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Regeneration and vegetative propagation of *Sphagnum palustre* as factor of population stability

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Abstract

The stability of the *Sphagnum palustre* populations on the meadows of the Kampinos National Park situated north-west of Warsaw was investigated in the period 1971-1974. Laboratory cultures were also started to establish the regenerative ability of various gametophyte parts of *Sphagnum:* the main stem, branches, leaves and spore germination.

The green stems and apical branches of the plants showed the highest regeneration ability. Brown stems and white branches developed less intensively. Leaves showed no tendency to develop into new plants. Gametophores were found to form quicker and more effectively by way of regeneration than from spores.

In natural conditions more intensive growth of branchings (new shoots) from the apical and green parts of *Sphagnum* was also observed, whereas the brown parts did not exhibit this ability.

INTRODUCTION

Sphagnum belongs to the bryophytes which seldom produce sporogonia, therefore for the stability of their swards the ability of vegetative propagation and regeneration is all the more important. The growth of these plants occurs apically by branching of single individuals into 2 or 3 shoots and dying back of the lower ones.

In field investigations it was endeavoured to establish the dates and frequency of branch formation and the correlation between this phenomenon and the density of the sward. The laboratory cultures were initiated at the same time to investigate the regenerative ability of various parts of the *Sphagnum* gametophyte.

METHODS

Sphagnum palustre served as experimental material.

Field studies were conducted in the period 1971-1974 in the Kampinos National Park on the Łąki Sierakowskie and Korfowe (meadows) in broshwood communities.

The experimental plots on both meadows were similar as far as their bryoflora is concerned. *Sphagnum palustre, S. apiculatum* and *Polytrichum commune* prevail here, with a large contribution of *Calliergon cuspidatum, C. stramineum, Drepanocladius aduncus, Aulacomnium palustre*. From among higher plants *Betula pubescens, Alnus glutinosa, Salix cinerea, Caltha palustris, Comarum palustre* are found here and *Calluna vulgaris* and *Vaccinium vitis-idaea* in drier places.

In 1973 and 1974 on the permanent plots chosen in pure *Sphagnum palustre* swards 30 plants on each of 5 sites were tagged (each plant was tied around right under the head). In the course of each vegetation season observations were recorded several times concerning the length of the branchings and the appearance of the tagged individuals, as for instance vigour of growth, dying back, and dying of particular plants. Simultaneously, on the same sites 5 circular *Sphagnum* patches with surface area 100 cm^2 were cut out, as far as possible of similar density, with plants of similar size. Each sample was sorted in the laboratory and the particular plants were counted. As single plant was considered each individual with a head. The mean density of the *Sphagnum* sward was calculated per 100 cm^2 surface area. At the same time the particular plants were analysed in reference to similar traits as those of the tagged individuals.

For evaluating the ability of regeneration of the particular *Sphagnum* parts and the rapidity of these processes, 4 experiments, were started in the laboratory.

The different parts of *Sphagnum* were cultured as well as spores:

1. the main stem deprived of branches and leaves was divided into 1-cm segments, separately the green parts (younger, apical, assimilating ones) and the brown parts (older, nonassimilating). They were placed in dishes 200 in each;
2. whole foliated side branches of *Sphagnum* were placed in dishes, 100 in each, separately the green assimilating and the white nonassimilating ones;
3. leaves taken from side branches, were placed on agar;
4. the spores were sown.

Experiment 4 (spores sown) was performed for comparing the rate of production of young gametophores from spores and from various parts of the stem (exp. 1, 2, 3). The experiments were started on 25 October, 1973 (series I) and repeated on 8 Jan., 1974 (series II). The plants were

cultured on Petri dishes half filled with Knop's medium (20 ml per 1000 ml agar), illuminated from above with fluorescent tubes of 7500 lux for 10 h daily over the whole experimental period.

Observations of the culture material were made at 5-day intervals under microscope. The appearance of gametophore buds was recorded and their development followed.

As green parts of shoots were considered the main stem and the lateral shoots covered with leaves on which living cells contained chloroplasts, thus capable of assimilation. On the other hand, the white and brown parts consist of main and lateral shoots covered with white leaves the cells of which are deprived of chloroplasts.

