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REVUE
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SOMMAIRE

ÉTUDES DE PROGNOSE SUR LES CONDITIONS PÉDOCLIMATIQUES, BIOÉDAPHIQUES, ÉCOPHYSIOLOGIQUES ET DE LA COUVERTURE VÉGÉTALE DU PÉRIMÈTRE DU SYSTÈME D'IRRIGATIONS HORIA-LIVENI-MANOLEASA, DÉPARTEMENT DE BOTOŞANI

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The improvement of the relation between cultivated plants and the hydric regime has been provided also through irrigations; complex ecological researches were effectuated in the irrigation system from Horia-Liveni-Manoleasa (Botoşani district). The researches aimed at performing some interdisciplinary studies such as of climatology, pedology, phytosociology, plant physiology, mesofauna and soil microbiology.

The complexity of these conclusions shows that the application of irrigation without a rigorous control may have negative effects on plants.

The conclusions have been substantiated on comparative studies between irrigated and unirrigated cultures.

Five tables and two figures constitute the iconography of this work.

L'activité agricole, comme préoccupation ayant pour but l'amélioration des relations des plantes cultivées avec le milieu environnant pour l'obtention de grandes productions de qualité supérieure, se réalise par des mesures d'amélioration et agrotechniques parmi lesquelles les irrigations jouent un rôle primordial.

Les recherches climatiques ont démonté qu'entre 1975–1988 dans la zone étudiée le déficit pluviométrique a affecté la plus grande partie de la période de végétation, ce qui justifie l'utilisation de irrigations pour assurer la croissance des plantes et l'augmentation des récoltes agricoles.

Mais, l'application de l'irrigation, sans un contrôle rigoureux, peut avoir dans certaines conditions des effets négatifs. En vue de l'établissement des meilleures conditions pour le maintien de la fertilité du sol et l'application des arrosages pendant les périodes critiques de la croissance des plantes, on a entrepris des études écologiques complexes dont les résultats sont présentés dans cet ouvrage.

DONNÉES SUR LE CADRE NATUREL

Le système d'irrigations Horia-Liveni-Manoleasa est situé au Nord-Est de la Plaine Moldave, dans la sous-unité la Jijia supérieure et du Başeu. Du point de vue géologique, le territoire est formé de depôts ordoviciens, siluriens, crétacés et néogènes sans plis, étendus sur un fondement cristallin qui appartient à la plateforme est-européenne.

REV. ROUM. BIOL.–BIOL. VÉGÉT., TOME 40, N° 2, P. 75–86, BUCAREST, 1995

La vallée du Prut sectionne les plus anciennes formations géologiques qui apparaîssent dans le Plateau Moldave, à savoir les formations crétacées (cénoméniennes), au-dessus desquelles se trouvent des sédiments d'âge néogène.

Le relief est largement ondulé, avec des interfluves collinaires ou bien sous forme de petits plateaux bas, dont l'altitude se répète sur de grandes surfaces. Le long du Prut et de ses affluents, plusieurs terrasses fluviales se sont formées à de diverses altitudes, la mieux développée étant la terrasse de 50 – 60 m(*2*).

Le climat a des caractéristiques tempérées-continentales, à nuance excessive. Les principaux facteurs génétiques du climat (radiation solaire, circulation générale de l'atmosphère et la surface active sujacente) ont déterminé l'enregistrement d'une température moyenne annuelle de 8°3 *C* et d'une quantité de précipitations d'à peu près 500 mm/an (*Tableau n° 1*).

Le régime pluriannuel des éléments climatiques a été caractérisé par de grandes déviations par rapport à ces valeurs moyennes. Ainsi, dans la période 1975–1989 la plus grande quantité de précipitations a été enregistrée à Avrămeni pendant l'année 1985 (718,3 mm) et à Ripiceni en 1980 (574,5 mm). La quantité la plus réduite a été enregistrée à Avrămeni en 1986 (393,5 mm) et à Ripiceni en 1982 (281,0 mm).

Pendant la période de végétation, l'évapotranspiration potentielle a eu des valeurs élevées, dépassant du point de vue quantitatif les précipitations tombées (*Tableau n° 2*). La différence entre les precipitations et l'évapotranspiration nous révèle que la plupart de l'année il y a eu un déficit pluviométrique important (*Tableau n° 3*).

Dans les années 1982 et 1986 tous les mois ont enregistré un déficit de précipitation, et dans la période 1975–1977 et 1981–1988 le déficit pluviométrique a englobé la plupart de la période de végétation. Les sécheresses ont été nombreuses et prolongées (*Figure n° 1*), une utilisation maximale du système d'irrigation étant nécessaire. La fréquence des périodes de sécheresse a été de 20% en mai et juin, de 25% en août, de 30% en avril et septembre et de 40% en octobre. Le nombre des périodes de sécheresse a été en moyenne de 2 par an et au maximum de 4 par an. Le nombre moyen de jours sans précipitations a été de 231 par an. La durée moyenne des périodes de sécheresse a été de 18 jours et celle maximale de 38 jours.

La grande fréquence des sécheresses a déterminné l'utilisation maximale du système d'irrigation. Seulement pendant la saison froide il y a eu un excédent pluviométrique et l'accumulation d'une réserve d'eau dans le sol a été possible. L'existance, dans le substrat, de l'argile, des marnes, du gypse et des sels solubles a déterminé une minéralisation accentuée des eaux souterraines, de type bicarbonatées, sulfatées, chlorosodiques et sulfureuses. Les eaux de surface ont elles-aussi une minéralisation accrue, contenant entre 1000 et 3000 mg/l sels solubles.

Dans les conditions des temperatures élevées et de l'évapotranspiration intense, il s'est produit une accumulation dans le sol des sels transportés vers la surface par l'eau phréatique ou bien par l'eau utilisée dans le système d'irrigations. De cette manière on a contribué à la croissance de la salinité des sols.

Tableau nº 1

Précipitations atmosphériques (mm). Quantités moyennes mensuelles et annuelles (1975–1989)

Station météo	I	F	M	A	M	I	I	A	S	O	N	D	An
Avrămeni	26,1	24,7	24,1	43,1	67,7	81,3	79,4	57,9	39,6	26,0	37,8	29,7	537,3
Ripiceni	19,6	21,8	21,2	36,3	60,6	85,6	84,9	48,0	35,7	28,0	31,0	26,1	498,9

Tableau nº 2

Évapotranspiration potentielle (mm). Quantités moyennes mensuelles et annuelles dans la période de végétation (1975–1989)

Station météo	M	A	M	I	I	A	S	O	N
Avrămeni	24,0	58,3	81,6	93,2	97,3	97,8	80,8	53,9	25,3
Ripiceni	25,5	58,1	81,1	91,5	96,8	96,4	80,3	53,0	23,7

Tableau nº 3

Le déficit el l'excédent des précipitations en relation avec le potentiel d'évapotranspiration (mm) pendant la période de végétation (1975–1989)

Station météo			M	A	M	I	I	A	S	O	N
Avrămeni	Excédent	PP	0,1								12,5
	Déficit	PP		15,2	13,9	11,9	17,9	39,9	41,2	27,9	
Ripiceni	Excédent	PP									7,3
	Déficit	PP	4,3	21,8	20,5	5,9	11,9	48,4	44,6	25,6	

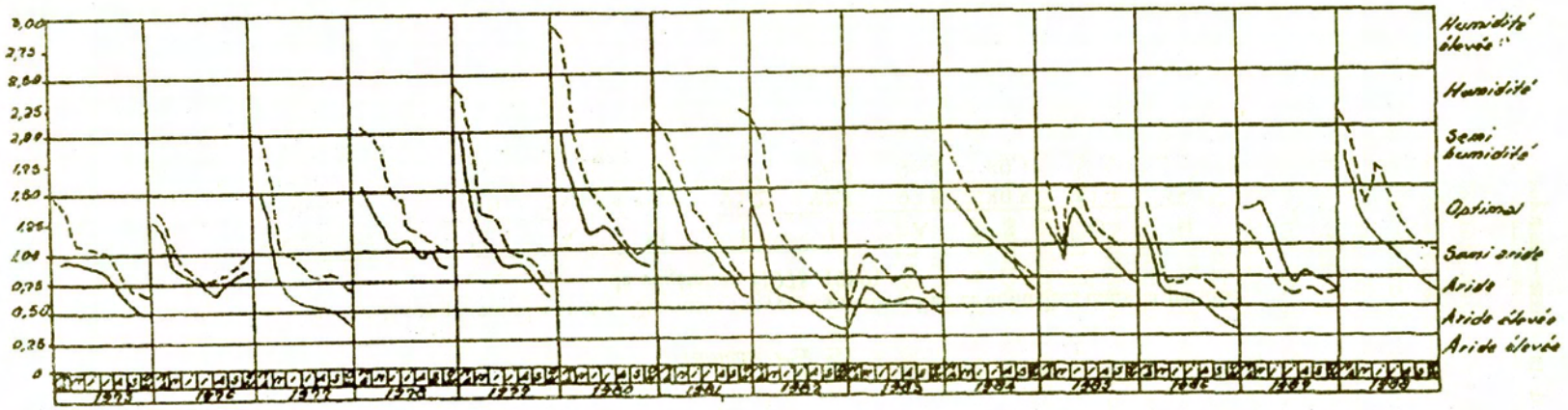

Fig.1. – L'index hydrothermique chez les stations Avrămeni et Ripiceni.

Les sols prédominants sont ceux du type des tchernoziomes cambiques, des tchernoziomes vertiques ou ceux typiques, ayant une fertilité élevée. L'utilisation des irrigations sur ces sols exige une attention accrue pour éviter le phénomène de salinisation.

Suite aux intenses processus d'érosion et de dénudation sur les versants, la turbidité des eaux courantes est très élevée, dépassant la quantité de 3000 g/m^3.

