INCORPORATION OF ^{14}C LABELLED METABOLITES INTO THE DEVELOPING SUNFLOWER CAPITULUM

L.F. Hernández[1] and J.H. Palmer[2].

1 Departamento de Agronomía-*CERZOS*, Universidad Nacional del Sur. Bahía Blanca, 8000. ARGENTINA

2 School of Biological Science. University of New South Wales, Sydney, 2033. AUSTRALIA

SUMMARY

The incorporation of ^{14}C labelled compounds from mature source leaves into the developing sunflower capitulum was studied. Plants grown under controlled environmental conditions were labelled during early stages of capitulum development. Foliar application of ^{14}C-labelled sucrose was used. Plants were sampled 24, 36 and 48 h after labelling and the activity incorporated into different parts of the young capitulum was determined. The percentage of activity incorporated into the capitulum was higher in the peripheral regions and decreased towards its centre. It is concluded that the floret primordia and the pith parenchyma had become the main sinks for ^{14}C by the time the capitulum development was completed.

INTRODUCTION

The effect of leaf position on the direction of movement of photoassimilates in the mature sunflower shoot was investigated by Prokofiev *et al.*, (1957). The assimilates imported by the youngest leaves and mature florets, follow a pathway which is related to the plant's phyllotaxy and to the pattern of vascular connections between source leaves and sinks. Considerable attention has also been focused on the failure of seed set particularly in the central part of the mature sunflower capitulum. This phenomenon has generally been attributed to poor development of the vascular anatomy in that region of the receptacle (Durrieu *et al.*, 1985).

Investigations by Thoday (1922) and confirmed later by Priestley and Scott (1936), showed that vascular differentiation commences in the vegetative shoot of the sunflower seedling within few days from germination. Although it is possible to detect strands of procambium originating from the young leaves in the terminal bud, they do not extend into the vegetative apical meristem and there is no vascular system present in the capitulum in the early stages of its development. In the maturing sunflower capitulum, it is known that assimilate acquisition is more efficient in the peripheral fruits than in the central ones (Patil *et al.*, 1976; Yegappan *et al.*, 1982). However the relative importance of the young capitulum and last formed leaf primordia in the sunflower plant as assimilate sinks and the distribution of assimilates within the developing capitulum has not yet been investigated.

In this investigation ^{14}C-sucrose was applied to a fully grown leaf in either the mid-stem or the upper-stem region of the shoot, in the early stages of flowering (20 to 45 days from emergence), and the subsequent pattern of incorporation of the ^{14}C label into the major regions of the young capitulum was followed.

MATERIALS AND METHODS

Helianthus annuus L. plants cv. Sunfola 68-2 were reared in 1 liter pots containing a sand/peat/ vermiculite mixture (ratio 1:1:1) and grown in a controlled artificial environment at a constant air temperature of 28 °C and 60-70% relative humidity. Fluorescent tubes and incandescent lighting provided a photon flux density of 550 $\mu mol\ s^{-1}\ m^{-2}$ at the top of the plants and an 18 h long-day photoperiod. Mineral nutrients were added periodically. The transition from vegetative growth to capitulum formation was recorded by frequent sampling and scoring the appearance of the apical region, using the 10-stage classification of Marc and Palmer (1981) for the floral stages (FS).

Plants were used for ^{14}C labelling when sampling showed they had attained one of the following FS: 4, 5, 6, 8 or 10. ^{14}C-sucrose was applied to one donor leaf on each plant. As the floral stage advanced, the position of this donor leaf in the leaf sequence was advanced towards the apical bud, to ensure that it was always a fully grown mature leaf at approximately the same distance with respect to the apical bud (Table 1).

Plants were kept in darkness for 24 h before use, to deplete assimilate content. Forty µl of the ^{14}C labelled sucrose solution (^{14}C specific activity: 0.925 $KBq\ \mu l^{-1}$) was applied as 20, 2 µl drops to the upper surface of the chosen leaf of each plant (Table 1) using a microsyringe, to provide 2.2×10^6 dpm per plant. After 15 min the plants were returned to the controlled environment and maintained under continuous illumination. This experiment was repeated once.

