

Changes in the chlorophyll *a* and *b* content in different-aged leaves of *Spergula vernalis* Willd.

EWA SYMONIDES

Department of Plant Systematics and Geography, Institute of Biology, N. Copernicus University

(Received: October 20, 1973.)

Abstract

The results are reported of investigations on the seasonal variations in the chlorophyll *a* and *b* content in the successive leaf tiers of *Spergula vernalis* Willd. growing in two different dune habitats. It was endeavoured to determine the influence of habitat conditions on the concentration of green pigments. The eventual relationship between their amount and the production of organic matter by the plants is discussed.

INTRODUCTION

The present paper is a part of more extensive studies on the influence of abiotic and biotic environmental factors on the biology, ecology and morphological variability of the dune species *Spergula vernalis* Willd. (Symonides 1974a, b, c, d). The investigations were carried out in the Toruń Basin in five different habitats constituting successive stages of overgrowing of dunes with vegetation. The differences between these habitats concerned in the first place the abundance of water and nutrient components and in some small degree the microclimate (Symonides 1974a).

In the present study it was endeavoured to establish whether and to what extent different microhabitat conditions influence the total chlorophyll (*a*+*b*) content and the ratio of chlorophyll *a* to chlorophyll *b* in the particular leaf tiers of *Spergula vernalis* in the course of the complete life cycle of this plant.

In order to save space the results are given only for the two extreme sites between which the differences in chlorophyll content were the widest.

METHODS

Chlorophyll *a* and *b* was determined during the whole vegetation season of the plant at 3—5-day intervals, except for the winter dormancy period of the one-whorl leaf rosette. Intact plants for analysis were taken from their natural habitats together

with the soil adhering to their roots. Each time the leaves of the successive tiers were analysed separately.

The procedure of quantitative analysis of chlorophyll *a* and *b* and the calculation of their content were based on the widely applied method described by Bruinsma (1963) and Steubing (1965). Extinction was measured in the chlorophyll extract in 80 per cent acetone at wavelengths of 663 and 645 nm in a Spekol colorimeter.

Since the leaves of *Spergula vernalis* are small and rodlike, it was difficult to calculate the surface area of the leaf blade. Therefore the results of analysis were converted to the dry weight unit. These results shown in the graphs are means of 3—4 determinations for the same leaf tier. The error of the determinations in replicates did not exceed 3 per cent.

The terrain, surface investigated and the climatic factors are described in detail in the paper of Symonides (1974a). A synthetic characteristics of the sites is given in the Table.

Table 1
Synthetic characteristic of the investigated area

Site	1	2
General physiognomy	Strongly wind-blown sands on slope exposed to the south	Extensive moors (heather) on flat land; fixed sands
Overgrown with vegetation: vascular (%)	20	75
sporulating (%)	—	75
Plant community	<i>Spergulo-Corynephorum</i>	<i>Arctostaphyllo-Callunetum</i>
General character of substrate	Soil profile under developed, consisting of unchanged matrix; very arid and dry sands	In soil profile distinct humus horizon 6 cm thick, underneath little changed matrix; soil richer in nutrient components and moister than on site 1
Physical properties of soil: hygroscopic water, %	0.20	0.53
true moisture, %:		
minimal	0.9	3.2
maximal	10.3	14.7
most frequently noted	3—5	7—10
Chemical properties of the soil:		
humus %	0.23	1.40
S mval/100 g soil	0.200	0.322
H mval/100 g soil	0.240	0.522
composition of cation exchangers:		
P ₂ O ₅ (mg/100 g soil)	4.0	8.0
K ₂ O (mg/100 g soil)	1.1	1.5
MgO (mg/100 g soil)	trace	trace

Explanation: x — refers to depth of 5-10 cm within which the main, mass of *Spergula vernalis* roots

S — alkaline cation exchangers content

H — exchange acidity

RESULT AND DISCUSSION

Seasonal changes in chlorophyll content ($a+b$) in the successive leaf tiers of *Spergula vernalis* are illustrated in Fig. 1. It should be mentioned, in view of the use for extinction measurements of the Spekol colorimeter, instead of a spectrophotometer, that the values obtained may be burdened with certain error. Nevertheless, they make possible a comparison of the chlorophyll concentration in plants from various sites.

