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EFFECTS OF FERTILIZERS AND OF THE USE OF SOWN SWARDS ON YIELD COMPONENTS IN TWO PERENNIAL GRASSES

BY
LUCIA STOICOVICI

The number of vegetative living tillers and their dry weights as well as the weight of nonliving tillers (including the senescent material) were examined in *Dactylis glomerata* L. and *Festuca arundinacea* Schreb. at the beginning and at the end of the growing season. Together with their cultivars, these two perennial grasses were grown in the field in mixture with *Trifolium repens* (Ladino) under an NPK nutrient treatment. In some plots the herbage of the sward was cyclically removed (defoliated) by sheep grazing. It is shown that the ratio of "yield components" per 400 cm² of ground area exhibits significant differences between species, cultivars and the use of the experimental sward unit.

The paper deals with the study of the growth and productivity of *Dactylis glomerata* L. and *Festuca arundinacea* Schreb. perennial grasses under the influence of several factors. The changes are evaluated from the yield components. The numbers and weights of tillers expressed per plant or per unit area are dynamic entities influenced by the genotype, environment and by the stage of development [5].

MATERIALS AND METHODS

Data for this paper are drawn from a field experiment at Juc-Bontida experimental station (Cluj county) including a sown sward. The determinations are part of the theme: the testing of perennial grasses and leguminous cultivars by sheep grazing.

From a total number of field experiments set up in 1978, three plots were chosen depending on their destination: 1. hayfield; 2. hayfield + pasture; 3. pasture. Each of them were identically subdivided in order to comprise *Dactylis glomerata* species with two cultivars of different maturity types, medium early Goliat and medium late Gotar, and *Festuca arundinacea* species with two cultivars, early Pandur and medium early Parnas. The species with their cultivars were grown in pure and in mixed swards with 10% *Trifolium repens* Ladino cultivar. Some of the plots (variants) were unfertilized and others were fertilized with 33.5% (N₅₀ kg/ha) ammonium nitrate, applied in spring and after each cycle of cutting or grazing defoliation. 20% superphosphate P₂O₅ (P₅₀ kg/ha) and 40% K₂O (K₅₀ kg/ha) potassium salt were added in autumn once every two years.

The plant material, the living and nonliving vegetative tillers, including the senescent, yellowing material derived from grasses and from white clover, stolons, shoots and leaves were harvested on May 5 and October 8, 1980. In this paper we insist upon the results obtained on the first harvest which is considered between the most important harvests in regard of forage yield distribution (herbage mass, e.g. dry and fresh materials) in the field. During the vegetation period, the herbage mass must be appreciated within three series of cuttings (herbage harvested) and within five series of sheep grazing with Romney Marsh breed (herbage consumed).

RESULTS

In the fertilized variant of *Dactylis glomerata* species, the effect of sward use upon the intensity of tillering is evident irrespective of cultivars and of whether the species is growing in pure or in mixed swards. There is a tendency of increase in the number of tillers from the hayfield toward the pasture. Significant ($P \leq 5$) and very significant ($P < 0.1$) differences were recorded. In Table 1, the significance between the average values subjected to the t test distribution is emphasized. But there are also differences between the cultivars imposed by the genotype. Thus, in the hayfield, either in pure or in mixed swards, distinct ($P \leq 1$) and very significant differences ($P < 0.1$) are obtained. In a similar way, in the hayfield in combination with the pasture plot (only in pure sward) or in the pasture, differences ($P \leq 1$) between the cultivars are obvious. These differences show a greater number of tillers with the medium late cultivar. The pure or mixed growth of these cultivars has no effects on the degree of tillering, no matter the destination of the sward. In these cultivars, in the unfertilized variant, only a slight decrease of the tiller number per unit area, especially in the hayfield and in the pasture (between 113 and 196/400 cm²), is noticed. Apart from the greater number of significant differences at tillering, significant differences between the dry weights of the same tillers are only seven. In the unfertilized variant, the same situation is recognized except that in both cultivars all the average values of dry weights are lower (between 7.5 and 14.4 g/400 cm²). The examination of *Dactylis* cultivars (including the Goliat cultivar) in Romania has proved a good distribution of production by the grazing series (about five series) [6].

In *Festuca arundinacea* species and in the treated variant tillering is not significantly influenced (unlike in *Dactylis*) by the way of sward use, irrespective of cultivars and the cultivation type. But between Pandur and Parnas cultivars there are five significant differences ($P \leq 1$) and one very significant difference ($P < 0.1$) in pasture and in mixed sward. The tillering process is stronger in Parnas cultivar. Neither cultivar changes significantly its number of tillers in pure or in mixed sward. In the unfertilized variant the tillering process is lower (between 105 and 230 mean number of tillers per 400 cm²). In both cultivars there are no significant differences between the dry weight of the tillers as influenced by the way of sward use. Between cultivars there are seven significant dif-

Table 1
Yield components in *Dactylis glomerata* and *Festuca arundinacea* at the beginning of the growing season (May 6, 1980)

Components	Hayfield				Hayfield + Pasture				Pasture			
	<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar		<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar		<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar	
No. tillers	1	2	1	2	1	2	1	2	1	2	1	2
	134.5 ±34.2	130.7 ±44.3	205.9 ±35.6	211.6 ±49.1	116.0 ±22.9	139.9 ±33.9	177.1 ±41.7	140.2 ±67.1	166.7 ±24.5	182.2 ±26.7	253.0 ±55.7	235.5 ±33.6
g tillers	13.7 ±2.9	18.4 ±4.0	14.6 ±2.6	15.8 ±5.2	12.6 ±3.4	16.9 ±3.7	13.1 ±3.5	11.3 ±6.2	16.4 ±5.2	16.7 ±4.7	17.0 ±3.3	14.0 ±3.8
Components	<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas		<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas		<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas	
No. tillers	1	2	1	2	1	2	1	2	1	2	1	2
	151.5 ±30.0	146.6 ±61.6	186.7 ±44.2	195.0 ±39.9	147.7 ±43.7	176.9 ±56.6	204.0 ±58.2	213.7 ±74.6	181.2 ±27.5	154.9 ±33.8	218.0 ±52.6	233.6 ±64.6
g tillers	12.8 ±2.6	13.8 ±4.0	16.1 ±4.0	16.5 ±2.6	10.7 ±2.9	15.2 ±6.6	17.4 ±4.7	19.4 ±8.5	12.6 ±2.5	12.3 ±3.6	18.8 ±4.3	22.1 ±7.6

Note: in Table 1 are shown the mean numbers of living tillers and their mean dry weights/400 cm² in the fertilized variant. The mean values include 8 individual values (samples) and are accompanied by the standard error (S.E. ±). 1. pure sward. 2. mixed sward.

ferences, most of them in the pasture. The dry weight increase is due to the Parnas cultivar. The tillers dry weight was not significantly influenced by the cultivation type. This problem is fully analysed by Mc Bratney (1978) in perennial ryegrass cultivars. It is asserted [3] that *Festuca arundinacea* has cultivars with high yields of green herbage and with a good regrowth capacity. Well appreciated are the late cultivars. In spring, the height of the vegetation in the hayfield was between 45 and 85 cm and in the pasture between 15 and 30 cm.

The results concerning the living plant material harvested in the fall is shown in Table 2, and it is noteworthy, that there is an important decline in the tillers number and their dry weights from spring until autumn. The decline in the tillers average number goes as far as 41/400 cm² with 7 g/400 cm² dry weight in *Dactylis* and 86/400 cm² with 8 g/400 cm² dry weight in *Festuca*. In autumn, the tillering process does not differ significantly in *Dactylis* or in *Festuca* cultivars between the pasture and the hayfield. Similarly, between the cultivars there are only two significant differences ($P \leq 5$) in *Dactylis* and a marked significant difference ($P \leq 1$) in *Festuca*. The tillers dry weights have given significant differences but between *Festuca* cultivars. In the hayfield as well as in the pasture, the sward canopy is interrupted by numerous gaps and the grass tussocks are isolated. The height of the vegetation ranges between 10 and 15 cm.

As has been assumed, the losses of nonliving material are greater in autumn than in spring due to plant ageing (Table 3). The causes of tiller death in a grass sward are well studied by Ong (1978). There are significant and very significant differences within the cultivars of both species. With two exceptions (in *Festuca*) the losses of nonliving material particularly in the fall are not significant between the hayfield and the pasture. In this respect it is concluded [2] that pasture production is likely to be good only when grazing or cutting management ensures that dry matter losses by decay are kept to a minimum, whilst gains by photosynthesis continue at a high level.

Trifolium repens, which enters into combination with grasses, does not show significant changes due to fertilization or other factors at the beginning of the growth season. But in autumn a great change is recorded. Thus, if in the hayfield the species occurrence is still noteworthy, in the unfertilized variant, with 0.24 or 0.87 g/400 cm², in the pasture the species is almost non-existent (between 2 and 11 leaves/400 cm²). This fact suggests that the regrowth of white clover is slowed down by grazing or/and after a long period of pasture management.

CONCLUSIONS

The analysis of yield components in *Dactylis glomerata* and *Festuca arundinacea* within the experimental trial of grasses and leguminous cultivars by sheep grazing, has revealed the following.

1. In spring, in *Dactylis glomerata* species the mean number of living tillers per 400 cm² significantly increase in the fertilized plot being used as a pasture irrespective of cultivars and the cultivation type (pure or

Table 2
Yield components in *Dactylis glomerata* and *Festuca arundinacea* at the end of the growing season (October 8, 1980)

Components	Hayfield				Hayfield + Pasture				Pasture			
	<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar		<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar		<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar	
	1	2	1	2	1	2	1	2	1	2	1	2
No. tillers	74.5 ±32.0	84.9 ±37.3	98.1 ±31.3	120.2 ±23.6	100.1 ±17.1	91.6 ±19.6	123.5 ±24.4	138.9 ±28.8	92.0 ±25.9	107.0 ±15.1	126.9 ±32.4	114.0 ±23.2
g tillers	3.8 ±1.7	4.2 ±2.4	3.8 ±1.7	4.4 ±1.6	5.7 ±1.9	6.5 ±1.1	8.7 ±1.7	11.8 ±3.0	5.1 ±1.7	5.5 ±1.7	3.9 ±1.3	3.7 ±0.9
Components	<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas		<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas		<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas	
	1		1		1		1		1		1	
	2		2		2		2		2		2	
No. tillers	131.9 ±42.0	116.1 ±23.3	102.4 ±30.3	60.4 ±32.9	109.2 ±22.8	90.4 ±22.1	87.6 ±23.5	83.4 ±16.2	135.0 ±31.6	147.1 ±25.8	125.9 ±31.6	136.1 ±39.2
g tillers	5.9 ±1.3	7.2 ±1.9	5.5 ±2.1	2.8 ±1.5	11.3 ±3.4	9.1 ±3.8	13.2 ±2.5	12.3 ±3.6	9.4 ±1.8	9.1 ±1.9	7.0 ±1.8	5.7 ±2.5

Note: in Table 1 are shown the mean numbers of living tillers and their mean dry weights/400 cm² in the fertilized variant. The mean values include 8 individual values (samples). 1. pure sward; 2. mixed sward.

Table 3

The mean dry weight of nonliving tillers (including the senescent material) (g/400 cm²) in the fertilized variant recorded on May 5 and October 8, 1980

Month	Hayfield				Hayfield + Pasture				Pasture			
	<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar		<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar		<i>D. glomerata</i> Goliat		<i>D. glomerata</i> Gotar	
May	1	2	1	2	1	2	1	2	1	2	1	2
	1.1 ±0.3	1.6 ±0.8	1.8 ±0.5	1.7 ±0.4	1.1 ±0.4	0.9 ±0.4	0.9 ±0.3	1.6 ±0.9	1.3 ±0.4	1.6 ±0.6	2.5 ±0.6	1.9 ±0.8
October	3.2 ±2.3	2.9 ±1.4	3.5 ±1.5	3.4 ±1.2	2.8 ±0.9	3.6 ±1.2	1.9 ±0.9	1.9 ±0.7	4.1 ±1.0	3.6 ±1.4	2.4 ±0.9	2.1 ±1.0
Month	<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas		<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas		<i>F. arundinacea</i> Pandur		<i>F. arundinacea</i> Parnas	
	1	2	1	2	1	2	1	2	1	2	1	2
May	1.5 ±0.7	0.8 ±0.6	1.3 ±0.5	1.6 ±0.4	0.6 ±0.4	0.4 ±0.2	1.6 ±0.6	1.3 ±0.5	0.7 ±0.3	0.6 ±0.2	1.4 ±0.7	1.8 ±0.8
	1.1 ±0.5	2.1 ±1.2	1.9 ±0.9	1.2 ±0.7	1.8 ±0.6	2.2 ±0.7	1.5 ±0.8	1.3 ±0.5	2.3 ±0.6	2.9 ±1.8	2.5 ±1.0	2.3 ±0.8

Note: The mean values include 8 individual values. 1 pure sward 2. mixed sward.

mixed swards). The yield components in *Festuca arundinacea* were not influenced by the way of sward use.

2. Between *Dactylis* cultivars as well as between *Festuca* cultivars significant and very significant differences were identified.

3. In autumn, the numbers and dry weights of living tillers decrease in both species and their cultivars, and the tillering process does not differ significantly in the way of sward use.

4. The losses of nonliving material in both grasses are greater in autumn and, with some exceptions, there are no differences between the hayfield and the pasture.

5. The behaviour of *Trifolium repens* species grown in mixture with grasses is different in spring and in autumn.

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LA CROISSANCE DE LA SURFACE FOLIAIRE CHEZ DEUX CRUS: ALIGOTÉ ET FETEASCĂ NEAGRĂ

PAR
ANCA ANTOHE

In the ecological conditions from northeastern Romania (Iasi county) the author studied the growth of the foliage surface of two group varieties: Aligoté — native from France and Fetească Neagră — native from Romania.

The growth rate of the foliage surface and of the maturity of leaves reflects the biological differences between the two varieties and the eco-physiological features of the Romanian variety.

La variation des conditions climatiques imprime un caractère spécifique à la croissance et au développement de la vigne. Parmi les facteurs qui limitent la croissance végétative et le rythme de développement et de maturation de cette plante il faut mentionner la température et la longueur de la journée.

En Roumanie, les études concernant la croissance de la surface foliaire de la vigne ont révélé certains aspects relatifs à la surface foliaire sur une plante [4], [5], [7], sur un drageon [2], [3] ou l'accumulation de substance sèche, glucides, acides aminés, etc. dans les feuilles [4], [7].

Dans les conditions écologiques spécifiques au nord-est de la Roumanie (département de Iași) on n'a pas entrepris jusqu'ici de pareilles recherches. Nous avons observé la croissance de la surface foliaire chez deux crus de vigne: Aligoté — cru français — et Fetească neagră — cru autochtone, tous les deux prépondérants dans la culture de la vigne de cette région.

MATÉRIEL ET MÉTHODE

L'étude s'est déroulée dans le cadre de la Station expérimentale hortiviticole Iași.

Les parcelles expérimentales sont situées sur la terrasse supérieure de la rivière Bahlui, à une altitude de 175 m, sur un versant ayant une inclination de 7—8°, exposé au sud-ouest.

Le sol est un tchernoziom lévigé et carbonaté à réaction neutre et faiblement alcaline. Les recherches se sont déroulées dans des conditions identiques d'expérimentation entre 1974 et 1977.

Le climat est tempéré-continentale à nuances excessives. La moyenne annuelle de la température de l'air est de 9°7 C, la moyenne des maxima au mois de juillet atteint 21°4 C et la moyenne des minima au mois de janvier atteint —3°5 C.

Le minimum thermique absolu est descendu jusqu'à $-32^{\circ}6$ C et le maximum absolu a enregistré $40^{\circ}0$ C.

La quantité moyenne annuelle de précipitations atmosphériques est de 515 mm; au printemps on enregistre 132,8 mm et en été 190,2 mm.

La dynamique de l'atmosphère se caractérise par la prédominance des masses d'air qui se déplacent du nord et du nord-ouest de l'Europe. Les mois à la plus grande fréquence des vents sont février et mars.

Nous avons employé comme matériel expérimental deux crus de vigne : Aligoté et Fetească neagră. Nous avons travaillé sur trois catégories différentes de drageons selon la position qu'ils occupaient sur le sarment : les drageons de la base, du milieu et du bout.

La croissance de la surface foliaire sur les drageons a été déterminée en trassant le contour de chaque feuille à part et elle a été exprimée en cm^2 .

Les échantillons ont été pris le 10 mai, le 10 juin, le 10 juillet et le 10 octobre.

Les indices physiologiques des mêmes feuilles (le poids vif, la substance sèche et l'eau) ont été déterminés comme suit : le poids vif de la feuille a été établi avec la balance analytique; la substance sèche a été déterminée en séchant la feuille à la température de 105°C et en la pesant plusieurs fois jusqu'à ce qu'on ait obtenu un poids constant; l'eau de la feuille a été calculée en faisant la différence entre le poids vif de la feuille et sa substance sèche. Lorsqu'on a représenté graphiquement la substance sèche et l'eau, chaque feuille a été considérée unité (100) et on a fait le pourcentage respectif.

L'intensité de la croissance des feuilles a été calculée en partageant la valeur de la surface foliaire au nombre de jours compris entre les deux déterminations respectives.

RÉSULTATS ET DISCUSSIONS

La croissance des feuilles et de la surface foliaire des drageons de la base du sarment (fig. 1 et 2) se produit selon une courbe en cloche qui se trouve en corrélation avec celle de la croissance en longueur des drageons.

Les internœuds avec la plus grande croissance en longueur correspondent aux feuilles qui ont le limbe foliaire avec la plus grande surface.

Chez les deux crus, les feuilles des nœuds 6–15 présentent les plus intenses croissances; chez le cru Fetească neagră les surfaces foliaires réalisées sont plus grandes.

Les feuilles de la base des drageons (1–6) ont la plus grande croissance au mois de mai; les feuilles des nœuds 6–18 atteignent la croissance maxima au moins de juin. Vers le bout des drageons, la croissance maxima a été trouvée au mois de juillet ou d'août; l'intensité de la croissance est beaucoup plus réduite et les surfaces foliaires réalisées de plus en plus petites, ce qui s'explique par leur apparition tardive sur les drageons.

L'intensité de la croissance des feuilles varie selon la position sur le drageon. On observe des valeurs accrues de l'intensité de croissance en commençant avec la 4^e ou la 5^e feuille jusqu'aux feuilles 14–15, ensuite elle diminue.

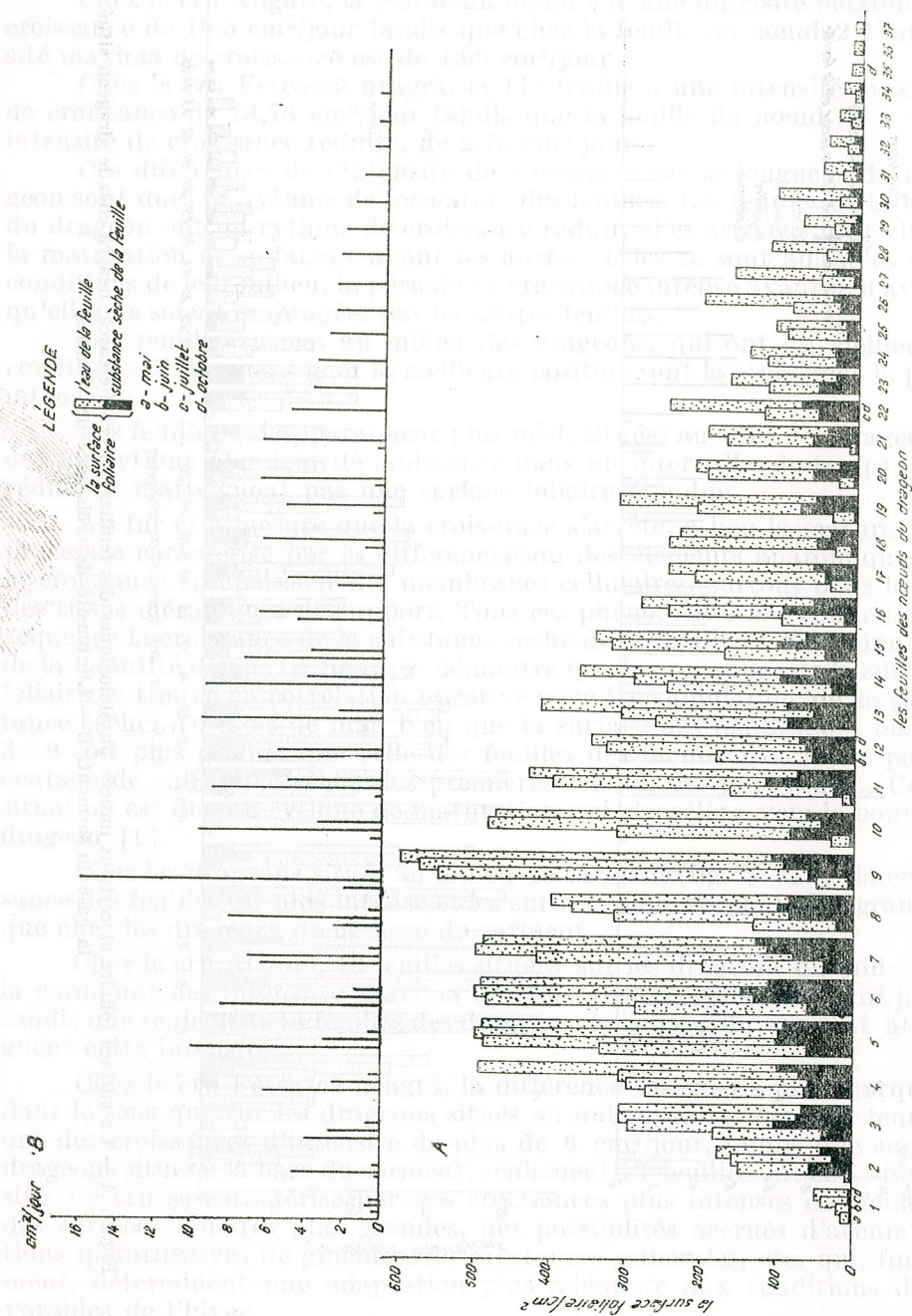


Fig. 1. A — La surface foliaire et certains indices physiologiques des feuilles du cru Aligoté chez le drageon situé à la base du sarment. B — L'intensité de croissance des feuilles.

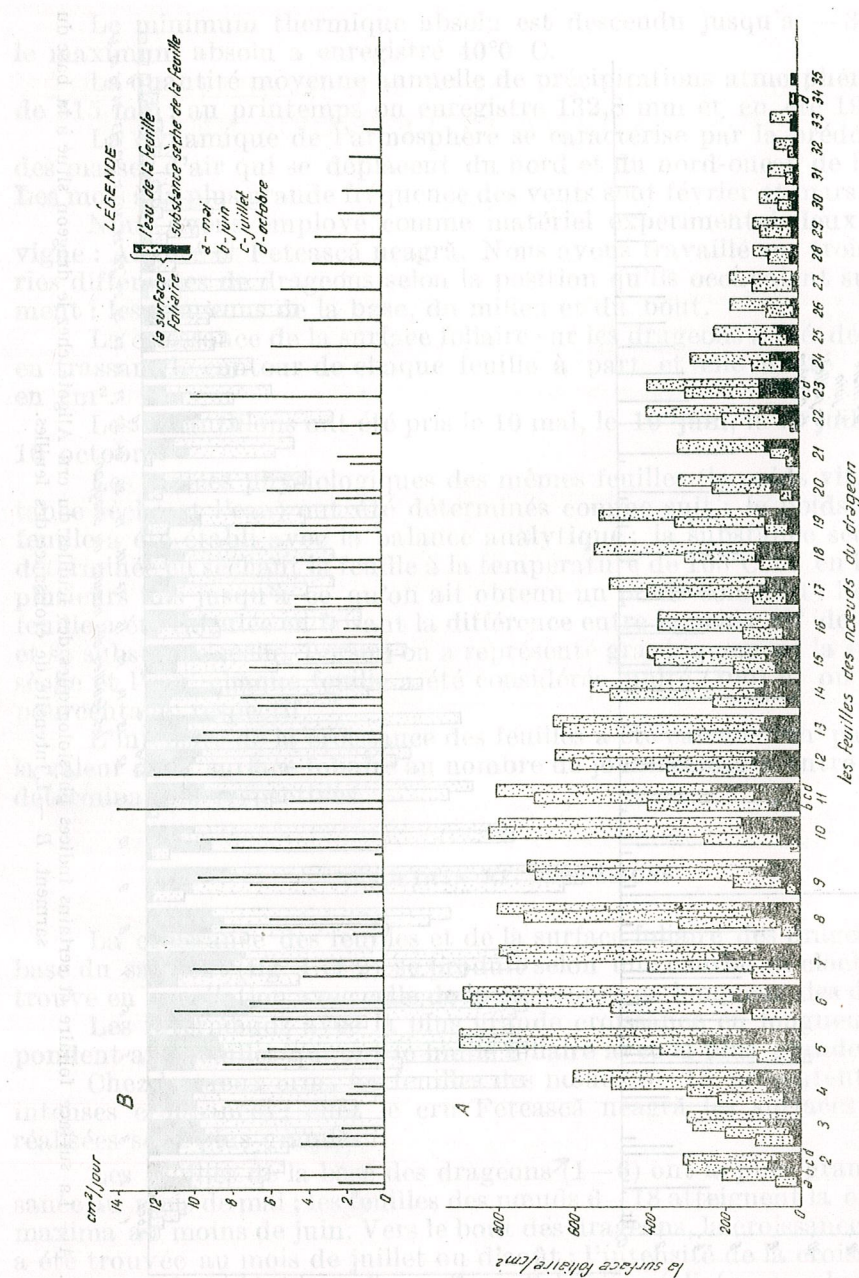


Fig. 2. A — La surface foliaire et certains indices physiologiques des feuilles du cru Fetească neagră chez le drageon situé à la base du sarment. B — L'intensité de croissance des feuilles.

Chez le cru Aligoté, la feuille du nœud 9 a une intensité maxima de croissance de $16,5 \text{ cm}^2/\text{jour}$ tandis que chez la feuille du nœud 2 l'intensité maxima de croissance est de $4,96 \text{ cm}^2/\text{jour}$.

Chez le cru Fetească neagră, la 11^e feuille a une intensité maxima de croissance de $13,73 \text{ cm}^2/\text{jour}$ tandis que la feuille du nœud 30 a une intensité de croissance réduite, de $2,75 \text{ cm}^2/\text{jour}$.

Ces différences de l'intensité de croissance sur la longueur du drageon sont dues au rythme de formation des feuilles. Les feuilles de la base du drageon ont un rythme de croissance réduit, elles arrivent plus vite à la maturation et se fanent avant les autres. Elles se sont adaptées aux conditions de leur milieu, la période de croissance intense ayant lieu avant qu'elles ne soient ombragées par les autres feuilles.

Les feuilles situées au milieu des drageons, qui ont les meilleures conditions d'illumination et la meilleure position, ont la croissance la plus intense.

Les feuilles qui apparaissent plus tard, situées au bout des drageons, ont un rythme plus lent de croissance dans un intervalle de temps plus réduit et n'atteignent pas une surface foliaire étendue.

Au fur et à mesure que la croissance s'arrête, a lieu la maturation, processus caractérisé par la différenciation des éléments anatomiques et la croissance en épaisseur des membranes cellulaires, surtout dans le cas des tissus mécaniques de support. Tous ces phénomènes ont comme conséquence la croissance de la substance sèche de la feuille et la diminution de la quantité d'eau. La figure 2 démontre que la croissance de la surface foliaire se trouve en corrélation négative avec l'accumulation de la substance sèche. Au mois de mai, bien que la surface des feuilles des nœuds 3—9 soit plus grande que celle des feuilles des nœuds 16—20, le pourcentage de substance sèche des premières feuilles est plus réduit. Cette situation est due au rythme de maturation qui s'accélère vers le bout du drageon [1].

Chez les drageons situés au milieu du sarment (fig. 3 et 4) la croissance des feuilles est plus intense et les surfaces foliaires sont plus grandes que chez les drageons de la base du sarment.

Chez le cru Aligoté, 18 feuilles situées sur les drageons du milieu de la corde ont des intensités maxima de croissance de plus de $6 \text{ cm}^2/\text{jour}$, tandis que seulement 14 feuilles des drageons de la base du sarment atteignent cette intensité.

Chez le cru Fetească neagră, la différence est encore plus marquée, dans le sens que sur les drageons situés au milieu du sarment 24 feuilles ont des croissances d'intensité de plus de $6 \text{ cm}^2/\text{jour}$, tandis que sur les drageons situés à la base du sarment, seulement 13 feuilles ont cette intensité. Ce cru se caractérise par des croissances plus intenses des feuilles, des surfaces foliaires plus grandes, des possibilités accrues d'accumulations quantitatives de glucides, de substances minérales, etc. qui, finalement, déterminent une adaptation plus adéquate aux conditions défavorables de l'hiver.

A mesure que les feuilles arrivent à la maturation, leur substance sèche augmente. Ce phénomène se trouve en corrélation négative avec la croissance de la surface foliaire.

