STOMATAL DENSITY IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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INTRODUCTION

Carbon dioxide exchange rate of C_3 species is influenced by mesophyl resistance and to a lesser extent by stomatal resistance (Miskin et al., 1972 ; Gaskell and Pearce, 1983).

Studies have been made in different species to ascertain stomata functions (Heickel, 1971 ; Turner and Begg, 1973) and the possible use of stomatal density in the selection of high yielding and drought-tolerant genotypes (Dobrenz et al., 1969 ; Teare et al., 1971 ; Miskin et al., 1972 ; Jones, 1977 ; Tanzarella et al., 1984).

Among the morpho-physiological characteristics of stomata, their density affects plant water and gas exchange. As breeding and agronomic researches on stomatal density require great amounts of time, it is useful to have preliminary information on the trait's variability as a function of genotype, environment and sampling unit.

The literature on stomatal frequency in sunflower (*Helianthus annuus* L.) is rather limited (Lovett and Campbell, 1973 ; Rawson and Craven, 1975 ; Dhopte and Aher, 1976 ; Gelfi and Blanchet, 1980). This study investigates the variability of stomatal density as related to different sampling units in six sunflower hybrids grown at two locations.

MATERIALS AND METHODS

The experiments were conducted in 1983 at two locations in the Po valley, Gandazzolo (near Bologna) and Monselice (near Padova). In each trial six commercial single crosses were evaluated to a randomized block design replicated four times. Each plot included 120 plants grown in six rows at a stand density of 4.8 plants/m^2. Seeds were hand planted at three times the required density and thinned after emergence to one plant per hill. Field techniques usually employed in Northern Italy for sunflower were adopted.

Leaf prints were taken after flowering in order to sample fully expanded leaves, using the technique described by Schoch and Silvy (1978). In each plot four competitive plants were randomly chosen. One print was made for both the abaxial and adaxial surfaces of one leaf for the lower, central and upper sectors of the plant canopy. In order to minimize the sampling variation due to different stomatal densities within leaf, great care was taken to sample the same leaf portion. Stomatal frequency was determined for each of the 1152 prints as the mean of the readings of five randomly chosen microscopic fields, each covering a surface of 0.132 mm^2. Fields heavily blurred by epidermal tears, or as a consequence of an inadequate pressure were not considered.

Plant height and head diameter were measured on 15 plants per plot ; flowering date and seed yield were determined on a plot basis after discarding border plants.

Error variances were homogeneous between locations for all traits except flowering. Analysis of variance for stomatal density was computed at each canopy sector and then combined over sectors. Plants within plot and microscopic fields within print were considered as random and locations, genotypes and sectors as fixed factors. Variance component estimates for sources of variation within plot were calculated by equating observed mean squares to their excepted values and solving for the variance components.

RESULTS AND DISCUSSION

Plants showed a regular and uniform growth during the vegetative and reproductive phases.

Table 1 reports mean stomatal densities of the abaxial and adaxial epidermis for each

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location, measured at different sectors of the plant canopy. Owing to the presence of numerous trichomes, some prints were blurred and consequently not utilized. Stomatal density was always lower in the adaxial epidermis, analogously to what reported in *Vicia faba* L. (Nerkar et al., 1981 ; Tanzarella et al., 1984) and soybean (Ciha and Brun, 1975). Stomatal frequency decreased from leaves of the upper canopy sector. In other species, upper leaves generally have higher frequencies compared to lower ones (Teare et al., 1971 ; Liang et al., 1975 ; Tanzarella et al., 1984), which is probably related to differences in water availability during leaf development. In wheat Quarrie and Jones (1977) reported that increasingly water-stressed leaves differentiated more trichomes and less stomata. The lower frequencies observed in our study for leaves of the upper sector could be related to greater water stress during their development.

Table 1

Mean stomatal densities (nr./mm²) and illegible prints (% in parentheses) at three canopy sectors in two locations

Location	Canopy sector					
	Lower		Central		Upper	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Gandazzolo	137 (1.6)	114 (1.0)	123 (3.1)	98 (2.6)	115 (6.3)	89 (24.0)
Monselice	146 (0.5)	122 (4.2)	152 (0.0)	117 (12.5)	135 (3.1)	105 (18.8)

For the variance analysis (Table 2), only data of the abaxial epidermis were used because of the high percentage (10.5%) of blurred prints taken on the adaxial epidermis. Highly significant effects were detected for locations and blocks, thus indicating a strong environmental influence on stomatal density as reported in other species (Ormrody and Renney, 1968 ; Shearman and Beard, 1972 ; Quarrie and Jones, 1977). The significant effects observed for canopy sectors can be interpreted as developmental variability mediated by the environment. Differences among hybrids showed a good repeatability over locations as indicated by the non-significant location × hybrids interaction. Highly significant effects were detected for locations × sectors and hybrids × sectors interactions, probably owing to the growth of sampled leaves within each sector under different environmental conditions, i.e. water availability, temperature and light intensity. These results indicate the utility of sampling leaves at the lower, central and upper canopy sectors in order to compare correctly stomatal density measured at different locations and in different hybrids.

