is poorly understood. Seed set and seed filling respond to environmental factors (e.g., water supply, mineral nutrients and radiation) in a wide range (Yegappan et al., 1982; Steer et al., 1988; Dosio et al., 2000). Deficient seed development may also arise from defects in fertilization (Nur, 1978; Rawson et al., 1984; Saranga et al., 1996). Furthermore, seeds are sinks, i.e., they assimilate consumption and storage organs without photosynthesis. Therefore, seed growth relies upon the import of assimilates, which either originate from the current photosynthesis in

source leaves (photoassimilates) or from vegetative storage tissues in the stem. This paper addresses the response of seed set and seed filling to changes in photoassimilate supply.

In previous work, pulse chase experiments with an exposure of leaves to 13CO2 or 14CO2 gave insights into the movement of carbohydrates to the capitulum (Alkio et al., 2002). The upper 10 to 15 of 24 fully expanded green leaves are involved in photoassimilate transport to the capitulum during seed filling. As chromatographic analysis revealed sucrose is the only moving compound (phloem transports sugar) in sunflower (Ito and Mitsumori, 1992; Alkio et al., 2002). During anthesis staminate florets and young achenes are strong sinks. When flowering was completed import levels to the achenes in peripheral whorls were almost uniform, whereas the import increased in the intermediate whorls and declined in the very centre of the capitulum. The distribution of photoassimilates in the capitulum is sectorial; typically, an upper source leaf supplies a 2/8- to 3/8-sector of the capitulum, whereas the midvein is connected with the middle 1/8-subsector. Both the insertion site of the source leaf and the middle 1/8-subsector have the same orientation. The lateral 1/8-subsectors are connected with the side veins of the source leaf. Hence, a single achene obtains photoassimilates from the leaves of three neighbouring ortostichies (Alkio et al., 2002).

Poor seed filling has often been attributed to a lack of vascularization of empty achenes (Yegappan et al., 1982; Durrieu et al., 1985; Goffner et al., 1988). However, Alkio and Grimm (2003) showed that vascular strands extend from the receptacle into empty achenes. Although the sink activity of empty achenes was very low, a slight movement of fluorescent probes and of 14CO2-derived photoassimilates in the phloem was detected.

Using different experimental approaches it was previously shown that seed filling and seed set in sunflower can be altered by changes in the source–sink ratio (Alkio et al., 2003). Among the treatments used (e.g., chilling during leaf initiation, short daylight regime) the non-invasive method of shading during floret initiation seems promising for testing sunflower yield. This method will be described here. The physiological background will be discussed in terms of photoassimilate distribution. Conclusions for sunflower breeding will be drawn.

Materials and Methods

Sunflower hybrids cultivars Rigasol and Kintasol; Deutsche Cargill GmbH, Salzgitter, Germany, and Monsanto S.A.S, Monbéqui, France were grown on the field experimental station of the Martin-Luther-Universität Julius-Kühn-Feld in Halle (Saale), Germany; 51°4´N, 11°7´E. Plants of 10–11 fully expanded true leaves (30–40 days after sowing) were shaded for ten days by covering with non-transparent plastic sheets, which reduces the incident photosynthetic active radiation by 90–95%. Non-shaded sunflowers served as a control. Open flowers were pollinated manually with the pollen of the same cultivar using a brush every other day. Leaf areas were measured at the end of flowering (leaf area = leaf width x leaf length x factor). Factors were determined using an image analyser. At maturity leaf number, head diameter, and the number and dry mass of filled and empty achenes were measured. Achenes were sampled in three concentric zones of the capitulum equal in width. Zones 1 and 3 referred to the most peripheral and the central one, respectively. Results are displayed as medians. Differences of medians with the 95%-bootstrap confidence interval were calculated (bootstrap simulation; 2Samples software, freely available from Mudelsee: http//www.uni-leipzig.de/~meteo/MUDELSEE/). Significant differences ($P < 0.05$) were marked with asterisks.

