Acta Bot. Neerl. **41**(2), June 1992, p. 143–149

Sink–source interactions: accumulation of sucrose in the apoplast and symplast of the source leaves as a result of sink strength reduction in *Pisum sativum* L.

M. A. VAN OENE, C. KOLLÖFFEL and P. WOLSWINKEL

Transport Physiology Research Group, Department of Plant Ecology and Evolutionary Biology, University of Utrecht, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

SUMMARY

The effect of sink-strength-reduction on the carbohydrate content in apoplast and symplast of source leaves was examined in *Pisum sativum* L. Sink strength was reduced by removing developing seeds or by means of the 'open seed coat technique'. After seed removal, an increase in the sucrose content could be observed, especially in the apoplast. Starch content did not change during the course of the experiments. Fixation of $^{14}CO_2$ was not influenced by sink-strength-reduction. The increase in the sucrose content can be explained as a consequence of a change in the balance between production and export of sucrose in the source leaves. Reduction in phloem loading may have caused an increase in sucrose content in the leaf apoplast, followed by an increase in sucrose in the leaf symplast, without a change in photosynthesis.

Key-words: carbon fixation, phloem loading, sink–source interactions, sink strength, starch, sucrose.

INTRODUCTION

During the development of large seeds a considerable quantity of nutrients is transported from the source regions of the plant to the seeds. In annual crop plants, such as pea, developing seeds become the major sinks (Thorne 1985). In legumes, the most important carbon source for developing seeds is sucrose (Zimmermann & Ziegler 1975) which is transported from source leaves to the sink regions via the sieve tubes. During the last 10 years, the study of sucrose transport into developing seeds has been stimulated by the development of the 'empty seed-coat technique' (Thorne 1985; Wolswinkel 1985; Patrick 1990). By means of this technique, the import of assimilates into the seed coat can be manipulated (Wolswinkel 1990, and references therein). Sink strength reduction reduces ^{14}C-photo-assimilate loading into the veins of source leaves and ^{14}C-photo-assimilate export to sink regions (Van Oene *et al.* 1992). In these experiments no effect of sink strength reduction on carbon fixation was observed.

When carbon fixation in the source leaves does not change, but the export decreases, an accumulation of carbohydrates is to be expected. In pea, photo-assimilate loading into the sieve tubes involves an apoplastic step (Turgeon & Wimmers 1988; Gamalei 1989). When reduction of sink strength does affect the loading of sucrose, a rise in sucrose content can

first be expected in the apoplast in the proximity of the loading sites and later on, more indirectly in the symplast.

A reduction in sucrose export might also lead to an increased starch synthesis in the source leaves. In *Vicia faba* a rapid rise in the synthesis of starch was observed after blocking of the petiole by means of cooling or by heat girdling, (Ntsika & Delrot 1986; Grusak *et al.* 1990). However, in *Glycine max* heat-girdling of the petiole or depodding caused no increase in starch content of the source leaf (Setter *et al.* 1980).

The aims of our experiments were to investigate changes in carbohydrate content of source leaves after reduction of sink strength. Furthermore, the influence of sink strength reduction on carbon fixation was examined, a factor that may have a great influence on the carbohydrate balance in the source leaves.

MATERIALS AND METHODS

Plant material

Plants of *Pisum sativum* L. cv. Argenteum, a cultivar from which the leaf epidermis can be easily removed, were grown in the laboratory garden during the early summer season. One flower was removed from each pair of flowers at the fruiting nodes, leaving only one developing fruit at each fruiting node. For pulse-labelling experiments in the winter season, plants of two other cultivars of pea (Marzia and Yoko) were grown in a greenhouse as described previously (Van Oene *et al.* 1992). From these plants all flowers were removed, except the first one. One pod with five to seven seeds developed.

Preparation of plants

Garden plants with similar looking pods and leaves were selected and placed in the laboratory in front of large windows. All pods except one were removed and all leaves except the one subtending the remaining pod were removed. Pod walls were opened and 2, 4 and 6 hours before collection of the apoplast fraction seeds. In control plants, after opening the pod wall, seeds were not removed. The pods were placed in a trough on a moist tissue. The percentage dry weight of the removed seeds was 20–25%. In this stage of development, the seeds were growing rapidly and contantly. In other experiments, large plants with several fruiting nodes were used, to obtain explants with a piece of the main stem (one internodium), one source leaf and one pod.