RESULTS

Field observations

Field observations demonstrated and confirmed the known natural phenomenon that new individuals of *Sphagnum* arise above all by apical branching of the main stem into 2 or, less frequently, 3 shoots showing almost equal growth ability (Fig. 1) with dying back of the lower segments.

Examination in the field of the tagged *Sphagnum* plants showed that from May to July the number of branching individuals increased. On the average 38 of 100 individuals branch in June and July. In August this number is somewhat smaller. Towards the end of the vegetation period, in october there occurs a period of intensive production of apical branchings, about 66 of 100 individuals from new shoots. The first period, June-July coincides with the most intensive individual growth of the whole population (S o b o t k a, 1974). In the second period, October, the mature apical parts of *Sphagnum* which developed in the current vegetation period branch intensively. This period may be considered as phase of renovation of the population, a tendency to supplement the sward in the places left empty after the death of some individuals during the vegetation season. (Table 1).

If we analyse the tagged plants in the field, it is seen that beside old branchings there appear on them new young shoots beginning with August (Fig. 1B). In August these short shoots are not yet numerous, on the average about 9 per 100 examined individuals, whereas in October there are 27 per 100 plants, thus almost one half of the 66 per 100 branchings appearing in this month.

The diagram (Fig. 2) shows the density of *Sphagnum* individuals in the particular months per 100 cm^2 against the background of precipitation. The number of individuals is highest in June (120, 140, 133, 84 per 100 cm^2), and in October (166, 182, 125, 103). These two periods when

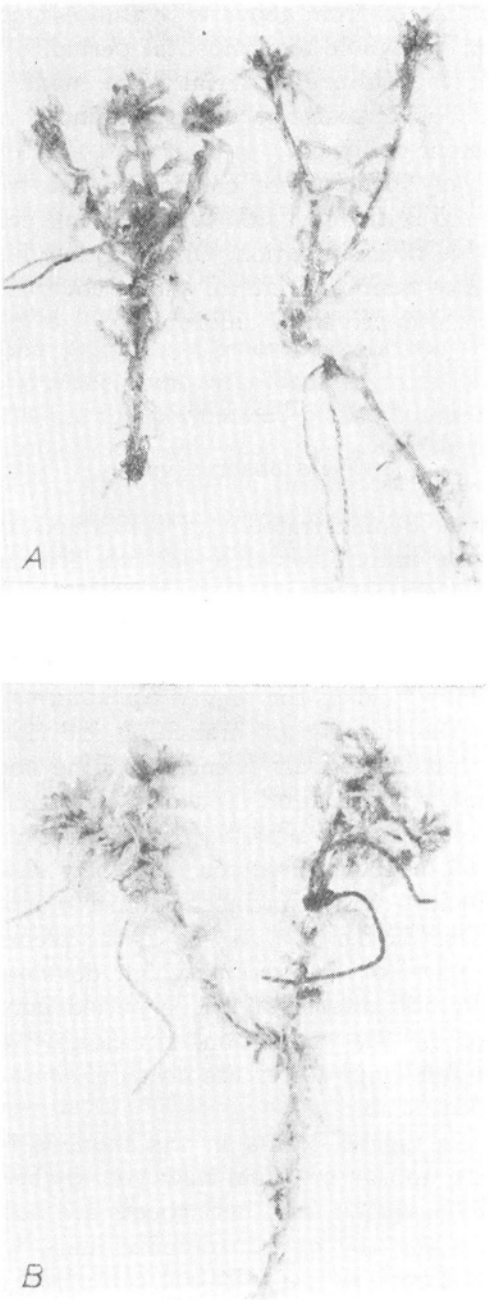


Fig. 1. Apical branching of tagged *Sphagnum palustre*
A — stage in July; *B* — two places of branching visible; the lower from July, the upper from October

Table 1

Number of tagged *Sphagnum palustre* individuals (%), producing new shoots

Localities	Years	Months				
		V	VI	VII	VIII	X
Sieraków	1973	5	38	40	30	50
	1974	9	46	42	36	71
Korfowe	1973	23	30	30	28	70
	1974	16	37	41	40	72

the *Sphagnum* swards are most dense coincide with the period of most intensive shoot production observed in the field. In the remaining months (March, Apr., May, Aug., Sept.) the mean density is lower. It would seem that in these months there occurs a gradual dying back of the plants. This is also confirmed by the observation of the tagged plants. Among the plants examined in the particular months, individuals were found showing no growth, dried up or dying back.