The data obtained indicate that, on all days when measurements were performed, the plants from site 2 contained more chlorophyll ($a+b$). This regularity concerned all leaf tiers and the differences were most pronounced when the chlorophyll amount was compared in the II i III tiers.

Since both areas studied lay on an open unshaded area it would seem that light was not the factor responsible here for the differences in the chlorophyll amount. This view is supported by the fact that on the compared sites the course of the successive phenophases, which as known are closely related with light intensity, was simultaneous (Symonides 1974 c).

Higher chlorophyll ($a+b$) concentrations on site 2 were probably due to the better edaphic conditions. Although the soil on both sites may be considered as extremely dry and arid (Table) the soil moisture at site 2 was always twice higher (this in turn ensuring a higher water content in the leaves), and the humus content six times higher than at site 1.

A simple relation was found between chlorophyll and water contents in the leaves by I. D. Zelepukhin and V. D. Zelepukhin (1967). A direct or indirect relationship between chlorophyll concentration in the plants and the presence of a number of elements in the soil is well known (Górski 1952; Bonner and Galston 1962). It results from a number of investigations that, under conditions of deficient nitrogen, phosphorus and potassium supply, chlorophyll synthesis and accumulation in leaves is diminished (Lebiedev, Litvinienko 1966, Nichiporovich et al. 1967).

It is interesting, however, that the ratio of chlorophyll a to chlorophyll b was practically identical on both sites (Fig. 2). This result confirms the view of Egle (1960), according to which the factor deciding of the ratio of a/b chlorophyll are the light conditions prevailing in the given environment.

The rate of synthesis and breakdown of the green pigments was also similar on both sites in the particular leaf tiers in the ontogenesis of the plants (cf. Figs 1 and 3). The fall of the chlorophyll level in the older tier at the moment when in the younger tier it reaches its maximum was also observed in other plant species and has been discussed in the literature (Jeffrey and Griffith 1947; Birecka and Dakić-Włodkowska 1964; Gej 1966, 1967).

Comparison of the weight of an average specimen from the sites examined (Symonides 1974a) suggests that in *Spergula vernalis* there exists some direct or indirect connection between the chlorophyll content in the leaves and production of organic matter.