The concentration of ^{14}C in regions of the developing capitulum was determined in five plants 24, 36 and 48 h after leaf labelling. The apical bud was removed and the capitulum

Table 1. Leaves used for ^{14}C-sucrose labelling in relation to floral stages

Floral Stage	^{14}C Labelled Leaf Number
4	7
5	10
6	12
8	18
10	22

exposed under a dissecting microscope by removing embryonic leaves and involucral bracts. The capitulum was divided into the regions shown in Fig. 1. Representative samples of involucral bracts and sub-apical receptacle tissue were oven dried at 30 °C and the dry weight obtained (Fig. 2). The ^{14}C activity in the tissue samples was determined using a Packard Tri-carb 460-C liquid scintillation counter. The results expressed as percentage of the ^{14}C specific activity of the capitulum tissue for both replicates are presented in Fig. 3. The time course results were also combined and expressed as the ^{14}C relative specific activity (RSA, Fig. 4) to eliminate bias resulting from variation in weight of the capitulum segments (Al-Hamdani and Todd, 1990). The radioactivity and dry mass of the source leaf were excluded in the calculations.

RESULTS AND CONCLUSION

The appearance of floret primordia on the newly formed receptacle of the capitulum in FS 5 marked the beginning of a phase of rapid expansion of the receptacle which was accompanied by a pronounced increase in dry weight of the involucral bracts, the pith parenchyma, and the receptacle surface, including the disc florets (Fig. 2).

By FS 4 when the receptacle structure was about to form, the sub-apical meristem became an active sink for ^{14}C. The involucral bracts also became active sinks for ^{14}C after their initiation in FS 3 but their relative importance declined once floret primordia production commenced in FS 4 (Fig. 3). Then most of the ^{14}C label entering the capitulum was preferentially taken up by the florets, initially in the peripheral zone and later in the central zone as florets occupied all the available space on the surface of the receptacle in FS 8 (Fig.4).

There is some experimental evidence that photoassimilate transfer from one point to another in the shoot system may be hormone-directed (Sovonick *et al.*, 1974; Gifford and Evans, 1981). However it is unclear whether the influence of hormones on the translocation of assimilates is due to their stimulation of metabolic activity in the accumulating organ, with consequent activation of long-distance transport, or is due to their effect on the rate of movement of assimilates to the sink organs (Patrick, 1976; Patrick and Wareing, 1976).

It is shown here that the stage of plant development strongly influenced the allocation of photosynthate to the regions of the capitulum studied. So that there is a positive relationship between the appearance of incorporation of assimilates into the floral organs and its developmental stage (Fig. 4). At each successive ^{14}C labelling the first rows of florets incorporated more labelled assimilates than the central zone, suggesting that the outer rows of florets in the capitulum may be able to monopolise the current supply of photosynthate. This does not preclude the possibility that the older florets could exert an influence on the development of younger tissues and organs through hormonal action or sink competition (Kinet *et al.*, 1985). It can be concluded from the labelling results that the florets and the pith