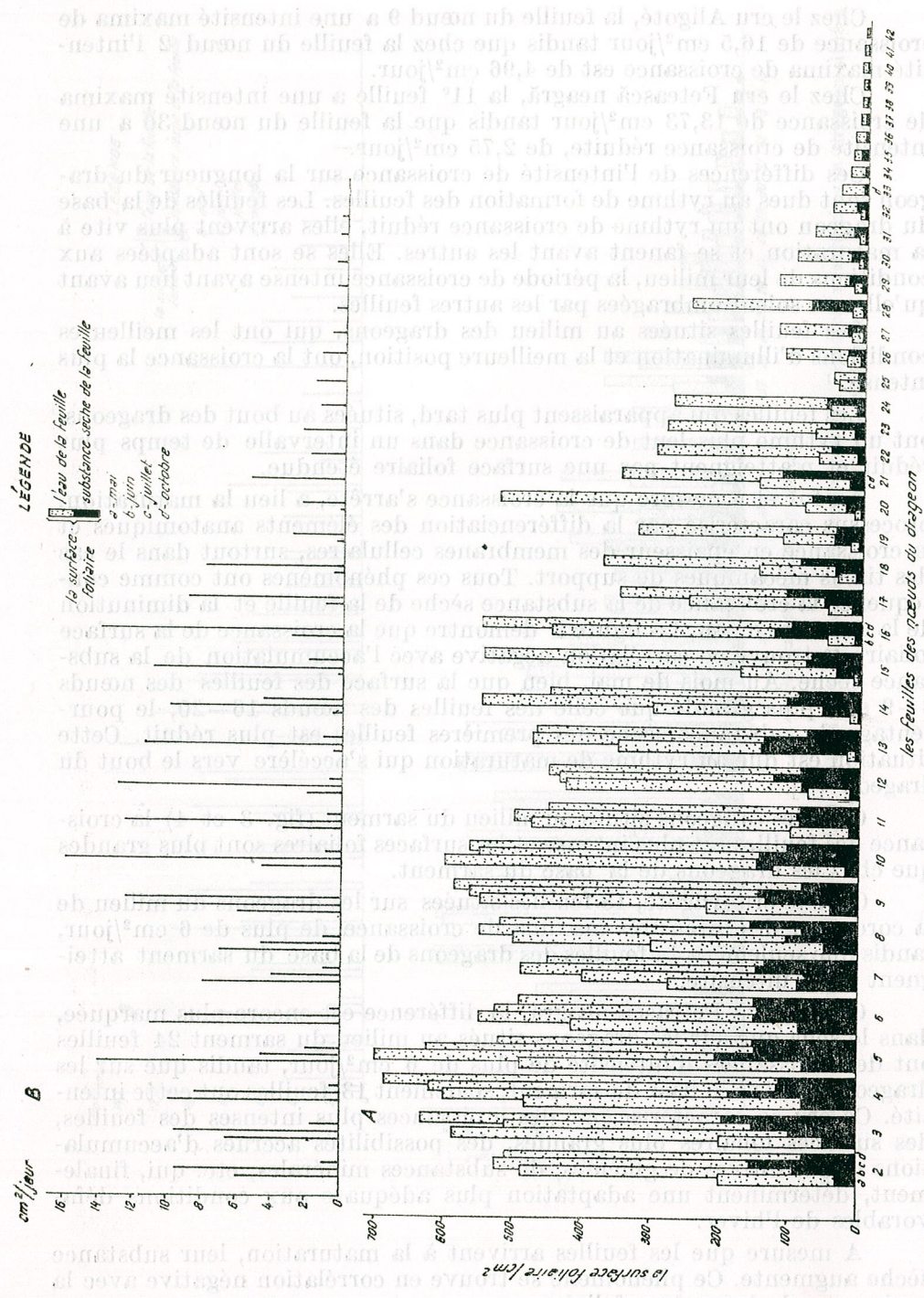


Fig. 3. A — La surface foliaire et certains indices physiologiques des feuilles du cru Aligoté chez le dragon situé au milieu du sarment. B — L'intensité de croissance des feuilles.

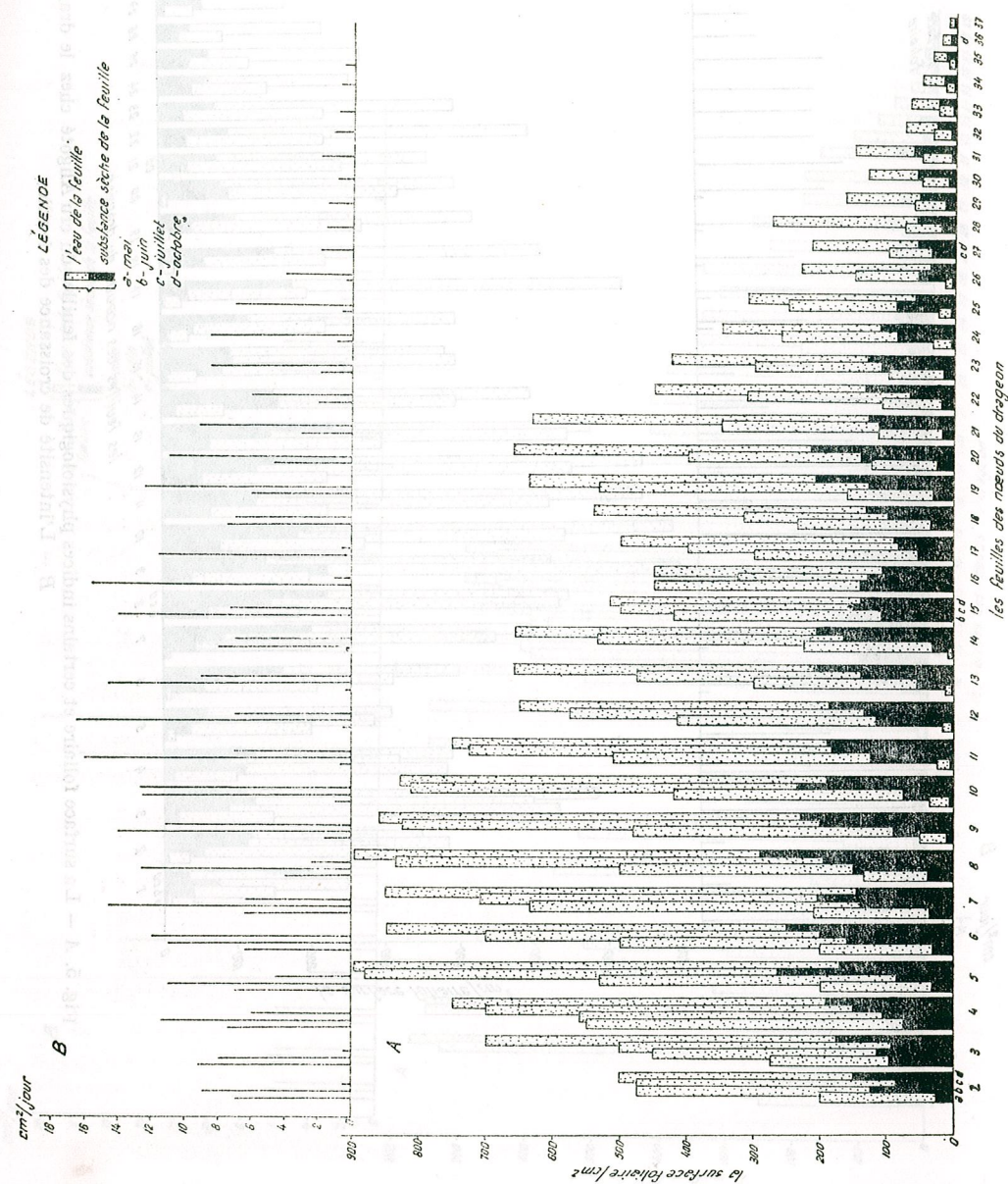


Fig. 4. A — La surface foliaire et certains indices physiologiques des feuilles du cru Aligoté chez le dragon situé au milieu du sarment. B — L'intensité de croissance des feuilles.

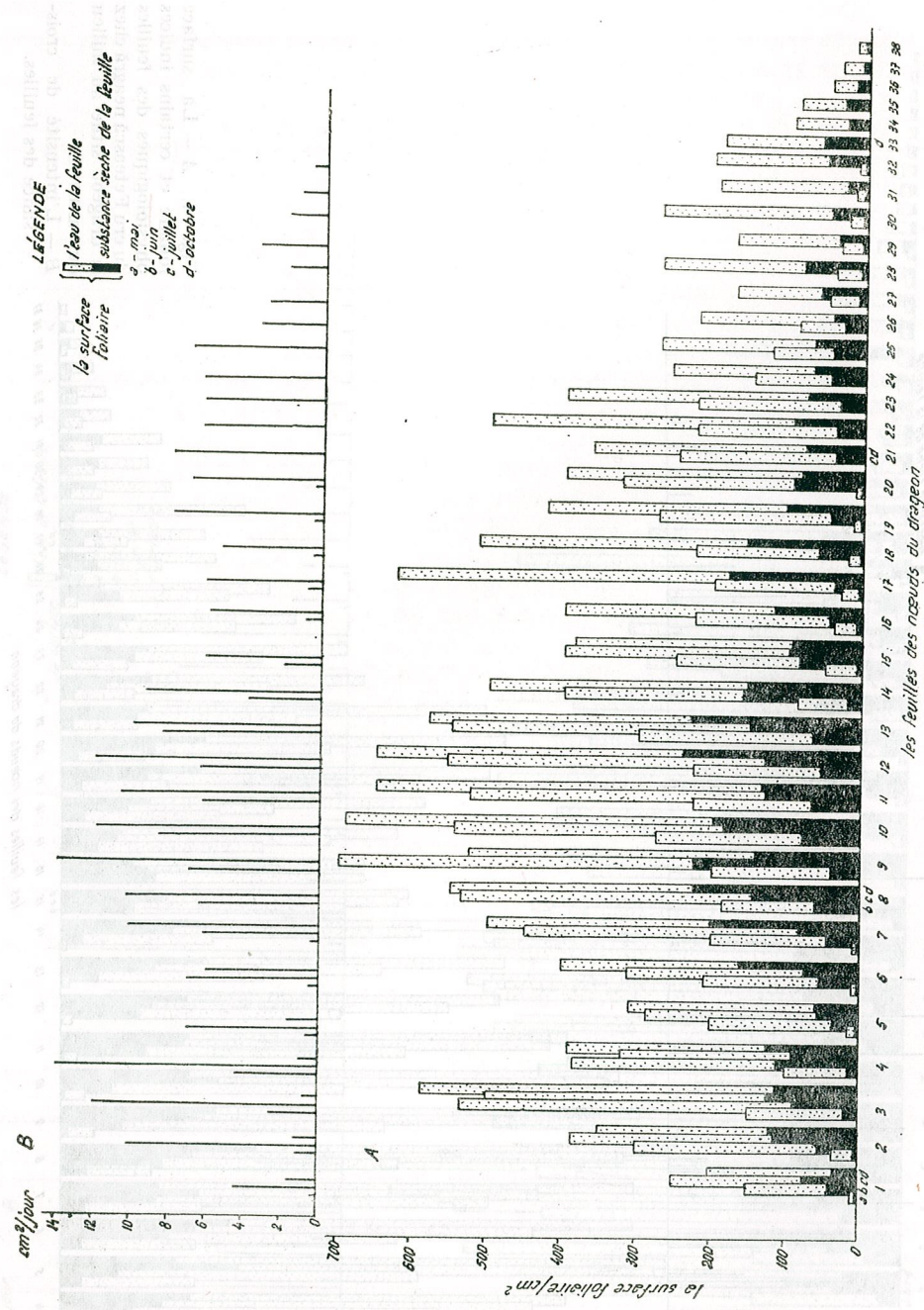


Fig. 5. A — La surface foliaire et certains indices physiologiques des feuilles du cru Aligoté chez le dragon du bout du sarment.
B — L'intensité de croissance des feuilles.

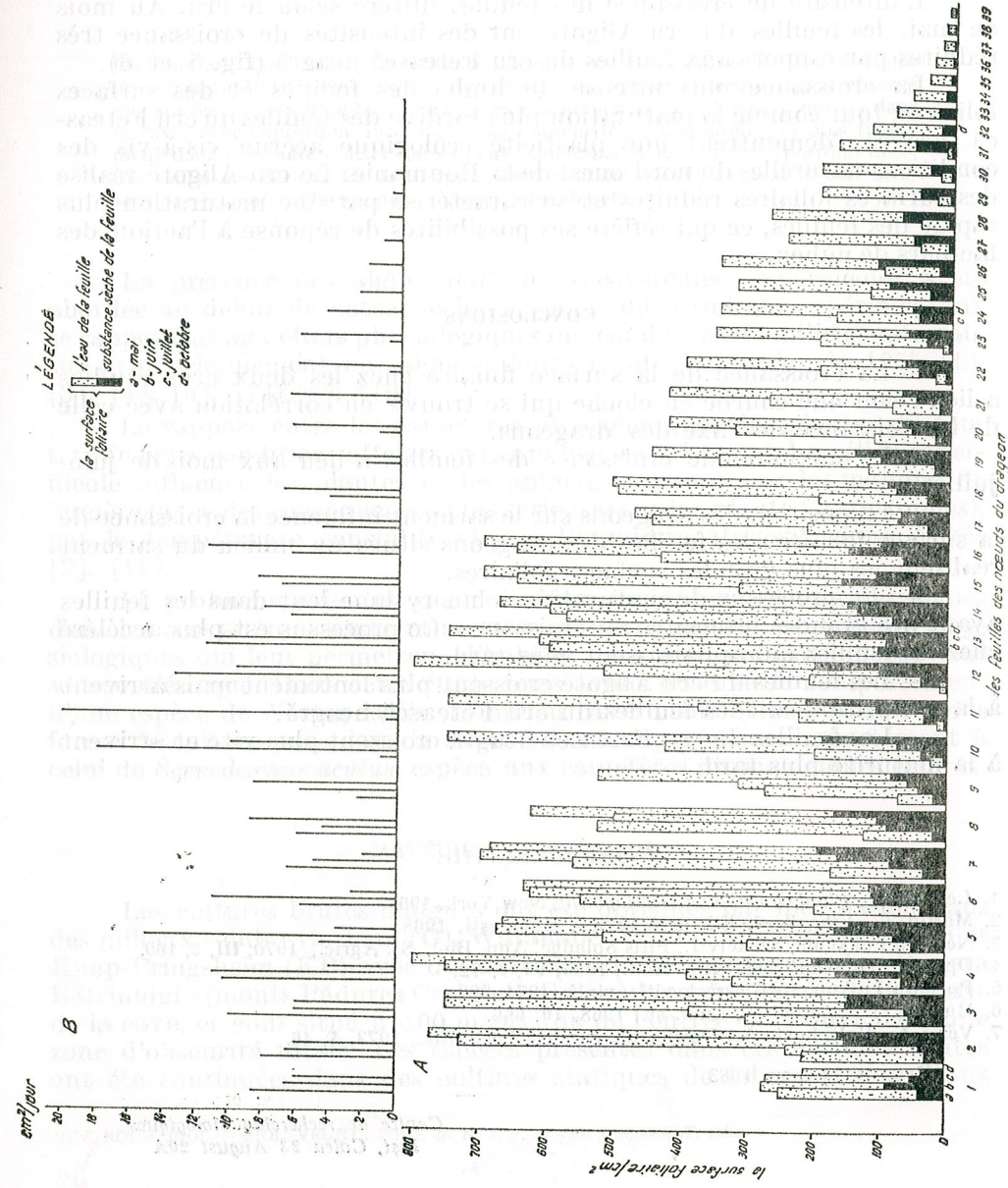


Fig. 6. A — La surface foliaire et certains indices physiologiques des feuilles du cru Fetească neagră chez le dragon du bout du sarment.
B — L'intensité de croissance des feuilles.

Les feuilles des drageons du bout du sarment (fig. 5 et 6) présentent les mêmes caractéristiques de croissance durant une période de végétation : la courbe de croissance de la surface foliaire est une courbe en cloche, les feuilles situées à la base et au bout des drageons ont des croissances réduites, la plus grande surface foliaire est réalisée par les feuilles 7-19 (cru Aligoté) et 5-21 (cru Fetească neagră).

L'intensité maxima de croissance des feuilles a lieu au mois de juin : chez les feuilles 1-8 pour le cru Aligoté et chez les feuilles 5-17 pour le cru Fetească neagră.

L'intensité de croissance des feuilles diffère selon le cru. Au mois de mai, les feuilles du cru Aligoté ont des intensités de croissance très réduites par rapport aux feuilles du cru Fetească neagră (fig. 5 et 6).

La croissance plus intense du limbe des feuilles et des surfaces foliaires, tout comme la maturation plus tardive des feuilles du cru Fetească neagră, démontrent une plasticité écologique accrue vis-à-vis des conditions naturelles du nord-ouest de la Roumanie. Le cru Aligoté réalise des surfaces foliaires réduites et se caractérise par une maturation plus rapide des feuilles, ce qui reflète ses possibilités de réponse à l'action des facteurs de milieu.

CONCLUSIONS

— La croissance de la surface foliaire chez les deux crus étudiés a lieu selon une courbe en cloche qui se trouve en corrélation avec celle de la croissance de l'axe des drageons.

— Le maximum de croissance des feuilles a lieu aux mois de juin-juillet.

— La position des drageons sur le sarment influence la croissance de la surface foliaire : les feuilles des drageons situés au milieu du sarment réalisent les plus grandes surfaces foliaires.

— Le processus de maturation a un rythme lent dans les feuilles ayant des grandes intensités de croissance. Ce processus est plus accéléré chez les feuilles qui apparaissent plus tard.

— Les feuilles du cru Aligoté croissent plus lentement mais arrivent à la maturité avant les feuilles du cru Fetească neagră.

— Les feuilles du cru Fetească neagră croissent plus vite et arrivent à la maturité plus tard.

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LA CROISSANCE ET LA MULTIPLICATION DES ALGUES *NOSTOC* SP. ET *SCENEDESMUS ACUTUS* MEYEN DANS LE MILIEU CAVERNICOLE

PAR

V. CRISTEA

En étudiant l'action des conditions cavernicoles sur l'espèce de *Nostoc* isolée de « Peștera Bătrînului » comparativement à *Scenedesmus acutus*, on a observé que sur les milieux liquides inorganiques ces algues se développent mieux dans la cave, avec exposition libre, que dans l'obscurité de laboratoire, et plus faiblement dans une boîte d'acier dans la cave que dans la cave avec exposition libre. Les radiations bêta de faible énergie observées dans la cave pourraient être utilisées par les chloroplastes pour exercer leurs fonctions.

La présence des algues dans les écosystèmes cavernicoles a été abordée au début de notre siècle [6], mais des résultats expérimentaux se rapportant aux effets physiologiques des conditions du milieu souterrain sur le développement des algues n'ont été obtenus qu'après 1955 [1], [3], [4], [5], [7], [8], [10].

Le rapport entre les caractères physiologiques des plantes du souterrain et les conditions offertes par ce milieu est saisissant. Le milieu cavernicole influence les plantes et les animaux surtout par le manque de lumière (avec des conséquences plus sérieuses sur les plantes autotrophes), par la température constante et par l'humidité atmosphérique élevée [2], [11].

Le problème essentiel qui doit être élucidé par l'étude des algues dans les écosystèmes souterrains est représenté par les mécanismes physiologiques qui leur permettent d'habiter dans ces conditions et par leur source d'énergie. Dans cette étude nous avons observé le comportement d'une espèce de *Nostoc*, isolée du milieu cavernicole et réintroduite sous forme de culture pure dans la cave. On a comparé son comportement à celui de *Scenedesmus acutus*, espèce aux caractères physiologiques connus.

MATÉRIEL ET MÉTHODE

Les cultures brutes d'algues ont été obtenues par inoculation dans des milieux solides Gorham (G), Watanabe (WAT), Allen-Arnold (AA) et Knop-Pringsheim (KP) avec du matériel provenant d'un gour de « Peștera Bătrînului » (monts Pădurea Craiului). Grâce à la conformation de la galerie de la cave, ce gour situé à 100 m environ de l'entrée se trouve dans une zone d'obscurité totale. Les espèces présentes dans ces cultures brutes ont été continuées dans des cultures statiques de laboratoire sur/dans

des milieux solides et liquides. De ces cultures on a récolté *Nostoc* sp., employé dans notre expérience.

Pour bien observer l'action des conditions cavernicoles sur les espèces *Nostoc* sp. et *Scenedesmus acutus* on a réalisé des cultures pures de ces algues sur deux types de milieux liquides : Kuhl-Lorenzen (KL) et Benecke avec K_2HPO_4 (BK₂) [9].

Les variantes expérimentales proposées pour l'étude ont été :

1. dans la lumière diffuse de laboratoire (LL) ;
2. dans l'obscurité de laboratoire (dans la boîte phototropique) (LI) ;
3. dans la cave, avec exposition libre (PF) ;
4. dans la cave, dans une boîte d'acier inoxydable, avec un système d'aération (PB). Cette variante a été effectuée pour écraner les faibles radiations α et β , qui pouvaient se trouver dans la cave.

L'expérience a été faite dans « Peștera cu Apă din Dealul Cornilor » (mont Pădurea Craiului). Dans la station où l'on a fixé les éprouvettes on a enregistré une température de 10°C et dans le laboratoire les valeurs moyennes quotidiennes ont été de 12–18°C ; 14 jours après, on a comparé les variantes expérimentales de la cave à celles de laboratoire. On a observé le développement des algues par la détermination des extinctions des suspensions algales. On a déterminé aussi l'activité spécifique (As) de la roche et des fragments de stalactite provenant de la cave. L'activité spécifique a été déterminée au moyen d'un spectromètre de scintillation dans le liquide type BETASZINT 5003. C'est un appareil à trois canaux énergétiques. Le canal A, de bande énergétique large, enregistre dans l'intervalle 1–1000 Kev, le canal B enregistre dans l'intervalle 100–750 Kev, correspondant généralement à ^{14}C et le canal C dans l'intervalle 20–350 Kev, correspondant à 3T .

RÉSULTATS ET DISCUSSIONS

Du matériel récolté de « Peștera Bătrînului » on a isolé deux types de *Clorococcaceae* et une forme de *Cyanophycée* qui appartient à la famille *Nostocaceae* (Pl. I).

L'action du milieu cavernicole en comparaison des conditions de laboratoire est présentée dans la figure 1. Comme on le voit dans cette figure, les variantes qui se sont développées dans l'obscurité naturelle ou artificielle ont eu une évolution plus faible que l'épreuve témoin dans la lumière diffuse. Mais il est à remarquer que toutes les deux algues dans les cultures sur les milieux inorganiques, à l'exception de *Scenedesmus* dans le milieu BK₂ dans la cave avec exposition libre, présentent une meilleure évolution que dans la boîte phototropique. Par calcul statistique on a établi que les différences sont significatives. Cette évolution n'a pas été déterminée par une illumination accidentelle des cultures dans la cave, parce que les plaques photosensibles exposées à côté n'ont pas été impressionnées.

Les cultures d'algues dans la boîte d'acier inoxydable de la cave n'ont pas présenté une évolution plus importante que dans le laboratoire à l'obscurité à l'exception de *Nostoc* sp. sur milieu KL. En examinant l'évolution des deux variantes de cultures de la cave et en les comparant à celles de la boîte phototropique, il résulte que dans la cave il y a un

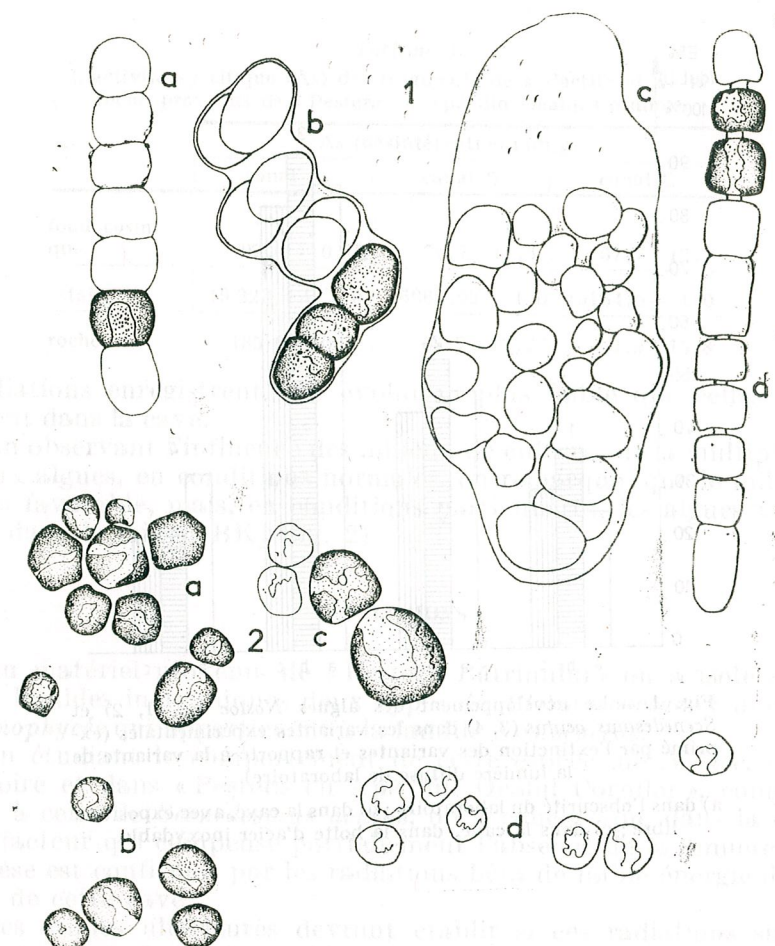


Planche I. *Nostoc* sp. et *Chlorella* sp. isolées des cultures brutes provenant de « Peștera Bătrînului ».

1. *Nostoc* sp. : a) trichome et b) thalle, développés en milieu solide Myers ; c) thalle et d) trichome, développés en milieu solide Benecke avec K_2HPO_4 ;
2. *Chlorella* sp. : a) et b) développée en milieu solide Knop-Pringsheim ; c) et d), développée en milieu solide Benecke avec K_2HPO_4 .

facteur qui permet le développement des algues en remplaçant dans une certaine mesure le manque de lumière et qui est écrané évidemment par la boîte d'acier inoxydable.

Par les analyses de radioactivité sur les épreuves de stalactite de la zone d'exposition des cultures (tableau 1), on a constaté qu'il a effectivement des radiations bêta de faible énergie, mais qui, lors de leur pénétration de l'atmosphère dans le milieu de culture liquide et même de leur transfert du milieu de culture dans le protoplasme cellulaire produisent, par le phénomène de scintillation, une luminescence réduite mais probablement suffisante pour que la photosynthèse ait lieu. Les cultures placées dans la cave, dans la boîte d'acier inoxydable, étant privées de

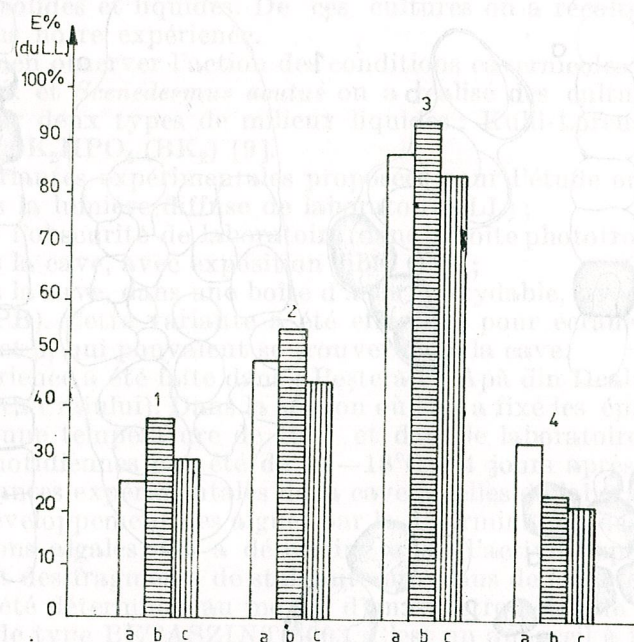


Fig. 1. — Le développement des algues *Nostoc* sp. (1, 2) et *Scenedesmus acutus* (3, 4) dans les variantes expérimentales (exprimé par l'extinction des variantes et rapporté à la variante de la lumière diffuse de laboratoire).

a) dans l'obscurité du laboratoire ; b) dans la cave, avec exposition libre ; c) dans la cave, dans la boîte d'acier inoxydable.

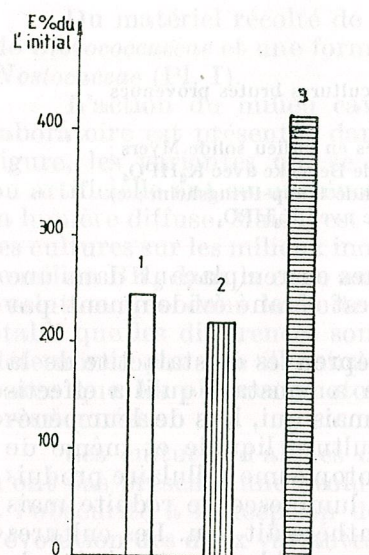


Fig. 2. — L'influence des milieux de culture sur le développement des algues *Nostoc* sp. et *Scenedesmus acutus*, dans la variante de la lumière diffuse de laboratoire.

1) *Nostoc* sp. sur le milieu liquide Kuhl-Lorenzen ; 2) *Nostoc* sp. sur le milieu liquide Benecke avec K_2HPO_4 ; 3) *Scenedesmus acutus* sur le milieu liquide Benecke avec K_2HPO_4 .

Tableau 1

L'activité spécifique (As) des fragments de stalactite et de la roche provenus de « Peștera cu Apă din Dealul Cornilor »

	As (désintégration/min/g)		
	canal A	canal B	canal C
fond cosmique	97,0 ± 10,1	76,7 ± 11,4	67,7 ± 12,1
stalactite	19 229,4 ± 1,0	16960,99 ± 1,0	15164,5 ± 1,0
roche	185,0 ± 10,8	66,7 ± 12,2	44,2 ± 15,8

ces radiations enregistrent une évolution plus faible que celles placées librement dans la cave.

En observant l'influence des milieux de culture sur la multiplication des deux algues, en conditions normales, on remarque que le milieu KL est plus favorable, mais, en conditions particulières, les algues végètent mieux dans le milieu BK₂ (fig. 2).

CONCLUSIONS

Du matériel provenant de « Peștera Bătrînului » on a isolé sur des milieux liquides inorganiques deux types de *Clorococcaceae* et une forme de *Cyanophycée* qui appartient à la famille *Nostocaceae*.