In pulse-labelling experiments with the cultivars Yoko and Marzia sink strength in the pod was manipulated by means of the 'empty seed-coat technique' as described previously (Wolswinkel & Ammerlaan 1983, 1984).

Collection of carbohydrates

To avoid problems with nycthemeral changes in sugar concentrations in the apoplast (Delrot *et al.* 1983), all samples were collected in the late afternoon over the whole series of experiments. The source leaf subtending the fruit was removed and the lower epidermis was carefully removed with a fine forceps. Each leaflet of the source leaf was rinsed immediately after removal of the epidermis in 5 cm^3 ice-cold buffer for 5 min. The buffer solution contained 2 mM 2-[N-morpholino] ethanesulfonic acid (Mes)-NaOH buffer (pH 5·5) and 0·5 mM $CaSO_4$. The rinsing solution was lyophilized in order to concentrate the sugars. The lyophilized material was dissolved in a proper amount of water for sucrose and glucose analysis. Immediately after rinsing, the leaf surface was measured with an area meter (Li-Color model 3100). Leaflets were stored at −40°C. Frozen leaflets were

boiled 2 × 20 min in 5 cm^3 water for extraction of the soluble carbohydrates in the leaf symplast. The extract was used to measure sugar content of the symplast fraction of the leaf. The residue was used for starch analysis.

Sugar analysis

The glucose oxidase/peroxidase method was used for the measurement of sucrose and glucose (Bergmeyer & Bernt 1974; Wolswinkel & Ammerlaan 1984). The glucose concentrations were determined before and after enzymatic hydrolysis of sucrose by β-fructosidase (invertase; EC 3.2.1.26). The sucrose content was calculated from the difference between the glucose concentration before and after enzymatic hydrolysis of sucrose.

Starch analysis

The residue of leaflets was homogenized in 3 cm^3 water. Three cm^3 of a 0·2 M sodium acetate buffer solution (pH 4·8) containing 0·2 mg amyloglucosidase (EC 3.2.1.3) and 0·2 mg α-amylase (EC 3.2.1.1) (Stitt *et al.* 1978) was added. After incubation at 37°C (2 h) and centrifugation, the glucose content in the supernatant was determined. Longer incubation times with the enzymes did not raise the glucose concentration.

Pulse-labelling experiments

Source leaves subtending the fruiting node of Yoko and Marzia plants from the greenhouse were incubated with $^{14}CO_2$ as described previously (Van Oene *et al.* 1992). The incubation time (10 min) was followed by a chase period of 20 min. Leaflets were extracted four times in 1 cm^3 of 80% (v/v) ethanol at 70°C over a total period of 4 h. The residue was destructed in 0·5 cm^3 of a 1:1 (v/v) mixture of 50% perchloric acid and 30% H_2O_2. After addition of 5 cm^3 lumagel (Lumac Systems AG, Basel, Switzerland), the amount of ^{14}C was counted in a liquid scintillation analyser. Before ^{14}C-counting extracted samples were bleached in strong light to reduce fluorescence.

Statistical analysis

The number of samples are indicated for each data point in the figures. Average values are given and the standard error of the mean. Differences in sugar and starch content are compared using the Student *t*-test with a 95% confidence interval.

RESULTS AND DISCUSSION

In whole plants of *Pisum sativum* the removal of seeds resulted in a gradual increase in the sucrose content in the leaf apoplast (Fig. 1). A similar trend could be observed in the data on sucrose in the leaf symplast, but the effect was less pronounced and occurred later (Fig. 1). Reduction of phloem loading due to sink reduction can explain the primary accumulation of sucrose in the apoplast. When phloem loading is reduced, an accumulation of sucrose can first be expected in the apoplast regions of the minor veins, from where phloem loading predominantly occurs. The amount of glucose measured in the apoplast and symplast was much lower than that of sucrose. Only minor changes in glucose content could be detected. A rise in glucose concentration in the leaf apoplast was only clear 6 hours after seed removal (Fig. 1). Experiments with explants with one source leaf and one pod and experiments on sink reduction by means of depodding showed the same trends (data not shown). Other authors have reported similar results. In *Vicia faba* L.