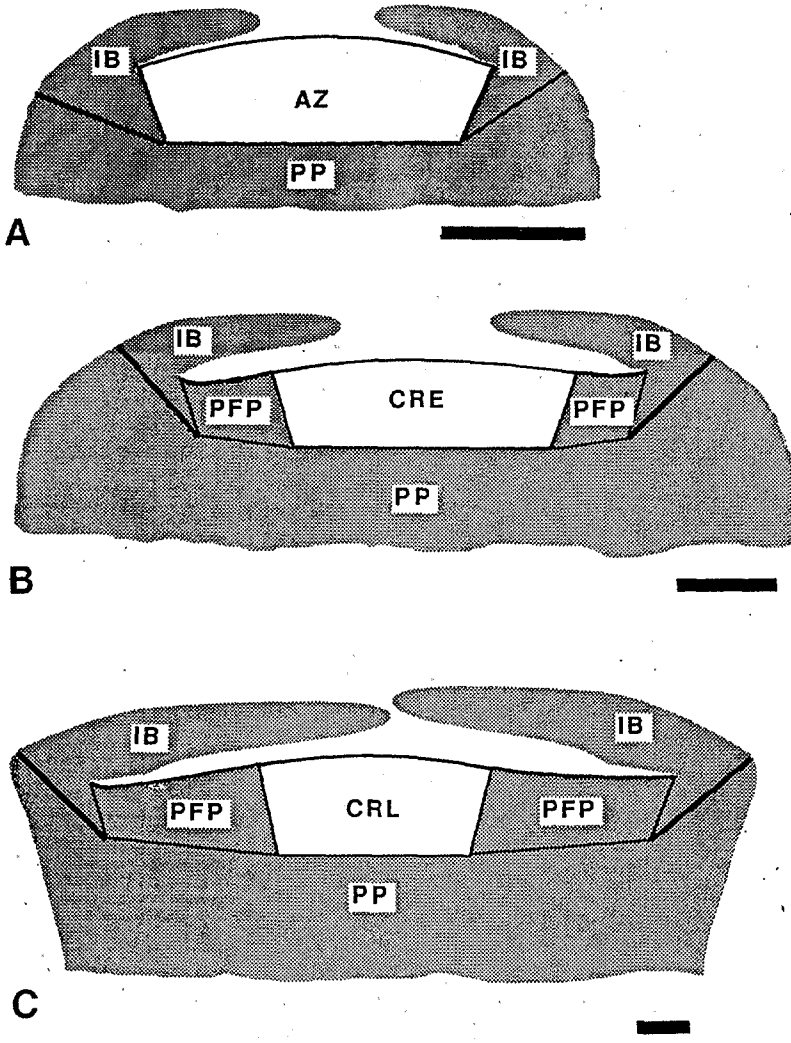


Figure 1: Schematic vertical section of the developing capitulum, showing regions sampled. A. FS 4 (Bar = 50 μm), B. FS 5 or 6 (Bar = 1 mm), C. FS 8 or 10 (Bar = 1 mm). (AZ) apical zone; (IB) involucral bracts; (PP) pith parenchyma; (PFP) peripheral region of the receptacle with floret primordia; (CRE) central region of the receptacle; early stage before appearance of visible floral primordia; (CRL) central region of the receptacle; late stage after appearance of floret primordia.

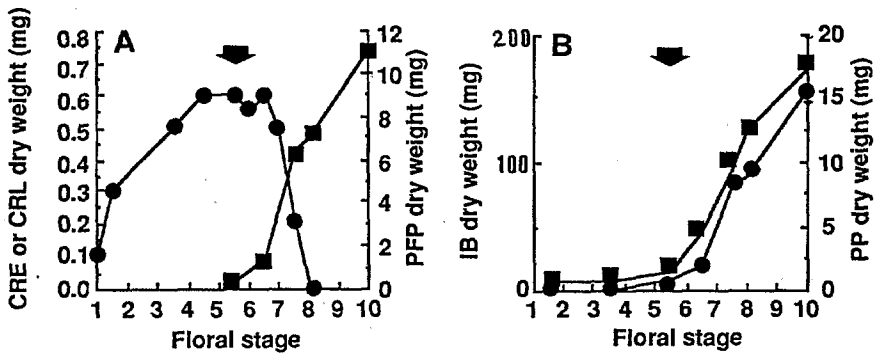
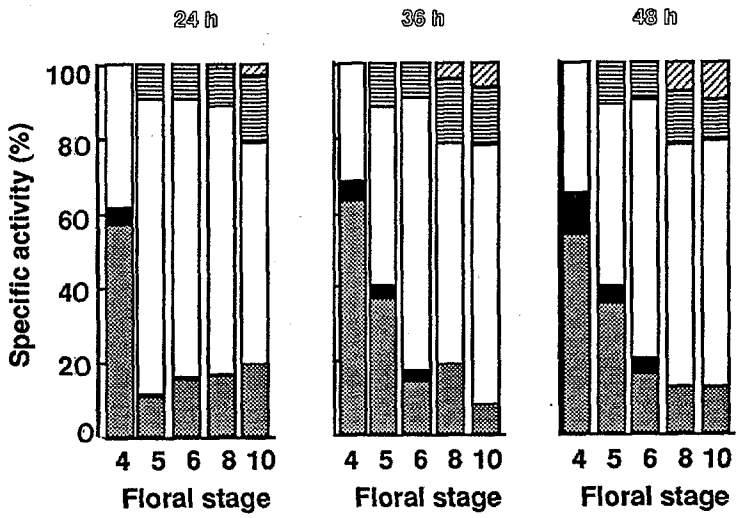


Figure 2: Change in dry weight of sampled regions of the capitulum during floral development. Each point is a mean for 10 values. Vertical bars = ± S.E. A. Change in dry weight of the central zone of the receptacle, before (CRE) and after (CRL) appearance of florets (●) and after the appearance of the peripheral floret primordia (■). B. Increase in dry weight; (■) involucral bracts, (●) pith parenchyma. Arrows indicate the time of floret appearance.