En étudiant le comportement de cette espèce de *Nostoc*, dans le laboratoire et dans « Peștera cu Apă din Dealul Cornilor », comparativement à celui de *Scenedesmus acutus*, on a conclu que dans la cave il y a un facteur qui compense partiellement l'absence de la lumière. Cette hypothèse est confirmée par les radiations bêta de faible énergie des stalactites de cette cave.

Des études ultérieures devront établir si ces radiations suffisent pour le processus de photosynthèse.

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LA VARIABILITÉ INDIVIDUELLE DE QUELQUES PROVENANCES DIFFÉRENTES DE *PAPAVER* *SOMNIFERUM* L., DANS DES CONDITIONS DE MILIEU SIMILAIRES

PAR

ION I. BĂRA, ELVIRA GILLE, GEORGETA PINZARU
et CONSTANTIN GRIGORESCU

Some varieties of poppy were investigated for morphological and biochemical traits.

All varieties were grown in similar environment and reproduced both by self-pollination and free pollination.

The morphological data were analysed by statistical methods and correlated with morphine production.

Le pavot, qui a été cultivé au moins depuis le néolithique [6], a été soumis aux pressions continues et diversifiées de la sélection artificielle.

Compte tenu de son aire de culture et de l'intérêt qui a dynamisé l'activité des sélectionneurs (ornement, aliment, médecine), les recherches de ceux-ci visant à l'amélioration du pavot ont eu des buts différents.

Par conséquent, les diverses cultovariétés, lignes ou variétés de pavot se sont bien adaptées aux conditions concrètes du milieu où elles ont été créées et ont vécu de longues périodes, ce processus d'adaptation étant semblable à celui d'autres groupes taxonomiques similaires de plantes ou d'animaux subissant l'influence de l'activité humaine de sélection et d'amélioration.

Comme le programme de recherches de notre laboratoire comprend aussi la connaissance et la caractérisation les plus complètes de la biologie de *Papaver somniferum* L., nous avons trouvé opportun d'étudier un grand nombre de variantes des plus différentes provenances, afin de trouver les méthodes les plus adéquates pour l'amélioration de ses qualités bioproductives.

Pour commencer nous nous sommes proposé de constater s'il y avait des différences morpho-anatomiques marquantes entre les diverses variantes de pavot, et ensuite si ces différences se maintenaient longtemps au cas où la culture était effectuée dans les mêmes conditions de milieu.

On pouvait ainsi établir si les dissemblances entre les variantes, cultivées au début dans des aires différentes, étaient dues au génotype ou bien aux facteurs de milieu (autrement dit, si ces dissemblances étaient une expression de la norme de réaction individuelle, une résultante de l'interaction génotype-milieu).

Parallèlement, nous nous sommes proposé d'étudier la stabilité de certains caractères dans les mêmes conditions de milieu, sous l'influence de l'autofécondation et de la pollinisation naturelle (libre).

Le dernier but de nos recherches a été l'hybridation entre diverses cultovariétés; lignes et variétés pour obtenir un matériel à précocité, résistance et productivité accrues.

MATÉRIEL ET MÉTHODES DE TRAVAIL

Les variantes utilisées dans l'expérimentation déclenchée en 1981 dans le champ expérimental de Secuieni, près de Roman, dans le département de Neamț, sont de provenances extrêmement différentes.

Une des variantes représente un matériel obtenu de l'université de Kichinev; d'autres ont été créées à partir des cultovariétés Kapeczky, Jemtchouzhnyi, Eckendorf, Modoroneez et des hybrides de celles-ci. Il y a aussi un cultivar autochtone proposé pour un brevet (« Mureș ») et d'autres variantes, créées à partir de certaines provenances propres, se trouvant depuis quelques années sous observation pour leur teneur accrue en alcaloïdes.

Les investigations visant à établir les différences entre les variantes mentionnées (tableau 1) ont porté sur les paramètres morphologiques (les dimensions des capsules: hauteur, diamètre et nombre de rayons stigmatiques) et biochimiques (teneur en alcaloïdes).

Les valeurs des paramètres morphologiques ont été établies par l'évaluation statistique des données selon la méthode préconisée par I. Tudose [7], et la teneur en morphine a été établie par la méthode Gyéresy et Rácz [1].

Au moyen des calculs statistiques on a établi la moyenne de la hauteur, du diamètre et du nombre des rayons stigmatiques des capsules ($\bar{x}$), la déviation de la moyenne calculée de la moyenne idéale ($s\bar{x}$), la déviation standard individuelle (s), le coefficient de variabilité ($s\%$) et les corrélations (r) entre la hauteur et le diamètre de la capsule, ainsi qu'entre le diamètre et le nombre des rayons stigmatiques.

On a récolté aussi, pour la génération à suivre, deux catégories de semences de chaque variante: une provenant des capsules de tous les individus d'une même variante qui ont bénéficié de pollinisation naturelle et l'autre provenant des individus isolés et autofécondés (les semences étant récoltées de chaque individu autofécondé séparément).

LES CONDITIONS PÉDO-CLIMATIQUES DU CHAMP EXPÉRIMENTAL

Au cours de l'année agricole 1980—1981, l'expérience a eu lieu sur un tchernoziom typique ayant les caractéristiques suivantes: pH (en eau) —6,4; la teneur en humus 2,8%; P_2O_5 3,9 mg/100 g sol; et K_2O mobile 23,6 mg/100 g sol. Le tchernoziom typique de Secuieni fait partie du groupe des sols peu acides, pauvres en phosphore mobile et riches en potassium mobile.

Sous rapport climatique, l'année agricole 1980—1981, assez instable a été caractérisée par un hiver à faibles précipitations, par un printemps

Tableau 1

La variante	Le caractère étudié										La teneur en morphine %		
	L'hauteur de la capsule					Le diamètre de la capsule							
	$\bar{x}$	$\pm$	$s\bar{x}$	s	s %	r	$\bar{x}$	$\pm$	$s\bar{x}$	s		s %	
Kichinev	34,00	1,67	3,74	11,00	0,82	18,40	0,86	1,94	10,54	0,12	10,00	1,00	10,00
Kapecky ♀	42,00	2,75	6,72	16,00	0,84	26,00	1,84	4,51	17,34	0,84	12,00	0,25	5,25
Jemtchouzhnyi ♂	36,00	3,20	7,82	21,72	0,94	23,00	2,90	7,09	30,82	0,74	10,33	0,80	1,96
Jemtchouzhnyi ♂	45,15	1,33	4,81	10,65	0,26	23,38	1,44	5,20	22,24	0,84	9,38	0,40	1,44
Jemtchouzhnyi ♂	44,60	1,58	3,54	7,93	0,92	24,26	1,03	2,31	9,52	0,19	10,80	0,37	7,68
Jemtchouzhnyi ♂	35,55	1,45	6,18	17,38	0,54	21,55	1,04	4,42	20,51	0,64	10,44	0,28	1,14
Jemtchouzhnyi ♂	42,44	1,39	4,40	10,36	0,38	28,15	1,53	4,85	17,22	0,60	12,90	0,54	1,72
Eckendorf ♀	36,00	2,87	7,59	21,08	0,23	27,00	1,17	3,10	11,48	0,97	11,28	0,52	1,38
Eckendorf ♀	44,78	0,91	3,53	7,88	0,04	27,72	1,49	5,77	20,81	0,28	10,06	0,29	1,16
Eckendorf ♀	46,58	1,39	5,73	12,34	0,31	25,94	1,31	5,40	20,81	0,84	11,11	0,40	1,65
Eckendorf ♀	45,37	1,24	3,50	7,71	0,21	32,12	1,65	4,67	14,53	0,50	13,37	0,37	1,06
Modoroneez ♀	34,16	1,40	3,43	10,04	0,09	20,66	2,56	6,25	30,25	0,85	9,83	1,35	3,31
Modoroneez ♀	45,50	1,40	5,24	11,51	0,67	27,50	0,91	3,41	12,40	0,47	11,71	0,30	1,13
Modoroneez ♀	29,50	2,21	4,43	15,01	0,87	16,50	1,98	3,87	23,45	0,13	8,75	0,47	0,95
Mureş	43,10	1,89	6,00	13,82	0,81	25,00	1,54	4,87	19,48	0,55	10,60	0,47	1,50
Mureş	45,26	0,98	3,53	7,79	0,65	28,96	1,16	4,21	14,53	0,77	10,92	0,23	0,86
Mureş	45,00	0,59	1,58	3,51	0,15	25,42	0,94	2,50	9,83	0,22	10,57	0,29	0,78
Mureş	45,11	2,11	6,33	14,03	0,39	26,44	1,30	3,90	14,75	0,89	10,22	0,57	1,71
Mureş	36,80	3,84	8,58	23,31	0,95	20,20	2,01	4,49	22,22	0,56	8,40	0,87	1,14
Notre matériel	33,16	1,26	6,21	18,72	0,69	25,70	1,07	5,27	20,50	0,71	11,45	0,30	1,47
Notre matériel	35,13	1,63	5,16	14,68	0,39	23,00	1,45	5,24	22,11	0,83	10,23	0,48	1,73
Notre matériel	44,46	1,51	5,44	12,23	0,72	27,46	0,80	2,90	10,56	0,11	13,46	0,40	1,45
Notre matériel	50,40	0,81	2,59	5,13	0,47	27,90	0,88	2,80	10,03	0,63	12,50	0,58	1,84
Notre matériel	36,57	0,62	2,73	7,46	0,71	33,60	1,09	4,75	14,13	0,56	11,78	0,27	1,18
Notre matériel	40,62	1,04	4,16	10,24	0,15	32,37	1,05	4,20	12,97	0,42	11,81	2,11	0,98
Notre matériel	37,35	0,73	3,29	8,80	0,54	32,05	1,18	5,31	16,56	0,43	12,00	0,20	0,91
Notre matériel	47,00	1,90	5,70	12,12	0,17	30,44	1,69	5,07	16,65	0,85	13,44	0,44	1,33
Hybride Modoroneez × Jemtchouzhnyi	42,29	1,77	5,31	12,55	0,38	24,32	0,76	2,29	9,41	0,33	10,77	0,36	1,09
Hybride Kapecky	35,52	1,03	3,57	10,05	0,50	26,89	1,02	3,55	13,20	0,83	11,27	0,34	1,19
Hybride Eckendorf × Jemtchouzhnyi	44,01	3,31	8,08	18,35	0,79	27,93	1,02	7,37	26,38	0,91	11,16	0,54	1,32
													11,82
													0,43—0,19

Tableau 21
Les conditions climatiques de l'année agricole 1980-1981

	Mois												Annuellement
	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	
	10	15	15	13	10	12	12	15	11	10	13	5	
Nombre des jours à précipitations	18,6	23,8	17,6	8,5	0,1	4,2	2,7	72,4	—	24,1	—	0,3	8
Première décade	25,0	8,6	9,4	8,4	8,0	23,0	22,8	17,6	26,4	9,6	8,5	63,0	—
Deuxième décade	11,7	5,4	22,1	2,9	0,2	2,5	19,4	20,0	34,5	18,5	15,7	3,0	558,5
Troisième décade	55,3	37,8	49,1	19,8	8,3	29,7	44,9	110,0	60,9	52,2	24,2	66,3	529,0
Total	36,8	32,6	26,8	27,7	21,1	25,6	44,3	62,7	77,8	71,3	59,7	42,4	+29,5
Moyenne pour 55 années	+18,5	+5,2	+22,3	+7,9	-12,8	+4,1	+0,6	+47,3	-16,9	-19,1	-35,5	+23,9	
Déviations													
Première décade	10,0	-0,9	-6,0	-4,3	2,6	0,8	6,6	11,2	21,7	17,2	21,7	14,9	
Deuxième décade	12,6	2,7	1,2	-6,4	-3,2	4,4	6,1	13,8	18,3	20,5	19,4	12,2	
Troisième décade	7,4	4,2	1,4	-4,2	-4,5	6,4	7,8	16,8	20,4	18,6	14,8	17,0	
Moyenne mensuelle	9,8	2,0	-1,1	-4,9	-1,5	3,9	6,9	14,1	20,1	18,7	18,5	14,7	8,4
Moyenne pour 55 années	9,0	2,9	-1,9	-5,1	-3,1	2,2	8,9	14,7	18,0	19,9	19,1	14,9	8,3
Déviations	+0,8	-0,9	+0,8	+0,2	+1,6	+1,7	-2,0	-0,6	+2,1	-1,2	-0,6	+0,2	+0,1

pluvieux et froid (surtout aux mois d'avril et de mai), suivi par une grande sécheresse durant l'été. Aux mois de juin, de juillet et d'août, l'humidité du sol a baissé et s'est maintenue au-dessous du coefficient de flétrissure (tableau 2).

Ces conditions ont déterminé une production plus réduite par rapport aux autres années en ce qui concerne la plupart des plantes (médicinales).

RÉSULTATS ET DISCUSSIONS

Sur le tableau synoptique, à part la provenance Kichinev et les hybrides, il y a plusieurs variantes pour le même lot de semences.

Les données n'ont pas été cumulées, justement pour rendre plus éloquente la variabilité individuelle dans le cadre de chaque cultivar ou provenance et pour nous permettre d'écarter le plus rapidement possible, durant l'action de sélection et d'amélioration, les catégories et les individus inadéquats.

C'est pour cela que nous allons discuter tant les dissemblances entre les variantes de la même provenance, que celles entre toutes les variantes analysées.

La hauteur des capsules, élément caractérisant la bioproduktivité de n'importe quelle espèce de pavot, a révélé d'amples différences tant entre les individus d'une même variante, qu'entre les diverses variantes.

Ainsi, la variante ayant la plus grande valeur moyenne de ce paramètre ($\bar{x} = 50,40$ mm) a été représentée par des individus provenant de notre propre matériel (obtenu par sélections en masse et successives au long de cinq années d'expérimentations) où l'on a identifié aussi une variante à la valeur bien plus petite de ce paramètre ($\bar{x} = 33,16$ mm). C'est en fait la plus petite valeur de toute notre expérience, et elle s'approche des variantes de la provenance Kichinev ($\bar{x} = 34$ mm) et de la cultovariété Modoroneez ($\bar{x} = 13,16$ mm).

La différence de 17,24 mm enregistrée entre la variante à valeur maximum et celle à valeur minimum, toutes les deux faisant partie de notre propre matériel mentionné ci-dessus, démontre que le matériel obtenu n'est pas encore uniforme, que la sélection doit être encore poursuivie. Cette même différence caractérise aussi notre expérience tout entière.

On a enregistré cependant de grandes différences entre les valeurs maximums et minimums du cultivar Modoroneez ($\bar{x} = 45,56$ et $\bar{x} = 34,16$ mm), qui est assez uniforme (tout au moins en théorie) sous l'aspect des potentialités génétiques.

On peut donc affirmer que notre matériel est d'une valeur élevée et qu'une sélection de longue durée pourrait restreindre la norme de réaction populationnelle. Cette assertion est soutenue tant par la valeur réduite de la déviation de la moyenne calculée ($s\bar{x} = 0,81$ pour la meilleure variante) et des déviations standard individuelles ($s = 1,26-5,70$ mm pour notre matériel), que par la valeur entre moyenne et basse de l'indice de variabilité.

Un autre groupe de variantes d'un intérêt bien particulier pour nos recherches est celui créé à partir de la cultovariété « Mureş ». On peut remarquer que cette espèce est assez uniforme en tant que norme de

réaction et qu'elle se situe vers la limite supérieure des valeurs de toute l'expérience. Toutefois, ce cultivar est recommandable surtout pour les conditions pédo-climatiques du plateau de Transylvanie.

Les autres espèces ont eu, en dernière analyse, le même comportement que le cultivar Modoroneez.

Parmi les hybrides (où le cultivar Jemtchouzhnyi a toujours joué le rôle de partenaire-mâle) celui qui a eu le meilleur comportement a été obtenu par la combinaison avec Eckendorf, le plus faible comportement revenant à la combinaison avec Kapeczky. Mais il est à remarquer que les hybrides ne se sont pas haussés, en tant que valeur absolue, au-dessus de leurs géniteurs.

Le diamètre des capsules. Le meilleur comportement a été présenté également par une de nos variantes ($\bar{x} = 33,60$ mm), le plus faible comportement revenant à une variante du cultivar Modoroneez ($\bar{x} = 16,50$ mm). Les variantes obtenues de notre matériel se sont manifestées de façon non uniforme, étalant une norme de réaction étendue ($\bar{x} = 23,01 - 33,60$ mm, donc une différence de 10,59 mm) et prouvant par cela qu'elles ne se sont pas encore stabilisées.

D'ailleurs la même situation a été trouvée chez les cultovariétés « Mureș », Jemtchouzhnyi, Modoroneez, etc., quoique d'amplitudes bien plus réduites.

Mais, la mesure la plus exacte de l'amplitude de la variabilité individuelle nous est donnée par le coefficient de variabilité (s%) dont les valeurs sont entre moyennes et basses chez les variantes qui nous intéressent; l'assertion est soutenue par le fait que les déviations standard individuelles sont également petites. Cependant, le cultivar Modoroneez, par exemple, a eu aussi des variantes à grande variabilité (s% = 30,25).

La corrélation entre la hauteur et le diamètre des capsules a été positive, à l'exception de deux variantes seulement, ce qui démontre que la croissance d'une dimension réclame la croissance de l'autre.

Malheureusement, on n'a pas pu constater la meilleure corrélation entre les deux dimensions chez les variantes dont soit le diamètre, soit la hauteur des capsules a eu des valeurs maxima. Et c'est bien naturel, puisqu'il n'y a pas de capsules à hauteur maximum chez les mêmes variantes qui ont le plus grand diamètre. Mais, étant donné que la variabilité des deux caractères est moyenne, on peut espérer que soit leur croissance simultanée (par sélection), soit leur combinaison par hybridation (par exemple, entre les variantes 23 et 24) sera possible.

Le nombre de rayons stigmatiques. Le meilleur comportement a été constaté une fois de plus, chez les variantes créées à partir de notre matériel ($\bar{x} = 13,46$; 13,44), ainsi que chez une variante du cultivar Eckendorf ($\bar{x} = 13,37$). Le plus réduit nombre de rayons stigmatiques a été enregistré chez l'une des variantes du cultivar « Mureș ».

Les déviations moyennes et standard individuelles et surtout le coefficient de variabilité révèlent que la variabilité du nombre de rayons stigmatiques est tout au plus moyenne (à rares exceptions près).

Quoique la valeur du coefficient de corrélation soit positive (avec trois exceptions), nous n'avons pas pu constater une corrélation parfaite entre le nombre de rayons stigmatiques, le diamètre et la hauteur des

capsules. Autrement dit, la variante ayant la plus élevée valeur moyenne de la hauteur des capsules n'a pas aussi les plus hautes valeurs du diamètre et du nombre de rayons stigmatiques. L'existence d'un pareil spécimen présuppose une activité prolongée de sélection, combinée avec des croisements entre les variantes, lignes, cultivars, etc. caractérisés par l'une ou plusieurs des qualités énumérées.

Du total des observations et des données groupées jusqu'ici il résulte clairement que, en ce qui concerne les trois paramètres morphologiques étudiés, le comportement des variantes réalisées à partir de notre matériel a été très bon. Mais il faut à regret renouveler la constatation qu'il n'y a pas eu de variante à valeurs maximums pour tous les paramètres (le diamètre a été maximum chez la variante 24, la hauteur chez la variante 23, le nombre des rayons stigmatiques chez la variante 22).

Quant à la teneur en morphine, qualité d'une importance particulière pour l'appréciation de la valeur d'une variante, la situation est la suivante :

Les plus grandes valeurs ont été constatées chez les cultivars Eckendorf (0,51%) et Jemtchouzhnyi (0,51%), Kapeczky et Modoroneez ayant aussi de grandes valeurs (0,40% et 0,43%).

Les variantes obtenues à partir de notre matériel ont eu un comportement variable, la teneur en morphine variant entre 0,29% et 0,44%. La même variabilité a été constatée aussi pour les autres provenances (les cultivars étrangers et « Mureș »), démontrant que la norme de réaction individuelle reste encore assez étendue.

D'autre part, on a constaté que le cultivar « Mureș » a enregistré des valeurs similaires à notre matériel mais d'une amplitude de variabilité plus grande (0,25%—0,46%).

La production de morphine étant déterminée génétiquement [2], [3], [4], [5], la sélection de notre matériel aussi bien que l'hybridation avec les lignes et les cultovariétés à haute valeur doivent être continuées.

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CHEMICAL CHANGES OF CIRCUMLACUSTRINE VEGETATION AND THE PELOIDOGENESIS IN THE SALT, PELOGENOUS LAKE VERDE (SOVATA, ROMANIA)

BY

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During the limnogeological researches carried out in 1976 in the pelogenous lake Verde (Sovata), there were also followed the relations existing between circumlacustrine vegetation and the lake, in order to estimate the possible contribution of the plant material to peloidogenesis. The 16 species from plant associations of circumlacustrine area, lake water and lake sediments, show different degrees of decomposition; the most intense decomposition was observed in pelogenous sediments (unctuous muds) and is peculiar to *Parmelia furfuracea*, *Alnus incana*, *Tilia cordata*, and *Betonica officinalis*, which are also peloidogenous species; the most decay-resistant are *Salix cinerea*, *Rhamnus cathartica* and *Senecio nemorensis*. Some plants have a decay-resistance — qualitatively estimated — differing from that mentioned in the literature, fact explained probably by the very different features of saline milieu of lake Verde. The chemical changes undergone by the plants fallen in the lake (especially those referring to K and Ca) are comparable with those mentioned in the literature. The statistical comparison of the chemical composition of fresh plants and of those found in lake water and sediments shows the highest similarities between lake mud and lake slope vegetation, which prove the doubtless contribution of the above-cited plant association to peloidogenesis.

Lake Verde in Sovata spa is an anthroposaline ecosystem with chlorosodic waters (water dry residue: 101.94—248.26 g l⁻¹) and sediments which are pelogenous to a great extent. Lake Verde is located between lake Roșu, which it drains and lake Ursu, which it supplies (Fig. 1).

During the limnogeological¹ and biological² researches carried out in 1976, there were also followed the relations existing between circumlacustrine vegetation and the lake with the view to estimating a possible contribution of the plant material to peloidogenesis. For that purpose, there were collected coincident samples (Fig. 1) both of fresh plant material from the circumlacustrine area (the samples collected from the lake slopes were denoted by "f₂" and those situated out of "f₂" area by "f₁")

¹ Bulgăreanu C. Valentin-Alexandru (1976), *Studii limnogeologice complexe în zona lacurilor sărate Roșu și Verde — Sovata* (Mureș), Research report, I.P.G.G. Bucharest.

² Venera Ionescu, Maria Năstăsescu, George Ignat (1976), *Studiu privind biologia lacului Roșu și Verde (Sovata, Mureș county)*, Research report, Faculty of Biology, University of Bucharest.

and of plant material (partially decomposed) from the lake (the plants taken from water were called "w" samples and those found in sediments "n" samples).

From the sampling points A, B, C, D and E there were collected both plant samples "w" and "n" and sediment samples denoted by "N".

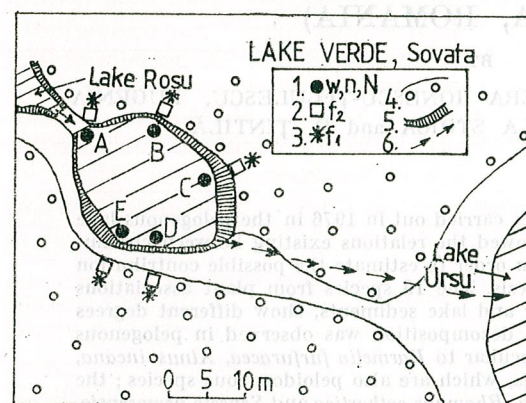


Fig. 1. — Location of lake Verde, Sovata; 1, plant samples (from lake water (w) and lake sediments (n)) and also sediment samples (N); 2, 3, plant samples from circumlacustrine area; 4, wooded area; 5, lake slopes; 6, outputs of lakes.

The sediments were described directly in the field and their chemical characteristics are shown in table 1; it is obvious that the intensity of peloidogenesis increases starting from A sampling point towards D + E sampling points.

Table 1

Chemical composition (% dry weight) of lake sediments (Sept. 1976) in sampling points A, B, C, D and E (see Fig. 1)

Samples	A	B + C	D + E
Sediments	Yellowish/blackish silts with gravels	Sandy grey silts, sometimes yellowish, with gravels and black muds, sometimes sandy	Black, grey muds and yellowish silts partially argillaceous
pH	6.5	6.5	6.5
Total C	3.83	2.68 — 3.01	3.34 — 3.42
Organic C	1.30	1.39 — 1.56	1.25 — 1.31
Inorganic C	2.53	1.12 — 1.62	2.03 — 2.17
Cl	7.92	13.27 — 14.87	13.05 — 14.98
SiO ₂	50.36	45.32 — 46.53	43.80 — 48.30
SO ₄	0.535	1.009 — 1.709	0.72 — 1.112
Ca	4.358	2.705 — 2.905	2.304 — 2.655
Mg	2.11	1.984 — 1.997	1.908 — 2.06
Mn	0.15	0.079 — 0.133	0.106
Total Fe	3.422	3.92 — 4.20	3.64 — 3.92
Na	3.529	6.05 — 6.403	5.829 — 7.728
K	0.971	1.553	1.747 — 2.330
S (by ignition)	1.16	1.56 — 1.68	1.64 — 1.84

The qualitative specific structure of the plant material from both the circumlacustrine area (f_1 , f_2) and the lake (w, n) is mentioned in table 2; it results that for the total number of 16 species recorded, the ratio between the species number from "w" and "n" phases is increasing parallelly with the increase of peloidogenesis intensity, i.e. from the A towards D + E sampling points: $3 : 5 = 0.6$ (in A point), $7 : 6 = 1.16$ (B + C) and $6 : 2 = 3.0$ (D + E). This fact indicates a more rapid decomposition of plant material in pelogenous sediments from D + E points.

Table 2

Plant species from circumlacustrine area (f), lake water (w) and sediments (n), Sept. 1976; A, B, C, D, E sampling points of plants and sediments (see Fig. 1)

Species	A	B	C	D	E
* <i>Parmelia furfuracea</i>	— w —	— — —	— — —	— — —	— — —
<i>Carpinus betulus</i>	— — —	— — —	— wn	f — —	— w —
<i>Alnus incana</i>	— — —	— — —	— — —	— w —	— — —
<i>Fagus sylvatica</i>	— — n	— wn	— wn	fwn	fwn
** <i>Quercus petraea</i> +					
<i>Q. robur</i>	fwn	— wn	fwn	— wn	— wn
<i>Salix cinerea</i>	— — n	— — —	— — —	— — —	— — —
<i>Atriplex sp.</i>	f — —	— — —	— — —	— — —	— — —
<i>Sorbus torminalis</i>	— — —	— w —	— wn	— w —	f — —
<i>Tilia cordata</i>	— — —	— — —	— w —	— — —	— — —
<i>Acer platanoides</i> +					
<i>A. campestre</i>	— wn	fwn	fwn	— w —	— w —
<i>Rhamnus cathartica</i>	— — —	— — n	— — —	— — —	— — —
<i>Betonica officinalis</i>	— — —	— w —	— — —	— — —	— — —
<i>Senecio nemorensis</i>	— — n	— — —	— — —	— — —	— — —

(—) absent; (*) thallus, only; (**) leaves and fruits (for other species, there were sampled leaves, only)

For the qualitative estimation of the decomposition degree (and so of the contribution to peloidogenesis) of the 16 species listed in table 2, it is necessary to accept the following premises:

— the plant material from the circumlacustrine area (f_1 and f_2) should necessarily pass through the "w" and "n" phases;

— supposing that the same species enter the lake every year, the plant material from sediments "n" has been buried in lake sediments for at least a year;

— the absence of a certain taxon in "n" phase and its simultaneous presence in "w" phase show that the plant material from water, "w", has been totally decomposed as a consequence of its burial into the sediment;

— the absence of a certain taxon in "w" phase and its simultaneous presence in "n" phase may signify — in terms of a low or non-existent allochthonous input of plant material — the persistence in sediments of plant material, i.e. its reduced decay rate;

— the absence of a plant species in both "w" and "n" phases seems to show that it does not participate to both decay and peloidogenesis processes (for example *Atriplex* sp.).

It results that the minimum or null values of the ratio between the number of sampling points with "n" plant material and those with "w" plant material would characterize the pelogenous species that are also easily decomposable; the latter, according to table 2, are as follows: *Parmelia furfuracea*, *Alnus incana*, *Tilia cordata* and *Betonica officinalis* (for these, the above-mentioned ratio has zero value).

Another series of plants includes the relatively decay-resistant species *Sorbus torminalis* (with a ratio 0.33), *Carpinus betulus* (0.50), *Acer* spp. (0.60), *Quercus* spp. (1.00) and *Fagus silvatica* (1.25).

Among the most decay-resistant plants (whose ratio tends ad infinitum) there can be cited *Salix cinerea*, *Rhamnus cathartica* and *Senecio nemorensis*.

The above-cited results coincide only partially with those mentioned in literature; thus, *in vitro* and *in situ* experiments (carried out in running waters and in their sediments and also in freshwater lakes), using different types of litter introduced in netted plastic bags, have shown the following decreasing order of the decay rate [3–5]:

Salix(*Acer*) > *Acer*(*Salix*) > *Quercus* > *Alnus* > *Fagus*

In comparison with this sequence, the placing by us of species *Alnus incana* before *Salix* and of this latter after the *Fagus* position, is contradictory and seems to be unexplainable; but it is possible that decomposition processes are developing in a different way — for the same plant species — in salt lacustrine milieus; thus, Egglisshaw (from ref. [4]), cited a higher breakdown rate of litter in hardwater streams as compared with that in softwater streams. Hodgkinson [4] suggests the effect, probably important, of sediments pH index on some litter types when it is greatly influenced by water chemistry.