L'INFLUENCE DES IRRIGATIONS SUR LE SOL

Les sols rencontrés dans le département de Botoşani sont répartis en deux catégories. La première catégorie est representée par les sols bruns, bruns luviques et gris forestiers, et la deuxième catégorie est representée par les tchernozioms typiques, les tchernozioms cambiques, tchernozioms cambiques-vertiques, tchernozioms argiliques et les sols vertiques. On rencontre aussi des sols halomorphiques et hydromorphiques qui apparaissent isolement dans le plaine de la rivière Başeu et sur les versants où on trouve aussi des regosols érodisols.

Les recherches sur l'influence des irrigations sur les propriétés des sols ont été réalisées sur des terrains irrigués pendant 15 ans, 10 ans et 5 ans, dans la zone des tchernozioms typiques, des tchernozioms cambiques et ceux cambiques-vertiques.

Des recherches réalisées, on a constaté que le tchernoziom cambique-vertique de Săveni, irrigué pendant 15 ans avec de l'eau de la rivière Başeu, présente la nappe phréatique élevée, surtout dans les zones dépressionnaires, près des surfaces, ayant un degré de minéralisation de 2,344 g/l, donc elle est moyennement saumâtre.

Les analyses chimiques montrent que le sol présente une faible salinisation contenant des sulfates à une profondeur de 0 à 100 cm, entre 6,4 mg/100 sol et 8,76 mg/100 sol, tandis que les chlorures sont comprises entre 3,5 mg et 5,0 mg/100 sol. On constate un lavage du carbonate de calcium jusqu'à la profondeur de 75 cm.

Le contenu total en sels solubles (Ctss) est au-dessus de la limite d'un sol non irrigué (32–37 mg/100 g sol) atteignant des valeurs qui varient entre 64 mg et 130,9 mg/100 g sol. On constate une accumulation des sels solubles dans le profil du sol.

Si on analyse la réaction du sol, on constante que sur le terrain non-irrigué les valeurs du pH varient entre 6,2 et 7,0 et sur celui irrigué il y a une tendance d'alcalinisation, le pH variant entre 7,2 et 8,0.

En analysant les propriétés physiques des sols non-irrigués et irrigués, on a constaté un tassement des sol irrigués à la profondeur de 20–30 cm. La densité apparente du sol irrigué est de 1,40 g/cm^3 tandis que celle du sol non-irrigué à la même profondeur est de 1,29 g/cm^3. Ce fait nous conduit à la conclusion que de 4 en 4 ans le sol doit être ameublé avec le MAS jusqu'à une profondeur de 60 cm pour améliorer sa perméabilité.

Nos recherches ont compris aussi les sols irrigués pendant 10 et 5 ans avec de l'eau du Prut. On a constaté qu'après 10 ans, respectivement 5 ans, on n'observe

pas une accumulation de sels solubles dans le profil du sol. On observe la même tendance de croissance de la densité apparente qui atteint 1,50 g/cm^3, par rapport au sol non-irrigué, pour lequel la valeur est de 1,12 g/cm^3. Puis, on observe aussi un début de lavage des sels aisément solubles de la couche de sol jusqu'à 40 cm de profondeur, où les sels solubles varient entre 14 et 23 mg/100 g sol, tandis que dans le sol non-irrigué les sels solubles varient entre 28 et 35,5 mg/100 g sol. En plus, on constate un lavage du carbonate de calcium de 50 à 60 cm de profondeur.

Le tchernoziom typique du secteur Ripiceni, irrigué pendant 5 ans n'a pas subi de modifications significatives suite aux irrigations, en dehors du fait que le même phénomène de tassement a eu lieu en s'imposant un ameublement jusqu'à 60 cm de profondeur, de 4 en 4 ans. Toujours par suite aux irrigations, on a constaté un lavage de carbonate de calcium jusqu'à la profondeur de 50 – 60 cm.

LA CARACTÉRISATION DU PHYTOGÉNOFOND ET DE LA VÉGÉTATION

Le phytogénofond de la zone étudiée est riche et varié, caractérisé par la prédominance des xérophytes comme expression du caractère steppique de la région. Du riche fond génétique identifié, bien des éléments sont d'une grande importance phytogéographique, certaines espèces étant rares, préservées seulement dans des enclaves réduites de végétation steppique: *Adonis varnalis, Astragalus austriacus, Asyneuma canescens, Chamaecytisus austriacus, Centaurea orientalis, Echium russicum, Helichrysum arenarium, Linum flavum, Pulsatilla montana, Phlomis pungens, Salvia nutans, Stipa tirsa, Teucrium montanum, Teucrium poliu* etc. Dans la zone étudiée on remarque aussi, un grand nombre d'espèces ségétales et rudérales comme expression de la prédominance des agroécosystèmes qui ont déterminé une large expansion de cette catégorie de plantes et l'apparition de certaines espèces à une fréquence élevée, souvent en grandes populations.

La prédominance des cultures agricoles, les surfaces réduites de prairies et leur état avancé de détérioration n'ont permis que la préservation d'enclaves de la végétation naturelle zonale. La végétation des prairies xérophiles, spécifique à la zone, se trouve seulement sur les versants inclinés et sur de petites terrasses, étant représentée par les phytocoenoses des associations *Medicagini-Festucetum valesiacae, Bothriochloetum ischaemi, Stipetum capillatae, Stipetum lessingianae* etc.

L'effet de l'irrigation prolongée va produire des modifications de la structure du phytogénofond dans le sens de la diminution et de la limitation de l'aire des espèces xérophiles, celles-ci étant remplacées peu à peu et constamment par des espèces mésophiles, mésohygrophiles et hygrophiles, suite à la croissance du degré d'humidité. Le spectre des mauvaises herbes changera dans le sens de la prédominance des espèces adaptées à un degré accru d'humidité du substrat: *Echinochloa crus-galli, Setaria lutescens, Setaria viridis, Hibiscus trionum, Solanum nigrum, Bidens tripartita, Galinsoga parviflora, Polygonum lapathifolim, Elytrigia repens, Tripleurospermum inodorum* etc.

Une large distribution et sur de grandes surfaces, quel que soit le type de culture, ont les espèces: *Phragmites australis, Tussilago farfara, Equisetum arvense* etc., mauvises herbes difficiles à combattre car, en dehors du fait qu'elles sont typiques aux terrains à humidité accrueelles indiquent aussi le tassement du sol.

Sur les terrains irrigués depuis plus de 15 ans –, dans les stations dépressionnaires, en dehors du phénomène d'apparition des terrains marécageux, il y a aussi des phénomènes de salinisation mis en évidence aussi, par les espèces indicatrices (halophiles): *Bolboschoenus maritimus, Juncus gerardi, Chenopodium glaucum, Inula britanica, Chamomilla recutita, Plantago cornuti, Rumex crispus, Ranunculus arvensis, Ranunculus sceleratus, Potentilla supina, Carex hirta, Polygonum persicaria* etc. Afin de garder et de conserver la plus importante partie du phytogénofond existant et de la végétation xérophile caractéristique pour la zone, on recommande d'éviter les travaux d'amélioration foncière dans les enclaves de végétation xérophile appartenant aux associations *Medicagin-Festucetum valesiacae, Stipetum Iessingianae, Stipetum capillatae.*

INDICES ÉCOPHYSIOLOGIQUES

Les indices écophysiologiques viennent à accomplir les recherches effectuées au niveau du sol, en complétant la relation "climat-sol-plante" par la détermination du rythme de croissance, du degré d'approvisionnement avec de l'eau et des éléments nutritifs absorbés par les principales plantes agricoles cultivées dans la zone: blé, orge, maïs, tournesol, soja, haricot et betterave.

Les déterminations ont été faites par les méthodes d'investigation usuelles (gravimétrie, titrimétrie spectro et flamenphotométrie), dans les principales phases de végétation (15.V., 27.VI et 6.IX.1988).

Le besoin d'eau des plantes est différent, en fonction de l'éspece, de la phase de végétation et du niveau de fertilisation. Ainsi, on constate que chez les plantes irriguées, l'intensité de la croissance en hauteur est supérieure pour toutes les espèces, quelle que soit la zone où on a réalisé les irrigations. La plus intense croissance végétative a été enregistrée pour le tournesol et le maïs, suivis par le blé, l'orge, le soja, les haricots, la betterave et les herbes pérennes des prairies permanentes, situées dans la localité Manoleasa-Ripiceni (*Tableau n° 4*).

L'accumulation de matière sèche par plante a été quand même supérieure chez les plantes non irriguées. Le degré d'hydratation des plantes irriguées a été toujours supérieur par rapport à celles non irriguées, le plus élevé contenu en eau totale étant remarqué chez les plantes cultivées sur des terrains plus tassés, suite à l'irrigation de longue durée, telles les légumineuses, le soja et les haricots cultivés dans la zone Săveni.

Le maintien d'un contenu normal d'eau dans tous les tissus des plantes agricoles, c'est une condition de base de la productivité. Mais, on observe que sur le parcours des premières phases de végétation, les graminées réalisent une augmentation de la concentration de matière sèche, tandis que le pourcentage d'eau des tissus baisse. La perte d'eau des tissus se réalise par la transpiration. Cela dépend des facteurs climatiques, du niveau de l'eau dans la plante et de la force radiculaire d'absorption d'eau du sol. Le bilan hydrique des plantes étudiées a varié entre des limites normales, étant optimal chez les plantes irriguées, mais, même chez les plantes non irriguées il n'a pas baissé sous 50% de leur poids frais. Chez toutes les espèces analysées, les plantes non-irriguées ont un régime hydrique plus bas et plus équilibré par rapport à celles irriguées, chez lesquelles le rythme de la perte d'eau este supérieur à celui des plantes non-irriguées, des mêmes zones étudiées.