(▩) pith parenchyma; (■) central zone of the receptacle (floret free); (□) involucral bracts; (▤) floret primordia in the peripheral zone of the receptacle; (▨) floret primordia in the central zone of the receptacle.

Figure 3: Time-course of distribution of ^{14}C specific activity in re gions of the capitulum after 24, 36 and 48 h after labelling. Values in the histograms are the mean for 5 plants and 2 repetitions.

parenchyma had become the main sinks for ^{14}C by the time that capitulum development was complete in FS 10 (Fig. 4). These results lead to the hypothesis that there may be a positive relationship between the extent of vascular development and the localized accumulation of assimilates in the capitulum. The ability of the young florets to compete strongly for ^{14}C may be the result of their rapid growth and cell division activity as they develop into mature florets.

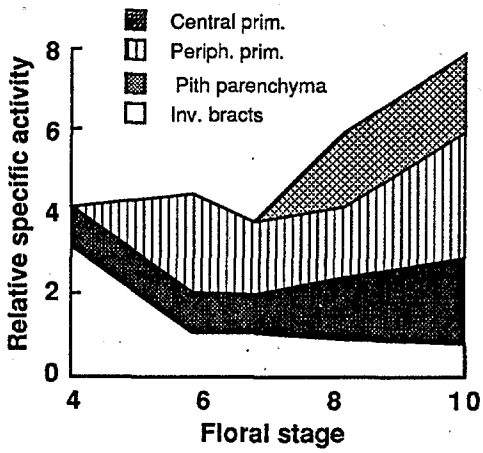


Figure 4: Mean relative specific activity (RSA) for ^{14}C in named regions of the capitulum for floral stages 4-10

ACKNOWLEDGEMENTS

L.F.H. is member of the Scientific Career of the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC, Buenos Aires, Argentina). Financial support to LFH from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) is gratefully acknowledged.

REFERENCES

DURRIEU, G., PERCIE DU SERT, C. and MERRIEN, A. 1985. Anatomie du capitule de tournesol. Consequences sur la nutrition des akenes. XI International Sunflower Conference Proceedings, Mar del Plata, Argentina, pp. 7-12.

GIFFORD, M. and EVANS, L.T. 1981. Photosynthesis, carbon partitioning and yield. Annu. Rev. Plant Physiol. 32: 485-509.

AL-HAMDANI, S. and TODD, G.W. 1990. Effects of temperature on photosynthate partitioning in alfalfa. Can J. Plant Sci. 70:203-208.

MARC, J. and PALMER, J.H. 1981. Photoperiodic sensitivity of inflorescence initiation and development in sunflower. Field Crops Res. 4: 155-164.

PATIL, V.A, BANGAL, D.B. and GOSWAMI, P.B. 1976. A note on interfloret competition in sunflower. Indian J. Plant Physiol. 19: 28-31.

PATRICK, J.W. 1976. Hormone-directed transport of metabolites. In: I.F. Wardlaw and J.B Passioura (Eds.), Transport and Transfer Processes in Plants, pp. 433-446. Academic Press, London.

PATRICK, J.W. and WAREING, P.E. 1976. Auxin-promoted transport of metabolites in stems of Phaseolus vulgaris L. Effects at the site of hormone application. J. Exp. Bot. 27: 969-982.

PRIESTLEY, J.H. and SCOTT, L.I. 1936. The vascular anatomy of *Helianthus annuus* L. Proc Leeds. Phil. Lit. Soc. 3: 159-173.

PROKOFIEV, A.A., ZHDANOVA, L.P. and SOVOLEV, A.M. 1957. Certain regularities in the flow of substances from leaves into reproductive organs. (In russian with English summary). Fiziol. Rast., 4: 425-431.

SOVONICK, S.A., GEIGER, D.R. and FELLOWS, J. 1974. Evidence for active phloem loading in the minor veins of sugar beet. Plant Physiol. 54: 886-891.

THODAY, D. 1922. On the organization of growth and differentiation in the stem of sunflower. Ann. Bot. 36: 489-510.

YEGGAPAN, T.M., PATTON, D.M., GATES C.T. and MULLER, W.J. 1982. Water stress in sunflower (*Helianthus annuus* L.) 3. Responses of cypsela size. Ann. Bot. 49: 69-75.