★

The results of the comparative plant ash chemical analysis of circumlacustrine vegetation (f_1 , f_2) and of the partially decomposed vegetation from lake Verde (w, n) are mentioned in table 3; the mean chemical content variation of plant material and lake sediments (see tables 1 and 3) is shown in figures 2a and 2b. It results that the decay of circumlacustrine vegetation, starting from " f_1 , f_2 " phase through "w" and "n" phases to "N" phase, means:

- an increase of ash, SiO_2 , total Fe, Ti and Pb contents;
- a maximum content in Na and Cl in "w" phase, probably caused by the "impregnation" with NaCl of plant material;
- decreasing contents in HCO_3 , Ca, K, V and Ag, probably due to their passing into lake sediments and/or lake water;
- quasi-constant contents in sulphides, Ni, Cr and Sn as well as variable ones in SO_4 , Mg, Mn, Mo and Co.

Some chemical changes of the leaf litter fallen in a freshwater lake [4], include K content decrease about 11 times during the decomposition of plant material (in our case, about 30 times); as concerns the macrophytes (more decomposable than leaf litter), there was noticed a decrease

Table 3

Chemical composition of plants from circumlacustrine area (f_1 , f_2), lake water (w) and sediments (n), Sept. 1976 (% in ash)

Components	f_1	f_2	w	n
Ash(%)	5.00–15.60	4.35–11.00	28.80–83.30	41.00–66.60
Cl	1.77–34.69	1.77–4.95	37.94–45.49	32.33–44.49
SiO_2	1.20–16.35	3.00–19.70	3.05–12.30	5.10–19.90
HCO_3	7.05–31.04	17.40–30.40	3.00–5.00	0.90–2.80
SO_4	0.78–8.22	0–9.66	1.23–3.03	1.74–5.25
Ca	4.70–21.00	11.80–20.20	3.60–5.70	2.50–3.40
Mg	3.30–4.80	3.30–5.88	0.24–0.78	0.30–1.02
Mn	0.80–0.96	0.08–0.80	0.88–2.32	0.10–0.56
Total Fe	0.28–0.56	0.14–0.56	0.70–3.64	0.78–4.06
Na	1.43–18.87	0.83–3.22	24.97–30.52	22.20–29.60
K	8.42–18.20	12.24–25.20	1.00–1.08	0.36–1.00
S (by ignition)	0.78–2.79	1.13–2.00	1.20–2.94	1.38–3.25
Ti*	80–230	80–240	180–1400	650–2600
Cr	0–15	3–15	0–20	0–25
Ni	3–20	5–30	10–30	10–25
Co	0–3	cca 3	5–10	3–5
V	3–5	cca 3	20–55	30–100
Mo	10–30	3–40	3–10	3–15
Pb	35–150	60–130	25–170	40–200
Ag	cca. 0.3	0.3–0.4	cca 0.3	cca 0.3
Sn	0–5	0–3	3–5	0–3

* Ti, Cr, ... Ag, Sn contents in ash are expressed in ppm.

Table 4

The comparison between "mixing phases" (N, f_1), (N, f_2), (N, w) and (N, n), made by calculating the Pearson variation coefficient sum (Σ cv, %) for 10 chemical components (for symbols, see explanations in the text)

Components	N, f_1	N, f_2	N, W	N, n
Cl	81.887	61.752	57.167	54.914
SiO_2	70.048	66.462	78.559	59.055
SO_4	109.122	118.394	50.120	71.938
Ca	83.064	76.916	29.162	20.262
Mg	37.446	45.162	64.203	58.814
Mn	120.147	102.829	102.771	76.281
Total Fe	83.881	86.501	43.043	38.924
Na	88.649	69.544	69.413	67.953
K	89.098	95.576	33.450	55.226
S (by ignition)	35.169	21.503	29.871	33.711
Σ cv, %	798.511	744.639	557.759	537.078

of K content of 10–30 times [1] and of Ca content of about 3 times [6]; in our case, Ca decreased about 5.5 times. A similar case is that of K decrease (ca 32 times) in the mud on which *Potamogeton pectinatus* is growing, in comparison with the amount of K in this species [2].

The successive comparison of the chemical composition of fresh plants (f_1 , f_2), of plants found in lake water (w) and in sediments(n) with the chemical composition of lake sediments(N) was made by calculating

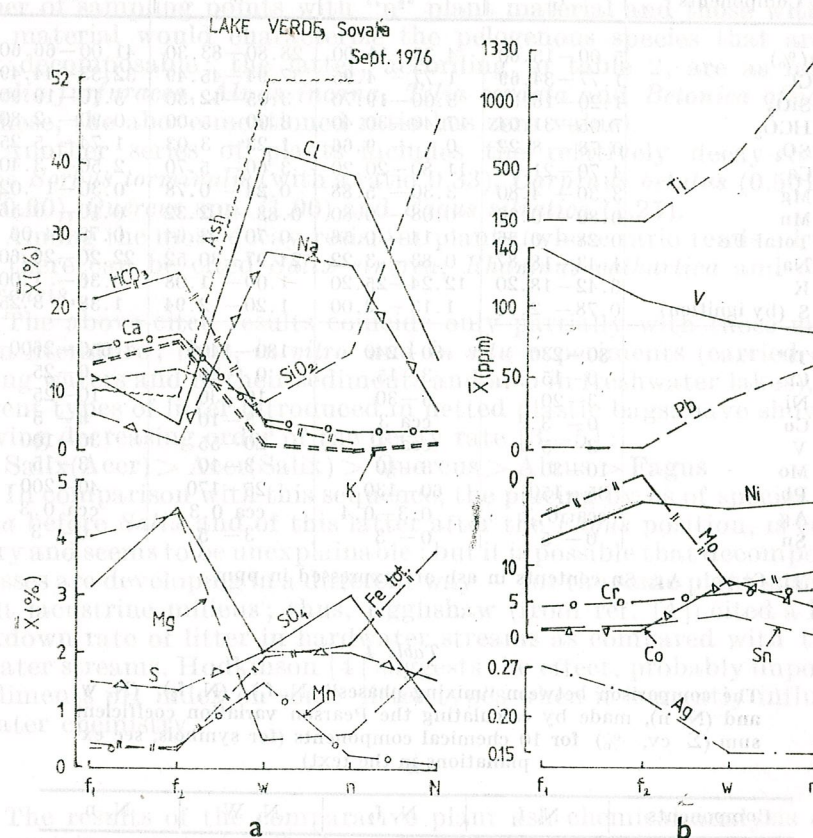


Fig. 2. — Variation of chemical contents in macro- (a) and microelements (b) in plants (from circumlacustrine area (f_1 , f_2), lake waters (w) and sediments (n)) and also in lake sediment samples (N).

the Pearson variation coefficient(cv), for every chemical component and for every "mixing phase" $N + f_1$, $N + f_2$, $N + w$ and $N + n$ (table 4). The sum of all Pearson coefficient values for all 10 components and for every mixing phases above-cited gave increasing Σcv values in the following sequence:

$$N, f_1 \rightarrow N, f_2 \rightarrow N, w \rightarrow N, n$$

This sequence shows greater chemical similarities between lake sediments (N) and lake slope vegetation (f_2) than between the same sediments(N) and the circumlacustrine vegetation (f_1); at the same time there is an increased similarity between the chemical composition of lake sediments(N) and that of plant material from sediments(n) than between

the same sediments(N) and the chemical composition of the plant material from lake water (w).

The Σcv values so obtained are comparable with those calculated for the same 10 chemical components from "mud-water" mixing phases in pelogenous, brackish lake Balta Albă [2]: Σcv values for *Potamogeton pectinatus* (761.78%) range between Σcv values of N, f_1 and " N, f_2 " phases in the present paper and Σcv values for *Cladophora fracia* (564.05%) range between Σcv values of " N, f_2 " and " N, w " phases.

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STRUCTURES RESEMBLING CELL WALL DEFICIENT FORMS OF BACTERIA ASSOCIATED WITH ASTER YELLOW DISEASE AND ISOLATED IN AXENIC CULTURE

BY
P. G. PLOAIE

From periwinkle plants, experimentally infected with AYA, and from the vector *Macrostes laevis*, an organism resembling cell wall deficient bacteria was isolated and subcultured on liquid and solid media with increasing concentrations of sugar (12–50%). The colonies of the agent have a natural bluish fluorescence and retain Diene's stain. The organism is Gram negative and resembles MLOs in liquid media, grows on solid media with thallos acetate, but grows also on Neopeptone and reverts to bacteria.

Aster yellows was first detected in Europe by Heinze and Kunze [11] and proved to be a disease distinct from other European yellows type diseases as stolbur, clover phyllody, Crimean yellows, clover dwarf, parastolbur or potato witches' broom [1], [21], [34]. The most frequent symptoms of this group of diseases are virescence, phyllody, reduction of leaves, dwarfing, proliferation and chlorosis [22], [33].

The discovery of mycoplasma-like organisms (MLO) in periwinkle plants experimentally infected with European yellows type diseases [3], [7], [19], [26], stimulated work in many laboratories for cultivating these microorganisms in artificial media. In some cases, structures resembling MLOs, that reverted to bacteria, were isolated from plants infected with stolbur and clover phyllody agent [2], [5], and other times, *Acholeplasma laidlawii* was detected in association with clover phyllody [8], [12], alone or in mixed infection [9].

A large scale trial was attempted in our laboratory for the cultivation of aster yellows agent (AYA), and almost all the media used for cultivation of plant mycoplasmas in different laboratories were carefully examined. These studies clearly established that AYA cultured on artificial media induced virescence and chlorosis when experimentally introduced into periwinkle [27], [28]. The first doubt concerning the mycoplasma etiology of aster yellows disease arose in 1976 when sediments of established culture were examined on thin sections and structures resembling MLOs and bacteria were detected together suggesting the existence of a small bacterium and its L-variant [23], [24], [25]. This paper reports the results of extensive investigations carried out by us starting from this hypothesis.

MATERIALS AND METHODS

Materials. a. *Plant material.* The following isolates of aster yellows agent were used for cultivation in cell-free media: periwinkle proliferation isolated in 1963 from cultivated *Vinca rosea* (*Catharanthus roseus* (L) G. Don) plants, in Romania, and also from leafhopper *Macrosteles quadripunctulatus* (Kirsch) [20]; onion proliferation detected in the southern part of Romania in 1971 on onion plants and transmitted by *Cuscuta campestris* Yunk. to periwinkle [18]; wheat chlorosis isolated from wheat through the vector *Macrosteles laevis* (Rib.) [29]; gladiolus yellows obtained in 1975 from cultivated "Flowersong" and "Life flame" gladiolus introduced in Romania, and transmitted to *Vinca rosea* by *C. campestris* and *M. laevis* [30]; aster yellows obtained from *M. laevis*, collected in 1980, from a field with *Callistephus chinensis* Nes., strongly contaminated with aster yellows disease, and transmitted to periwinkle plants [32] (see b). These isolates produced typical aster yellows symptoms in *Vinca rosea*, were indistinguishable from each other and were similar to the aster yellows agent isolated in the United States [6] and examined by us in 1968 on an exchange programme visit at Dept. of Entomology of the University of California, at Berkeley.

b. *Insect material.* Adult leafhoppers, *M. laevis*, were collected from a natural focus with aster yellows disease on *C. chinensis*. Groups of 50 leafhoppers were caged on individual healthy periwinkle seedlings for 3 days, to determine their capacity for transmission of AYA, and then these groups of tested leafhoppers were used to extract AYA for its primary isolation in cell-free media. After being exposed to insect feeding, the test plants were held in a greenhouse (20–24°C) for appreciation of symptoms.

Methods. a. *Purification of AYA.* The periwinkle plants having strong symptoms (1–2 months after their appearance) (Fig. 1 a) were sectioned in the middle and the top part (Fig. 1 b, c) sequentially surface sterilized, by immersion, in the following solution: 5% calcium hypochlorite 10 min, 1% chloramine 5 min, 10% cetyl-pyridinium bromide 5 min, 1% formaldehyde 5 min, 70% ethanol 5 min. After each agent of sterilization, the plant material was thoroughly rinsed in 1–2 liters of sterile distilled water and then placed between two sheets of sterile blotting paper. The midribs and stem were separated and triturated in sterile buffer solution, either 0.1 M phosphate buffer + 0.85% NaCl + 0.01 M $MgCl_2$ + 0.1 M glycine [14]; 0.1 M phosphate buffer + 0.33 M NaCl; GKNP solution: 0.1% glucose + 0.04% KCl + 0.009% $Na_2HPO_4 \cdot 7H_2O$ + 0.006% KH_2PO_4 + 0.8% NaCl [4]; or 0.1 M phosphate buffer + 5% sucrose + 1% PPLO broth [30], using a sterile pestle and mortar. After clarifying in sterile centrifuge tubes at 5000 rpm for 10 min at 4°C in a MSE centrifuge, supernatant fraction was collected aseptically, centrifuged at 20,000 rpm for 30 min in a VAC 601 Janetzki preparative ultracentrifuge, also in sterile conditions. The sediment was dispersed in 5 ml of sterile buffer and was then assayed for biological activity (potential of growth) and for morphological study by electron microscopy. Groups of 50, 70, 130 viruliferous leafhoppers were immersed in 70% ethanol, washed with sterile water and similarly processed, and the extract was used as inoculum.

b. *Culture media.* The composition of the media was as follows: PPLO broth (Difco) 2.1 g, Bacto-tryptone (Difco) 1 g, sugar 12 g, fresh yeast extract 10 ml, distilled water 100 ml, phenol red 0.1% 2 ml, and pH 7.6, 7.8, and 8.0. After sterilization by autoclaving at 121°C for 20 min, 20 ml horse serum (inactivated 60 min at 56°C) was aseptically added. This medium was denominated R (reversion). A series of media (R1–R5) were also used, the R medium being supplemented with 0.2% $MgSO_4 \cdot 7H_2O$ and increasing concentrations of sugar, as follows: R1 (17 g), R2 (27 g), R3 (35 g), R4 (42 g), R5 (50 g). The same media with 0.8% Noble agar and in some experiments with 0.1–0.5% thallous acetate were used as solid media. 2% Neopeptone (Difco) with 0.8% Noble agar was used as bacteriological medium, and C_3G medium (15) for isolation of spiroplasmas.

c. *Inoculation.* An amount of 1 ml of buffered extract from plants or insects was diluted with 10 ml of growth medium and then passed through a Millipore HA or Sartorius SM 11306 filter (0.45 μm pore diameter) using a syringe and Swinnex-25 filter holder attached to a receiving flask. Sometimes 2, 3, or 4 Swinnex-25 filter holders with filter in place were mounted together, sterilized for 20 min at 1 at. and used for filtration in cascade. The cultures obtained in liquid media were diluted in buffer and a few small drops introduced on solid media, in petri dishes. The cultures were incubated at 31°C. All operations were carried out under sterile room and all liquid and solid cultures were examined in bright and dark field with an optical microscope, using the usual methods of staining in microbiology (Gram, Giemsa, Safranin).

d. *Electron microscopy.* The culture cells were harvested from solid media and from culture flasks after centrifugation at 20,000 for 30 min and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed in OsO_4 2% in 0.1 M phosphate buffer and embedded in Epon 812. The blocks were sectioned on a Tesla 490A ultramicrotome and the sections stained with uranyl acetate and lead citrate and examined in a Tesla BS 500 electron microscope. For a better appreciation of the morphology, colony-impressions were taken on electron grids and examined without staining under an electron microscope.

RESULTS AND DISCUSSION

Quality of inoculum. In the sediments obtained from plants and insects, and used as inoculum, ovoid or spherical bodies 200–600 nm in diameter, resembling MLOs, were always detected by high resolution electron microscopy (Fig. 1 d). Sometimes, larger round cells predominated in the sediment. Some of them were filamentous cells (Fig. 1 e) or dilated filaments (Fig. 1 f). No spiroplasmalike structures or bacteria were observed in the extract of plants or insects. The mentioned buffers, at pH 7.0–7.4, ensured a good preservation of presumed AYA by the time of purification.

Growth in liquid media. First isolation of presumed AYA in C_3G , R media or in media used by us before [27], [28], had a slow and poor growth. A visible growth always appeared after 1–6 months. The C_3G

medium did not support growth when inoculated with extract of insects at first isolation but when few milliliters were transferred by filtration in R media, positive serial subcultures were obtained.

In numerous experiments of direct isolation of AYA from plants or insects, a filtrable microorganism was always isolated that showed a better growth at 31°C, on media with high level of sugar (R3, R4) and pH 7.6–8.0. In serial subcultures, the cultures developed within 10–15 days on R4, but a strong culture appeared after 2 months. The pH changed to acid after 10–25 days, but in some cases the changes appeared after 4–5 months. A bluish fluorescent ring formed on the flask and numerous small colonies having a bluish fluorescence and retaining Dienes' stain grew on the flask in R2–R5-type media. The microorganism grew in mucoid masses, like a grape. The smears, prepared from solid or liquid media using a gentle heat fixation, stained with gentiana violet, Giemsa's stain or safranin, and examined under the optical microscope showed only small coccoid cells, at limit of visibility. The organism was Gram negative, and filtrable through a 0.45 μ m pore diameter filter, in Swinney-type adaptors in cascade of 2–4 filters.

Growth on solid media. The microorganisms passed from liquid to solid media grew as coccoid elements abundantly on R–R4 media. The colonies were not typical "fried egg", they grew like "fried egg" on R3–R4-type media, were 0.1–0.3 mm in diameter, had a bluish auto-fluorescence and retained for a long time the Dienes' stain. The cells grew embedded in mucus [25] and the microorganisms were not inhibited by 0.1–0.5% thallos acetate. When the cultures from solid and liquid media were transferred on Neopeptone the organism easily reverted to bacterial form. The colonies retained Dienes' stain, the central part of the colony had coccoid elements but bacteria appeared at the periphery and the natural fluorescence changed to a pale yellow and only bacteria could be detected in colonies. Old cells had an electron dense nucleoid suggesting a developmental cycle.

Electron microscopy. Extremely electron dense bodies (no staining was necessary) 200–500 nm were detected in all cultures on solid media (Fig. 1 g). At the periphery of colonies long filaments like a string of beads (Fig. 1 h) were very often detected. These electron dense bodies or beaded filaments were always detected by us on thin sections of periwinkle infected with European aster yellows diseases (see Ploaie and Maramorosch [26], Figs. 3 and 4; Ploaie [24], Fig. 7). These coccoid electron dense cells grew up to larger cells, divided and became less electron dense. Some of them comprised an electron dense nucleoid (Fig. 2 a). On thin sections the electron dense bodies exhibited a strong cell wall, less evident in the larger cells with a central nucleoid.

In liquid media, structures resembling MLOs, electron dense bodies and giant bodies (Fig. 2 c, d) were detected in numerous sediments analysed. In R medium (low concentration of sugar), MLOs and bacteria were detected together (Fig. 2 e). Cultures from R medium reverted more easily on Neopeptone (Fig. 2 f).

Isolation and culture of AYA are extremely difficult. In all our studies only plants experimentally infected with AYA and having strong

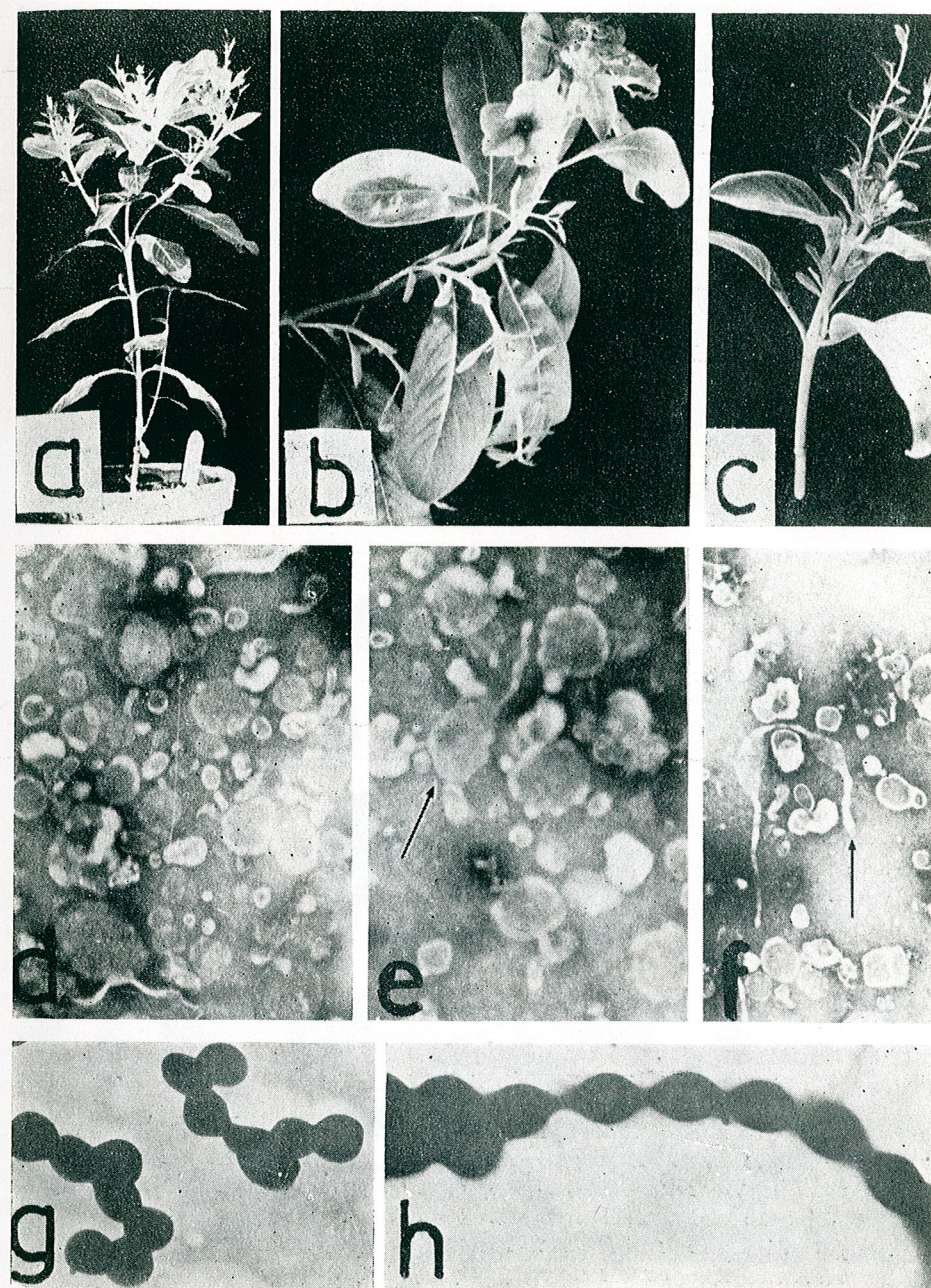


Fig. 1. — Symptoms of aster yellows disease on periwinkle. a, A plant at 2 months after symptoms have appeared; b and c, top of plant with strong symptoms used for preparation of inoculum; d, MLOs purified from plants. Numerous small, round cells, or larger cells with filament (e, arrow) or dilated filaments (f, arrow); g and h, electron dense bodies developed on solid media, in groups or beaded filaments (d–f, negative staining with 6% ammonium molybdate; g–h, no staining).

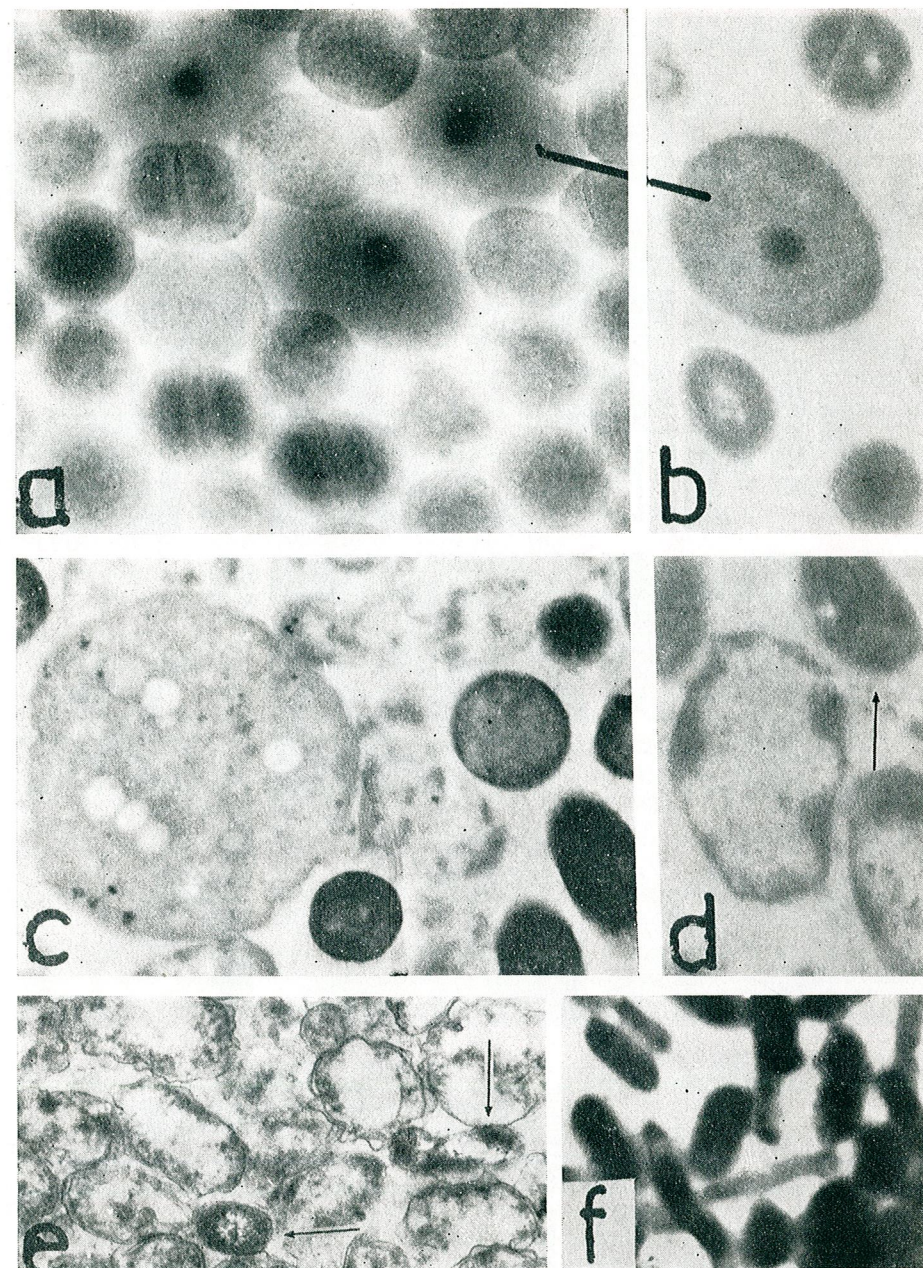


Fig. 2. — Culture at third passage on solid media. The electron dense bodies are enlarged and divide and some of them have a central nucleoid (a). This culture on thin sections (b) shows clearly the nucleoid in larger cells and the presence of the cell wall of electron dense bodies ($\times 25,000$). c, Aspect of cultures in R4 liquid medium, with MLOs, giant bodies and electron dense bodies; d, typical MLOs, in the same culture together with cells having cell wall (arrow), $\times 12,800$; e, MLO and bacteroid forms (arrow) isolated on R medium from insect *M. laevis*, $\times 20,000$; f, bacteroid cultures on Neopeptone, $\times 6,400$.

symptoms were used. All steps of isolation and culture were verified by electron microscopy. Although *S. citri* was isolated from plants purposely infected with AYA [13], no structures resembling spiroplasmas appeared on dark field examination of our cultures, or by other methods. Spiroplasma-induced diseases are not of the virescence type, and spiroplasmas can easily be isolated from plants or insects while maintenance *in vitro* of AYA for more than 72 hr in a buffer solution is a difficult problem [31]. In our opinion, AYA is a distinct microorganism, most probably a cell wall deficient bacterium (L-phase variant). All characteristics of growth resemble cell wall deficient bacteria, as bluish autofluorescence, growth in string and mucoid masses, elementary bodies, giant bodies [17]. Mixed infections (MLOs and walled organism) in the same plant cell [10] or vector cell [16] were detected several years ago only in association with European aster yellows diseases. This suggests that AYA may be a small bacterium that reverts to CDW as a function of microecological conditions.

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MICROPROPAGATION IN *VITIS VINIFERA* L.: III STUDIES REGARDING 'IN VITRO' STIMULATION OF MULTIPLE AXILLARY SHOOT DEVELOPMENT IN SOME GRAPEVINE CULTIVARS FOR CLONAL MULTIPLICATION

BY

ANA ROȘU, AURELIA BREZEANU and MARGARETA IORDAN

This paper deals with the effect of some plant hormones on 'in vitro' multiple shoot development from nodal stem explants of 5 *Vitis vinifera* L. cultivars, in order to evaluate the potential significance of this procedure for clonal multiplication of grapevine. The stimulative effect of 6-benzyl-aminopurine (2.25 mg/l) in combination with 3-indoleacetic acid (0.18 mg/l) or 4-(3-indolyl)-butyric acid (0.18 mg/l) on multiple shoot development is demonstrated. A maximum multiplication rate of 20 shoots per one axillary bud was attained over a three-month period, with variations in the reactivity degree of the explants belonging to different cultivars, as well as of the explants from the same cultivar.

The 'in vitro' culture techniques enable plant regeneration from pieces of tissues, or even from single cells, by expressing the regenerative potential of the plant cells when exposed to rigorously controlled nutritional, hormonal and physical conditions. These techniques, which offer now a promising way for rapid clonal propagation in many plant species are based on plant regeneration by meristem tip cultures, somatic embryogenesis or adventitious shoot induction. Use of callus cultures as intermediary stages of the micropropagation techniques often results in chimeric elements and genetically aberrant plants [8]. This disadvantage can be avoided by using meristem tips or nodal segments with pre-existing meristems as primary explants and evading the conditions which favour the callus development. The plants regenerated from meristems preserve the genetic traits of the parental plant because of the more uniform diploid nature of the meristematic cells [7].