Tableau nº 4

La variation des indices écophysiologiques des plantes agricoles

Plante		Croissance cm./pl.	Mat. sèche	Eau totale	Glucides totaux	Protéines brutes	Éléments minéraux	Localité
blé d'automne	non-irrigué	58,52	44,20	55,80	50,05	9,53	6,70	Ripiceni après 5 ans d'irrigations
	irrigué	66,20	39,91	58,58	47,68	9,93	7,07	
maïs	non-irrigué	38,60	20,21	79,79	50,13	5,00	3,11	
	irrigué	41,90	15,43	84,53	44,97	5,57	3,81	
tournesol	non-irrigué	–	–	–	–	–	–	
	irrigué	1,93 m	19,55	80,45	23,93	5,00	2,54	
soja	non-irrigué	35,12	21,26	78,73	30,37	4,53	4,03	
	irrigué	40,58	20,11	79,89	30,00	4,86	4,44	
prairie	non-irrigué	7,55	22,05	77,95	38,23	5,88	6,37	
blé d'automne	non-irrigué	57,24	40,24	59,76	48,69	3,70	5,41	Stânca après 10 ans d'irrigations
	irrigué	60,92	34,89	65,11	44,58	5,55	6,84	
riz	non-irrigué	52,16	24,73	75,27	57,00	9,20	6,08	
	irrigué	91,72	20,82	79,18	50,00	9,62	7,45	
maïs	non-irrigué	74,20	25,56	74,44	38,30	6,43	5,53	
	irrigué	91,90	15,40	84,60	36,00	6,62	5,82	
tournesol	non-irrigué	1,08 m	25,33	74,67	30,30	4,08	8,20	
	irrigué	1,90 m	10,59	89,46	26,90	4,47	8,58	
betterave	irrigué	–	20,63	79,37	20,60	10,30	8,67	
blé d'automne	non-irrigué	82,68	40,76	59,24	46,93	7,85	6,32	Săveni après 15 ans d'irrigations
	irrigué	92,72	38,50	61,50	40,55	8,60	7,50	
riz sur sol salin	non-irrigué	20,55	24,87	75,13	58,60	6,40	5,81	
	irrigué	76,77	21,20	78,80	53,58	9,20	8,37	
soja	non-irrigué	50,02	20,22	79,78	30,15	4,58	4,20	
	irrigué	51,80	15,04	84,96	25,77	5,57	4,55	
petit pois	non-irrigué	15,21	16,41	83,58	36,37	6,07	4,68	
	irrigué	20,20	15,12	84,88	34,28	6,92	5,25	

L'élimination de l'excès hydrique se réalise par la transpiration, processus par lequel les plantes règlent leur métabolisme dans les limites normales, permettant une bonne assimilation chlorophyllienne, mise en évidence par le contenu élevé en protéines assimilées comme produits primaires. On a constaté que chez les plantes non-irriguées et surtout chez l'orge cultivé sur les terrains salins de la zone Săveni on trouve les plus élevées valeurs en glucides osmotiques actifs et totaux. Ces substances contribuent à l'augmentation des forces d'absorbtion de l'eau du sol et de la rétention de l'eau liée au niveau des tissus et des organes des plantes.

Les plus grandes valeurs des glucides totaux ont été mises en évidence chez l'orge cultivé sur des sols salins et sur ceux non-irrigués (57–58%), suivi par le maïs, le blé, les haricots, le soja et d'autres.

La protéine brute présente des valeurs supérieures chez les plantes irriguées, par comparaison à celles non-irriguées. Ce fait este mentionné, aussi, par d'autres chercheurs, lorsqu'ils se rapportent à l'absorption des éléments minéraux nutritifs du sol, ce qui facilite le métabolisme des protéines, plus actif pour les plantes irriguées (*8, 13*).

Dans nos déterminations on a analysé aussi le contenu en macroéléments (N, P, K, Na et Ca) comme somme des éléments minéraux.

En comparant les indices écophysiologiques des plantes non-irriguées, on constate que chez les plantes cultivées dans la zone Ripiceni et Stânca-Ştefăneşti, ayant une période de 5 et 10 ans d'irrigation, donc un sol bien approvisionné en eau, les plantes irrigueés ont un habitus plus vigoureux, un régime d'eau plus actif, une absorption des éléments nutritifs plus intense et une synthèse des substances organiques supérieure aux plantes cultivées dans la zone Săveni, ayant une période 15 ans d'irrigation, et par rapport à celles non irriguées.

INDICATEURS DE L'ACTIVITÉ BIOLOGIQUE DU SOL

a. L'EFFET DES IRRIGATIONS SUR LES MICROARTHROPODES ÉDAPHIQUES

Réagissant vite à l'intervention du facteur anthropique surtout dans les cultures agricoles intensives, les microarthropodes édaphiques peuvent servir comme indicateurs efficaces des conditions du milieu modifiées, ce qui justifie la nécessité des prognoses zoopédologiques (*2, 3, 5*).

L'évaluation de l'impact de l'irrigation du sol sur les microarthropodes édaphiques a eu pour but l'établissement de certains éléments de prognose utile pour optimiser l'équilibre écologique de l'aménagement hydrotechnique analysé.

La littérature spécialisée fournit peu de données sur l'effet de l'irrigation sur la faune édaphique (*7*), mais on connaît le fait que l'humidité du sol constitue un facteur abiotique décisif pour les animaux édaphique (*5*).

On a analysé, par la méthode Berlese-Tulgren, des lots égaux, chacun formé de 5 preuves de sol (S preuve = 100 cm^2) prélevées dans la période végétative estivale des agroécosystèmes irrigués, par comparaison aux agroécosystèmes similaires non-irrigués. Du total de 112 preuves, on a dressé un inventaire d'à peu près 15 400 individus de microarthropodes édaphiques, les uns ont été déterminés jusqu'au niveau de la famille tandis que les oribatides et les collemboles jusqu'au

niveau de l'espèce (*1, 4, 6*). Dans l'analyse statistique des données on a appliqué le test «t» Student et l'indice de corrélation simple.

L'étude des communautés des microarthropodes édaphiques des périmètres irrigués a mis en évidence que les pratiques agricoles appliquées et l'apport hydrique mettent leur empreinte sur la densité globale, la densité des divers taxons composants et sur leur domination numérique.

L'effet de l'irrigation sur ces mailles de la chaîne de décomposants de la nécromase n'est pas uniforme, il est conditionné par la durée de l'entretien du sol en système d'irrigation et la dimension des modifications coenotiques est reliée au type de la culture.

Les différences qualitatives et quantitatives plus évidentes apparaissent parmi les coenoses qui peuplent le sol cultivé avec des plantes dont la tige est une paille et celles du sol avec des cultures sarclées (*figure n° 2*). Dans le sol cultivé avec de l'orge, il y a une période courte, estimée à 5–6 années, d'amélioration de la structure coenotique, conséquence des irrigations, suivis par un déclin progressif dans le sol irrigué depuis 15 années.

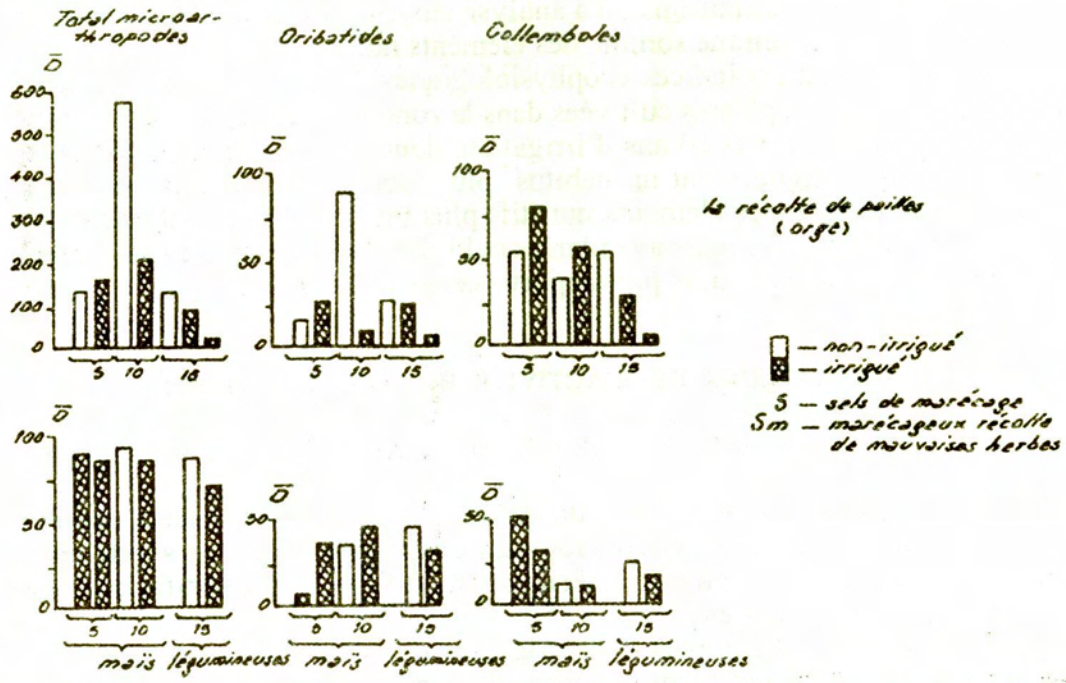

Fig. 2. – La moyenne des densités/100 cm² des microarthropodes édaphiques des périmètres irrigués.

Les divers taxons de microarthropodes ont présenté des réactions différentes, en fonction de leurs limites de tolérance envers les conditions bioédaphiques induites par l'irrigation. Le rôle positif de l'irrigation s'est fait sentir surtout sur les collemboles du sol avec des cultures non-sarclées et sur les oribatides de celui cultivé de maïs, à une irrigation de 5–10 années. La stimulation numérique de ces groupes détritomicrophytophages a été accompagnée par la croissance du nombre des prédateurs (gamasides et actinédides) qui assure leur réglage numérique. Dans le périmètre irrigué depuis 15 années, on a constaté une réaction négative de ces groupes, surtout dans les sols salins depressionnaires, où apparaissent les éléments

salzotolérants (*Zygoribatula cognata, Punctoribates hexagonum* - oribatides, *Proisostoma minuta* - collembola).