Results and Discussion

Shading during floret initiation changed the habit and seed development in sunflower significantly. The leaf area was reduced whereas the number of leaves remained constant (Table 1). Leaf growth stagnated during shading. In later stages leaves grew more vigorously, partly compensating for the loss of area. Furthermore, the floret (achene) number was considerably reduced. However, this decrease was more pronounced than that of the leaf area. Hence, shading increased the upper green leaf area per floret referred to as the source–sink ratio. Present results show that the effect on seed development was similar in both hybrids; seed set, measured as the percentage of empty achenes decreased, especially in the centre of the capitulum. Seed filling, measured as average dry mass of filled achenes increased (Table 1). Quantitative differences are due to the different genotypes. Rigasol and Kintasol derive from an identical cms parent, but different restorers. Kintasol produces more but smaller achenes than Rigasol (Table 1).

Source–sink ratio and seed set are negatively related (Figure 1). Plots in Figure 1 show data from the recent shading experiments (Table 1), data obtained from other field and growth chamber treatments (floret removal, defoliation, chilling during leaf initiation, short day and shading during floret initiation [Alkio et al., 2003]), as well as data re-calculated from literature. Results of these analyses consistently reveal that seed set improves when source–sink ratio increases. At a low source–sink ratio (green leaf area per floret = 2 cm sq.) seed set is poor and highly variable (12–64% empty achenes; [Alkio et al., 2003]). In contrast, at a high source–sink ratio (green leaf area per floret = 10 cm sq.) the minimum and maximum percentage of empty achenes converges towards 5% and 13%, respectively (Alkio et al., 2003). Furthermore, a higher source–sink ratio results in a higher dry mass per filled achene, i.e., better filling of the individual achene (Table 1; Alkio et al., 2003).
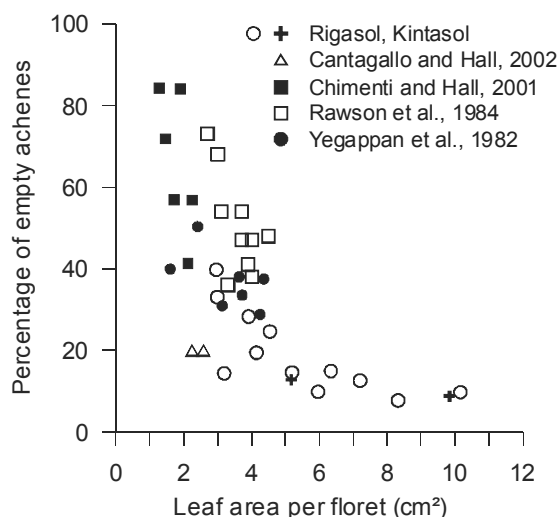

Figure 1. Response of seed set to the source–sink ratio. Seed set was measured as percentage of empty achenes in the capitulum. The source–sink ratio was measured as upper green leaf area per floret at the end of flowering. Data are from the present (Table 1) and previous investigations (Alkio et al., 2003) and recalculated from the literature.

Table 1. Effect of shading during floret initiation on source and sink characteristics in the sunflower hybrids Rigasol and Kintasol. Plants of 10–11 fully expanded true leaves were shaded for ten days. For analysis leaf areas were measured at the end of flowering. Achenes were counted and weighed in three concentric zones of the capitulum at maturity; zone 1 denotes the peripheral one. Data are given as medians. Significant differences ($P < 0.05$) were marked with asterisks; ns = not significant.

Characteristics	Rigasol			Kintasol		
	Control $n = 48$	Shaded $n = 47$		Control $n = 48$	Shaded $n = 48$	
Leaf number	30	30	ns	35	35	ns
Total green leaf area (cm²)	13917	9776	*	15948	12248	*
Upper green leaf area (cm²)	10827	7844	*	11640	9483	*
Stem height (cm)	131	134	*	129	136	*
Stem volume (cm³)	1218	1134	*	1230	1350	*
Capitulum diameter (cm)	23.5	19.0	*	26.0	21.5	*
Floret (achene) number	1524	781	*	2192	901	*
Upper green leaf area per floret (cm²)	7.2	10.2	*	5.2	9.8	*
Empty achenes (%) zone 1	6.1	7.8	*	6.4	7.2	ns
Empty achenes (%) zone 2	9.1	6.5	*	9.7	7.7	*
Empty achenes (%) zone 3	43.4	21.8	*	37.0	15.4	*
Empty achenes (%) zones 1–3	12.6	9.8	*	12.7	8.8	*
Dry mass per achene (mg) zone 1	124.3	130.4	*	91.7	99.1	*
Dry mass per achene (mg) zone 2	123.1	132.1	*	85.5	91.3	ns
Dry mass per achene (mg) zone 3	106.0	120.2	*	77.8	90.6	*
Dry mass per achene (mg) zones 1–3	122.2	129.8	*	88.2	95.5	*