In natural conditions the meristems originating in the leaf axils are able to develop in a limited number of lateral shoots, because of the inhibitory effect exerted by the main shoot by the apical dominance phenomenon. The degree of apical dominance can be changed by the environmental factors, an important role in this respect being played by the plant hormones.

The effect of the exogenously supplemented plant hormones on 'in vitro' stimulation of multiple shoot development from meristem tips was demonstrated for many plant genera [1], [2], [3], [4], [5], [7], [11], [12].

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In our experiments we studied the effect of some exogenously supplied hormones on the multiple axillary shoot development in some grapevine cultivars, in order to elaborate a method for rapid and efficient clonal propagation with the aid of the 'in vitro' techniques.

MATERIAL AND METHOD

Elongating shoots from cuttings forced in the laboratory, belonging to cultivars: Riesling italian, Fetească regală, Cabernet Sauvignon, Perlette and Chasselas d'Oré were cut in nodal segments containing one

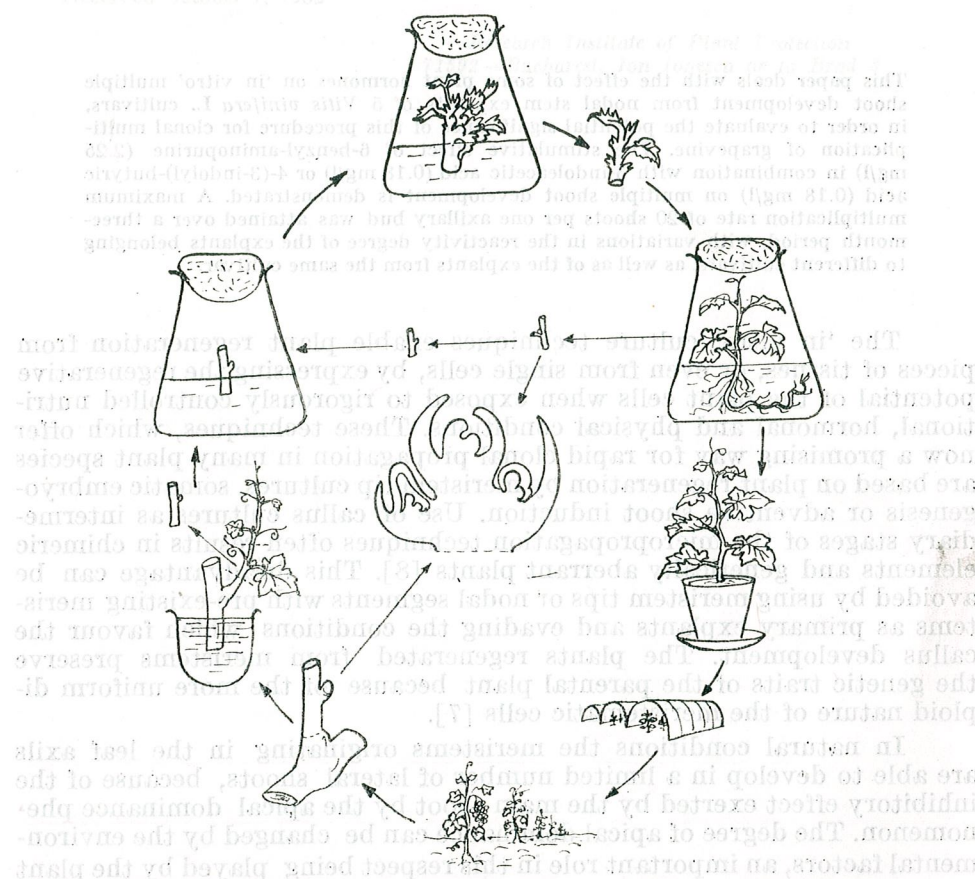


Fig. 1. — Diagrammatic representation of the principal phases of the grapevine propagation experiments by 'in vitro' multiple shoot development.

axillary bud and an internode piece of about 1 cm in length. The explants were surface sterilized in an aqueous solution of HgCl_2 (0.1%) for 15 minutes, followed by three rinses with a solution of CaCl_2 (10 mg/l)

and then by three changes of sterilized distilled water. In some cases, in order to eliminate the operations for attaining asepsy, the nodal explants were excised from plantlets obtained by the 'in vitro' rooting of the axillary shoots (Fig. 1). The nutrient substrate was represented by variants of the Murashige and Skoog basal medium [10] with 8 gr/l agar and various hormonal combinations (Table 1). The cultures were maintained at a

Table 1

Nutrient media for stimulation of multiple shoots develop-
ment

Variant	Basal medium	Cytokinins (mg/l)	Auxins (mg/l)			
		BAP	IAA	NAA	IBA	IPA
1	MS	—	0.18	—	—	—
2	MS	2.25	—	—	—	—
3	MS	2.25	0.18	—	—	—
4	MS	2.25	—	0.18	—	—
5	MS	2.25	—	—	0.18	—
6	MS	2.25	—	—	—	0.18

MS — the basal medium Murashige and Skoog (1962)

BAP — 6-benzyl-aminopurine

IAA — 3-indoleacetic acid

NAA — 2-naphthylacetic acid

IBA — 4-(3-indolyl)-butyric acid

IPA — 3-(3-indolyl)-propionic acid

temperature of 25°C ($\pm$ 2°C) and a photoperiod of 16 hours with a light intensity of 4,000 lx. The detached axillary shoots rooted on the MS basal medium with 1.8 mg/l IAA and 0.022 mg/l kinetin. The plantlets with a well-developed root system were transplanted first to pots with an autoclaved mixture of sawdust and sand (2 : 1) and after that to an unsterilized soil mixture in the greenhouse. Watering was carried out with a solution of half-strength of the MS macro- and microelements during the first week and afterwards with tap water.

RESULTS AND DISCUSSION

The elongation of the main shoots and the development of the first primordia of additional buds occurred one week after the culture was started (Pl. I, Fig. 2). The reactivity degree of the nodal region varied both with the composition of the nutrient medium and with the cultivar (Tables 1, 2).

After three weeks of incubation the majority of the explants from the variants 3 and 5 developed 2—3 shoots and 1—4 additional buds (Pl. I, Figs. 3, 4). By detaching the first elongated shoots and transferring them to the rooting medium, the first rooted plantlets were obtained a month after the experiment was started. The best results were recorded on variants 3 and 5, in which BAP was administered in combination with IAA or IBA, the combined effect of these hormones being expressed in a

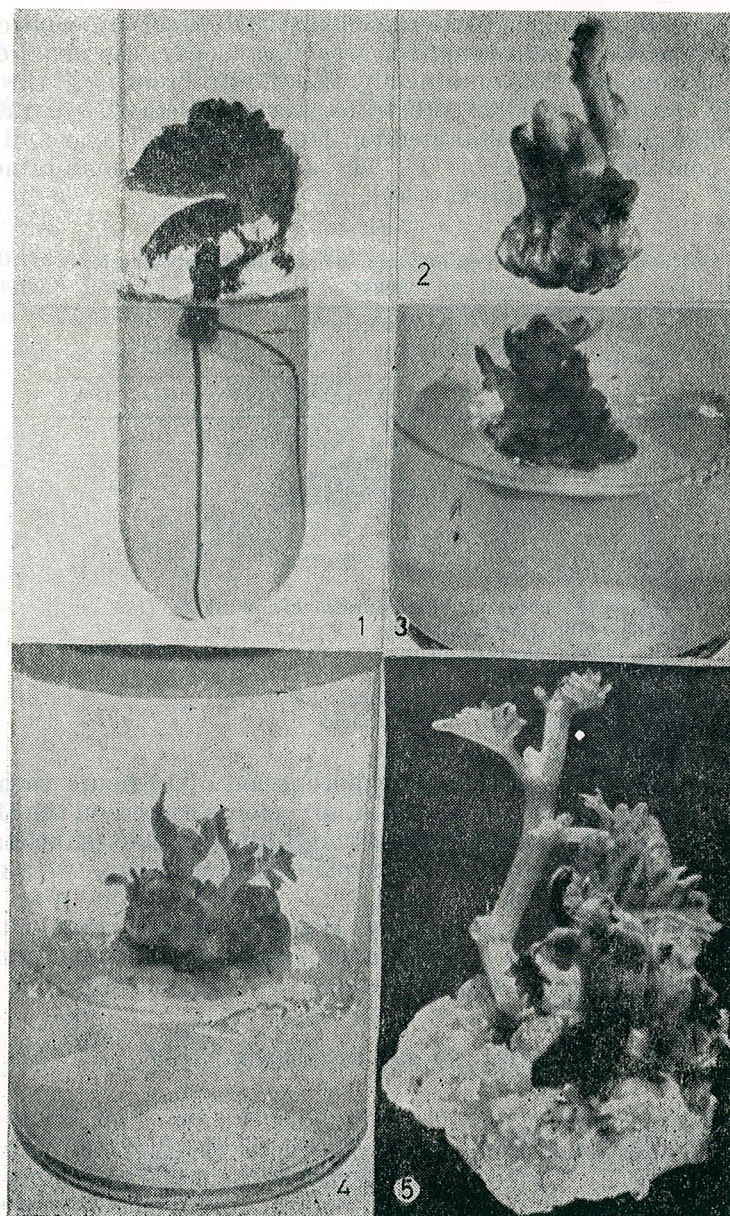


Plate I — The effect of plant hormones on multiple shoot and bud development from nodal explants.

- 1 — Nodal explant on variant 1 (0.18 mg/l IAA) with a single shoot elongation and differentiation of adventitious roots from the internode.
- 2 — Elongation of the first shoot and differentiation of additional buds one week after culture initiation on variant 3.
- 3,4 — Explants after 3 weeks of culture on variant 3 (Fig. 3) and 5 (Fig. 4).
- 5 — Explant on variant 4 with callus proliferation at the basal end of the explant and with a low number of shoots and buds.

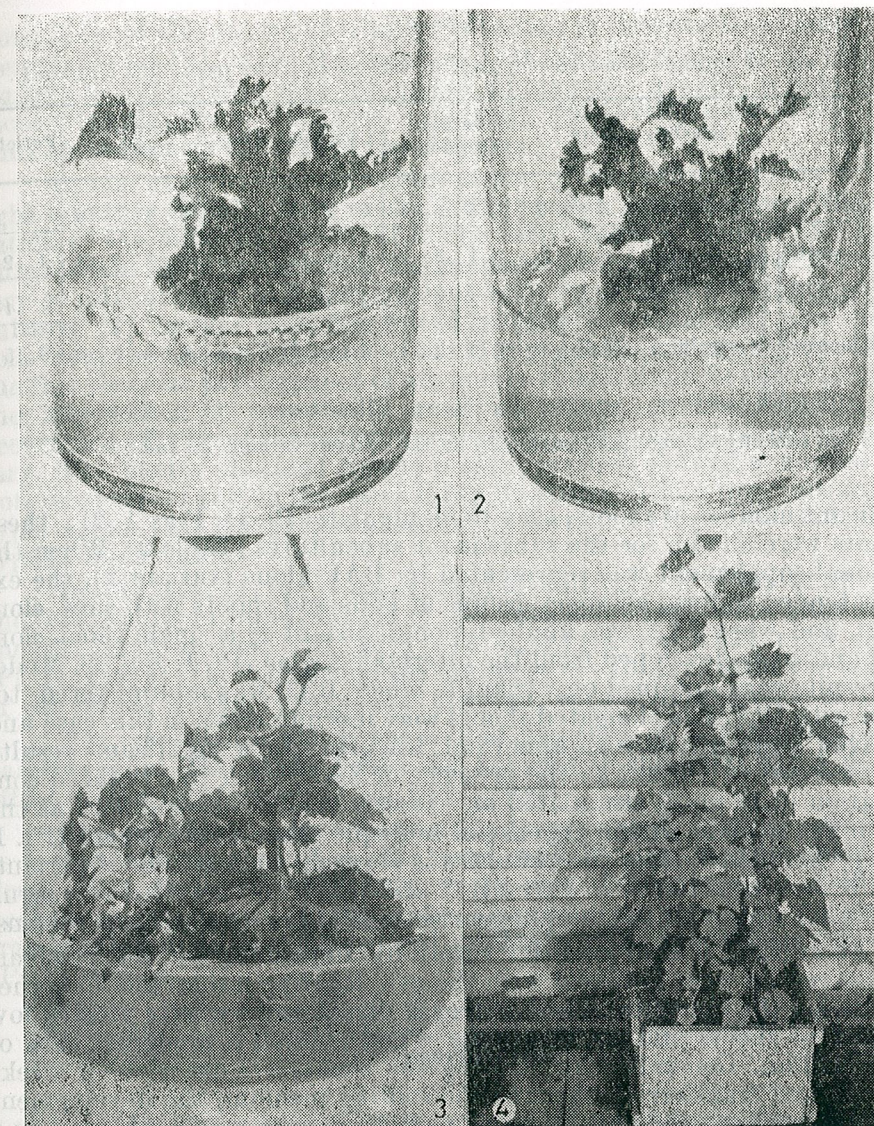


Plate II — Plant regeneration from multiple shoot differentiation 'in vitro'.

- 1, 2 — Explants with multiple shoots after two months of culture on variants 3 and 5.
- 3 — Whole plants obtained by detaching the shoots and cultivating them on the rooting medium.
- 4 — Plants transferred to soil under greenhouse conditions.

Table 2

Maximal rate of multiple shoot development in various cultivars from *Vitis vinifera* L.

Cultivar		Riesling italian				Cabernet Sauvignon				Chasselas d'Oré				Fetească regală				Perlette	
Variant		2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	3	4	5
Time since culture initiation	1 month*	3	5	2	5	3	1	3	1	3	2	2	3	1	2	1	2	1	2
	2 months	6	8	3	8	5	1	6	2	5	3	3	4	2	5	3	3	1	3
	3 months	13	20	8	15	9	4	10	6	9	7	4	6	4	7	4	5	4	5

* All subcultures were performed only on the optimal variants (3 and 5).

significant number of shoots, easy to manipulate (Pl. II, Figs 1, 2); these variants were chosen for the subsequent subcultures (Table 2). When the hormonal supplement was represented by BAP alone (variant 2), the explants tended to form compact groups of buds and shoots with slow elongation, and when IAA was applied alone (variant 1) a single shoot elongated and roots developed from the internode region (Pl. I, Fig. 1). Moderate results were obtained on variant 6, where BAP was administered together with IPA. The elongated shoots were less numerous in this case and displayed some leaves with abnormal features. Less significant results were recorded on variant 4; the presence of NAA, even in a reduced concentration (0.18 mg/l) stimulated an excessive callus development at the base of the explant at the expense of multiple shoot development (Pl. I, Fig. 5). One month after the culture was started, the explants of variants 4 and 6 were transferred to variants 3 and 5, and this resulted in a significant amelioration of the morphogenetic capacity of the axillary regions.

As regards the differential reactivity depending on the cultivar, the best results were obtained with the cvs. Riesling italian and Cabernet Sauvignon; the explants of cvs. Fetească regală and Perlette yielded low numbers of shoots and cv. Chasselas d'Oré showed a moderate rate of multiplication. Generally, by excising the shoots ≥ 1 cm at 3–4 weeks intervals and transferring them to the rooting medium, the development of additional buds and elongation of new shoots was much enhanced as a result of attenuating the apical dominance. The morphogenetic potential of the axillary region varied from one explant to the other within the same cultivar, some explants proving a remarkable morphogenetic potential by developing more than 20 shoots and compact groups of buds over a three-month interval. By periodic subcultures on fresh media, the morphogenetic capacity of the explants increases and maintains itself a long time, generating permanently new shoots and buds; some cultures more than 1 year old retain their morphogenetic capacity unaltered. The frequent development of compact masses of many small shoots and buds needs further testings in order to establish the conditions for a suitable

elongation of the shoots. A similar situation is described for some ornamental plants [2], with the mention that the development of the bud clusters is comparable with the apical dominance; usually, a dominating shoot from the group elongates, suppresses the development of the other buds and exhausts the rest of the clump.

In our experiments the maintenance of the cultures under a lower light intensity (200 lx) for 4 weeks, stimulated in some instances the elongation of the multiple shoots. If in the early stages of culture the shoots differentiate from pre-existing meristematic loci a switch-over from axillary to adventitious bud development may occur after a number of subcultures [2]. In this case, the initial proliferative zone may also include cells from the tissues adjacent to the meristematic axillary region. Due to the fact that the development of adventive shoots could be correlated with the appearance of genetically modified plants, studies regarding the occurrence of this phenomenon amongst the regenerated plants are necessary. The plants obtained by the present method showed a normal phenotypical aspect and on this basis it can be supposed that they are true-to-type, a fact that has to be confirmed by further cytogenetic and phenotypical observations.

In the case of the 5 experimented cultivars of *Vitis vinifera* L., the best results in rapid rooting of the detached shoots were obtained on the MS basal medium [10] with 1.18 mg/l IAA and 0.022 mg/l kinetin (Pl. II, Fig. 3); the root primordia appeared 7–10 days after the transfer of the shoots to this rhizogenetic medium, with a rooting rate of 100%.

The transplantation of the rooted plantlets on a hormone-free MS basal medium (two weeks before the transfer to the pots) had a positive effect on the fortification of the root system.

The plantlets were subsequently transplanted from the test tube to a sterilized mixture of sawdust and sand (2:1); in order to prevent desiccation, during the first two weeks they were protected with glass beakers or transparent plastic cloth. The plantlets were then successfully transplanted in an unsterilized soil substrate under greenhouse conditions (Pl. II, Fig. 4).

CONCLUSIONS

- Under the culture conditions described multiple shoots developed gradually on nodal stem explants from all experimented cultivars;
- all explants produced shoots usable for rooting, but the yield of shoots varied especially with the hormonal supplement and with the cultivar;
- the most significant yield of shoots was obtained on MS basal medium with 2.25 mg/l BAP and 0.18 mg/l IAA or IBA;
- the shoots were readily rooted on MS basal medium with 1.18 mg/l IAA and 0.022 mg/l kinetin;
- this procedure can be successfully used for a rapid, efficient and probably true-to-type 'in vitro' propagation of grapevine.

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EVALUATION OF THE RADIOPROTECTIVE CAPACITY OF SOME CHEMICALS BY DIFFERENT CYTOGENETIC TESTS

BY

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The radioprotective capacity of some newly synthesized chemicals of the cystamin group was studied by using the modified *Allium* test (micronucleus and chromosomal aberrations) and the micronucleus test in *Mesocricetus auratus*. Several doses of gamma radiation (50, 150, 300, 400, 500, 600 r), as well as different concentrations of the chemicals were tested taking cystamin as reference substance. When referred to cystamin, both the results of the *Allium* test and those of the micronucleus test in *Mesocricetus auratus* showed two of the chemicals to have pronounced radioprotective potentials.

INTRODUCTION

Cytogenetic assays rank high among the procedures recommended in testing mutagenesis, carcinogenesis and radioprotection. Several reasons justify the importance of studying the chromosomal aberrations by cytogenetic assays. Firstly, these assays can be directly applied to humans and, when used on animals, the extrapolation of their results to man proves correct. Secondly, they allow the visualisation of the whole genome with the optical microscope. And thirdly, correlations can be established between the induction of chromosomal aberrations and the induction of mutations by irradiation and chemicals.

Another recommendation in testing mutagenesis is to use as decisional, testing systems *in vivo* on mammals so that their results could be extrapolated to man.

Finally, in testing certain environmental substances it is necessary to apply more than one testing system, since no chemical can be ascertained as mutagen if only one such system is made use of.

Thus, the testing systems can be considered to fall into two categories: 1. testing systems for monitoring and sorting out the numerous environmental mutagens, such as the *Ames* test, the *Drosophila* test, the *Allium* test, the tests *in vitro*, etc.; 2. decisional testing systems used to determine whether the substance under study is a mutagen or not, or if it is potentially mutagenic for man. Such testings are performed on mammals (mice, rats, hamsters).

Still, the use of more than one test does not only help to sort out the environmental substances and to decide whether they are noxious or not; it also leads to correlations, to the discovery of specific reactions to a certain agent of the different types of cells, and, finally, to the discovering of the molecular mechanism of action of the mutagens. When the specific

reactions of each type of cells and the molecular mechanism of action are known, new, faster and more efficient mutagenesis tests can be elaborated. In this study, for testing the radioprotective capacity of certain substances we have used the *Allium* test, i.e. chromosome aberration test, and the micronucleus test on the root tips of *Allium cepa*, and the micronucleus test on the bone marrow of *Mesocricetus auratus*.

The *Allium* test was first introduced by Levan in 1938 [4] and ever since it has been widely used in different mutagenicity studies. It is recommended as a screening test with many advantages: it is cheap, fast, easy to perform and has large possibilities for cell division. The capacity of plants to activate promutagens into mutagens is more and more talked about [5] and makes one of the essential conditions any test should fulfil. The use of plants to sort out the environmental mutagens [8] is also supported by the correlations that could be established between the chromosomal aberrations seen in root tips and those found in mammals [2].

In order to make it more efficient, the *Allium* test underwent several modifications [1]. The latest ones consists of germinating the onion bulbs directly in the treatment solution, so that both the morphological and the cytogenetic alterations could be watched simultaneously.

The scope of our study being the evaluation of the radioprotective capacity of some chemicals, we were compelled to modify the test ourselves, as we have used combined treatments with irradiation and chemicals. Thus, for an easier way of handling materials, taking the materials to the radiation source included, onion seeds were used instead of bulbs.

The modification brought over by Fiskesjö [1] was employed in testing the germ capacity of seeds in solutions that were to be tested as radioprotectors as well as in testing their possible mutagenic capacity.

The micronucleus test on mammals. This test was independently developed by Schmidt [6] and Heddle [3]. The assay is based on the observation that all the chromosomal aberrations, except for inversions and translocations, have an acentric fragment. Since these fragments, lacking centromeres, cannot shift to the poles with the aid of the nucleus spindle, they are frequently lost and are not included in the nuclei of the daughter-cells, forming micronuclei in the cytoplasm of these cells. Micronuclei can also result from retardatar chromosomes as well as from aneuploids [7].

MATERIALS AND METHODS

Biological test material: root tips of *Allium cepa* ($2n = 16$) and bone marrow from *Mesocricetus auratus* ($2n = 44$).

Chemicals: cystamin (C), substance gamma (G) and substance 5. A ^{60}Co source was used for γ irradiation.

In *Allium* test, two concentrations were tested for each chemical (0.05% and 0.1%). Samples of each concentration from the three chemicals were irradiated with 300, 400, 500, 600 and 700 r.

The seeds germinated in Petri pots on moisted filter paper, and when a growth of 1 cm was obtained, they were transferred into the treatment solution, 45 minutes before irradiation.

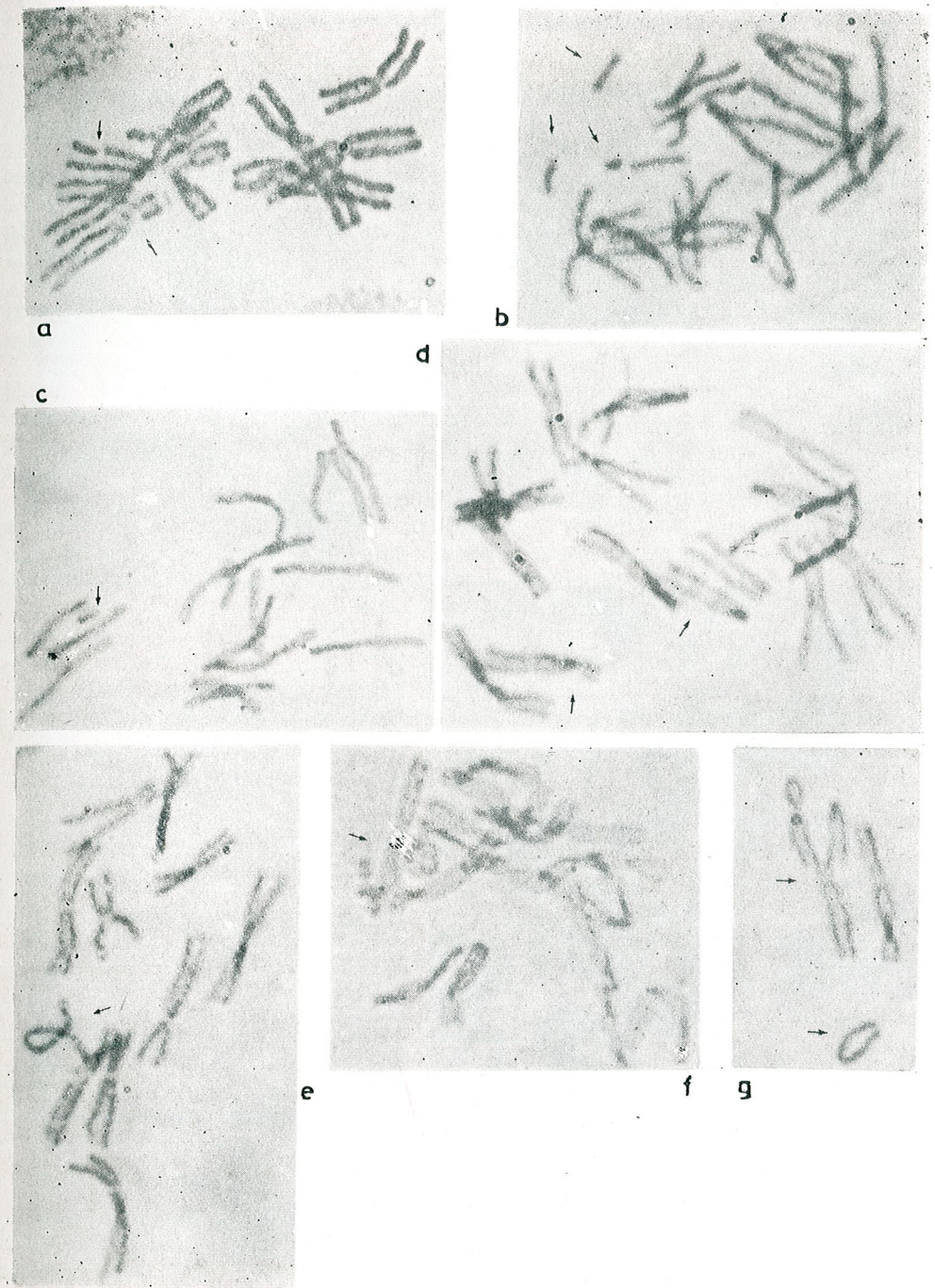


Plate 1. — The chromosome aberration test in *Allium cepa*. a, Chromatid breaks; b, chromatid fragments; c, chromosome fragments; d, isochromatid breaks and chromatid break; e, chromatid interchange on the same arm; f, ring and fragment from intrachromosome deletion; g, a triradial aberration configuration from two chromosomes via reciprocal recombination and erroneous reciprocal recombination.

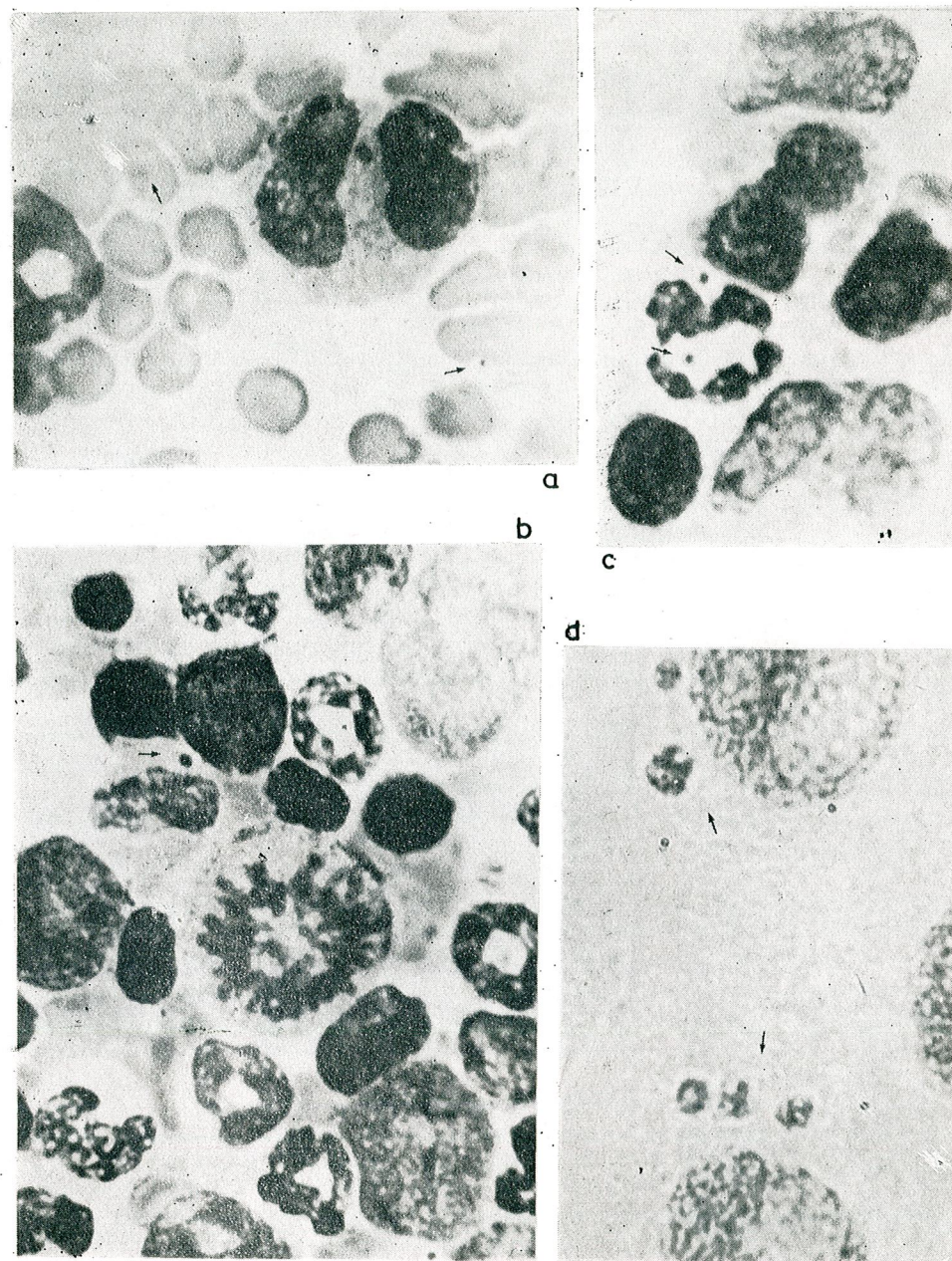


Plate 2. — The micronucleus test in *Mesocricetus auratus* and *Allium cepa*. a, b and c, Micronuclei in erythrocytes (a) and immature white cells (b, c); d, 5 micronuclei in 2 interphase cells in *Allium cepa*.