Le groupement trophique des détritomicrophytophages domine avec plus de 75% dans tout les agroécosystèmes analysés, mais le rapport numérique des principaux taxons composants a varié dans les trois périmètres étudiés. Ainsi, les microarthropodes du périmètre Ripiceni ont été dominés par les collemboles et les oribatides, les premiers ayant une plus grande importance dans les sols irrigués; à Stânca le rapport oribatides/collemboles a été supérieur à l'unité pour la culture d'orge non-irriguée et inférieure à l'unite pour celle d'orge irriguée, pendant que dans les cultures sarclées ce sont les oribatides qui ont été plus abondantes; à Săveni, les oribatides ont été prédominantes dans les cultures de légumineuses.

La dominance des oribatides a été en relation avec la quantité d'humus du sol (r = 0,748), celle des acaridides avec la quantité d'argile (r = 0,998) et celle des colemboles a dépendu, en grande mesure, de l'humidité du sol.

Les caractéristiques structuralles des communautés de microarthropodes identifiés dans les périmètres irrigués depuis 10–15 années prouvent que la pratique prolongée de l'irrigation mène à l'annihition de certaines mailles importantes de la chaîne saprophyte-saprophage, la diminution de la capacité biogène des sols ayant pour conséquence la baisse de leur fertilité.

b. L'EFFET DE L'IRRIGATION SUR LES MICROORGANISMES DU SOL

Nos recherches sur la microbiologie du sol ont poursuivi la détermination d'indicateurs biologiques (pH, humidité du sol) qui influencent et caractérisent l'activité métabolique des microorganismes du sol (activité déhydrogénazique, respiration globale du sol et densité de la microflore du sol). Les valeurs de ces indicateurs microbiologiques sont consignées dans le *Tableau n° 5.*

Tableau n° 5

Moyenne des valeurs des indicateurs microbiologiques des sols

Durée d'irrigation	Mode d'emploi	Culture	Facteurs abiotiques		Facteurs biotiques			
			pH	Humidité	AD	CO_2	Bact.	Fungi
Ripiceni	irrigué	blé	6,80	13,75	2,61	290	39	22
5 ans	non-irrigué	blé	6,05	13,75	1,70	270	34	16
Stânca	irrigué	mais	6,30	13,55	3,17	380	39	15
10 ans	non-irrigué	mais	6,80	13,00	2,05	290	38	13
Săveni	irrigué	orge	6,15	14,15	3,80	410	27	6
15 ans	non-irrigué	blé	6,40	15,20	3,10	350	11	11

Ces recherches ont révélé l'existence d'une relation étroite entre l'irrigation et la microflore du sol, surtout lorsque l'irrigation est de longue durée. Ainsi, on a constaté, par la diminution de l'humidité du sol à des valeurs d'à peu près 15% ou inférieures à celle-ci, suite à la sécheresse de l'été, que l'influence des irrigations sur

les microorganismes est évidente par la stimulation plus accentuée du nombre de microorganismes, de la production de CO_2 et de la minéralisation du N_2 (*5, 11*).

Les valeurs moyennes de l'activité enzymatique (déhydrogénasique) et de la respiration globale du sol sont plus élevées dans le sol des cultures irriguées par comparaison à celles non-irriguées du système d'irrigation de 5–10 années, et dans le système d'irrigation de 15 années l'activité déhydrogénasique a les valeurs moyennes les plus élevées, proches de celles du sol des cultures non-irriguées. Ce fait démontre que l'effet de l'irrigation sur la vie des microorganismes du sol est estompé dans le cas des irrigations de longue durée (dépassant 10 années), les différences des valeurs moyennes devenant insignifiantes entre les cultures irriguées et celles non-irriguées.

Des résultats obtenus, on constate la fait que, quels que soient le type des cultures et la durée d'irrigation des terrains, ainsi que le manque d'irrigation, le nombre des bactéries a varié entre millions et dizaines de millions de cellules/g de sol humide et celui des micromycètes entre milliers et dizaines de milliers de cellules/g du sol humide.

Le maximum d'efficacité de l'irrigation a été enregistré dans le système d'irrigation de 5 et 10 années lorsque la microflore a atteint des valeurs élevées de la densité numérique. Le nombre de bactéries et de micromycètes des cultures irriguées est supérieur à celui du sol des cultures non-irriguées, et qui démontre l'influence positive de la pratique des irrigations sur la densité des microorganismes et la structure équilibrée des populations microbiennes, ce qui contribue à la préservation de la fertilité élevée des sols.

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WHEN DOES A SIGNIFICANT CORRELATION EXIST BETWEEN THE ANNUAL GROWTH IN *ABIES ALBA* TREES AND CLIMATIC CHANGES ?

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The relation between annual tree growth and climate is a well known fact but direct mathematical values have not been usually given in articles. We have investigated the annual growth of an *Abies alba* population, in a natural, old (140 years), mixed (fir and beech) forest, in the Southern Carpathians (Bucegi Mountains, Prahova Valley) in relation with air temperature, relative humidity and precipitation. We have found four different types of individual growth curves in the whole population. No significant effect of climate parameters has been detected for the mean growth of population, nor for any individuals along the entire period of life, nor for any period in certain individuals. No correlation has been distinguished between air temperature and the growth ($r = -0.24–(+0.25)$).

We have noticed the significant correlation between individual tree growth, only in the mature period, particularly with relative air humidity ($r = 0.74–0.49$) and a lower correlation with annual precipitation ($r = 0.52–0.47$). The correlation exists only for the individuals that have a normal growth curve and do not change their position in the general canopy (light conditions) and for the individuals that show a well-balanced development (a circular stem with about equal cross radii).

The tree populations have first come to the attention of sylviculturists, obviously for economic reasons, but they have also focussed the interest of researchers, who tried to discover some of their physiological, morphological and ecological laws. Several dendrochronological, dendroecological and dendroclimatic interpretations have been put forward by an increasing number of authors (V. Soran et al. 1978, 1985, D. Tătăran et al. 1988, Al. Tissescu et al. 1991, P. Stoll et al. 1992) concerned with the possible correlation between the thickness of annual ring growth and the changes of the climate over the time. Although they have been following this correlation in different species (spruce fir, pine and oak) and many catalogues listing average growth/species have been worked out (dendrometric catalogues, USSR), no significant relationship between growth and environmental factors could be detected.

Since the majority of works deal with individual trees, we have attempted to establish a correlation between annual growth and climatic factors in both the population as a whole and in each of its individual members.

MATERIAL AND METHOD

Investigations were carried out in the *Abies alba* population, because resinous species are known to grow thicker and therefore have better visible annual rings. Round sections from the tree trunk, cut up to 0.30 cm from the soil surface, were

machine-and-hand-polished and measured with an ocular micrometer attached to the MBS3 binocular. Measurements covered the shortest and the longest radii.

A competition was found between the fir and the beech population, in particular. They make up a mixed, zonal forest (as *Pulmonario* (*rubro*) *Abieti-Fagetum* Knapp 42) Soo 62, at the foot of the Vânturişu mountain, Bucegi massif (on the flank facing the Prahova Valley) at 840 m alt., UP III, u.a. 55. Several generations of this forest have developed naturally, the oldest one being represented by 120 – 140-year-old fir trees. This species appears to have found its optimum ecological environment here. The whole parcel contains 360 indiv./ha, having basically an average diameter of 54 cm (at 1.30 m from the ground) and height of 30 m in the oldest generation; the about 90-year-old generation of trees is 27 m tall, with a diameter of 36 cm. In the third generation, ca 60 years old, tree height and thickness stand at 26 m and 36 cm, respectively.

In 1992, the trees were trained and all mature specimens were felled.

The representative trees from each 60 – 120-year-old generation, 32-55.3 cm thick, were measured. The tree growth/age correlation coefficient was found to be 0.72.

The climatic factors followed were the annual temperature means, relative air humidity, the sum of precipitation/year and during dormancy (November-March). The data were provided by the records of the Sinaia-Mânăstire meteorological station beginning with the year 1886. The events of the 1961-1992 interval, registered at Cota 1500 station, were correlated with the Sinaia station altitude.

The data and the graphs were statistically processed with the help of the Excel 4.0 Program.

RESULTS AND DISCUSSION

Starting from the premise that all the individuals of a population are exposed to the same temperature and air humidity conditions and have the same moisture reserve from precipitation, we assumed that the mean annual growth of the whole population varies with the environmental factors, eliminating individual differences. In the case of our fir-tree population, several correlations were attempted: between annual diametral growth (Fig. 1) and air temperature = 0.04 (Fig. 2), between average growth and precipitation = 0.17, and between growth and air humidity = 0.29. As no relevant relationship was discovered, a fact confirmed by other authors, too (Stoll et al., 1992), we went deeper into this study and noticed that individual growth follows a peculiar trend, with growth rates in each life-stage varying according to some patterns. The generally known curve shows young trees to grow at a faster rate, stimulated by internal factors, then comes a steep fall and ultimately a very low-stability threshold. However, only 37.5% of the specimens from our population followed this pattern (Fig. 3a), the remaining ones presenting

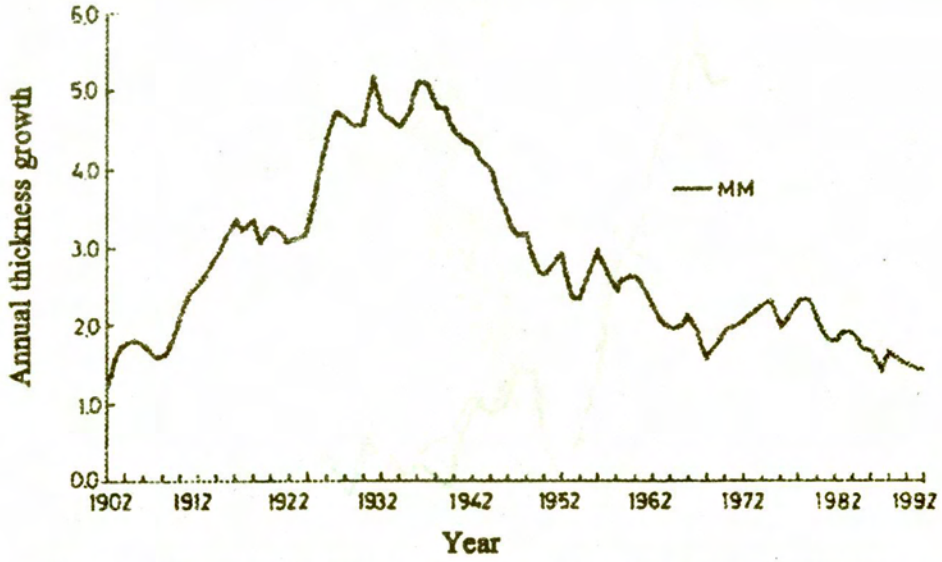


Fig. 1. – The annual thickness growth mean of noticed fir tree population.