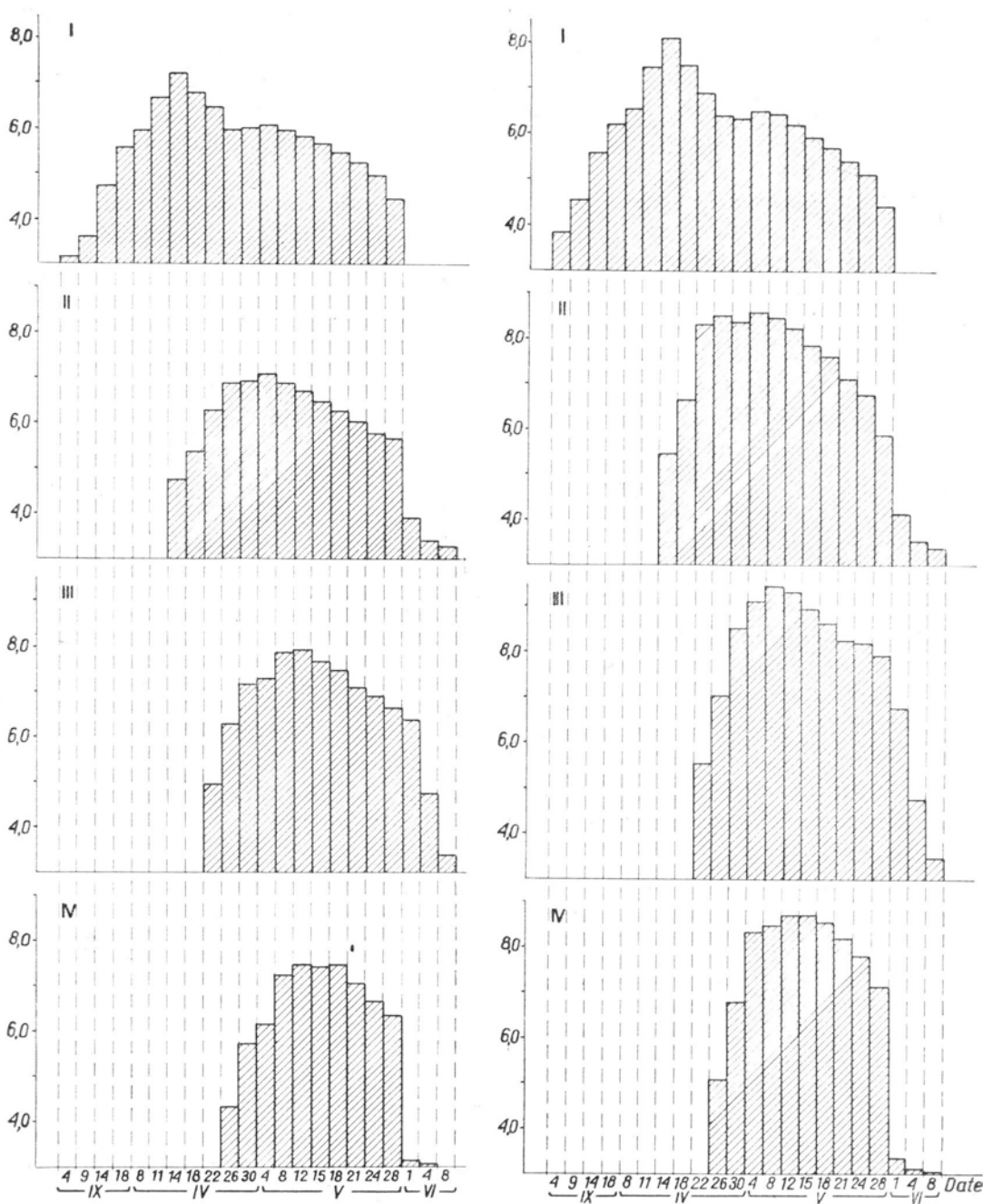


Fig. 1. Seasonal changes in chlorophyll (a+b) content in successive leaf tiers (I—IV); on left site 1, on right site 2

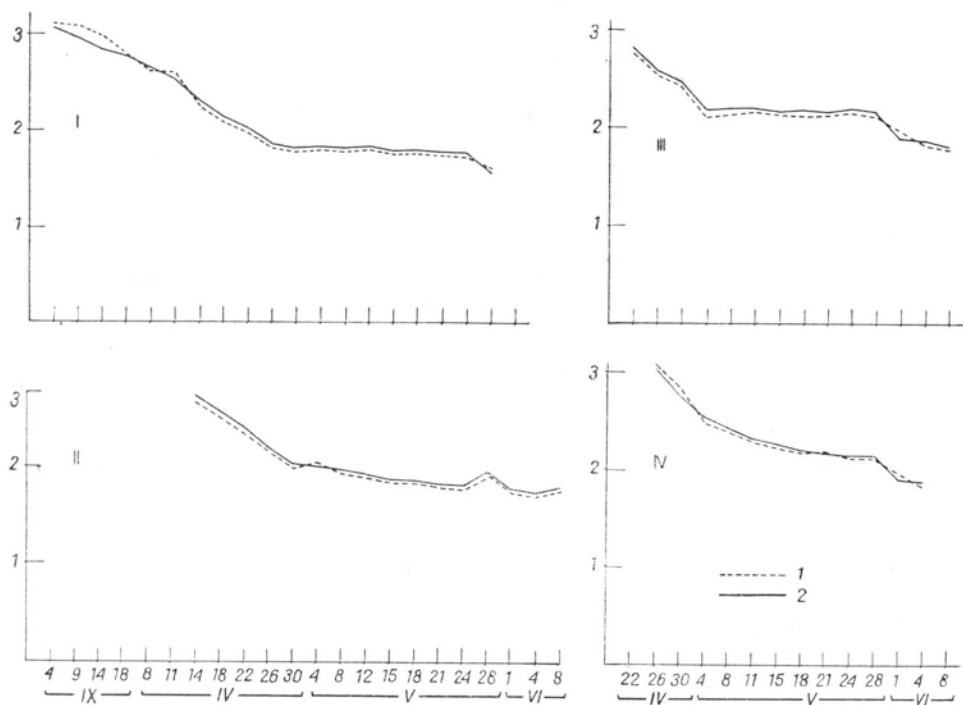


Fig. 2. Ratio of chlorophyll *a* to chlorophyll *b* in successive leaf tiers (I—IV) in sites 1 (dashed line) and 2 (continuous line)