After 10 minutes from irradiation, the treatment solution was replaced by ordinary tap-water. Prefixing and fixing were performed at 6 and 24 hours' recovery from irradiation. The preparations were obtained after the usual squash method and coloured with Feulgen pigments. The following chromosomal aberrations were analysed: fragments, metaphase interchanges and micronuclei during interphase (Plates I and II). In order to establish the mytotic index, the material was not prefixed. In the micronucleus test on *Mesocricetus auratus*, young animals (6–7 weeks) of approximately the same weight (50–80 g) and sex (males) were selected.

The radiation doses were 300, 400 and 600 r. The concentration of the solution was chosen function of the active substance percentage. Four animals per variant were analysed. The immolation of the animals occurred 24 hours after irradiation. The lamellas were prepared as follows: the femoral marrow was pinned out and quickly placed on a slide as prints. After drying, the slides were coloured with May-Grünwald-Giemsa as follows: 3 minutes in May-Grünwald, 1 minute in deionized water and 30 minutes in 10% Giemsa; 3 to 4 slides were prepared for each animal and at least 400 cells were read on each slide (Plate II).

RESULTS AND DISCUSSION

First of all the potential mutagenic effect of the substance under study (control with the substance) was analysed. Thus, in the modified

Table 1

The chromosome aberration test and the micronucleus test in control, control with chemicals and control with irradiation in *Allium cepa*

Treat-ments	Period of recovery	Metapha-ses analysed	Frag-ments	Inter-changes	Cells with micro-nucleus 0/100	Mitotic index %
Control	—	100	—	—	1,12	8.77
C-0.05	6	70	—	—	—	7.01
C-0.1	6	90	—	—	—	7.23
G-0.05	6	100	—	—	—	7.32
G-0.1	6	90	—	—	—	6.71
5-0.05	6	100	—	—	—	5.02
5-0.1	6	100	—	—	—	5.57
50 r	6	60	—	—	—	5.38
	24	70	—	—	5.0	6.5
150 r	6	70	—	—	—	6.26
	24	70	—	—	7.0	7.87
300 r	6	50	—	—	11.9	3.91
	24	50	—	—	28.6	5.82
400 r	6	80	11.2	9.1	21.9	3.12
	24	50	12.3	10.3	37.5	4.15
500 r	6	50	12.3	13.8	39.5	2.16
	24	60	12.8	13.9	47.5	5.83
600 r	6	50	13.8	14.1	42.0	2.81
	24	70	14.1	15.7	56.6	6.96
700 r	6	50	14.7	15.8	42.9	2.01
	24	60	14.9	16.2	67.5	6.07

Allium test [1], after the treatment with the test solutions, the germ power of seeds and the morphology and length of roots were analysed. No differences were noticed in this respect, when referring to the control.

The mytotic index, when referred to the control, ranges normally (4.5%). The metaphase and interphase analyses revealed that the studied radioprotectors do not produce fragments, interchanges and micronuclei (Table 1).

The same table shows data regarding the mutagenic effect of the gamma radiation doses employed. The frequency of fragments, interchanges and micronuclei was noticed to be in direct proportion to the radiation

Table 2

The micronucleus test in *Allium cepa*
(chemicals + irradiation)

Treatments	Cells with micronuclei ‰		Mitotic index, ‰, after 6 h
	after 6 h	after 24 h	
Control	1.12		7.55
300 r	11.9	28.6	3.91
C-0.05 +300 r	5.0	7.0	5.4
C-0.1 +300 r	0.4	1.0	5.44
G-0.05 +300 r	3.1	7.0	5.09
G-0.1 +300 r	4.1	5.8	5.39
5-0.05 +300 r	10.6	1.1	3.40
5-0.1 +300 r	5.9	1.1	5.69
400 r	21.9	37.5	3.12
C-0.05 +400 r	3.0	8.8	3.65
C-0.1 +400 r	5.9	13.0	6.38
G-0.05 +400 r	5.4	4.2	4.31
G-0.1 +400 r	7.3	12.5	3.51
5-0.05 +400 r	3.0	14.7	3.57
5-0.1 +400 r	1.5	5.0	3.53
500 r	39.5	47.5	2.16
C-0.05 +500 r	3.7	13.6	2.41
C-0.1 +500 r	6.0	14.5	1.34
G-0.05 +500 r	8.2	12.5	2.57
G-0.1 +500 r	1.8	12.6	3.41
5-0.05 +500 r	2.0	15.0	2.56
5-0.1 +500 r	1.8	2.3	3.00
600 r	42.0	56.6	2.81
C-0.1 +600 r	4.0	13.0	2.80
G-0.1 +600 r	1.6	20.5	4.29
5-0.1 +600 r	0	5.8	2.73
700 r	42.5	67.5	2.01
C-0.1 +700 r	5.2	5.5	3.86
G-0.1 +700 r	1.0	13.8	3.40
5-0.1 +700 r	1.3	11.4	2.71

dose. The mytotic index was found to be lower in the variants at 6 hours after irradiation. The variants at 24 hours after irradiation show a normal range of the mytotic index when referred to the control.

In combined assays, i.e. chemicals and irradiation (Table 2) the doses of 50 and 150 r were given up, since they did not induce chromosomal aberrations of significant frequency. A total lack of chromosomal aberrations in fragments and interchanges could be noticed with all the combined treatment variants. For all the combinations, the frequency of the micronuclei decreased in comparison with their frequency in the irradiated material. Thus, after 24 hours from irradiation, the material irradiated with 300 r had a micronuclei frequency of 28.6‰, while in combined treatment C + 300, G + 300, 5 + 300, especially for the variants with higher concentration of the chemical (0.1‰), this frequency decreased to 1‰ micronuclei with cystamin, and to 1.1‰ with substance 5. The significant decrease of the micronuclei frequency in the variants of 0.1‰ concentration of the chemical was observed for all the radiation doses (400r, 500r, 600r and 700r).

With respect to the radioprotective potential, when comparing C, G and 5, substance 5 is found to have a stronger effect. The table shows the lowest frequencies of micronuclei in the following cases: 5-0.1% + 300r = 1.1‰; 5-0.1% + 400r = 5‰; 5-0.1% + 500r = 2.3‰; 5-0.1% + 600r = 5.8‰. The dose of 700r was an exception, since here cystamin was of greater efficiency than the other two substances: C-0.1% + 700r = 5.5‰. The protective effect of chemicals was proportional to the radiation dose, i.e. for small doses the micronuclei frequency

approached the frequency of the control (1.13‰ micronuclei) and for large doses it decreased, for example, only to 5.8‰ or 11.4‰ in 5-0.1% + 600r and 5-0.1% + 700r, respectively.

Both in the irradiated material and in the one with combined treatment, the higher frequency of micronuclei after 24 hours from irradiation as compared to the frequency after 6 hours was due to the fact, quite normal, that after 24 hours all the aberrations during a whole cell cycle accumulated as interphase nuclei.

In recovering the mytotic index after irradiation, substance G seemed more efficient than 5 and C (Table 2).

When establishing the correlations between the radioprotective effect data from the three chemicals studied by micronucleus testing on vegetal and animal material (Table 3), substance 5 was found to be more efficient than G and C both in plants and in animals (e.g. 5+300 = 1.1‰

Table 3

The micronucleus test comparatively in the *Allium* roots and bone marrow of *Mesocricetus auratus*

Treatments	Micronuclei in roots ‰	Micronuclei in bone marrow ‰
Control	1.12	1.00
300 r	28.6	6.73
C+ 300 r	1.0	3.52
5+ 300 r	1.1	2.11
400 r	37.5	12.10
C 400 r	13.0	6.75
G+ 400 r	12.5	3.66
600 r	56.6	22.53
C+ 600 r	13.0	6.44
G+ 600 r	20.5	5.56

miconuclei in *Allium* test and 2.11‰ miconuclei in bone marrow). The radioprotective effect of G was equal to that of C.

The higher frequency of miconuclei in plant material (root tips) as compared to the animal material (bone marrow) was probably due to the different, specific, reactions to irradiation of the different biological materials.

CONCLUSIONS

— The modified *Allium* test ascertained that the germ power of seeds, the morphology and length of roots were not altered during the treatment with individual substances (C, G and 5), in comparison with the control.

— The metaphase and the interphase analyses pointed out that the gamma radiation doses (300r, 400r, 500r, 600r and 700r) employed induce chromosomal aberrations like fragments, interchanges and miconuclei.

— The mytotic coefficient, calculated at 6 hours from irradiation, decreases with the increase of the radiation dose. Yet, after 24 hours from irradiation, the coefficient recovers almost to normal (Table 1).

— In all variants with combined treatments (chemicals + irradiation) the frequency of chromosomal aberrations during metaphase is reduced to 0, while the frequency of miconuclei during interphase decreases significantly when compared to the irradiated material. The frequency of miconuclei decreases function of the chemical's concentration (concentration higher than 0.1% — reduced frequency of miconuclei) (Table 2).

— As regards the radioprotective potential, comparisons between substances C, G and 5 show that substance 5 has a stronger effect, producing the smallest values of miconuclei frequency. Small frequencies of miconuclei, comparable to those of the control, are noticeable only with small radiation doses (300r). With large doses (500r, 600r, 700r) these substances are efficient as radioprotectors, yet only by comparison with the very high frequency of the miconuclei induced by irradiation (Table 3).

— After irradiation, during the recovery of the mytotic index, substance G seems more efficient than 5 and C (Table 2).

— By correlating the miconucleus test on plant and animal materials with regard to the radioprotective effect of the three chemicals, substance 5 is found to be more efficient than G and C both with plants and with animals. Substance G has a radioprotective effect almost equal to the reference substance (C) (Table 3).

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DIE WIRKUNG VON CARBENDAZIM AUF DEN ZELLYKLUS IM WURZELMERISTEM VON *TRITICUM AESTIVUM* UND *VICIA FABA*

VON
GEORGETA LAZÄR-KEUL und M. KEUL

The action of Carbendazim — applied as Bavistin (50% Carbendazim), in doses of 0.5, 1, and 2 kg/ha — on the cell cycle in root meristems of wheat (*Triticum aestivum* ssp. *vulgare*) and horse bean (*Vicia faba*) was studied by using the cytophotometric determination of the relative DNA nuclear content. In wheat, the action of Carbendazim is rendered evident by a prolonged G₁-phase, delayed S- and G₂-phase and decreased mitotic index. With the horse bean, Carbendazim induces a delay of G₂-phase. Both effects determine the lowering of the mitotic activity in the root meristems.

Unter den systemischen Fungiziden finden die Benzimidazolcarbamate Carbendazim (Methyl-benzimidazol-2-yl-carbamate, MBC) und Benomyl [Methyl-1-(butylcarbamoyl)-2-yl-carbamate] eine breite Anwendung im Pflanzenschutz [1]. Carbendazim entsteht in Benomyl-behandelten Pflanzen als stabiles Umwandlungsprodukt von Benomyl und ist demnach für dessen fungistatische Wirksamkeit verantwortlich [3]. Beide Fungizide werden nach Boden- [4], [17], [25], [26], Samen- [2], [20] oder Pflanzenbehandlung [7], [15] durch die Wurzeln und Blätter aufgenommen und als Carbendazim in alle Pflanzenteile transportiert [7], [12], [24]. Carbendazim kann über das Wurzelsystem wieder ausgeschieden werden [18]. Benomyl wird dank seiner physikalisch-chemischen Eigenschaften leichter aufgenommen als Carbendazim [5] und ist fungitoxisch wirksamer [2].

Es wird vermutet, daß die fungitoxischen Eigenschaften der Benzimidazol-Derivate dank der chemischen Strukturähnlichkeiten zwischen der Benzimidazol-Gruppe und den Purinbasen Adenin und Guanin der Primärwirkung auf den Nukleinsäure-Stoffwechsel zu verdanken sind [3]. Nach anderen Autoren beruht der Wirkungsmechanismus auf der antimitotischen Aktivität der Carbamat-Gruppe, so daß die Beeinträchtigung der Nukleinsäure- und Proteinsynthese sekundäre Erscheinungen darstellen [9], [10], [16].

Carbendazim und Benomyl üben verschiedene Nebenwirkungen auf Kulturpflanzen auf die sich in der Beeinträchtigung der Zellteilung [16], des Wachstums [6], [17] und anderer physiologischer Prozesse [25], [26], sowie in der Beeinflussung der DNS-, RNS- und Proteinsynthese äußern [3], [20], [22]. Benomyl reduziert die PAL-Aktivität [19], während für Carbendazim cytokinin-ähnliche Wirkungen beschrieben wurden [15], [21], [22], [23]. Dagegen liegen relativ wenige Untersuchungen über den Einfluß dieser Fungizide auf den Zellzyklus in pflanzlichen Meristemen vor.

MATERIAL UND ARBEITSMETHODE

Winterweizenkaryopsen [*Triticum aestivum* L., ssp. *vulgare* (Vill., Host) Mac Key, cv. „Dacia“] und Ackerbohnenamen (*Vicia faba* L.) wurden in Linhardt-Schalen auf befeuchtetem Filterpapier bei konstanter Temperatur (21°C) und im Dunkeln zur Keimung angesetzt.

Carbendazim gelangte als Handelspräparat Bavistin (50% Carbendazim) zur Anwendung. Die für Aufwandsmengen von 0,5; 1 und 2 kg/ha pro Linhardt-Schale umgerechneten Bavistin-Dosen wurden in destilliertem Wasser gelöst und auf die Filterpapierunterlagen aufgetragen. Die Kontrollpflanzen wurden auf destilliertem Wasser angezogen.

Die Carbendazim-Wirkung auf den Zellzyklus im Wurzelmeristem wurde nach 72stündiger Behandlungsdauer untersucht. Wurzelspitzen der behandelten und unbehandelten Pflanzen wurden 2 Stunden in Äthylalkohol-Eisessig (3:1) fixiert und in 70% Äthylalkohol konserviert. Die Hydrolyse wurde bei 28°C in 5 N HCl vorgenommen. Die optimalen Hydrolysezeiten (15 min bei *Triticum*, 25 min bei *Vicia*) wurden in Vorversuchen ermittelt. Die Feulgen-Färbung im Schiff-Reagens dauerte 2 Stunden.

Der relative DNS-Gehalt der Interphasenkerne wurde Feulgen-cytophotometrisch anhand von Quetschpräparaten nach der Zwei-Wellenlängen-Methode bestimmt (vgl. [8], [11]). Die Messungen wurden mit dem Ortholux-Mikroskop mit Photometer-Aufsatz und Geradsicht-Spiegel-Monochromator (Leitz) durchgeführt. Die Wellenlängenpaare (484 und 520 nm bei *Triticum*, bzw. 500 und 530 nm bei *Vicia*) wurden aufgrund der mikrospektrophotometrisch aufgenommenen Absorptionsspektren von 10 Feulgen-gefärbten Interphasenkernen ermittelt. Je Variante wurden mindestens 100 Interphasenkerne gemessen und ihr DNS-Gehalt nach der Tabelle von Mendelsohn [14] in Arbeitseinheiten (AE) berechnet. Parallel wurden die diploiden (2 C) und tetraploiden (4 C) Referenzwerte an je mindestens 30 Telo- und Metaphasen-Kernen pro Variante bestimmt.

ERGEBNISSE UND DISKUSSION

Die Häufigkeitsverteilungen der cytophotometrischen DNS-Werte des Weizenwurzelmeristems zeigen nach Behandlung mit Bavistin (50% Carbendazim) je nach der angewandten Dosis mehr oder weniger stark ausgeprägte Veränderungen gegenüber der Kontrolle (Abb. 1.). Im allgemeinen kann festgestellt werden, daß die Häufigkeit der diploiden DNS-Werte nach Bavistin-Behandlung zunimmt, während der Anteil der tetraploiden Zellkerne entsprechend abnimmt. Die Bavistin-Konzentration von 0,5 kg/ha übt dabei die stärkste Wirkung aus. Es wurden keine Werte höheren Ploidiegrades gemessen.

Aufgrund dieser Ergebnisse kann gefolgert werden, daß Bavistin den Ablauf des Zellzyklus im Weizenwurzelmeristem verändert. Der Einfluß der Bavistin-Behandlung auf den prozentualen Anteil der Zellzyklusphasen geht aus Abb. 2 hervor. Es kann festgestellt werden, daß der relative Anteil der G₁-Phasenkerne insbesondere unter dem Einfluß von

0,5 kg Bavistin/ha stark erhöht wird, während die Häufigkeit der S- und G₂-Phasenkerne leicht abnimmt. Unter den gegebenen Versuchsbedingungen hat Bavistin keine polyploidisierende Wirkung.

Die Erhöhung des Anteils der Zellkerne die die G₁-Phase durchlaufen, weist auf eine verlängerte Phasendauer hin, so daß es zu einem

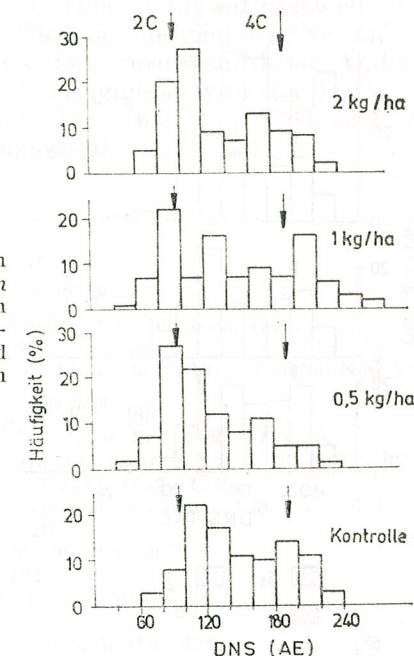


Abb. 1. — Die DNS-Häufigkeitsverteilungen im Wurzelmeristem von *Triticum aestivum* ssp. *vulgare* nach Einwirkung von Bavistin (50% Carbendazim) im Vergleich zur Kontrolle. Die Mittelwerte der diploiden (2 C) und tetraploiden (4 C) Referenzwerte sind durch Pfeile markiert.

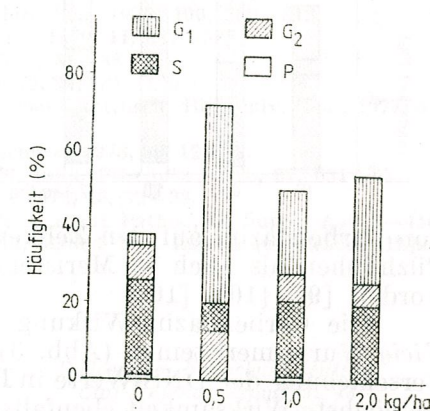


Abb. 2. — Die Wirkung von Bavistin auf die Verteilung der Zellzyklusphasen im Wurzelmeristem von *Triticum aestivum* ssp. *vulgare*. G₁ = Posttelophase; S = Synthesephase; G₂ = prämitotische Phase; P = polyploide Zellkerne.

verspäteten Eintritt in die S- bzw. in die G₂-Phase kommt. Die Abnahme des G₂-Gipfels kann in diesem Sinne als Ausdruck einer verminderten Proliferationsintensität aufgefaßt werden. Bestimmungen des Mitoseindex zeigen, daß der Prozentsatz der Kernteilungen unter Bavistin-Ein-

fluß herabgesetzt wird. So wird der Mitoseindex nach Bavistin-Behandlung von cca. 9 im Falle der Kontrollpflanzen bis auf Werte um 5–6 reduziert. Manche Autoren führen die Hemmung der Zellteilung durch Carbendazim auf eine primäre Beeinträchtigung der Nukleinsäure- und Proteinsynthese zurück [3], [20]. Andererseits ist eine direkte Wirkung

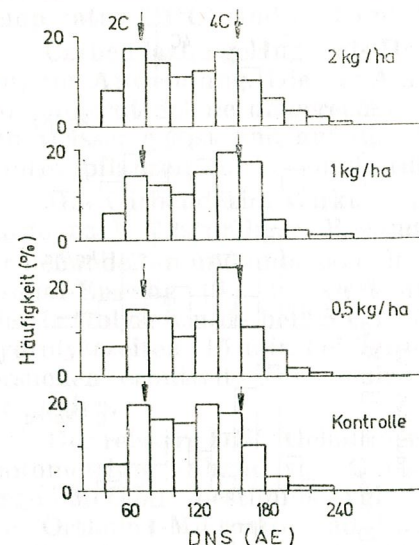


Abb. 3. — Die DNS-Häufigkeitsverteilungen im Wurzelmeristem von *Vicia faba* nach Einwirkung von Bavistin (weitere Erklärungen in Abb. 1.).

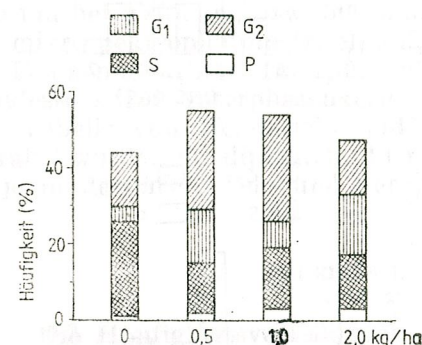


Abb. 4. — Die Wirkung von Bavistin auf die Verteilung der Zellzyklusphasen im Wurzelmeristem von *Vicia faba* (weitere Erklärungen in Abb. 2.).

von Carbendazim auf den Zellkern und auf die Kernteilung sowohl bei Pilzhypen als auch in Meristemzellen höherer Pflanzen nachgewiesen worden [9], [10], [16].

Die Carbendazim-Wirkung auf die DNS-Häufigkeitsverteilung in *Vicia*-Wurzelmeristemen (Abb. 3) zeigt im Gegensatz zu *Triticum* eine Verschiebung der DNS-Werte in Richtung der tetraploiden Werte, wobei die höchste Wirksamkeit ebenfalls bei geringeren Dosen (0,5 und 1 kg/ha) festzustellen ist. Der Einfluß von Bavistin auf den Zellzyklus (Abb. 4) zeigt einen relativ unveränderten Prozentsatz der G_1 -Phase. Dagegen nimmt der Anteil der S-Phasenkerne leicht ab, während der G_2 -Gipfel erhöht wird. Gleichzeitig wird eine leichte Erhöhung der polyploiden Kerne festgestellt.

Aus der Form dieser Verteilung der Zellzyklusphasen ist auf eine Verlängerung der Dauer der S- und G_2 -Phasen zu schließen. Eine Blockierung der G_2 -Phase wurde bei *Aspergillus nidulans* festgestellt und als Ausdruck der antimitotischen Aktivität von Carbendazim gedeutet [9]. Aufgrund seiner kanzerigenen und mutagenen Wirkung ist die Zulassung von Carbendazim im Obstbau eingeschränkt worden [13].

Die unterschiedliche Wirkung von Carbendazim auf den Zellzyklus im Wurzelmeristem von *Triticum* und *Vicia* ist möglicherweise auf artbedingte Aufnahme- und Resistenzunterschiede zurückzuführen. Andererseits ist bekannt, daß Carbendazim in Abhängigkeit von der Dosis Stimulierungen oder Hemmungen des Wachstums [6], [17], [26], bzw. der Nukleinsäure- und Proteinsynthese induziert [9], [21], [23].

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WACHSTUMSPHYSIOLOGISCHE UNTERSUCHUNGEN AN DICAMBA-BEHANDELTEN MAIS-KEIMPFLANZEN

VON

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The effect of certain superoptimal (1, 2, and 4 kg a.i./ha) and underoptimal (0.015, 0.030, and 0.060 kg a.i./ha) doses of dicamba on the growth of maize (*Zea mays*, cv. HD-95) seedlings in a prolonged and a 48-hour transfer experiment was studied. As compared to the growth pattern of the control plantlets approximating a sigmoid type curve, the treatment with dicamba changes this development pattern. With both variants of treatment, the dicamba induces aberrations in the plantlets' growth and a severe decreasing of the fresh and dry weight, depending on the dose. In the transfer experiments, the ceasing of cell elongation after 24 hours and the incapacity for the root to resume its growth at 48 hours after the herbicide action are rendered evident. With the underoptimal doses, in which the growth of the root system is slowed down, a dose-effect type response is observed.

Dicamba (2-Methoxy-3, 6-dichlorbenzoesäure) und verwandte Benzoesäure-Derivate gehören dank ihrer wachstumsregulierenden und phytotoxischen Eigenschaften zur Gruppe der sogenannten Wuchsstoff-Herbizide [9]. Durch die Beteiligung von Dicamba an der Zusammensetzung kombinierter Herbizide wird ein breiteres Wirkungsspektrum der Unkrautbekämpfungsmittel gewährleistet [2], [19].

Das in seiner Wirkung auxin-ähnliche Herbizid Dicamba [9], [11], wird sowohl durch die Blätter als auch durch die Wurzeln aufgenommen [5], [6], [8], und zeichnet sich durch eine hohe Mobilität im Pflanzenkörper aus. Es wird symplastisch und apoplastisch transportiert und reichert sich besonders in den Wachstumszonen an [4], [5], [6]. Dicamba wird im allgemeinen schwach metabolisiert [4], [6] und kann von den Wurzeln wieder ausgeschieden werden [5].

Im Vergleich zu anderen Auxin-Herbiziden, wie Picloram und 2,4-D, sind die Nebenwirkungen von Dicamba auf Kulturpflanzen relativ wenig untersucht worden. Dicamba beeinflusst das Wachstum [20] und andere physiologische Prozesse [15], sowie den Zellzyklus im Wurzelmeristem [12]. Die Primärwirkung von Dicamba beruht wahrscheinlich in der Abspaltung von Histon aus Nukleohiston, wodurch die Matrizenverfügbarkeit von DNS erhöht und die Proteinsynthese aktiviert werden [1], [9].

Vorliegende Arbeit vergleicht die Wirkungen kurzfristiger (48 Std.) und andauernder Behandlung mit supra- und suboptimalen Dicamba-Konzentrationen auf die Wachstumsdynamik von Mais-Keimpflanzen und beschreibt einige morphologisch-anatomische Effekte der Herbizid-Wirkung.

MATERIAL UND ARBEITSMETHODE

Als Versuchsmaterial wurden Mais-Keimpflanzen (*Zea mays* L., cv. HD-95) verwendet, die bevorzugt zur Untersuchung der pflanzenphysiologischen Wirkung chemischer Faktoren herangezogen werden [10]. Die Anzucht der Pflanzen erfolgte in Linhardt-Schalen auf befeuchtetem Filterpapier, bei konstanter Temperatur (22°C) und im Dunkeln.

Die Herbizid-Wirkung wurde für die Konzentrationen von 0,015; 0,030; 0,060; 1; 2 und 4 kg aktive Substanz/ha in zwei Varianten untersucht: 1. bei andauernder Behandlung, wobei sowohl die Keimung als auch die Anzucht auf mit Dicamba-Lösung durchtränkter Filterpapier-Unterlage erfolgte; 2. bei kurzfristiger Behandlung von 48 Stunden, wobei die Keimung 48 Stunden lang auf Filterpapier mit destilliertem Wasser vorgenommen wurde, wonach die Keimlinge für 48 Stunden auf Filterpapier-Unterlagen mit Dicamba versetzt und anschließend wieder auf destilliertes Wasser übertragen wurden. Die Kontrollpflanzen wurden auf destilliertem Wasser angezogen.

Die Wachstumsdynamik der Keimpflanzen wurde im Laufe von 6 Tagen verfolgt, indem die Länge der Primärwurzel und der Koeoptile (bzw. des 1. Blattes) täglich gemessen wurde. Die Versuche wurden in 3 Parallelversuchen durchgeführt. Am Versuchsende wurde die Frisch- und Trockenmasse bestimmt. Andere Einzelheiten der Versuchsdurchführung sind einer früheren Arbeit zu entnehmen [20]. Zur Untersuchung anatomischer Effekte wurden Handschnitte durch die Wurzel durchgeführt.

ERGEBNISSE UND DISKUSSION

1. *Die Wirkung andauernder Dicamba-Behandlung auf das Pflanzenwachstum.* Die Wachstumsdynamik der Primärwurzel und des Sprosses (Koeoptile+1. Blatt) der Kontrollpflanzen folgt einer sigmoiden Kurve (Abb. 1.), wobei die Maximalwerte des Wachstumsrhythmus am 3.—4. Tag nach dem Ansetzen verzeichnet werden (nicht dargestellte Angaben). Unter der Einwirkung supraoptimaler Dicamba-Konzentrationen (1; 2 und 4 kg a.S./ha) wird dieses typische Wachstumsmodell vollkommen verändert (Abb. 1). Das Wachstum und die Entwicklung des Wurzelsystems verlaufen außerordentlich langsam, die Primärwurzel bleibt kurz (unter 10 mm Länge) und ist stark verdickt. Die Ausbildung embryonaler Nebenwurzeln wird unterbunden (Tafel I/2). Im Bereich der supraoptimalen Dicamba-Dosen sind die Effekte auf das Wurzelwachstum unabhängig von der angewandten Konzentration praktisch identisch. Daraus kann geschlossen werden, daß die Schwelle der phytotoxischen Konzentrationen in allen Dosen überschritten wurde, wodurch Wachstum und Entwicklung stark beeinträchtigt werden.