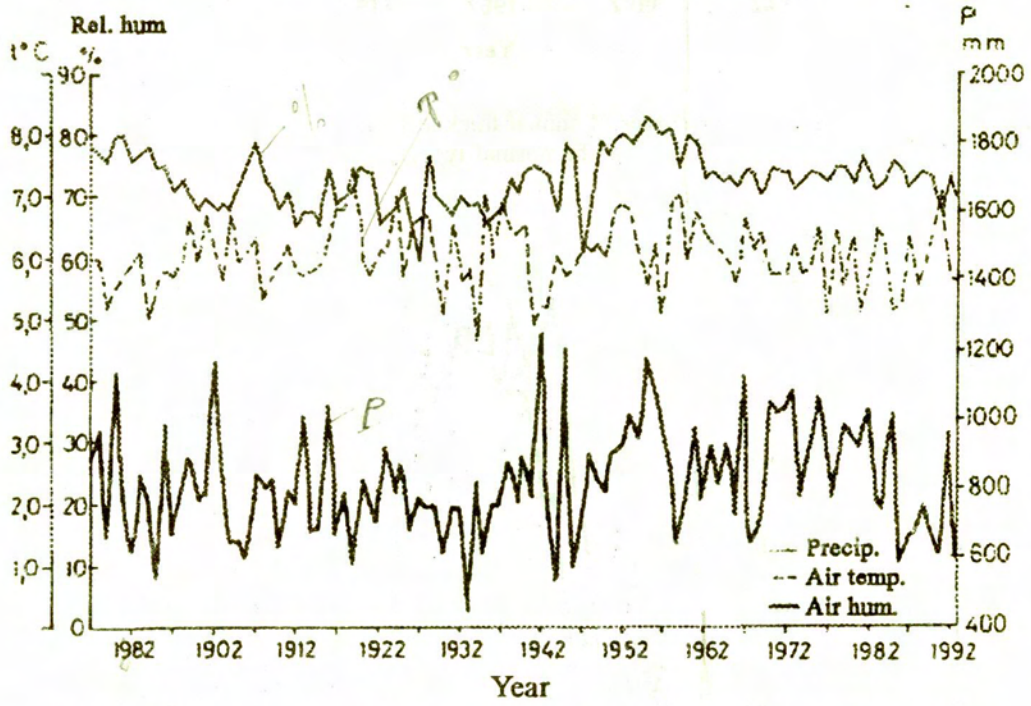


Fig. 2. – The annual mean dynamics of some climatic factors at Sinaia (Bucegi Mt.).

other growth behaviours during their lifetime. They fell into three groups: 1): slow, almost stagnant growth within the first 40 years; short-time explosive growth, subsequently regressing to moderate levels (Fig. 3b). The incidence of this pattern is fairly low - 12.5%.2); 2): two periods of intense growth: early and late in life (Fig. 3c). This group covers 25% of the cases. 3): relatively even growth rates - slow in early life, a very short span of intense growth, ending up in a long period of moderate growth (Fig. 3d). This group includes 25% of our specimens.

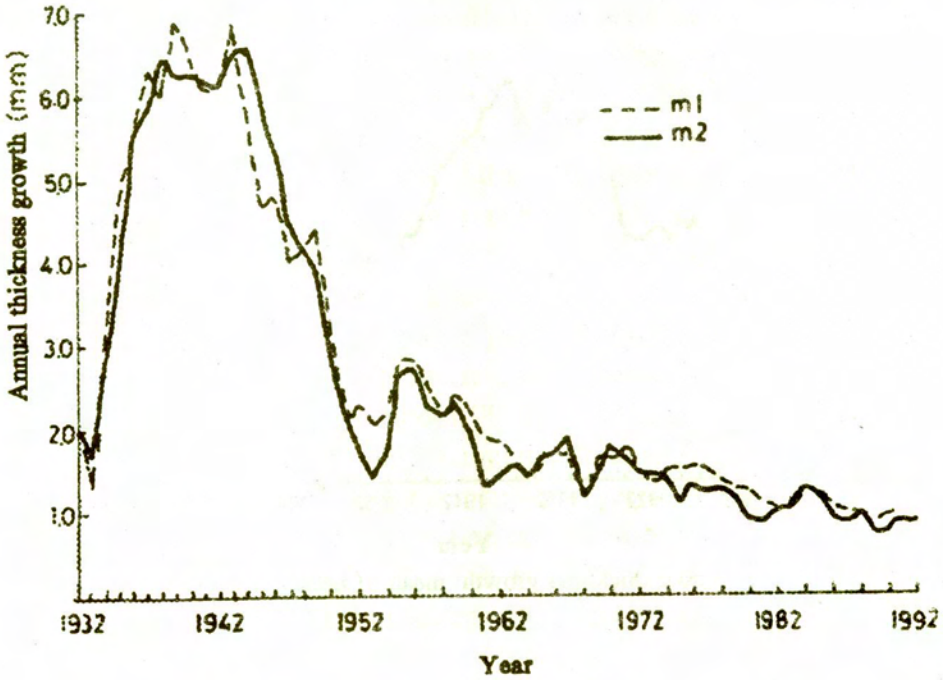
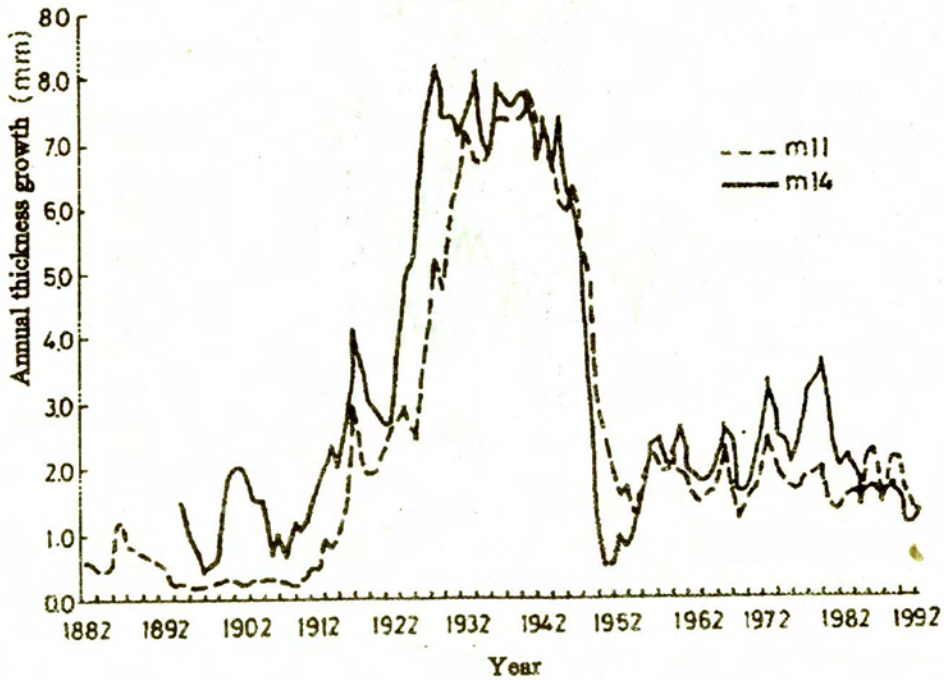
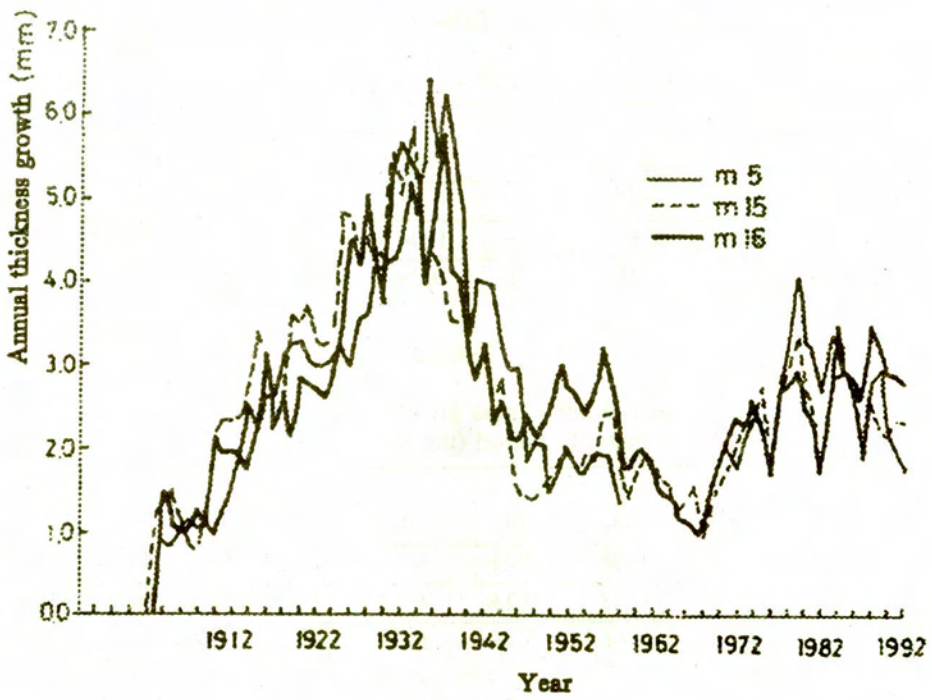