Precipitation exerts no doubt a strong influence on the population size in the swards and on branching. August and September are charac-

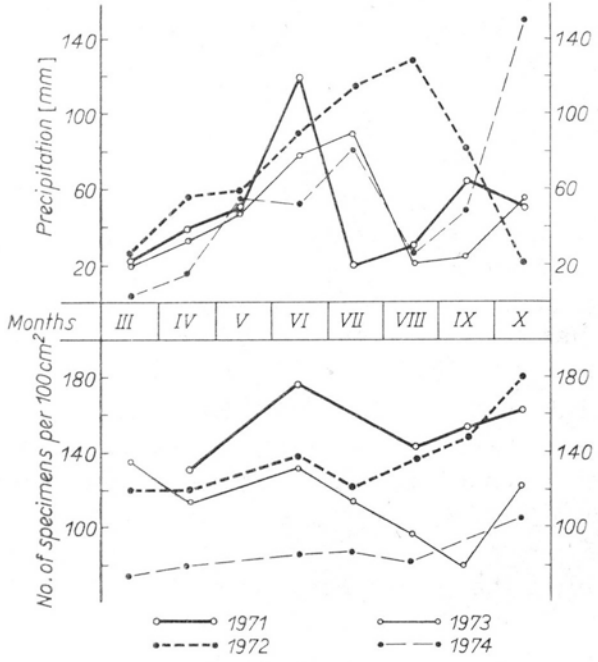


Fig. 2. Diagram of average values of *Sphagnum* density for 100 cm² in comparison with precipitations in consecutive months in the years 1971—1974

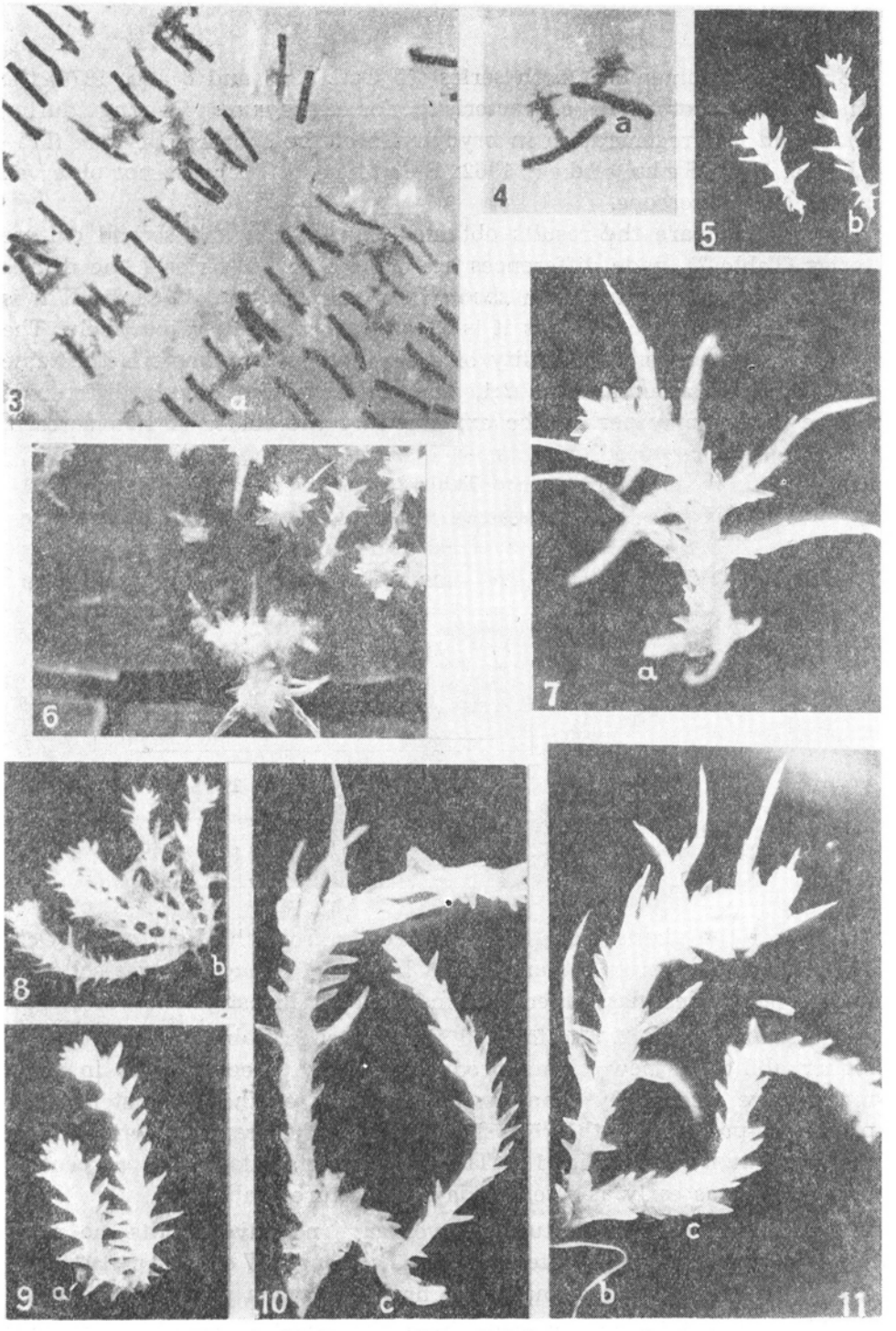
terized by a lower precipitation level, simultaneously the number of the *Sphagnum* plants in the swards decreases (August — 142, 135, 97, 81/per 100 cm^2). A different course was only observed in 1972. August and September were characterized by a higher density of individuals as compared with that in other months. As seen from the diagram (Fig. 2), rainfall in August reached in this month a maximal monthly value of 130 mm, thus it was 4 times higher than in the remaining years.