There is no direct experimental access to quantify the capacity of source leaves to export photoassimilates. The estimation of the upper green leaf area to reflect the source capacity is a strong simplification. It ignores physiological and structural factors, which are involved in fine-tuning of assimilate export, e.g., photosynthetic activity, thickness of photosynthetically active cell layers, carbohydrate allocation from storage tissues. However, the approximation seems to be sensitive enough to elucidate some basic phenomena of a source leaf dependent achene development. Interpreting our findings in terms of phloem transport, the increase of the export capacity of source leaves always improved both seed set and seed filling. Thus, achene development in sunflower is source limited. Depending on the source–sink ratio, seed

set varied over a wide range (36–95% filled achenes). However, the percentage of empty achenes did not fall below a lower limit of 5%. This implies that other factors apart from photoassimilate distribution also affect seed development.

Table 2. Estimation of potential achene yield per plant under improved photoassimilate supply in the sunflower hybrids Rigasol and Kintasol. Improved photoassimilate supply was obtained from increased source–sink ratio following shading during floret initiation. Experimental data (see also Table 1) are medians.

Characteristics	Symbols, formulas	Rigasol	Kintasol
Floret (achene) number, control	Fc	1524	2192
Empty achenes (%), control	Ec	12.6	12.7
Dry mass per filled achene, control (mg)	Dc	122.2	88.2
Achene yield per plant, control (g)	$Yc = Fc \cdot Dc \cdot (100-Ec) \cdot 10^{-5}$	162.7	168.7
Empty achenes (%), shaded	Es	9.8	8.8
Dry mass per filled achene, shaded (mg)	Ds	129.8	95.5
Achene yield per plant, improved (g)	$Yi = Fc \cdot Ds \cdot (100-Es) \cdot 10^{-5}$	178.4	190.9
Achene yield per plant, improved (%)	$Yi\% = Yi/Yc \cdot 100$	109.7	113.2
Potential increase in achene yield per plant (%)		9.7	13.2

It is evident that sunflower yield benefits from an optimum assimilate supply. Estimating the potential achene yield per plant on the basis of the floret (achene) number in control plants, an increase of 9.7% and 13.2% was calculated for Rigasol and Kintasol, respectively (Table 2). This supports the view that the assimilate supply and transport in sunflower might be the main factor for improving future sunflower yield (Lopez Pereira et al., 1999). However, the transformation of this potential (theoretical) yield into an actual yield per hectare is much more complex. Even though physiological research is often far from breeding programmes and crop management, present investigations might give some new ideas for sunflower breeding.

Firstly, temporary shading of isolated, field-grown sunflowers can be used to evaluate yield potential in different sunflower crosses by analysing the maximum seed set percentages and dry mass per seed under improved photoassimilate supply. Notably, the shading method provides high source–sink ratios which correspond with the lowest percentages of empty achenes (Figure 1; Alkio et al., 2003). However, sunflowers have to be shaded in the correct stage during floret initiation. In praxis, this method is robust and applicable under field conditions.

Secondly, seed set should be more intensively addressed as a selection criterion than the achene number. Since in modern sunflower cultivars the source–sink ratio has been decreased if compared with older ones (Lopez Pereira et al., 1999), source characteristics like leaf area per plant, leaf area duration and leaf area index may be interesting. These characteristics have

a high genetic variability and heritability (Pasda et al., 1995). A high source–sink ratio as a target in sunflower breeding may lead to a better seed development than to breed for a source or sink size alone. The source–sink ratio can be raised by increasing the leaf area per floret, and/or by delaying leaf senescence during the achene filling period. However, the "stay green" trait may bear additional problems (late-drying stands, susceptibility for diseases), which have to be solved for boundary cropping areas of sunflower.

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