Although it was claimed until lately that in natural conditions there is no distinct correlation between chlorophyll concentration and the intensity of photosynthesis, however, in recent years a number of papers have appeared expressing quite a different opinion. The existence of such a relationship was established among others by Sprague and Curtis (1933), Bray (1960), Brougham (1960), Šesták (1963), Lieth (1965), Šesták and Čatský (1966). In Polish investigations a linear relation

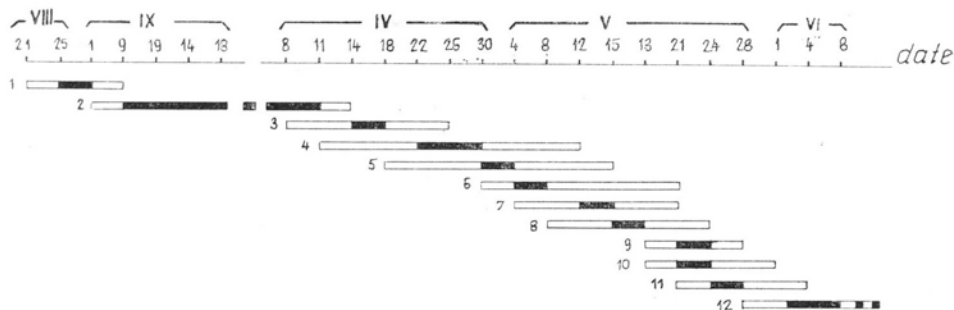


Fig. 3. Course of more important phenological phases for *Spergula vernalis*:

1 — seedling, 2 — one leaf whorl, 3 — two leaf whorls, 4 — three leaf whorls, 5 — inflorescence buds, 6 — flower buds, 7 — beginning of flowering, 8 — full bloom, 9 — beginning of fruiting, 10 — seed dissemination, 11 — seed dissemination, 12 — drying up of plants

was found by Więckowski (1967) between the intensity of photosynthesis and the amount of chlorophyll in the early developmental phases of the leaf of *Phaseolus vulgaris*. The problem of the practical consequences of this relationship in investigations of the productivity of whole ecosystems is discussed by Medina and Lieth (1964), Okubo et al. (1964) and Květ, Nečas and Ondok (1971). This problem, however, exceeds the limits of the present paper.

REFERENCES

- Birecka H. and Dakić-Włodkowska L., 1964. Photosynthesis, translocation and accumulation of assimilates in cereals during grain development IV. Acta Soc. Bot. Pol. 33 (2): 407—426.
- Bonner J., Galston A., 1962, Podstawy fizjologii roślin, PWRiL, Warszawa, 483 pp.
- Bray J. R., 1960. The chlorophyll content of some native and managed plant communities in Central Minnesota, Can. J. Bot. 38:313—333.
- Brougham R. W., 1960. The relationship between the critical leaf area, total chlorophyll content, and maximum growth-rate of some pasture and crop plants, Ann. Bot. N.S. 24: 463—474.
- Bruinsma J., 1963. The quantitative analysis of chlorophylls a and b in plant extracts. Photochem. and Photobiol. 2: 241—249.
- Egle K., 1960. Menge und Verhältniss der Pigmente, Handbuch der Pflanzenphysiologie (W. Ruhland) V(1): 444—496, Berlin Spring, Verlag.
- Gej B., 1966. Changes in chlorophyll a and b content in leaves of different age in some dicotyledones plants, Acta Soc. Bot. Pol. 35 (2): 209—224 (Polish, English summary).
- Gej B., 1967. Wzrost i intensywność fotosyntezy liści różnego wieku niektórych roślin dwuliściennych, Warszawa, 91 pp.
- Górski F., 1962. Fizjologia roślin, Warszawa, 611 pp.
- Jeffrey R. N., Griffith R. B., 1947. Changes in the chlorophyll and carotens of curing burley tobacco cut at different stages of maturity, Plant Physiol. 22: 34—41.
- Květ J., Nečas J., Ondok J. P., 1971. Studijní informace základní vědy v zemědělství. [In:] Metody rustové analýzy, Praha, 111 pp.
- Lebiediev S. I., Litvinienko L. G., 1966. O vzaimnosvjazi miezdu fotochimičeskoj aktivnostju, energetičeski i strukturnymi osobennostjami chloroplastov, Fizjol. Rast. 13 (3): 411—415.
- Lieth H., 1965. Ökologische Fragestellungen bei der Untersuchung der biologischen Stoffproduktion. I. Qualitas plantarum et materiae vegetabiles, 12: 244—261.
- Nichiporovich A. A. et al., 1967. Aktivnost fotosinteticheskogo apparata rastenij i azotnyj obmien, Fizjol. Rast., 14 (5): 849—859.
- Medina E. and Lieth H., 1964, Die Beziehungen zwischen Chlorophyllgehalt, assimilierender Fläche and Trockensubstanzproduktion in einigen Pflanzengemeinschaften, Beitr. Biol. Pflanzen, 40: 451—494.
- Okubo T., Oizumi H., Hishino M., Nishimura S., 1966—67. Chlorophyll amount for analysis of matter production in forage crops. [In:] Photosynthesis nad Utilization of Solar Energy, Level III, Experimente, Tokyo, 43—46.
- Sprague H. B., Curtis N., 1933, Chlorophyll content as an index of the productive capacity of selfed lines of corn and their hybrids, J. Amer. Soc. Agron. 25: 709—724.
- Steubing L., 1965, Pflanzenökologisches praktikum, Berlin und Hamburg 266, pp.
- Symonides E., 1973a, Populacje *Spergula vernalis* Willd. na wydmach Kotliny Toruńskiej, Polish Journal of Ecology (in press).
- Symonides E., 1973 b, Zmienność morfologiczna *Spergula vernalis* Willd. z różnych siedlisk wydmowych Kotliny Toruńskiej, Polish Journal of Ecology (in press).
- Symonides E., 1973 c. Fenologia *Spergula vernalis* Willd. na tle warunków mikroklimatycznych, Polish Journal of Ecology (in press).