Another factor important for the development and growth of *Sphagnum* is temperature (P e d e r s o n, 1975). High and low temperatures in the summer months have an inhibitory effect on the density of the populations. Both the summer and autumn intensive processes of branching of the main *Sphagnum* shoots may be considered as the decisive factor in renewal, and the agent ensuring stability to the population.

Laboratory observations

E x p e r i m e n t 1. The gametophyte of *Sphagnum palustre* exhibits a high viability manifested in a high regeneration ability, particularly of the main stem (Figs 3, 4) and branches (Tables 2, 3) The results listed in table 2 show that cut green assimilating shoots possess this capacity in a two times higher degree than the brown nonassimilating ones. After 65 days of culture 65 per cent of the green shoots in series I produced young gametophores and in series II 31 per cent, whereas the brown nonassimilating shoots gave in series I 34 per cent of new shoots and in series II only 16 per cent. The first buds on the cut shoots in culture appear after 18 days. They form on shoots at the wounded sites where the side branches and leaves were detached. The buds consist of 3-4 minute leaves in which all cells contain chloroplasts. It is only in later growth of the gametophore, beginning with the 4-th-5th leaf that differentiation occurs in the leaves to chlorophyll cells and hyaline ones. At the same time there develop from the lower parts of these young gametophores numerous rhizomes.

At this period the newly developed plants are but weakly attached to the mother stem and fall easily from it (Fig. 5). After 50 days, when the young plants reach a lenght of 2 - 2.5 cm, side branches begin to develop from them (Figs 6, 7) and the leaves differentiate to stem and branch ones. The plants take on a form characteristic for mature individuals. Very often the gametophores do not form singly on shoot segments but grow in bunches of several (Figs 8, 9). The process of one shoot branching into several is a known phenomenon (P i l o u s, 1971). It is owing to this ability that *Sphagnum* forms compact extensive swards.