Das Wachstum der Koeoptile und des 1. Blattes ist im Bereich der supraoptimalen Dosen von der Konzentration abhängig, ohne daß jedoch eine strenge Korrelation zwischen Dosis und Effekt besteht (Abb. 1). Im Gegensatz zum Wurzelsystem wird das Sproßwachstum zwar stark gehemmt, jedoch nicht völlig aufgehoben. Morphologisch sichtbare Veränderungen nach Dicamba-Einwirkung äußern sich durch mannigfaltige Verformungen und Drehungen der Koeoptile (Tafel I/2); diese sind

verbreitert und innen hohl, da die Ausbildung der Blätter unterbleibt. Elektronenoptische Untersuchungen belegen, daß Dicamba die Ultrastruktur der Chloroplasten verändert [17].

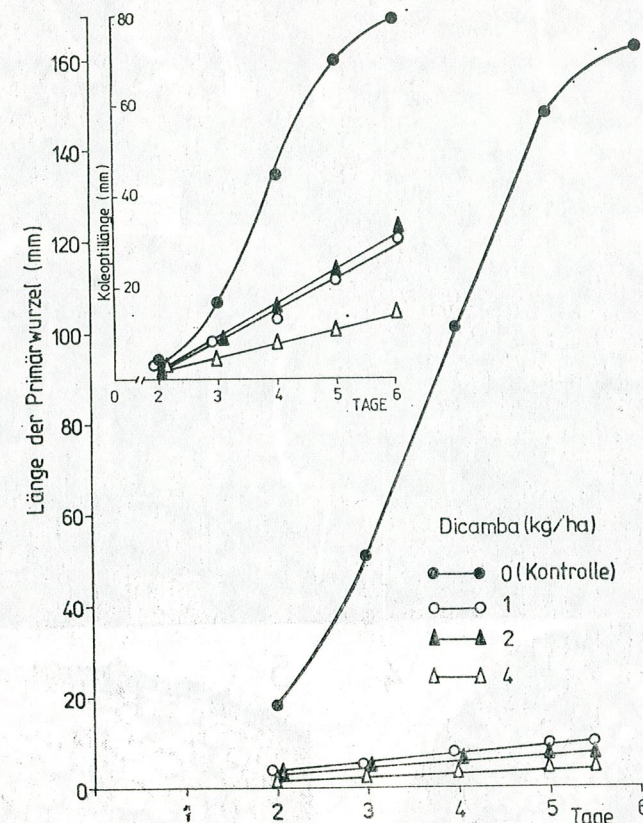
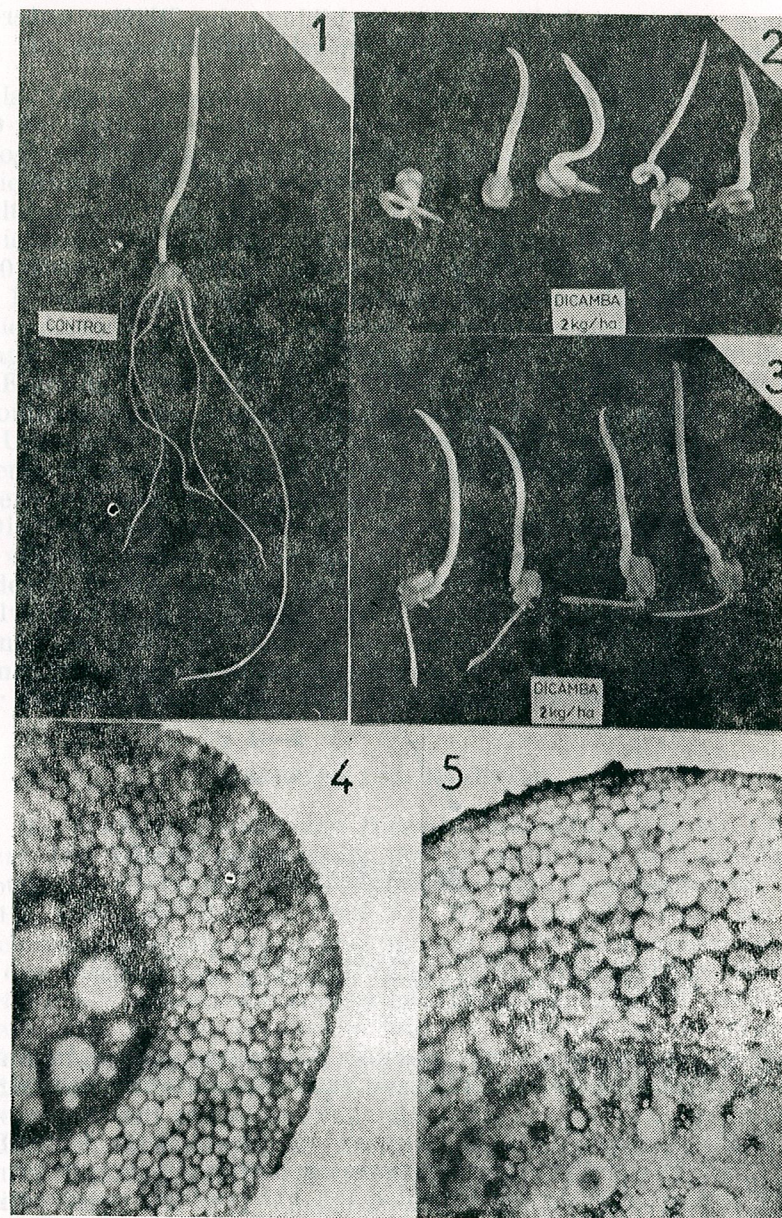


Abb. 1. — Die allgemeine Wachstumsdynamik von Mais-Keimpflanzen unter langfristiger Behandlung mit supraoptimalen Dicamba-Dosen im Vergleich zur Kontrolle.

Die Ergebnisse dieser Versuchsvariante unterstreichen die äußerst starke phytotoxische Wirkung langfristiger Dicamba-Behandlungen auf Mais-Keimpflanzen. Die unterschiedliche Sensibilität des Wurzelsystems und der Sproßachse gegenüber Dicamba bestätigt dessen Eigenschaften als Auxin-Herbizid, die aufgrund spezifischer Methoden zur Bestimmung der Auxin-Aktivität nachgewiesen wurden [11]. Die unterschiedliche Dicamba-Wirkung auf das Wurzelsystem und die Sproßachse geht eindeutig aus dem Verhältnis Sproß/Wurzel hervor. Im Falle der Kontrollpflanzen zeigt das Verhältnis mit Werten um 0,5 eine stärkere Entwicklung des Wurzelsystems in diesem Wachstumsstadium an, während diese Werte bei Dicamba-behandelten Pflanzen bis auf 3—4 zunehmen. Die morphologischen Anomalien der Sproßachse sind wahrscheinlich auf ungleichmäßige Vergrößerungen der Zelldimensionen durch Permeabilitäts- und Turgeszenzänderungen zurückzuführen [13], [18].



Tafel I

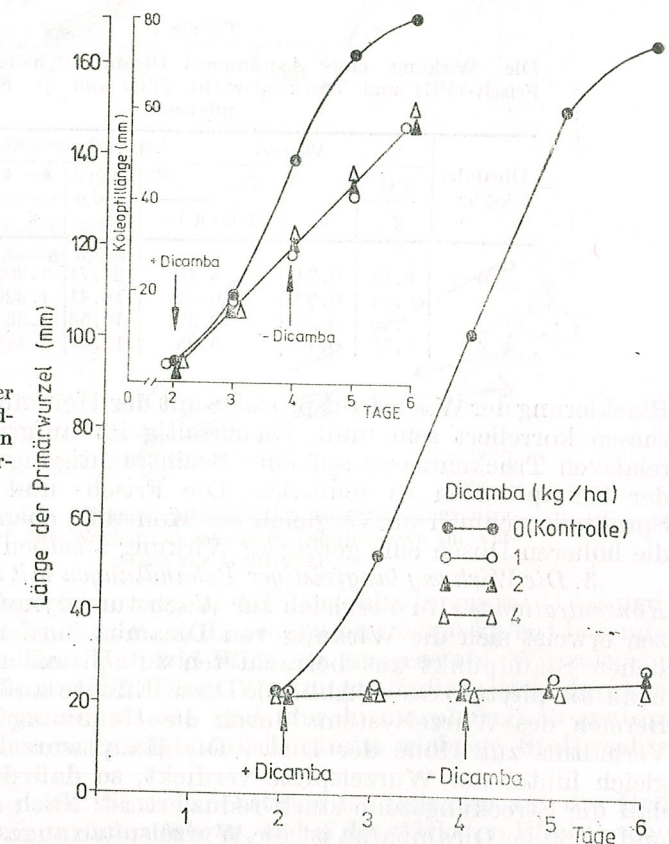
1. Kontrollpflanzen; 2. Wachstumshemmung und morphologische Effekte nach andauernder Einwirkung von Dicamba (2 kg/ha) auf Mais-Keimpflanzen; 3. Wachstumshemmung und morphologische Effekte nach 48stündiger Einwirkung von Dicamba (2 kg/ha) auf Mais-Keimlinge; 4. Querschnitt durch die Primärwurzel (Kontrolle); 5. Querschnitt durch die Primärwurzel nach 48stündiger Behandlung mit Dicamba (2 kg/ha).

2. Die Wachstumsdynamik nach kurzfristiger Dicamba-Behandlung.

Nach Übertragung der mit destilliertem Wasser vorgekeimten Pflanzen auf Dicamba wird das Wurzelwachstum drastisch gehemmt und im Laufe der gesamten Behandlungsdauer von 48 Stunden fast vollständig unterdrückt (Abb. 2). Eine Wiederaufnahme des Wurzelwachstums kann auch nach erneuter Übertragung der Keimpflanzen auf destilliertes Wasser nicht mehr festgestellt werden. Es muß demnach gefolgert werden, daß Dicamba die Zellstreckung auch nach kurzfristiger Behandlungsdauer vollkommen blockiert.

Außer der Hemmung des Wurzelwachstums induziert die kurzfristige Dicamba-Behandlung in Abhängigkeit von der Dosis verschiedene morphologische Effekte. Konzentrationen von 1 und 2 kg Dicamba/ha führen zu subapikalen Anschwellungen [8], die schon 24 Stunden nach Beginn der Behandlung auftreten und auch an den vorhandenen Nebenwurzeln festzustellen sind (Tafel I/3). Wurzelquerschnitte durch die subapikalen Anschwellungen zeigen eine chaotische Aktivierung der Zellteil-

Abb. 2. — Die Wirkung einer 48stündigen Dicamba-Behandlung auf das Wachstum von Mais-Keimpflanzen im Vergleich zur Kontrolle.



ungen im Perizykel und in der Endodermis [3], sowie eine Vergrößerung der Zelldimensionen im Rindenparenchym (Tafel I/5). Bei Dosen von 4 kg/ha sind die Wurzeln schlaff und nekrotisch verbräunt, die subapi-

kalen Anschwellungen dagegen nur schwach ausgebildet. Die Ausbildung von Nebenwurzeln unterbleibt bei der Mehrzahl der Pflanzen.

Die kurzfristige Dicamba-Behandlung auf das Sproßwachstum wirkt sich weniger drastisch aus. Wachstumsunterschiede zwischen Kontroll- und Dicamba-behandelten Pflanzen treten erst nach 48 Stunden Behandlungsdauer auf (Abb. 2). Nach Übertragung der Keimpflanzen auf destilliertes Wasser wachsen die Koleoptile mit verminderter Geschwindigkeit weiter, wobei die Reaktionen auf verschiedene Dicamba-Dosen praktisch gleich sind. Die kurzfristige Dicamba-Behandlung ruft ähnliche morphologische Anomalien der Sproßachse wie nach kontinuierlicher Einwirkung hervor.

Die Wirkung einer kurzfristigen Dicamba-Behandlung auf die Frisch- und Trockenmasse ist in Tabelle 1 zusammengefaßt. Das Frisch- und Trockengewicht des Wurzelsystems nehmen in Abhängigkeit von der Dosis stark ab. Die relative Trockenmasse nimmt dagegen mit größeren Dosen zu. Die Abnahme der Frisch- und Trockenmasse zeigt, daß die

Tabelle 1

Die Wirkung einer 48stündigen Dicamba-Behandlung auf das Frisch-(FG) und Trockengewicht (TG) von je 60 Mais-Keimpflanzen

Dicamba kg/ha	Wurzel			Sproß		
	FG	TG		FG	TG	
		g	g/100 g FG		g	g/100 g FG
0	8,81	0,744	8,45	21,71	1,802	8,30
1	3,01	0,303	10,07	16,41	1,326	8,08
2	2,26	0,234	10,37	16,54	1,364	8,25
4	1,59	0,148	9,28	17,07	1,442	8,45

Blockierung der Wachstumsprozesse mit der Hemmung wichtiger Stoffsynthesen korreliert sein muß. Gleichzeitig ist aufgrund der Zunahme der relativen Trockenmasse auf eine Beeinträchtigung des Wasserhaushaltes der Keimpflanzen zu schließen. Die Frisch- und Trockengewichte der Sproßteile nehmen im Vergleich zur Kontrolle ebenfalls ab, wobei jedoch die höheren Dosen eine geringere Wirkung ausüben.

3. Die Wirkung langfristiger Behandlungen mit suboptimalen Dicamba-Konzentrationen. Im Vergleich zur Wachstumsdynamik der Kontrollpflanzen erweist sich die Wirkung von Dicamba in den vom landwirtschaftlichen Standpunkt aus betrachteten suboptimalen Dosen noch weitgehend als phytotoxisch (Abb. 3). Diese Effekte äußern sich besonders im Bereich des Wurzelsystems durch die Hemmung der Primärwurzel im Verhältnis zur Höhe der Dosis. Die Hauptwurzel bleibt kurz und ist, gleich hinter der Wurzelspitze verdickt, so daß der Eindruck entsteht, daß die Streckungszone stark reduziert ist. Nach Behandlung mit 0,015 und 0,030 kg Dicamba/ha ist die Wurzelspitze angeschwollen; das Wachstum kann von diesen Anschwellungen von neuem, jedoch mit verminderter Geschwindigkeit, aufgenommen werden.

Das Längenwachstum der Sproßachse wird durch suboptimale Dicamba-Konzentrationen wenig beeinträchtigt. Bei 0,015 kg/ha sind die

absoluten Koleoptillängen denjenigen der Kontrollpflanzen vergleichbar. Durch Erhöhung der Dicamba-Konzentrationen wird das Längenwachstum der Koleoptile fortschreitend gehemmt. Außerdem sind Verformungen und Torsionen der Koleoptile festzustellen.

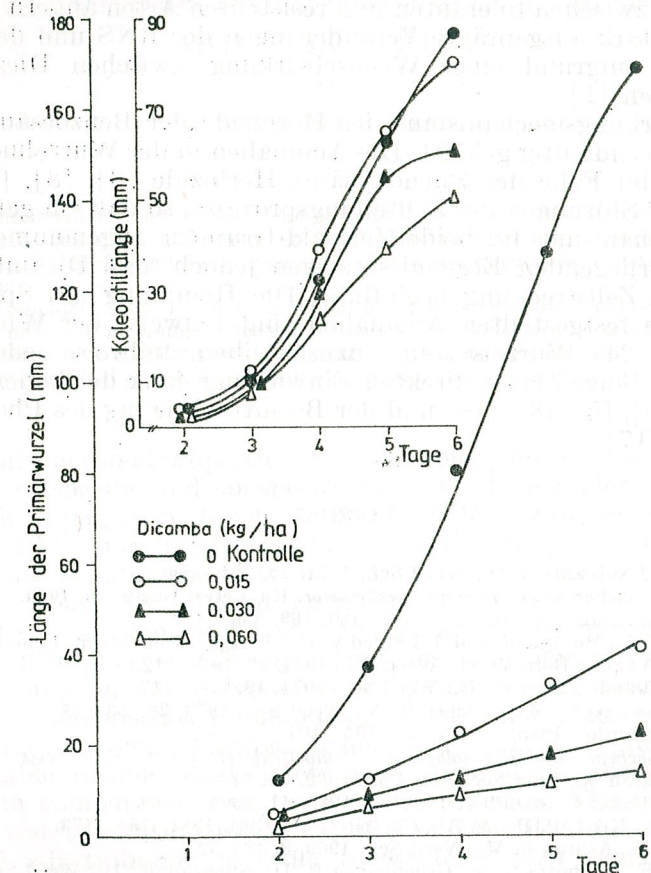


Abb. 3. — Die Wirkung suboptimaler Dicamba-Konzentrationen auf das Wachstum von Mais-Keimpflanzen im Vergleich zur Kontrolle.

Die erzielten Ergebnisse zeigen, daß Dicamba in supra- (1; 2 und 4 kg/ha) und suboptimalen Dosen (0,015; 0,030 und 0,060 kg/ha) besonders auf das unmittelbar der Herbizid-Wirkung ausgesetzte Wurzelsystem phytotoxische Effekte ausübt. Diese Wirkungen äußern sich durch die Hemmung der Wachstumsprozesse und durch morphologisch-anatomische Veränderungen, woraus folgt, daß Dicamba wichtige Stoffwechselprozesse beeinträchtigt.

Die Blockierung des Wurzelwachstums ist teilweise auf die Hemmung der Zellstreckung zurückzuführen, wobei die stärksten Effekte schon nach einer Behandlungsdauer von 24 Stunden auftreten. Die Abnahme der Frisch- und Trockenmasse läßt vermuten, daß die Blockierung der Wachstumsprozesse mit der Hemmung der Substanzproduktion ver-

bunden sein muß. Biochemische [1], [7], [14] und cytophotometrische Untersuchungen des Kern-DNS-Gehaltes [12] ergaben, daß Dicamba den Nukleinsäure- und Eiweißstoffwechsel beeinträchtigt. Dicamba verändert den Ablauf des Zellzyklus im Wurzelmeristem [12]. Selektivitäts-Unterschiede zwischen toleranten und resistenten Arten äußern sich durch verschieden stark ausgeprägte Veränderungen der RNS und des Proteingehaltes [7] aufgrund einer Wechselwirkung zwischen Dicamba und Nukleohistonem [1].

Der Wirkungsmechanismus der Herbizide der Benzoesäure-Gruppe ist noch nicht endgültig geklärt. Die Anomalien in der Wurzelmorphologie beruhen wie im Falle der Phenoxysäure-Herbizide [3], [8], [12] wahrscheinlich auf Störungen der Zellteilungsprozesse, so daß ein gemeinsamer Wirkungsmechanismus für beide Herbizid-Gruppen angenommen werden kann. Die vorliegenden Ergebnisse zeigen jedoch, daß Dicamba zusätzlich auch die Zellstreckung beeinflusst. Die Hemmung des Sproßwachstums und die festgestellten Anomalien sind teilweise der Wirkung von Dicamba auf das Wurzelsystem zuzuschreiben, teilweise jedoch wahrscheinlich die Folgen einer direkten Einwirkung dank der hohen Translokationsrate [5], [6], [8], [16] und der Beeinträchtigung des Photosyntheseapparates [17].

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TESTING THE ANTIMICROBIAL ACTIVITY OF SOME ACTIVE FACTORS ISOLATED FROM HUMAN MILK AND COLOSTRUM

BY

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The antimicrobial effect of some components of human milk: lysozyme, lactic peroxidase, lactoferrin, secretory IgA and α -lactalbumin used separately and associated is tested *in vitro* on Gram positive and Gram negative bacteria as well as on fungi.

The bactericidal action by mutual regulation of lysozyme and lacticperoxidase, and the increased antifungal effect of the association lactoferrin + α -lactalbumin were noticed. The specific component secretory IgA has an agglutination effect for all the microbial strains tested, without affecting finally their viability.

The antimicrobial properties of human colostrum and milk correspond to a system of specific and nonspecific factors: the complement and antibodies (such as secretory IgA); lactoferrin (LF); lysozyme (LZ); lacticperoxidase (LP); antiviral factors [4], [6], [13], [14]. This system exists in all mucous membranes of animals in order to defend them against various infections. An efficient antimicrobial system obtained *in vitro* and using the conditions and factors existing in the organism's humours and cells, can be successfully applied in therapy. Some of the mentioned components used in our previous investigations in the form of purified preparations, demonstrated microbiostatic and microbicidal actions on some bacteria and fungi. Various authors [5], [10], [12], [15], [16] point out remarkable antimicrobial effects when some of the above factors are associated in comparison with their separate action. The present paper intends to test the antimicrobial activity of LZ, LP, LF, secretory IgA (SIgA) and α -lactalbumin in order to establish their synergistic action for controlling and regulating their activity.

MATERIALS AND METHODS

Test microorganisms. Bacteria: *Staphylococcus aureus* Oxford and *Escherichia coli* 0 128 B 12 strains were obtained from the Epidemiological Laboratory of the Institute of Hygiene — Bucharest; *Salmonella enteritidis* was obtained from the "Cantacuzino" Institute — Bucharest; *Proteus mirabilis* was isolated from a patient and identified in the Epidemiological Lab. of the Institute of Hygiene.

Fungi: the yeast *Candida albicans* 704 was isolated from a patient in the Clinic of the "Filaret" Hospital.

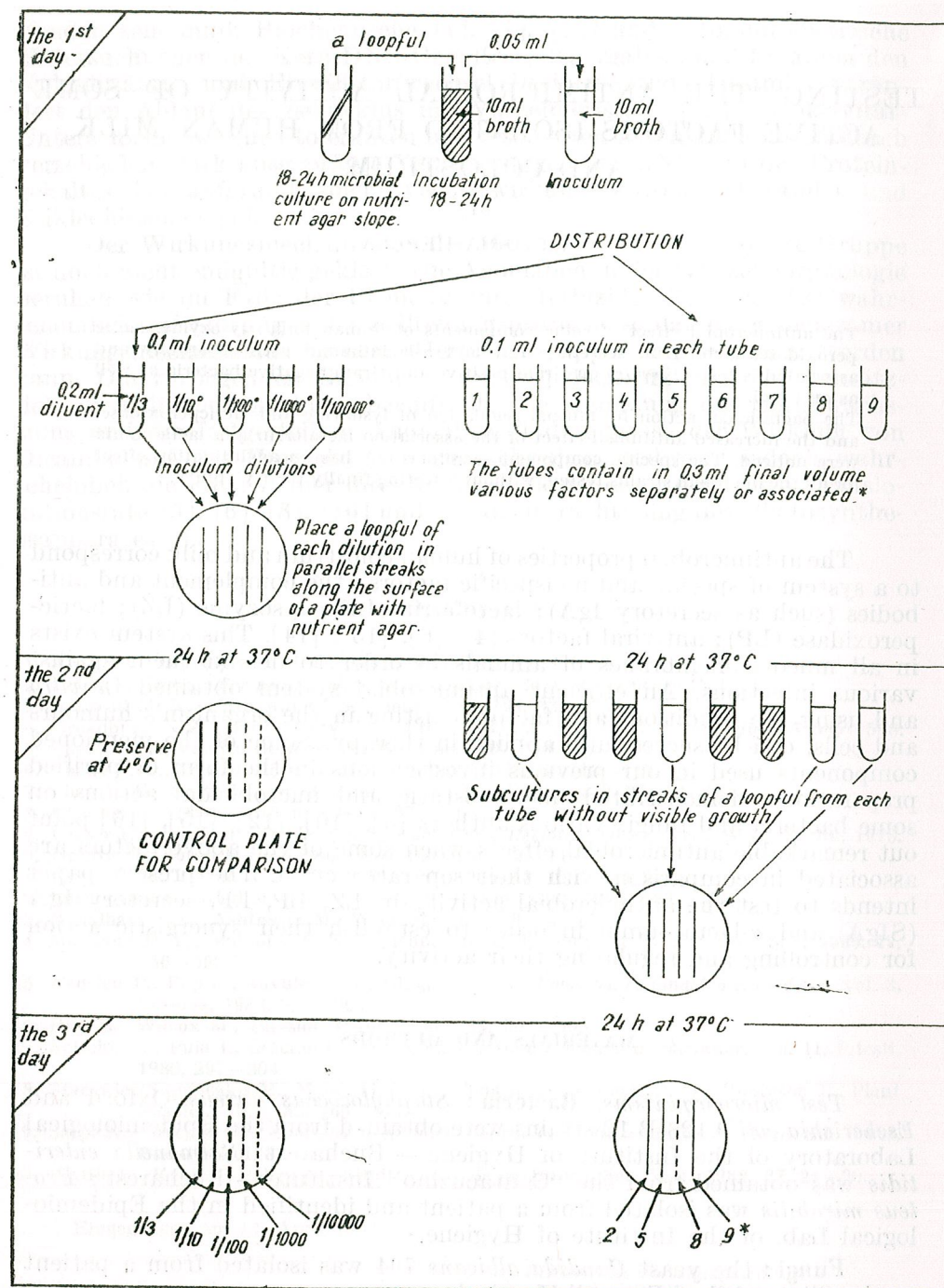


Fig. 1. — The study method in liquid medium of the bactericidal effect of active factors, separately or in association.

The bacteria stock cultures were kept on nutritive gelose in test tubes with paraffined stoppers at +4°C. Fresh cultures for analyses *in vitro* were obtained by passage on gelose slopes and incubation at 37°C. Before tests, the cultures' purity was controlled by obtaining single colonies isolated on gelose plates after 24 hours incubation at 37°C. The 24-hour pure cultures grown at 37°C were used for inoculum preparation. The yeast stock cultures were kept on agar YPG medium under a paraffin layer at +4°C. The YPG medium contains (in g/l) Difco Yeast Extract — 10 g; bacto-peptone — 10 g; glucose — 20 g; agar — 20 g. It is sterilised at 120°C for 20 min, with a final pH 5.6. The inoculum, made up of suspensions in saline or broth (for bacteria) and acetate acetic acid buffer (for yeasts), was standardised. The germs concentration was established in each case by reading the extinction value at 450 nm (colorimeter FEK—56 M) and accurately determining CFU/ml (according to [7]), or was obtained according to Duval and Soussy [8].

Active factors isolated from human milk and colostrum were used in the form of purified preparations of a given concentration and enzymatic activity in the case of enzymes. Lactic peroxidase had $c = 50 \mu\text{g/ml}$ and specific $A = 178 \mu\text{M/mg/min/25}^\circ\text{C}$, pH 5.7. For $10 \mu\text{g/ml}$ LP, $1 \mu\text{mol/ml}$ H_2O_2 and $2 \mu\text{mol/ml}$ KSCN were also introduced in system. The lysozyme (lytic $A = 54 \mu\text{g/ml}$ or 540 i.u./ml , pH 5.5—5.8) was added to samples in concentrations of $6 \mu\text{g/ml}$. SIgA was used as a solution of 5 mg/ml concentration, pH = 8, lactoferrin in a concentration of 3 mg/ml and α -lactalbumin in concentrations of 3.3 mg/ml .

The microbicidal activity was appreciated in samples containing reaction mixtures represented by the inoculum, the analysed factors alone or in certain combinations, and the medium or corresponding buffer. The samples were kept for various periods of time at 37°C and the viability of test microorganisms was determined in CFU/ml by collecting at certain periods given quantities from the samples. These were serially diluted and inoculated by dissemination of 0.1 ml from each dilution on two plates with agar medium; 24—48 hours after incubation at 37°C, CFU were counted, appreciating the viability in comparison with the control. For some of the samples, the microbicidal activity was analysed by appreciating the percentage of surviving germs after a 24-h contact with the associated factor or factors using the method recommended in the study of the bactericidal power of antibiotics in liquid medium, adapted by us (Fig. 1). The microbicidal anti-*P. mirabilis* effect of the association LZ with the system LP was also tested by the method of diffusion in agar used in the study of antibiotics synergism.

Bacteriolytic activity was evaluated by measuring OD at 450 nm, in samples incubated at 37°C for established periods of time.

RESULTS AND DISCUSSION

When testing the effect of the lactic peroxidase system (LP_s) and LZ used separately and in combination along a first experiment with *P. mirabilis*, a stronger inhibitory synergic activity after 60' incubation was found in the case of simultaneous introduction of the two factors

(Fig. 2). Analysis of the viability evolution in experimental samples revealed no effect of lysozyme when used alone, a bacteriostatic effect of lactic peroxidase system and no intensified lysozyme activity under the influence of LP_s alone in an initial stage. In our experimental conditions, this result does not substantiate the existence of a sensibilisation

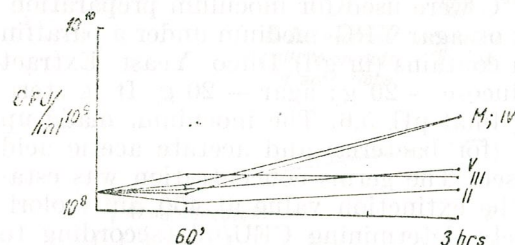


Fig. 2. — The effect of lactic peroxidase system (LP_s) and lysozyme (LZ), separately and associated, on the bacteria *Proteus mirabilis*. M = control; II = LP_s + LZ; III = LP_s ; IV = LZ; V = LP_s + LZ after a 30' period of initial incubation with LP_s only.

period by LP system for Gram negative bacteria, followed by a facilitated lytic action of LZ on its cellular wall, although the cells' aspect on smears supports this idea. It is possible that the destructive action of LZ

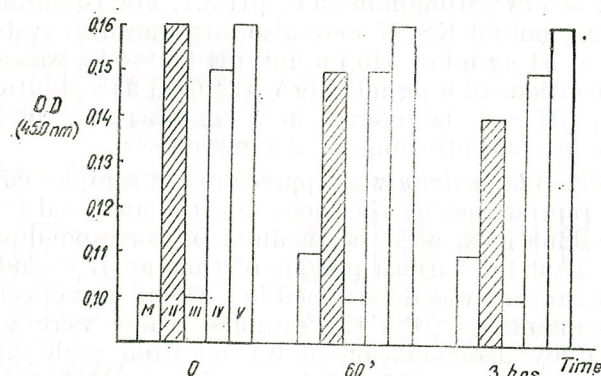


Fig. 3. — Changes in OD of the *P. mirabilis* suspension incubated at 37°C in the presence of tested factors. Same legend as for Fig. 2.

manifests itself by another mechanism (besides the enzymatic hydrolysis of β 1—4 glycosidic bonds in peptidoglycans) and also that a higher LZ concentration is necessary for a longer period of time. These suppositions were confirmed by other experiments as well.