- Symonides E., 1973 d. Gospodarka wodna *Spergula vernalis* Willd., Polish Journal of Ecology (in press).
- Šesták Z., 1963. Changes in the chlorophyll content as related to photosynthetic activity and age of leaves, Photochem. and Photobiol., 2: 101—110.
- Sesták Z., Čatský J., 1966. Metody studia fotosyntetické produkce rostlin, Academia, Praha.
- Więckowski S., 1967. Badania nad aktywnością i ultrastrukturą aparatu asymilacyjnego w rosnącym liściu *Phaseolus vulgaris*, Kraków, 75 pp.
- Zelepukhim W. D., Zelepukhin I. D., 1967. Soderżanie chlorofilla i vodnyj režim lista, Fizjol. Rast., 14 (1), 142—147.

Author's address

Dr Ewa Symonides

Institute of Biology, N. Copernicus University,
Department of Plant Systematics and Geography,
ul. Gagarina 11, 87-100 Toruń, Poland

Zmiany w zawartości chlorofilu a i b w liściach różnego wieku u Spergula vernalis Willd.

Streszczenie

W pracy zbadano zmiany w zawartości chlorofilu ($a + b$) oraz stosunku chlorofilu $a : b$ w liściach kolejnych pięter wydmowego gatunku *Spergula vernalis* Willd., porastającego dwa różne siedliska wydmowe Kotliny Toruńskiej.

Materiał do analiz pobierano w ciągu całego cyklu rozwojowego rośliny, co 3—5 dni. Analizy ilościowe wykonano według metody opisanej przez Bruinsma (1963) i Steubing (1965).

W wyniku przeprowadzonych badań stwierdzono:

1. Na wilgotniejszym i zasobniejszym w składniki pokarmowe siedlisku (tab. 1) roślina produkowała więcej chlorofilu ($a + b$), niż na skrajnie suchym i jałowym (ryc. 1). Różnice w koncentracji chlorofilu u roślin porównywanych stanowisk zaznaczały się w całej ontogenezie i we wszystkich piętrach liści.
2. Różnice siedliskowe nie wpłynęły na wielkość stosunku chlorofilu $a : b$ w analogicznych piętrach liści u roślin porównywanych stanowisk (ryc. 2).
3. Tempo syntezy i rozkładu zielonych barwników w poszczególnych piętrach liści było prawie identyczne na obu stanowiskach.
4. Porównanie ciężaru osobników wyrosłych w różnych warunkach siedliskowych z jednej strony (Symonides 1973a), a stężenia zielonych barwników z drugiej, sugeruje istnienie liniowej zależności między ilością chlorofilu w liściach a produkcją masy organicznej u *Spergula vernalis*.