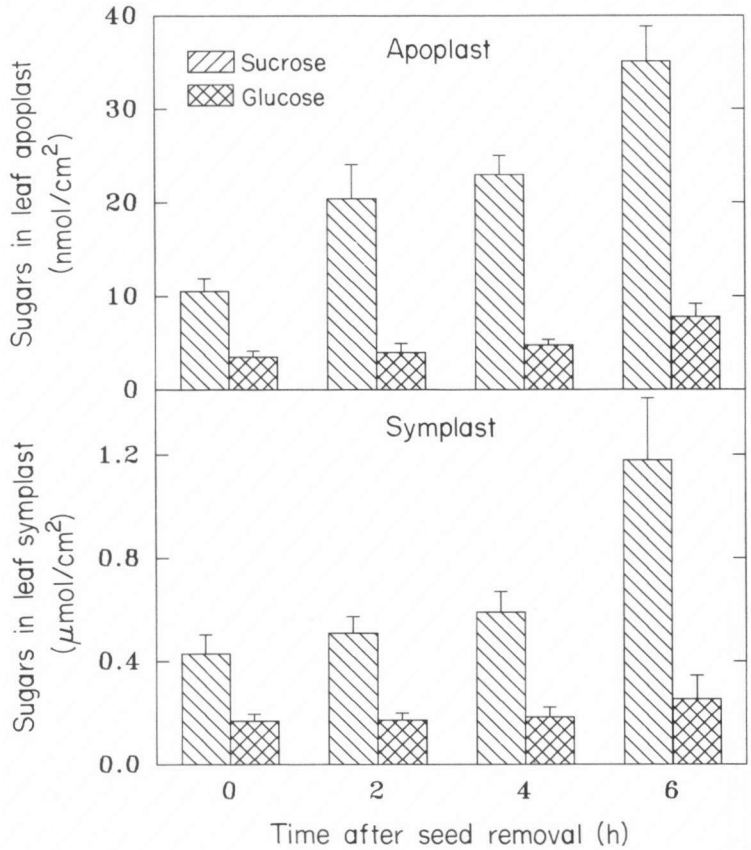

Fig. 1. Effect of seed removal on sucrose and glucose content in the leaf apoplast and symplast. Seeds were removed 2, 4 and 6 h before sampling. Control: plants with attached seeds (0 h). Scale bars indicate standard error of the mean; 14 samples per data point.

steam girdling of the petiole resulted in an increase in sucrose content, but not in that of glucose, especially in the leaf apoplast (Ntsika & Delrot 1986). In *Glycine max* a clear increase in sucrose could be observed in the leaf after depodding or girdling of the petiole, (Setter *et al.* 1980). In pea (Pate 1966) and other Fabaceae (Ziegler 1975), sucrose, and not glucose, is the sugar that is transported via the phloem to the sink regions.

With respect to the effect of sink strength reduction on the balance between production and export of sucrose other factors, such as carbon fixation and starch synthesis, have to be taken in account. An influence of sink strength reduction on ^{14}C-fixation in the source leaves could not be demonstrated 3·5 hours after sink manipulation, indicating no change in photosynthesis (Fig. 2a). Other authors also did not find an effect of a reduced export of photo-assimilates on photosynthesis over short time periods (Geiger 1976 and references therein, Plaut *et al.* 1987; Ntsika & Delrot 1986). Possible changes in photosynthesis as a consequence of sink strength reduction will take more time than the time of our experiments.

Even 6 hours after seed removal, the starch content in the source leaf had not increased significantly. The amount of starch found in the leaf residue after extraction of soluble