Figs. 3—11. Regeneration of *Sphagnum palustre*

3 — fragments of *Sphagnum* main shoots on agar in Petri dishes with new plantlets; *4* — regenerating shoots fragments; *5* — individual separated young plants; *6* — culture with branching *Sphagnum*; *7* — one enlarged branched *Sphagnum* plant growing from shoot fragment; *8* — bud of young plant separated from the shoot fragment; *9* — two young plants of *Sphagnum* growing from the shoot fragment; *10* — green side branch with two young gametophores; *11* — apical branch of *Sphagnum* with new branched plant
a — part of main shoot, *b* — rhizoids, *c* — side branch of *Sphagnum*

In the experiments of both series (25 Oct., 1973 and 8 Jan. 1974) the thallus-like protonema characteristic for *Sphagnum* forming during shoot and leaf regeneration in bryophytes of the subclass Bryinae (Misiura, 1964; Schneider, 1962; Berthier, 1972) was not observed under the microscope.

If we compare the results obtained in the first and second culture series (Table 2), wide differences are noticeable. In series I the degree of regeneration for the green shoots is 65 per cent and in series II it is 31 per cent. For brown parts it is 34 and 16 per cent, respectively. The ratio of the regenerative ability of green and brown shoots is the same in both series amounting to 2:1. The causes of these wide differences in the results may perhaps be explained by the choice of the material.

Table 2

Number of stem segments (%) producing young plants

Number of days of culture		20	25	35	45	55	65
I series 25.X.1973	green stem	14	24	31	40	52	65
	brown stem	12	14	17	17	24	34
II series 8.I.1974	green stem	6	16	16	26	29	31
	brown stem	5	8	8	16	16	16

In series I the plants were brought directly from the field on 25 Oct., 1973, whereas in series II started on 8 Jan., 1974 *Sphagnum* was collected from a bed under glass where the material for investigation was stored.

Experiment 2. The foliated side branches (Table 3) exhibit a still higher ability of new plants production. This process occurs in them much more intensively than in the segments of the main stem. New plants are produced with greatest rapidity by the branches derived from the *Sphagnum* heads (Fig. 11). The latter plants attain 100 per cent of regeneration as early as after 25 days of culture (Table 3).

Experiment 3. Cultured leaves gave negative results, no regeneration processes were noted in them, although Woester (1934) affirms that he observed formation of new *Sphagnum* plants from leaves.

Pilous (1971) reports that, from the vegetative cells of various parts of *Sphagnum*, protonema forms which then gives rise to new shoots. In the present experiments with main stems and side branches regeneration in *Sphagnum*, protonema formation was not observed.

Table 3

Number of branches (%) producing young gemetophores

Number of days of culture	23	25	35	45	55
Green branches	50	63	83	84	84
White branches	—	—	13	31	31
Apical branches	60	100	100	100	100

Experiment 4. Protonema only developed after spore germination. The germination ability of *Sphagnum* spores is variable. In series I of experiments some spores germinated by way of a short cellular filament after 7-8 days, in series II germination occurred as late as after 15 days. This different spore germination period does not, however affect the rapidity with which the gametophore develops from the protonema. In both cultures after 50-55 days buds of young foliated *Sphagnum* plants appear on the protonema (Fig. 12).

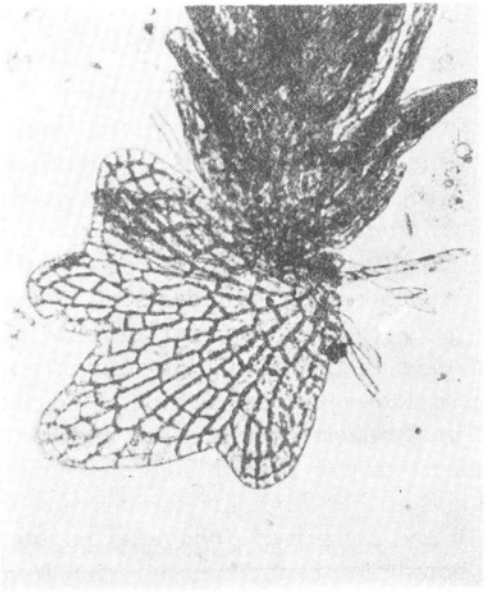


Fig. 12. Gametophore with leaves growing from the protonema

Comparison of the time of production of new plants from various *Sphagnum* parts and spores (Table 4) shows that the development of new individuals is much quicker in the case of stem and branch fragments than from spores. In stem regeneration there appear as early as after 18 days of culture young gametophores on the shoots and branches. Young gametophores derived from spores appeared after 50 days. During