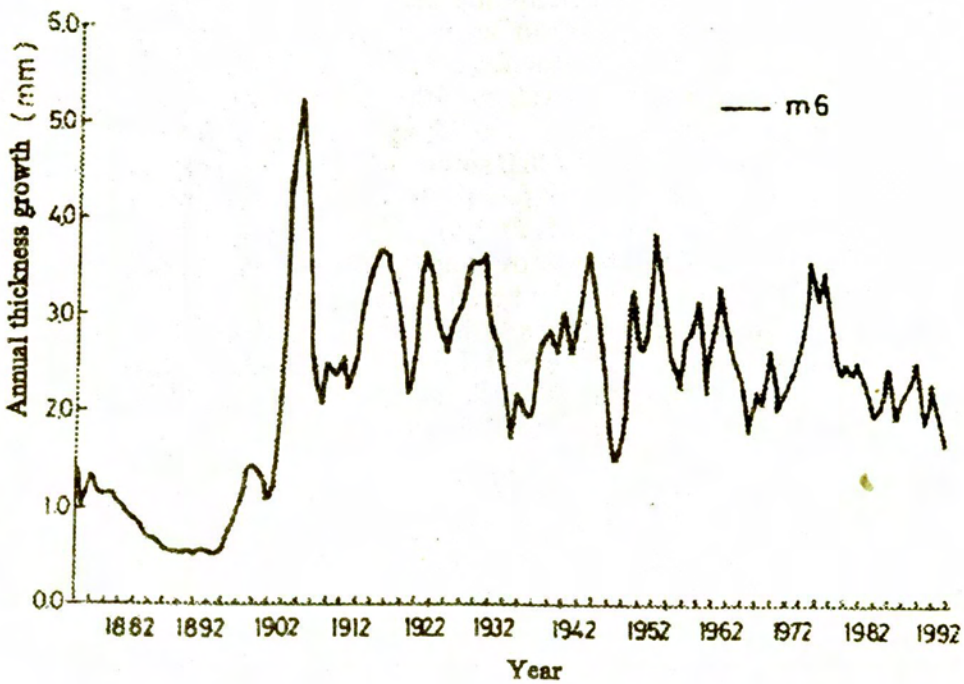
Fig. 3. – The curves of annual thickness individual growth:
a. the normal type;



b. the slowly initial growth type;



c. the two peaks type;



d. the relative temperate growth type.

Table 1

Index of correlation *r* between the annual thickness of trees and climatic parameters

Climatic parameters	Trees m_1	m_2	m_3	m_4	m_5	m_6	m_7	m_8
Air. temp.	0.02	-0.02	0.01	0.28	-0.08	0.25	0.01	0.08
Precipitation	-0.01	-0.02	-0.16	0.04	-0.16	0.15	-0.04	0.19
Air humidity	-0.08	-0.10	-0.26	0.09	-0.40	0.11	-0.09	0.33

Table 2

Index of correlation *r* between the thickness growth of trees and climatic parameters in the maturity period (the last 50 years)

Climatic parameters	Trees m_1	m_2	m_3	m_4	m_5	m_6	m_7	m_8
Air temp.	0.10	0.06	0.21	0.15	-0.20	0.17	0.09	0.20
Precipitation	0.43	0.38	0.16	0.33	0.03	0.37	0.50	0.37
Air humidity	0.75	0.66	0.49	0.29	-0.11	0.31	0.75	0.30

The shorter or longer period (25 up to 40-45 years) of very slow growth rates (Figs 3a-d) is obviously the result of an unfavourable position of some trees in the vertical forest structure. Standing deep into the shade, they barely receive the necessary light for survival. In this situation, the limiting environmental factor, namely light, rules over internal or climatic factors alike.

The individuals of a population were grouped by type of growth curve, according to the graphic method. But for a few exceptions, similar results were obtained when grouping them by the values of the diametral growth correlation, these values being extremely high (m1/m2 - r = 0.98; m11 - r = 0.86, or m3/m7 - r = 0.90; m9 - r=0.92; m14 - r = 0.90). The fact that some individuals register high correlation values (m11 or m14), but develop different growth curves, is the consequence of their being correlated with individuals that are of a different age (e.g. 60 years with 92 years), hence much of the curve overlaps. The remaining segment, though important, corresponds to the period when only one of the individuals stays alive.

The correlation between the growth of each individual tree (an average of two radii) throughout its lifetime and the different climatic factors is shown in Table 1. Not only are the correlation indicators (r) devoid of any significant value, but moreover, they fail to relate either with air temperature, precipitation or air humidity.

When plotting the growth curves we realised that, in most cases, after a certain age, more precisely after maturation, the rate of tree growth tended somehow towards uniformity. So, we attempted a new correlation, for the past fifty years, which proved the existence of a relationship between annual diametral growth and air humidity (r = 0.69), yet inconclusive in regard of annual precipitation (r = 0.49). Growth, however, was found to better relate to the sum of annual precipitation than to the sum of precipitation preceding the beginning of vegetation in trees. Individual scores (Table 2) show a clear-cut difference as far as the significance of the correlation is concerned. There are

continued table 1

m_9	m_{10}	m_{11}	m_{12}	m_{13}	m_{14}	m_{15}	m_{16}	m
0.01	-0.04	-0.07	0.35	0.14	-0.13	-0.15	0.00	
-0.07	0.12	-0.21	0.35	0.09	-0.16	-0.21	-0.23	
-0.12	0.13	-0.36	0.36	0.01	-0.41	-0.34	-0.23	

continued table 1

m_9	m_{10}	m_{11}	m_{12}	m_{13}	m_{14}	m_{15}	m_{16}	m_m
0.18	0.14	0.20	0.24	0.21	0.00	-0.18	-0.12	0.18
0.34	0.45	-0.09	0.50	0.40	0.16	-0.06	-0.09	0.49
0.64	0.72	0.03	0.49	0.41	-0.11	-0.03	0.16	0.69

two categories of individuals: some in which there is a fairly good correlation between growth and the climatic factors, and others in which the correlation is absent, or very weak.

Only 32% of all the individuals studies showed a significant relation between these two variables (> 0.64), and they all belong to the first, normal curve, group (Figs 3a, 4).

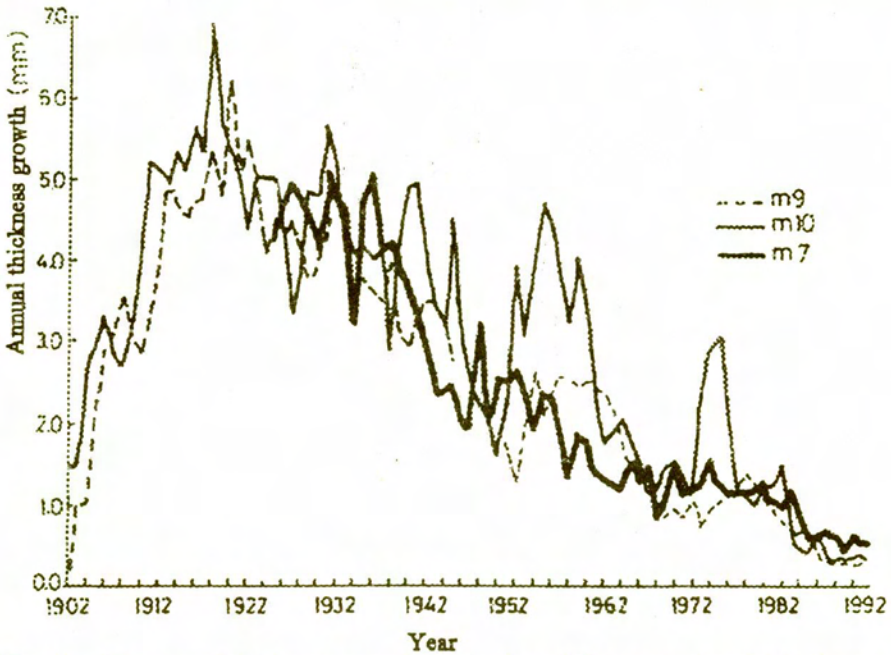


Fig. 4. – The annual thickness growth dynamics of the trees only which show a significant correlation with climate.