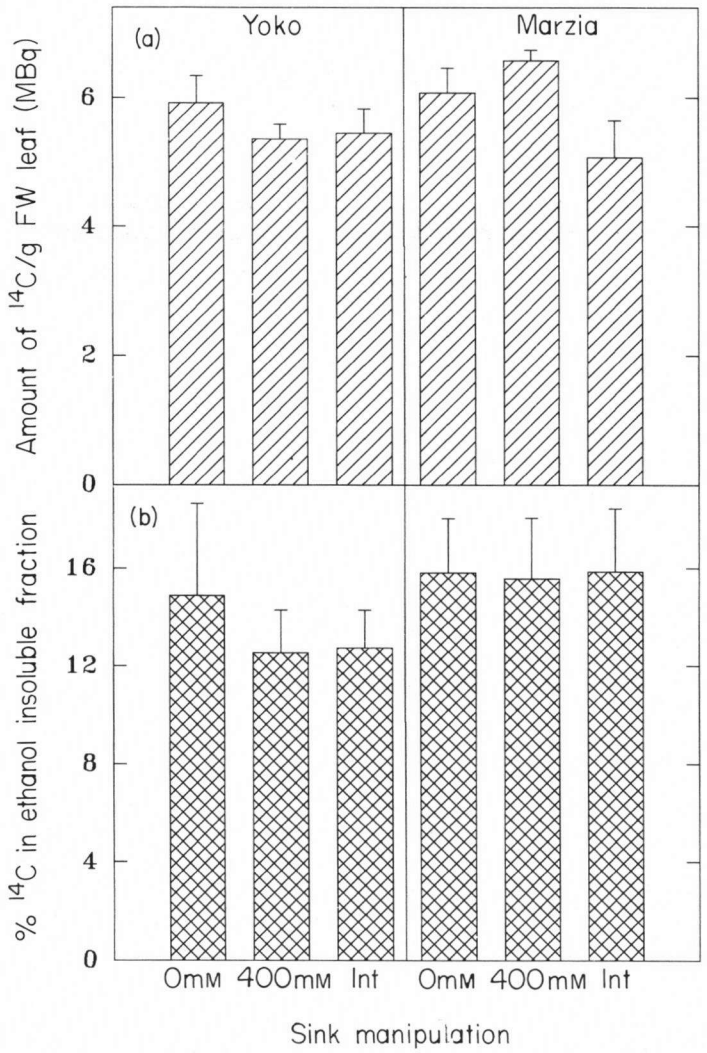

Fig. 2. The amount of ^{14}C-photo-assimilate in source leaves of *Pisum sativum*, cv. Yoko and cv. Marzia (2a) and the percentage of the ethanol insoluble fraction of ^{14}C based on the total ^{14}C-activity 30 min after start of $^{14}CO_2$ pulse-labelling in source leaves (2b). Plants were operated 3 h before the start of the pulse-labelling procedure. Sink treatments: intact seeds (Int), seeds with 400 mM osmoticum (high sink strength) and seeds with 0 mM osmoticum (low sink strength). Scale bars represent standard error of the mean, eight samples per data point.

sugars was 84 μg/cm^2 leaf (SE 38; 40 leaflets) for plants with attached seeds and 96 μg/cm^2 leaf (SE 47; 38 leaflets) for plants with removed seeds. These results were confirmed by measuring the amount of ethanol soluble and insoluble ^{14}C, after pulse-labelling with $^{14}CO_2$ of source leaves of *Pisum sativum* cv. Yoko and Marzia (Fig. 2b). In these experiments, sink strength was manipulated by means of the open seed-coat technique. The insoluble ^{14}C-fraction represents the amount of newly synthesized starch in the source leaflets during the experiment (Grusak *et al.* 1990). The ^{14}C-method measures the current starch synthesis without interference in the amount of starch already present. This makes the ^{14}C-method more sensitive. Three and a half hours following sink manipulation there

was no clear difference between the amount of ^{14}C in the insoluble fraction of plants with reduced sink strength (Fig. 2b, 0 mM) and plants with high sink strength (Fig. 2b, intact and 400 mM). Other authors have reported a rapid rise in starch content in leaves of *Vicia faba* after blocking the petiole by heat girdling (Ntsika & Delrot 1986; Grusak *et al.* 1990). The differences in the results from several research groups may be related to differences in the developmental stage of the leaves. In *Glycine max* depodding and the more brutal steam girdling of the petiole did not lead to an increase in the starch content (Setter *et al.* 1980).

The present results on carbohydrate content in the leaves and carbon fixation after sink strength reduction fit well with previous results on loading of ^{14}C-photo-assimilate and subsequent transport to sinks (Van Oene *et al.* 1992). All these results can most easily be explained by the effect of sink strength reduction on phloem loading of photo-assimilates in the sources leaves. This reduced loading will first lead to an accumulation of the transport sugar sucrose in the leaf apoplast and later in the leaf symplast, while photosynthesis is unaffected during the time of the experiments.

ACKNOWLEDGEMENTS

We thank Ankie Ammerlaan and Simone de Wolf for their technical assistance and Nunhems Zaden B.V., Haelen for supplying of pea seeds, cultivars Marzia and Yoko. The investigations were supported by the Foundation for Biological Research (BION), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

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