Table 4

Comparison of time necessary for gametophore formation from branches, stems and spores

Number of days of culture	10	20	30	40	50
Branches	o	—	—	+	+
Stems	o	—	—	—	+
Spores	o	o	o	o	—

Abbreviations:
o — no gematophore,
— — appearance of gematophore,
+ — branched gematophore.

this 50-day period the gametophores developed from shoots and branches produce side branchings. Thus, it would seem that, in the natural habitat, vegetative propagation and regeneration are more effective than multiplication by way of spores.

CONCLUSIONS

An increase in sward density and rejuvenation of *Sphagnum* populations occurs by way of development of young daughter individuals in the apical parts owing to branching of the mother plants. Then the lower parts of the plant die with simultaneous release and falling off of the young individuals from the mother plants.

The mumber of daughter individuals increases towards the end of the vegetation season, in October 66 individuals of 100 produce new shoots.

Simultaneously with the development of new plants death of the oldest and weakest ones in the population occurs, manifested by its reduced density. These losses are supplemented at later periods of the vegetation season, in October. Precipitation and air temperature exert a significant influence on the growth and development of *Sphagnum*. Low precipitation and rather high air temperature have an inhibitory effect on the growth and density of *Sphagnum* populations.

Renewal of the population can also occur from fragments of the main stem and side branches, living or dying back. The higest regenerative ability of 100 per cent was noted in the youngest side branches derived from the apical parts of the plants, that is the heads.

The formation of young gametophores is quicker and more effective in the course of regeneration of the stem and side branches than when they develop from spores. Renewal of *Sphagnum* populations is a factor ensuring their stability and existence for long years. The ability of vegetative propagation and regeneration are all the more important for *Sphagnum* since it belongs to bryophytes but seldom producing spores.

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Regeneracja i rozmnażanie wegetatywne torfowców jako czynnik trwałości populacji

Streszczenie

W sezonach wegetacyjnych 1971—1974 przeprowadzono obserwacje nad odnawianiem się i trwałością populacji *Sphagnum palustre*, na stałych powierzchniach znajdujących się na łąkach Sierakowskich i Korfowe w Kampinoskim Parku Narodowym. Poza tym prowadzono w pracowni hodowlę na agarze, w celu zbadania zdolności regeneracyjnych różnych fragmentów torfowca: łodygi głównej pozbawionej liści i gałązek, ulistnionych gałązek bocznych, liści. Wysiano również zarodniki torfowca, aby porównać szybkość wytwarzania się młodych gametoforów z różnych części torfowca i z zarodników.

Otrzymane wyniki wskazują, że największą zdolność regeneracji posiadają zielone, asymilujące łodygi i gałązki szczytowe roślin. Słabiej rozwijają się fragmenty brązowe nie asymilujących łodyg i białe, nie asymilujące gałązki (tabela 3,4). Nie stwierdzono zdolności tworzenia nowych roślin z liści.

Hodowle wykazały, że powstawanie młodych osobników jest znacznie szybsze z fragmentów łodygi i gałązek bocznych niż z zarodników. W drodze regeneracji łodyg i gałązek, już po upływie 18 dni w hodowli pojawiły się młode gametofory. Zarodniki zaś wydały młode gametofory dopiero po 50 dniach.

W terenie obserwuje się intensywne wyrastanie odgałęzień (nowych pędów) w częściach szczytowych torfowca (tabela 1). Liczba osobników potomnych zwiększa się w końcowym okresie sezonu wegetacyjnego, między wrześniem i październikiem; na 100 osobników pojawia się średnio 66 nowych pędów. Równocześnie w okresie sezonu wegetacyjnego dochodzi do zamierania najstarszych i najsłabszych osobników w populacji, co wyraża się jej mniejszym zagęszczeniem. Zagęszczenie to zostaje uzupełnione w późniejszym okresie wegetacji w październiku (tabela 2).