Table 2

Analysis of variance for stomatal density of the abaxial epidermis

Source of variation	df	MS	F
Locations (L)	1	13 258	165.7**
Hybrids (H)	5	2 889	36.1**
Sectors (S)	2	3 552	44.4**
L × H	5	139	1.7
L × S	2	1 115	13.9**
H × S	10	273	3.4**
L × H × S	10	53	0.7
Blocks	6	532	6.7**
Error	102	80	

** Significant at the % probability level.

Table 3 reports components estimate for within plot sources of variation, i.e. plants within plot and microscopic fields within leaf print. Since no significant correlation was detected between means and variances, the decrease observed from the lower to the upper canopy sector may be related to a greater influence of microenvironmental differences within plot on the lower leaves during their development. No definitive statement can be made as to which sampling unit was more variable, since the plant component, as compared to the microscopic field component, was greater in the lower and central canopy sectors, but not in the upper. It should be pointed out that the plant component may be overestimated by an unknown amount due to the variability in stomatal frequency present within the leaf surface. This amount should nevertheless be small, considering that leaves were sampled in the same portion.

Table 3

Components estimate for within plot sources of variation at three sectors of the plant canopy

Canopy sector	Plants $(\hat{\sigma}^2_P)$	Microscopic fields $(\hat{\sigma}^2_f)$
Lower	436	275
Central	278	203
Upper	158	183

Table 4 reports mean values of measured traits. Hybrids were highly differentiated for yield and morphologically. Stomatal density of the abaxial epidermis varied from 122 to 150 stomata/mm² and was characterized by a low coefficient of variation (3.9%). The only trait

significantly correlated on a mean varietal basis with stomatal density was plan height (r= —0.86**).

In terms of the utilization of stomatal frequency in sunflower breeding programmes, these results showed a good repeatability of the trait. Owing to the great amount of time required to measure stomatal density, its use will necessarily be limited to specific purposes, such as breeding for drought tolerance. Sunflower has a low water-use efficiency owing to a feeble stomatal resistance (Blanchet and Gelfi, 1978) and agronomic benefits might be obtained reducing stomatal frequency and size.

Table 4

Mean values, L.S.D., coefficient of variation (CV) and F ratio of the investigater characters

Cultivars	Stomatal density [1] (nr/mm²)	Height (cm)	Head diameter (cm)	Flowering (days) [2]	Seed yield (q/ha)
Genotype					
Romsun HS 52	150	167	24.8	31	33.7
Gloriasol	145	180	21.8	31	32.7
Novisol	137	177	21.7	28	33.9
Romsun HS 301	129	181	23.5	30	37.0
Stromboli	125	191	23.7	32	34.7
Arancio	122	185	23.6	26	31.6
L.S.D. (P=0.05)	5.4	5.7	1.3	1	2.6
CV (%)	3.9	3.1	5.4	2.1	7.4
F ratio for hybrids	**	**	**	**	**

[1] Measured on the abaxial epidermis.
[2] Starting from June lst.
** Significant at the % probability level.

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FRÉQUENCE STOMATIQUE DANS LE TOURNESOL (*HELIANTHUS ANNUUS* L.)

Résumé

On a rélévé la fréquence stomatique dans six hybrides commerciaux de tournesol (*Helianthus annuus* L.) cultivés dans deux endroits.

On a relevé l'empreinte de la surface supérieure et inférieure d'une feuille appartenante au secteur inférieur, moyen et supérieur de quatre plantes de chaque parcelle. Pour chacune de 1152 empreintes prises, on a fait cinq lectures au microscope. On n'a fait que l'analyse de la variance des données de la page inférieure parce que beaucoup d'empreintes (10.5%) de la page supérieure n'étaient pas limpides à cause des trichomes. La frequence stomatique moyenne des hybrides a été calculée entre les 122 et 150 stomates/mm².

Comme les interactions localité × secteurs et hybrides × secteurs se sont relevées significatives, il faut relever le caractère sur les trois secteurs de la

plante. L'interaction localité × hybrides n'était pas significative bien qu'elle eût été nettement differenciée.

Le bas coefficient des variations (3.9%) et la bonne constance de la densité stomatique dans les deux endroits nous indiquent la possibilité de son exploitation dans les programmes d'amélioration génétique.

DENSIDAD ESTOMATICA EN EL GIRASOL (*HELIANTHUS ANNUUS* L.)

Resúmen

Ha sido medida la densidad estomatica en seis hibridos commerciales de girasol (*Helianthus annuus* L.) cultivados en dos lugares. Ha sido relevada una impresion de las hepidérmicas abasales y adasiales en una hoja de los sectores inferiores, centrales y superiores de cuatro plantas por cada pedazo de terreno. Han sido ejecutadas cinco observaciones microscópicas por cada una de las 1152 impressiones relevadas. Han sido calculadas análisis de variabilidad empleando solamente los datos de la hepidermis abasal debido a que el 10,5% de las impresiones adasiales habia sido ofuscata por la presencia de muchos tricomas. La frequencia estomatica media de la hepidermis abasales ha sido calculada entre 122 y 150 estomates/mm^2. Por causa de la significativas interacciónes lugar por sector e hibrido por sector, se recomienda de mesurar la densidad estomatica sobre los diversos sectores de la planta. La interacción lugar por hibrido no era significativa aunque los lugares hubiese sido altamente diferenciado. El bajo coeficiente de las variaciones (3.9%) e la buena repetibilidad de la densidad estomatica nos hace sugerir su posible empleo en los programas de mejoramiento.