The modifications in OD of *P. mirabilis* suspension incubated at 37°C in the presence of tested factors confirm the results previously mentioned (Fig. 3).

The positive antimicrobial effect of LZ associated with LP system is also confirmed by the method of diffusion in agar. The aspect of the obtained inhibition area corresponds to an additional effect of LZ associated with LP system.

In order to confirm the results obtained on *P. mirabilis* and expand the tests on other factors and on several microorganisms, test experiments of the antimicrobial activity of LP_s , LZ, SIgA, α -lact. and LF, separately

or associated, have been performed on all investigated bacteria as well as on *C. albicans*.

In sample containing LP_s , LZ + LP_s , LP and H_2O_2 the absence of *S. aureus* survivors is noticed (Fig. 1) *.

The bactericidal effect of LP alone is very interesting; for the other types of strains it is: 10% in *S. enteritidis*, and 1% in *E. coli* and *P. mirabilis*. Potentiation of lysozyme with lactic peroxidase on *P. mirabilis* as well as on the other test bacteria is confirmed. The antimicrobial action of LP is also proved by the modified aspect (smaller dimensions) of colonies developed from surviving germs. The combinations LZ + SIgA and SIgA + α -lact. did not have any inhibitory effect on tested bacteria. SIgA alone or in the mentioned combinations caused bacterial agglutination without affecting bacterial viability.

In experiments testing the antimicrobial effect by appreciating the viability in CFU/ml, 3, 6 and 9 hours after introducing the factors into samples with *S. aureus* (Fig. 4) the bactericidal effect of LP and of the association LP with LZ is noticed at a lower concentration (25 μ g/ml) of LP than the one used in previous experiments. Lysozyme is bacteriostatic after 9 hours and SIgA after 3 hours, without affecting bacterial viability (as a matter of fact after 9 hours it exceeds the viability of the control). We consider that the delayed effect of LZ is due to its low concentration (85 μ g/ml). Appreciation of viability in the respective samples points out the absence of any antimicrobial effect of α -lact. and of the associations SIgA with LZ and α -lact., although the latter induces a reduction of CFU as compared to the control in the last three hours of action.

The effect of lactoferrin, SIgA, α -lact. and lysozyme, separately or associated, on *C. albicans* 704 is tested on 8 samples (Fig. 5) that contain acetate acetic acid buffer to ensure the optimum activity of lactoferrin at pH 5.

In samples used so far, the antimicrobial effect is due to LF alone or associated with α -lactalbumin. α -lactalbumin alone has no effect, the yeasts in this sample having a higher viability than the control. The other combinations of LF (with SIgA and lysozyme) does not modify the viability of *C. albicans* 704. The results obtained by other authors [15] show that SIgA does not increase the inhibitory effect of LF on the growth of some strains of *E. coli*, *Pseudomonas* and *Proteus*, and the selective bactericidal effect of LF on some bacteria can be intensified by α -lact. or serum albumin [1]. The effect of SIgA over 2 hours is to decrease the viability by agglutination. As well as with the bacteria tested in previous experiments in the presence of SIgA, agglutination is a result of action specificity. For *C. albicans* the testing time is of only two hours and therefore we can not appreciate the impact on viability which in fact does not occur in the tested bacteria (viability being estimated after 9 and 24 h).

These observations support the idea that the main function *in vivo* of SIgA is not to destroy the microbial agents alien to the human or animal body but rather to prevent their access to the general immunological system [9].

* The apparent absence of any survivor is appreciated in terms of the technique used for antibiotics as survivors percentage below 0.01 %.

The susceptibility of *C. albicans* 704 to LF is in agreement with data provided by other authors [2], [11]. It is interesting that the antimicrobial effect is given by the saturated LF. According to recent data

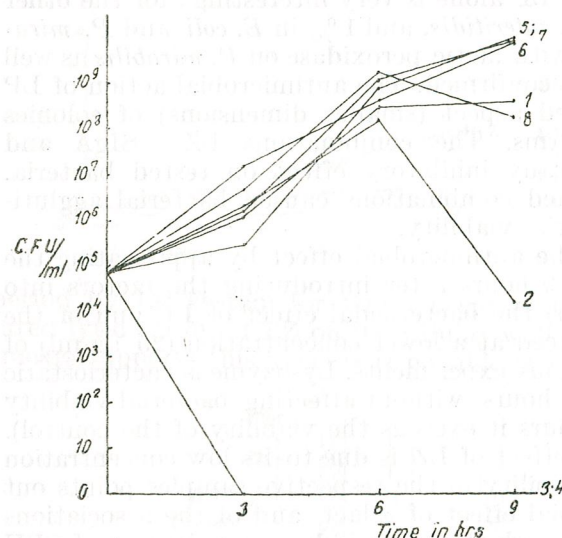


Fig. 4. — Susceptibility of *S. aureus* Oxford to the action of active factors in human milk used separately or in association. 1 = control (inoculum + saline); 2 = LZ; 3 = LP; 4 = LZ + LP; 5 = SIGA; 6 = LZ + SIGA; 7 = α -lact.; 8 = SIGA + α -lact.

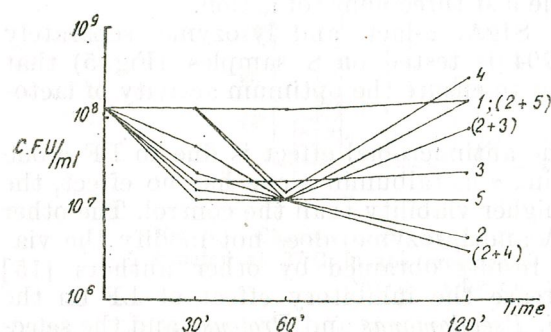


Fig. 5. — Viability of *C. albicans* 704 incubated for two hours at 37°C with lactoferrin (2), SIGA (3), α -lactalbumin (4), lysozyme (5) and with lactoferrin in association with SIGA (2 + 3), α -lactalbumin (2 + 4) and lysozyme (2 + 5).

[3], it is not so much the presence or absence of Fe in the medium (which seems not to be the target situs of the action) but rather the experimental physical conditions and the metabolic state of the target microorganism that are of importance for the mechanism of action of LF.

CONCLUSIONS

The results obtained so far allow the following conclusions:

- LP alone or added to LZ proves to be the strongest bactericidal factor in human milk.
- The antibacterial effect of LP is differentiated according to the tested microorganism.

— LP associated with LZ leads to:

- (a) reciprocal intensification of the bactericidal action on *S. aureus* in time;
- (b) intensification of LZ lytic activity on Gram negative bacteria such as *P. mirabilis*, by simultaneous associations;
- (c) intensification of the bactericidal action of LP on *S. enteritidis*, *P. mirabilis* and *E. coli*.

— α -lactalbumin enhances the effect of LF on *C. albicans*.

— Some associations of the active factors tested do not lead to a synergical action.

— SIGA agglutinates the tested microorganisms without affecting their viability along the experimental period.

These data are valid criteria to be considered in establishing the composition of preparations from human milk, specific for infections of mucous membranes and for the pathogenic agent, in order to be successfully used in the clinic.

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THE EFFECT OF GENOTYPE AND CULTURE AGE ON THE MORPHOGENETIC POTENTIAL OF *DATURA INNOXIA* MILL. CALLUS CULTURES AND ON THE PLOIDY OF REGENERANTS

BY

ELENA BADEA and PETRE RAIGU

Experiments were carried out to study the morphogenetic capacity of 23 genotypes of *Datura innoxia* with different ploidy levels (n , $2n$, $3n$, $4n$, $6n$, $8n$). It was found that the ability to differentiate is not dependent on the number of genomes, as cells with 1–8 chromosomal complements can undergo differentiation. By screening many genotypes it was possible to isolate some lines which do not lose their differentiation potential during prolonged culture. An estimation of the ploidy of regenerants has revealed the in vitro polyploidization during prolonged culture which is not always correlated with a loss in organ forming capacity.

Datura innoxia, as well as *Nicotiana tabacum*, has a high capacity of growth and differentiation on artificial culture media. There are only a few studies on the morphogenetic capacity of *Datura innoxia* and they refer to haploid and diploid material [3, 11, 13].

The loss of regenerative potential during prolonged subcultures was reported in cell cultures of many species and has been attributed to the increased incidence of aneuploidy and polyploidy [5, 6, 12, 15]. There are, however, reports in the literature about the regeneration of plants from long-term cultures in several species which suggests that the decline of the organogenic potential does not imply the loss of the genetic information necessary for cytodifferentiation [8, 9, 14, 16].

In this paper we reported the effect of genotype, ploidy level and culture age on the in vitro plant regeneration and the ploidy level of regenerants as function of these factors.

MATERIAL AND METHODS

The explants used for culture initiation were excised both from androgenetic plants as well as from plants regenerated via callus.

The induction and maintenance of callus were made on Murashige-Skoog [7] medium supplemented with 1 mg/l naphthylacetic acid (NAA) and the regeneration of plants on the same medium with 1 mg/l benzyl aminopurine (BAP) and 0.1 mg/l NAA.

The cultures were maintained by serial subcultures at 3–4 weeks intervals and incubated for 16 hours under light at 2500 lux.

The regenerated shoots were transplanted into the Murashige-Skoog medium without any growth regulators, for rooting.

Chromosomes were counted in the root tip squashes after Feulgen staining.

RESULTS AND DISCUSSIONS

The results concerning the differences observed in the regenerative capacity of the 23 genotypes with different ploidy levels are presented in Table 1. The regenerative capacity of calli derived from haploid, di-

Table 1

The effect of genotype and ploidy level on morphogenetic capacity of *Datura innoxia* callus cultures

Genotype of the explant	Morphogenetic capacity in the second subcultures	Morphogenetic capacity in the tenth subcultures
n=12 (L4)	+	—
n=12 (L30)	+++	++
n=12 (L6)	+	—
2n=24 (I1* 105)	+	—
2n=24 (I1 6)	+++	+++
2n=24 (I1 7)	+	+
2n=24 (I1 5)	++	+
2n=24 (I1 33)	++	++
2n=24 (I1 11)	+++	+++
2n=24 (I1 54)	++	+
2n=24 (I1 55)	++	+
2n=24 (I1 12)	++	+
2n=24 (I1 106)	++	+
2n=24 (I1 56)	+	—
2n=24 (I1 112)	++	+
2n=24 (I1 58)	+	—
3n=36 (L 113)	+	—
3n=36 (L 12)	+++	+++
3n=36 (L 2)	++	—
3n=36 (L 32)	++	—
4n=48	++	+
6n=72	++	—
8n=96	++	—

* isogenic line; +++ extensive differentiation; ++ moderate differentiation; + some differentiation; — no differentiation

reported that haploid and diploid calli from *Datura innoxia* show similar morphogenetic capacity with respect to shoot formation but this property is lost during culture.

Cytogenetic analyses of the plants derived from n, 2n, and 4n calli after a year of culture and from a 2n callus after a period of two years were also performed (Table 2).

ploid and polyploid plants of *Datura innoxia* is not dependent on the number of genomes, as cells with 1—8 chromosomal complements can undergo differentiation. Generally, the haploid and diploid calli produced more buds and shoots than the polyploid ones.

The genotype of the explant influences the regenerative capacity as there are differences between the calli initiated from different isogenic lines, haploid and triploid plants. So, from the 14 isogenic lines studied, only two had a high ability to differentiate which has been maintained for a long time. The totipotency of these cultures has not diminished during a prolonged culture period (10 subcultures of approximately 4 weeks each).

Our results are similar to those obtained by Forche et al. (1981) who noticed that differences concerning the morphogenetic capacity of haploid and diploid tissues are due more to genetic and/or other differences than to the ploidy level. Sharma and Chowdury (1977) also re-

The chromosome constitution of regenerated plants shows an increased frequency of polyploidy with time in culture. From the haploid calli n, 2n and 4n plants differentiated, after the second transfer, and after a one year period of culture, only 4n plants. The same increase of

Table 2

Chromosome number of the plants regenerated from different subcultures

Chromosome number of donor plant	No. of subcultures	No. of plants examined	No. of plants with different levels of ploidy			
			n	2n	4n	8n
n=12	2	46	2	36	8	—
n=12	12	10	—	—	10	—
2n=24	2	56	—	17	39	—
2n=24	10	10	—	—	10	—
2n=24	20	5	—	—	4	1
4n=48	2	20	—	—	20	—
4n=48	10	10	—	—	10	—

the frequency of polyploidy, during prolonged culture has also been noticed in the case of regenerants from diploid calli. The two years old cultures produced non-rooting plants, many of which developed aberrant and stunted shoots, none of which elongated more than a few centimeters. One of these plants, which rooted, was an octoploid.

The tetraploid genome is much more stable as all plants regenerated after culture for a year were tetraploid ones.

The chromosome counting of the regenerants reveals the chromosome constitution of the callus cells. The increase of the number of polyploid plants in time is a proof for in vitro polyploidization during prolonged cultures, but this progressive increase of polyploidy is not always correlated with a loss in organ forming capacity.

The absence of aneuploids among the regenerated plants suggests that with *Datura innoxia* the aneuploid cells do not undergo a cytodifferentiation process or that in calli there is a selective pressure against the plant regeneration of this type of cells. The aneuploid plants were regenerated from cultures of many species [1, 2, 4, 10] but not from those of *Datura innoxia*.

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LE PROFESSEUR TRAIAN I. ȘTEFUREAC À SON 75^{ème} ANNIVERSAIRE

Le professeur et homme de science Traian I. Ștefureac est né le 18 avril 1908 à Cimpulung Moldovenesc, département de Suceava.

Il étudia les sciences naturelles à la Faculté des sciences de l'Université de Cernăuți, se formant à l'école botanique de l'illustre professeur M. Gușuleac et des renommés savants E. Botezat, C. Hurmuzachi, Fr. Netolytzky, A. Penecke, P. Ionescu Bujor, N. Florescu, O. Marcea et autres. Il accomplit ses études en 1934 et fut nommé professeur de lycée; parallèlement à ses tâches didactiques, il déploya une riche activité scientifique de pionnier dans le domaine de l'écologie et de la phytosociologie des Bryophytes.

En 1940 il soutint sa thèse de doctorat « Recherches synécologiques et sociologiques sur les Bryophytes de la forêt séculaire de Slătioara (Bucovina) », un des premiers ouvrages de ce genre dans la bibliographie universelle et qui le consacra dès le début comme un pionnier des recherches de synécologie.

A partir de cette même année et jusqu'en 1973 il parcourut tous les degrés de la hiérarchie didactique universitaire, enseignant avec compé-

tence la botanique systématique phylogénétique, la botanique générale, la géobotanique et faisant des cours de spécialisation en algologie, mycologie, phylogénie. Il fut longtemps le chef de la chaire de botanique systématique, membre du conseil de direction du Jardin botanique, chef de laboratoire et de secteur à l'Institut de sciences biologiques; il eut une importante contribution à l'organisation, après la guerre, de l'herbier du Jardin botanique et de la chaire de botanique systématique.

A partir de 1973, en tant que professeur-conseil, Tr. I. Ștefureac dirige l'élaboration des thèses de doctorat de nombreux scientifiques; il déploie aussi une riche activité de recherche scientifique ainsi que de vulgarisation des sciences biologiques, de l'histoire de la botanique, de la protection de la nature.

Sa complexe activité se reflète dans les nombreux ouvrages publiés par les revues de biologie végétale de l'Académie de la République Socialiste de Roumanie, des universités et des institutions biologiques de Roumanie, ainsi que par les revues de l'étranger, comme « Travaux bryologiques », « Revue bryologique et lichénologique », « Pollen et spores », « Bulletin of Bryology », « Lejeunea », « Journal of Bryology » etc. Il a publié aussi des livres, dont certains ont un caractère monographique, concernant les recherches synécologiques et sociologiques sur les Bryophytes de la forêt séculaire de Slătioara, les alpages des monts Bucegi, les recherches bryologiques sur certaines formations de végétation de Roumanie. Il a collaboré à l'élaboration de la Flore de la République Socialiste de Roumanie, de la Flore cryptogamique de la Roumanie et de l'« Atlas des Portes de Fer » publiés par l'Académie, il rédige des conspécts des Bryophytes de diverses régions de la Roumanie.

En tant que taxonomiste et systématicien, il a décrit de nombreux taxons, nouveaux pour la science et pour la Roumanie (algues, Bryophytes, Cormophytes) et quelques cénotaxons, abordés du point de vue phytocénologique (bryocénoses, associations muscinales et cormophytiques). Il étudie les espèces relictées arctiques et sous-arctiques parmi les Bryophytes (*Sphagnum wulfianum*, *Helodium blandowii*, *Aulacomnium turgidum*, *Meesia triquetra*, *M. longiseta*, *Mnium cinclidioides*, *Conostomum boreale*, *Moerckia flotoviana* et autres) et les Cormophytes (*Cochlearia pyrenaica*, *C. p.* var. *borzaeana*, *Cobresia simpliciuscula*, *Carex chondrorhiza*, *C. loliacea*, *C. capillaris*, *Calla palustris*, *Trientalis europaea*, *Viola palustris*, *V. epipsila*, *Betula warnstorffii* et autres) ainsi que parmi les associations relictaires (*Sphagnum wulfianum* avec *Helodium blandowii*, *Aulacomnium turgidum* avec *Cobresia simpliciuscula* et autres) et il étudie aussi certaines relictées tertiaires (*Bucegia romanica*, *Andriala levitomentosa*, etc.).

L'aire large de ses préoccupations embrasse le domaine des Bryophytes, dont il est fondateur d'école, celui des Cormophytes, qu'il étudie sous l'aspect phytocénologique et floristique, de l'algologie et de la mycologie; il élabore des travaux d'analyse du caractère général de certaines régions de la Roumanie, des ouvrages sur les écosystèmes de végétation les plus variés (forêts, marais tourbeux, terrains salins et d'altitude) en mettant en évidence leur écologie, variabilité-polymorphisme-spéciation, cytologie-caryologie (polyploïdie), taxonomie et cytotaxonomie, morpho-

logie et anatomie, physiologie, morphogenèse, embryologie, tératologie, corologie, aréalographie, florogenèse, palynologie et sporologie, évolution et phylogénie.

Il étudie les endémities et leur corologie en Roumanie, l'évolution phylogénétique des algues vertes, le problème du toundra alpin dans les Carpates de Roumanie.

Dans le domaine de la protection de la nature, il remplit avec compétence la tâche d'inspecteur honoraire des réserves boisées de Bucovina; il publie des ouvrages sur la flore et la végétation des réserves naturelles de Roumanie, et surtout de Bucovina et Maramureș, relevant la nécessité impérieuse de protéger les espèces rares, les endémities, les relictées.

Il est membre de l'« International Association of Bryologists », de « l'Association de cartographie des Bryophytes de l'Europe », du comité de rédaction des revues de biologie végétale de l'Académie de la République Socialiste de Roumanie et rédige des comptes rendus des travaux des bryologistes roumains pour la « Revue bryologique et lichénologique » de Paris.

Il participe à de nombreux congrès et symposiums en Roumanie et à l'étranger, où il présente d'intéressantes communications scientifiques.

Les botanistes roumains lui ont dédié quelques taxons nouveaux: *Polytrichum ștefureacii* Plămadă, *Hieracium alpinum* L. var. *ștefureacii* Nyár., *Veronica urticifolia* Jacq. f. *ștefureacii* Zaharia et autres.

Son activité didactique, sa présence active dans la vie scientifique, ses recherches de grand intérêt lui valurent l'attribution des prix « Gheorghe Doja » (1957) et « Em. C. Teodorescu » (1974) de l'Académie de la République Socialiste de Roumanie et de l'ordre « Étoile de la République Populaire Roumaine » (1964).

A l'occasion de son anniversaire, les botanistes lui adressent leurs vœux de longue vie et lui souhaitent de continuer sa riche activité scientifique.

Venera Ionescu
Ioan Cristurean

NICHIFOR CEAPOIU, FLOAREA NEGULESCU, *Genetica și ameliorarea rezistenței la boli a plantelor* (Genetik und Amelioration der Resistenz gegenüber Pflanzenkrankheiten). Edit. Academiei, București, 1983, 298 S., 42 Abb.

Das vorliegende, planmässig besonders gut ausgearbeitete Buch umfaßt zwei Teile: „die genetischen Ameliorationsgründe der Resistenz gegenüber den Pflanzenkrankheiten“ (9 Abschnitte) und „die Amelioration der Krankheiten landwirtschaftlicher Pflanzen“ (7 Abschnitte).

Im ersten Teil handelt es sich vornämlich über die Genetik der Resistenz und Pathogenität der Pflanzen und die genetische Interaktion zwischen Wirt und Schmarotzerpflanzen, sowie über die bekannten Theorien der Gegenseitigkeit zwischen Wirt und Schmarotzer.

Es wird die Tätigkeit der Gene in gegenseitiger Aktivität Wirt — Schmarotzer, hinsichtlich der Notwendigkeit der Abwehr der Pflanzen behandelt, sowie die Genetik der pathogenen Populationen. Es ist auch die Rede über die genetische Verletzlichkeit und deren Uniformität, sowie die Entwicklung aller pflanzlichen Schmarotzer. Besonders die ersten Abschnitte die sich damit beschäftigen sind von den Verfassern gut und weitgehend ausgearbeitet, indem hier das Verhältnis Genetik, Amelioration, Resistenz und Nützlichkeit vornämlich landwirtschaftlicher Kulturpflanzen einer näheren Betrachtung unterzogen werden.

Im zweiten Teil werden die vorläufigen nötigen Informationen über die Entwicklung der Ameliorationsarbeiten betreffend die Resistenz zu den Krankheiten, sowie im gleichnachfolgenden Abschnitte wird die objektive Amelioration gegenüber den möglichen Pflanzenkrankheiten gegeben. Der nachfolgende Abschnitt befaßt sich mit dem Grund des Keimplasmas für die Resistenz oder die genetischen Zentren, sowie der Ursprung der Pflanzen als Quellen der Pflanzenresistenz. Es wird auch der Amelioration, der Resistenz gegenüber den Krankheiten Rechnung getragen. Dieser bedeutende Abschnitt ist so ziemlich weit entwickelt betreffend sowohl die gradlinige Amelioration, sowie deren Methoden. Ein Abschnitt ist der Kontrolle der Krankheit und der Handhabung der Gene für die jeweilige nötige Resistenz und deren Toleranz gewidmet. Die Verfasser schenken ihr Interesse auch der Bestimmung der Resistenz der Pflanzen gegenüber den Krankheiten; in diesem Teil werden besonders die natürlichen bis zu den künstlichen Infektionen in den verschiedenen Stadien sowie die Testierung behandelt.

Letztlich widmen die Verfasser ihr Interesse den Ergebnissen die in der Genetik und in der Amelioration der landwirtschaftlichen Pflanzen und deren Resistenz gegenüber den Krankheiten in der Sozialistischen Republik Rumänien mit der Zeit erreicht worden sind, indem den wichtigsten Pflanzen Rechnung getragen wird.

Dieses Werk ist zunächst den Handhabern dieser Pflanzen gewidmet, kann aber allen Wissenschaftlern empfohlen werden, die darin eine richtige Orientierung in ihrer täglichen Tätigkeit finden können.

Ion T. Tarnavski

JOHN A. HEDDLE, *Mutagenicity, New Horizons in Genetic Toxicology* (Cell Biology: A series of monographs, Academic Press, 1982, 471 p.)

Lately, a new branch of mutagenesis, the environmental mutagenesis has captured the interest of the researchers. It does not endeavour to discover new mutagen substances, but to test the mutagen, carcinogen and teratogen potential of the naturally existent environmental chemicals and of the man-made ones.

Mutagenicity, New Horizons in Genetic Toxicology is a book discussing problems raised by testing environmental mutagenicity. Each of its 16 chapters, written by outstanding specialists

lists, deals with an independent problem, considering all the involved aspects, from specific methodology to specific testing systems, from advantages and shortcomings to interpretation. Though not a book of techniques, it presents however the most modern, efficient and adequate techniques in close connection with the biological material studied and the goals pursued.

Discussing general problems of mutagenicity testing, Chapter 1 (John Ashby) points out the necessity of using at least 3 tests to characterize genotoxic chemicals, as well as the need for taking into account the divergences among the results of certain testing systems and hence the difficulties in their interpretation.

Questions of wide interest, such as whether short-term tests can be compared to the data on carcinogenicity in humans, or used to establish the carcinogenic activity of chemicals, are largely debated in Chapter 2 (H. Bartsch, L. Tomatis and C. Malaveille).

The influence of environmental factors, of daily life, of lifestyle and food on the incidence of cancer, as well as cancer evaluation by employing mutagenicity assays, fall under the scope of Chapter 3 (Takashi Sugimura and Minako Nagao). It is generally accepted that the mutagens discovered during microbial testings can initiate tumors in mammals.

Chapter 4 (D. W. Bryant and D. R. McCalla) continues the argument on toxic pollutants as cause of point mutations and cancer in a steel foundry environment.

Chapter 5 (H. F. Stich, M. P. Rosin, C. H. Wu and W. D. Powrie) focusses on the usage of mutagenicity testing in evaluating food products. Thus, adequate methodologies, lists of chemicals with genotoxic activity found in foods, and further recommendations for approaching this problem, are all presented here.

The general usefulness and usage of short-term tests in types of cell transformations for the identification of chemicals that are putative carcinogens, are dealt with in Chapter 6 (Andrew Sivak and Alice S. Tu).

Mutagenicity testing with cultured mammalian cells using cytogenetic assays is presented in Chapter 7 (A. T. Natarajan and G. Obe) which also introduces all the classical and modern cytogenetic assays comparing them to each other and to other short-term tests. The correlation between gene mutations and chromosomal alterations, the application of cytogenetic assays to the identifying of mutagens and carcinogens, and the use of human lymphocytes in mutagenicity research are comprised in this chapter as well.

Following a logical sequence of ideas, Chapter 8 (Veronica M. Moher and J. Justin McCormick) deals with the measurement of mutations in somatic cells in culture and with the possibilities of extrapolating the mutagenesis and carcinogenesis data to humans.

Chapter 9 (Maria Kucerova) debates the question of chromosomal aberrations induced in occupationally exposed persons, the adequate cytogenetic assays to be used, their validity and the possibility of employing the results of cytogenetic studies in occupational health service.

The sensitivity, the correlations and the wide perspectives offered by the sister chromatid exchange assay led to the development of adequate analysis and statistic calculus methodologies, as well as to the discovery of the rationale of applying this assay to humans — Chapter 10 (Anthony V. Carrano and Dan H. Moore II).

The 6-thioguanine resistant blood lymphocyte assay for direct mutagenicity testing in humans is minutely exposed in Chapter 11 (Richard J. Albertini, David L. Sylwester and Elizabeth F. Allen). This way of detecting directly somatic cell mutations *in vivo* in man is described alongside with its results, debates and the most proper statistical analysis methods.

Sperm assays using the same parameters to fertility diagnosis and the new YEF test are important for highlighting the chemicals induced germ-cell damage in man. Their advantages and shortcomings, their value in detecting the antispermato-genetic effects in chemically exposed man, are some of the questions discussed in Chapter 12 (A. J. Wyrobek).

Chapter 13 (J. B. Brewen and D. G. Stetka) spotlights the importance of including the cytogenetic methods *in vivo* in any program of mutagenicity assessment, stressing the sensitivity, the capacity and the perspectives of the assay of sister chromatid exchanges.

The questions of genetic risk estimation by detecting the dominant skeletal mutations with the aid of the standard specific-locus and the sensitive-indicator methods, make the object of Chapter 14 (Paul B. Selby).

The best systems to detect and monitor the mutagenic airborne pollutants, are the plants. Chapter 15 (William F. Grant and K. D. Zura) introduces practically all the testing systems at *Tradescantia*, the pollen systems and other higher-plant mutagen assays systems. Due to a good correlation between mutagenicity data from higher plants and from nonmammalian and

mammalian systems, as well as to the similarity in genomic organization and cell heredity with humans, plants make sensitive detectors of atmospheric mutagens.

Finally, Chapter 16 (A. D. Kligerman) deals with the *in vitro* and mainly *in vivo* assays for detecting the effects of genotoxic agents in aquatic environment, using fishes as biological detectors. Cytogenetic assays *in vivo*, being most sensitive in fishes, are stressed upon. The most adequate fish species for such research studies are also listed here.

In conclusion, the high scientific qualities and the usefulness of this book should be considered here. It is interesting and stimulating both for experienced researchers and for the beginners, since it really offers new and large horizons in the field.

The variety of chapters, as the editor, J. Heddle, points out himself, was imposed by the necessity of developing the field research. Each chapter stresses the limitations and the desiderata of the studies, recommending also new further lines of approach. The rich bibliography outlines the vast documentation of the authors offering good guiding lines to the reader. Mention should also be made of the excellent graphical and technical conditions of the volume.

Veronica Stoian

AVIS AUX AUTEURS

La «Revue roumaine de biologie — Série de biologie végétale» publie des articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie végétale : morphologie, systématique, géobotanique, physiologie, écologie, génétique, microbiologie, phytopathologie. Les sommaires des revues sont complétés par d'autres rubriques, comme : 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc. ; 2. Comptes rendus des livres de spécialité parus en Roumanie. Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés en deux exemplaires. Les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes seront exécutés à l'encre de Chine noire, sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, dans les tableaux ou dans les graphiques sera évitée.

Les références bibliographiques, citées par ordre alphabétique, comporteront le nom de l'auteur, l'initiale du prénom, le titre de la revue, abrégé conformément aux usances internationales, l'année, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé, de maximum 10 lignes, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures). La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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