Since some of the trees grow randomly, with their shortest radius being very different from the longest one, we checked whether the correlation holds only for one of them, namely if it represents a specific relationship with the tree-trunk (Table 3). As a rule, a better correlation was obtained along one radius, e.g.a 90% value increase of the correlation index for the shortest radius of each tree, both in the specimens in which growth/climate relate significantly and in those with poorer scores. This value increase along a single radius is obvious in trees with very good correlations (m1, m2), as well as in some instances of trees which did not correlate at all. Huge differences between the two radii were usual, significant values being

Table 3

Index of correlation *r* between the thickness growth of trees on different radii and climatic parameters in the maturity period (the last 50 years)

Individual/radius (cm)		Climatic parameter		
		Air temperature	Precipitation	Air humidity
1	15.83	0.11	0.43	0.67
1a	16.48	0.09	0.40	0.75
2	15.27	0.00	0.27	0.56
2a	15.25	0.09	0.41	0.66
3	16.84	0.19	0.16	0.48
3a	16.06	0.22	0.17	0.50
4	23.72	0.21	0.21	-0.05
4a	18.94	0.00	0.26	0.45
5	25.20	-0.11	-0.05	-0.10
5a	21.86	-0.25	0.10	-0.10
6	25.79	0.10	0.35	-0.08
6a	17.44	0.15	0.20	0.56
7	15.50	0.09	0.47	0.74
7a	14.95	0.09	0.52	0.74
8	31.88	0.09	0.19	-0.03
8a	22.49	0.24	0.31	0.58
9	26.11	0.15	0.30	0.57
9a	20.28	0.20	0.36	0.65
10	29.86	0.17	0.38	0.62
10a	26.21	0.09	0.50	0.77
11	31.21	0.05	-0.13	-0.09
11a	21.17	0.28	0.10	0.25
12	22.20	0.15	0.34	0.04
12a	19.14	0.23	0.47	0.63
13	32.28	0.18	0.43	0.49
13a	18.47	0.20	0.24	0.16
14	34.97	0.03	0.29	0.09
14a	26.95	-0.07	-0.29	-0.52
15	26.54	-0.22	-0.19	0.00
15a	20.11	-0.05	0.20	-0.07
16	27.04	-0.16	-0.17	0.01
16a	20.76	0.03	0.16	0.49

obtained for one of them only. All these trees (m6, 8, 12 and even 4) have the same type of curve, indicating uniform growth throughout lifetime. Trees of this type are of distinct age and size. The imbalanced growth between the various parts of one and the same tree is presumably caused by a different quantity of sunlight falling upon some of its branches. All in all, this group of trees cannot ever exceed moderate growth rates in their lifetime.

The trees in which the relationship between growth and climate is very evident also have few biometric features in common (Table 4); they belong to the younger generation (the last two generations being mature); have diametral growth proportional to age and develop greater symmetry; growth along several radii is far more homogeneous both in the young and the old specimens of these trees than in the group with non-significant correlations (m13-42.44%, m11-32.37%).

Table 4

Biometrical values of trees significantly correlated with climatic parameters in the maturity period (the last 50 years)

Individual	Age	Mean diameter (cm)	Variability of radii to maximum	Correlation coeff. r	
				Precipitation	Air humidity
1	62	317	0.13%	0.43	0.75
2	62	305	7.88%	0.38	0.66
7	69	304	3.87%	0.50	0.75
9	91	464	22.59%	0.34	0.64
10	94	560	12.39%	0.45	0.72

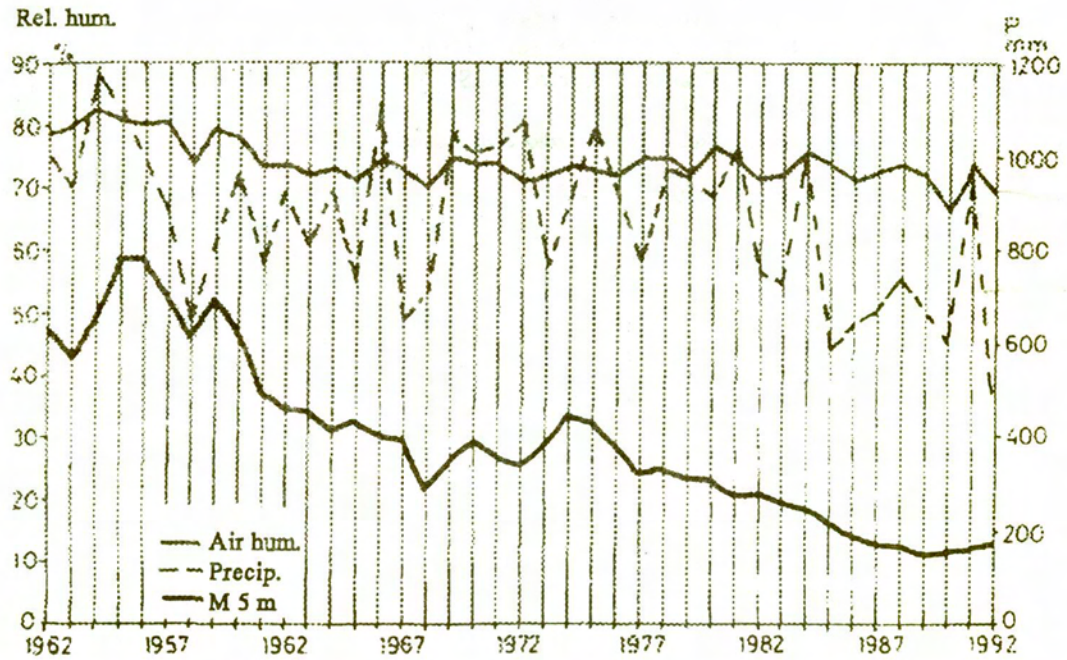


Fig. 5. – Annual growth mean of trees significantly correlated with climate (M5m).

To sum up we could say that fir-tree growth and air humidity correlate significantly, while growth and annual precipitation do not relate so well. However, the years of scanty or abundant precipitation have an obvious and immediate impact (Fig. 5). It is strange that no relationship to air temperature was detected, moreover so as they often are a limiting factor in the mountainous zone of this fir population. Perhaps it is not the temperature means that are involved, but other more important thermic parameters.

The trees in which growth relates to climate, a correlation between the two becomes obvious only after growth stability has been reached (during maturity) and only if the trees show relative diametral growth symmetry and hold constantly a favourable position in canopy – in relation to the sunlight. It is only this category of trees that can supply relevant information concerning climatic conditions.

Although the very old Sinaia tree population was supposed to be a "climate data base" for a long period of time, it failed to fall into the normal curve type and did not offer the expected evidence concerning the influence of all climatic factors on biological processes for the last period either.

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EFFECT OF CADMIUM ON THE GROWTH OF BLUE-GREEN ALGA *SPIRULINA PLATENSIS*

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Cadmium influence on the growth, chlorophyll and protein nitrogen accumulation in a *Spirulina platensis* alga of the Algal Collection of the Institute of Biology, Bucharest, was studied. The experiments lasted 9 days and were carried out in a chamber with 8.000 lux illumination. Cadmium was added as cadmium sulphate in Zarrouk nutritive medium of algal suspension in increasing quantities from 0.5 to 20mg/l nutritive solution.

The results showed that 10 and 20 mg/l cadmium doses were lethal for Spirulina, whereas 0.5; 1 and 2mg/l ones induced quantitative increase of the chlorophylls and stimulated cell division and protein synthesis. The dose of 5mg/l cadmium caused a decrease in the chlorophyll content and reduced the cell multiplication in still viable algal suspensions.

INTRODUCTION

Quite often, the final results of microelement action on algal suspensions depend on the used algal species, the microelement quantity in nutritive solution and the kind of microelement effect assessment (1, 2, 12). Rosko J.J and Rachlin J.W. (11) found that cadmium administration in algal culture medium induced the increase of the chlorophyll content. On the contrary, De Filippis F. (4,5), Mang S. (9), Conway H.L. (3), Gross R.E. (7) reported the decrease of chlorophylls by cadmium added in the culture medium. A reduction with 50% of cell multiplication promoted by 0.75 mg/l cadmium dose was observed by Stratton G.W. (*12*). Also, Dragos et al. (6) studying the effects of zinc and cadmium on the growth of Monoraphidium and Kirchneriella algae observed that the cell number in suspension was reduced with 50% at 1.5 mg/l cadmium dose, after 14 days of culture growth.

The purpose of the present investigation was to study the physiological reactions to cadmium sulphate administration of *Spirulina platensis* cultured in modified Zarrouk medium.

MATERIALS AND METHODS

Spirulina platensis (Nordst.) Geitl. alga was cultivated on modified Zarrouk medium (10) with cadmium, added as cadmium sulphate in quantities of 0.5; 1; 2; 5; 10 and 20 mg/l nutritive solution. The experiments were carried out in a chamber with artificial illumination of 8.000 lux. To avoid mutual shadowing, the algal suspensions, in cylindrical glass recipients of 1000 ml, were bubbled with steady stream of air produced by vibratory devices. The culture medium was inoculated with an amount of algal biomass producing a 100.000 cells/ml suspension in all experiment variants. The ambient temperature varied between 21 – 22°C. The

results represent average values of three repetitions for each variant. At the end of each culture cycle (established at 9 days) algal suspensions were filtered and the resulted biomass was analysed in fresh or dried (at 105°C) state. Assimilatory pigments (mg/g.d.w.) were determined from the fresh biomass, by the Holm method (1954) (8), while nitrogen from the dry biomass, by the Kjeldahl method. The protein was calculated using formula: N × 6.25.

RESULTS AND DISCUSSIONS

The carried out experiments showed that *Spirulina platensis* easily adapted to the low cadmium doses of 0.5; 1 and 2 mg/l, but not to high ones such as 5; 10 and 20 mg/l. In general, the accumulation of dry weight (g/l/day) (Table 1) was similar to quantitative synthesis (mg/g.d.w.) of chlorophylls (Fig. 1).

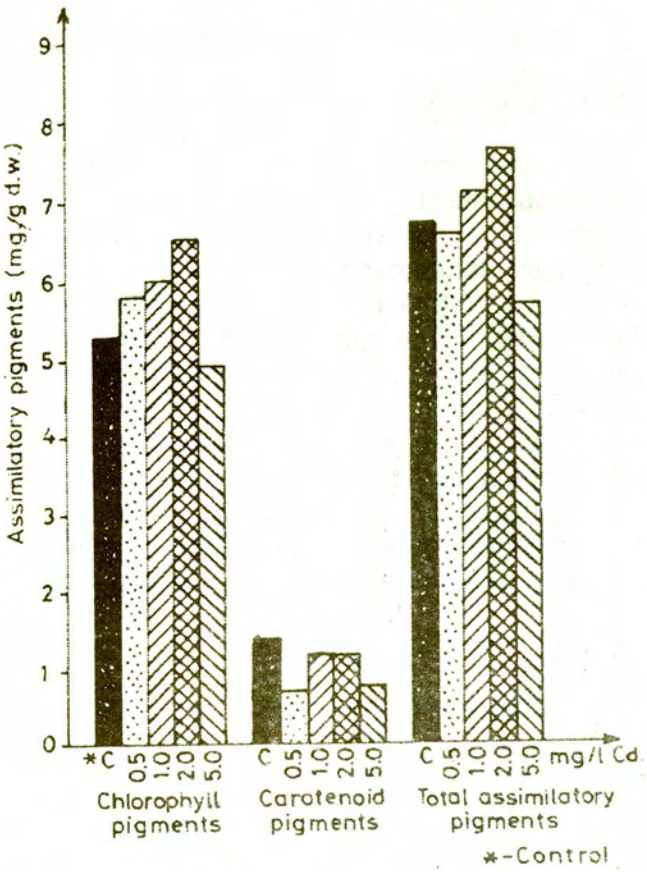

Fig. 1. – Cadmium effect on assimilatory pigment synthesis in Spirulina alga.

A decrease of carotenoid quantity in algal biomass was noted in all experimental variants with added cadmium, as compared to the control (Fig. 1).

The dose of 0.5 mg/l cadmium administered in growth medium of Spirulina alga resulted in an amount of 0.148 g/l dry weight therefore a 0.07 g/l/day increase as against control. Also an amount of 5.58 mg/g.d.w. chlorophylls, 0.83 mg/g.d.w. carotenoids and 6.41 mg/g.d.w. total assimilatory pigments were determined. These data indicated a chlorophyll increase with a concomitant carotenoid decrease, which

resulted in the decrease of total assimilatory pigments as compared to the control (Fig. 1). Microscope examination revealed that algal filaments had an average of 3.72 ± 0.18 helix spires (Fig. 3), a value close to control (4.3 ± 0.2) (Fig. 2).

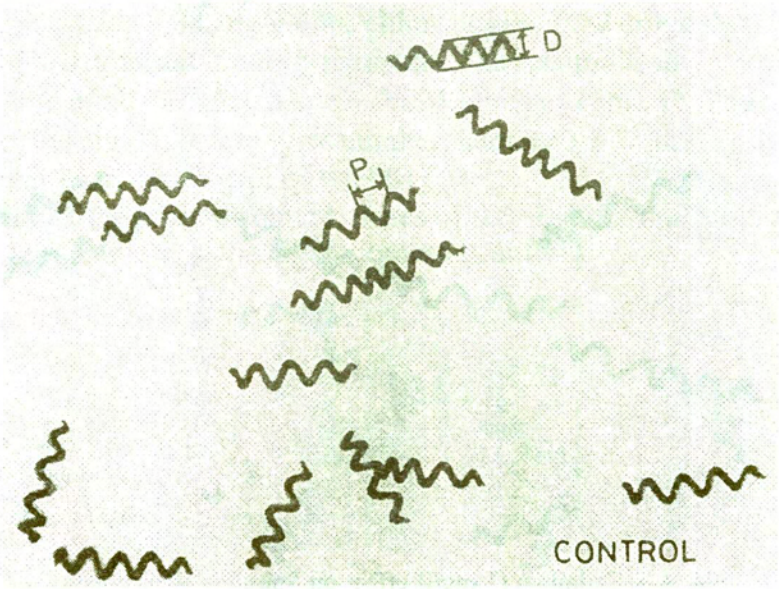

Fig. 2. – Spirulina culture (control).

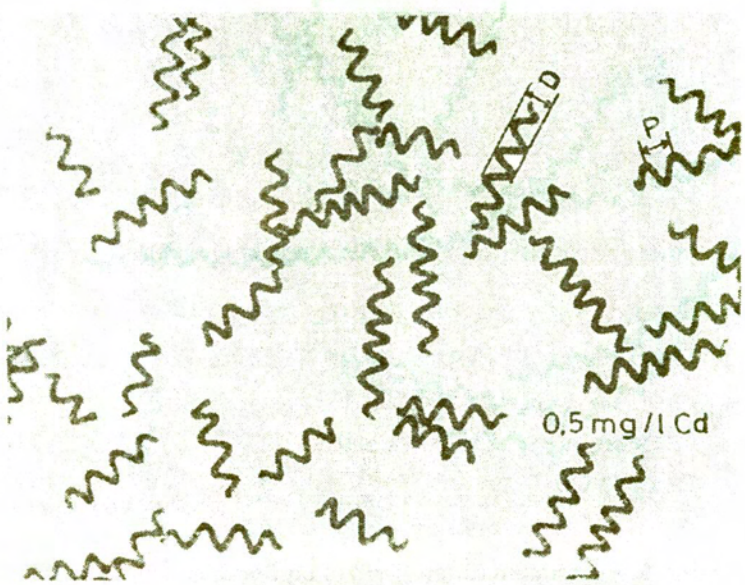

Fig. 3. – Cadmium (0.5 mg/l) effect on Spirulina alga growth.

Estimation of dry weight in variant with cadmium dose of 1 mg/l Zarrouk medium showed an amount of 0.158 g/l dry weight, that was a 0.07 g/l/day increase as against the control. In this case cadmium stimulated quantitative synthesis of chlorophylls, which together with carotenoids resulted in an ob-

vious rise of total assimilatory pigments relative to control (Fig. 1). The average value for algal filament spire number was 6.75 ± 1.25 (Fig. 4).

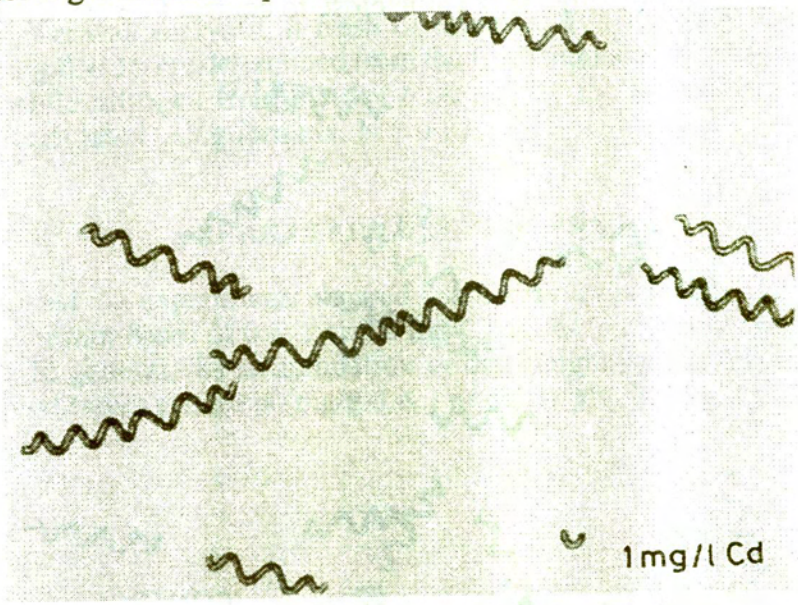

Fig. 4. – Cadmium (1 mg/l) effect on Spirulina alga growth.

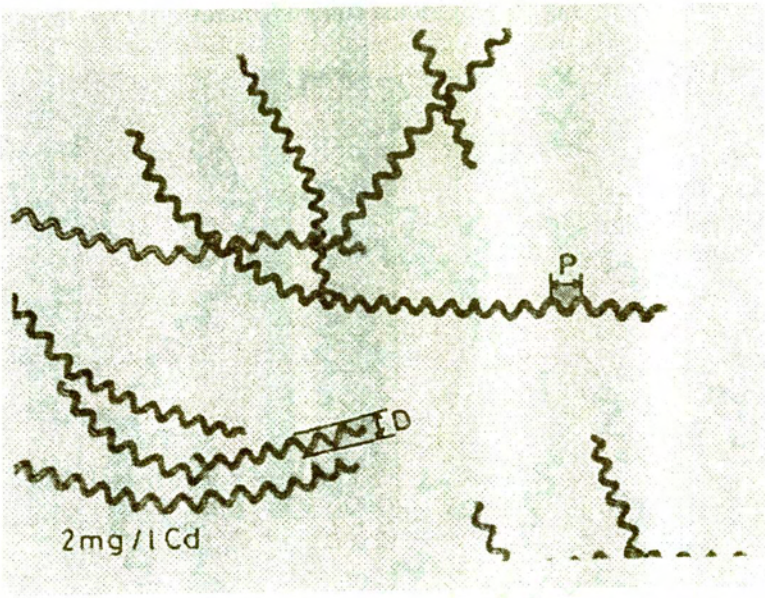

Fig. 5. – Cadmium (2 mg/l) effect on Spirulina alga growth.

The dry weight accumulation amounted 0.168 g/l, at the 2 mg/l cadmium dose, which represented 0.026 g/l/day increase as against control. The chlorophyll accumulation exhibited the greatest values, so that total assimilatory pigments reached a maximum of 7.71 mg/g.d.w. (Fig. 1). Also, chlorophylls increased with 1.11 mg/g.d.w. related to the control. With regard to the morphological features of the algal filaments, the number of helix spires was the greatest, an average value 11.37 ± 1.73 (Fig. 5), remarkably different from the control (Fig. 2) ($p < 0.001$).

The addition of cadmium to Zarrouk medium in a dose of 5 mg/l negatively affected assimilatory pigment synthesis (Fig. 1), dry weight accumulation (Table 1) and algal cell multiplication. The following data on assimilatory pigment synthesis were obtained: 4.88 mg/g.d.w. chlorophylls, 0.82 mg/g.d.w. carotenoids and 5.70 mg/g.d.w. total assimilatory pigments. The accumulated dry weight showed the lowest value of all variants, namely 0.094 g/l/day (Table 1). The lowest value indicated also the algal filament spire number, namely 3.37 ± 0.22 (Fig. 6), significantly different from the control ($p < 0.001$).

In regard of the protein content, doses of 0.5; 1 and 2 mg/l cadmium stimulated the protein increase in the algal biomass (Table 1).

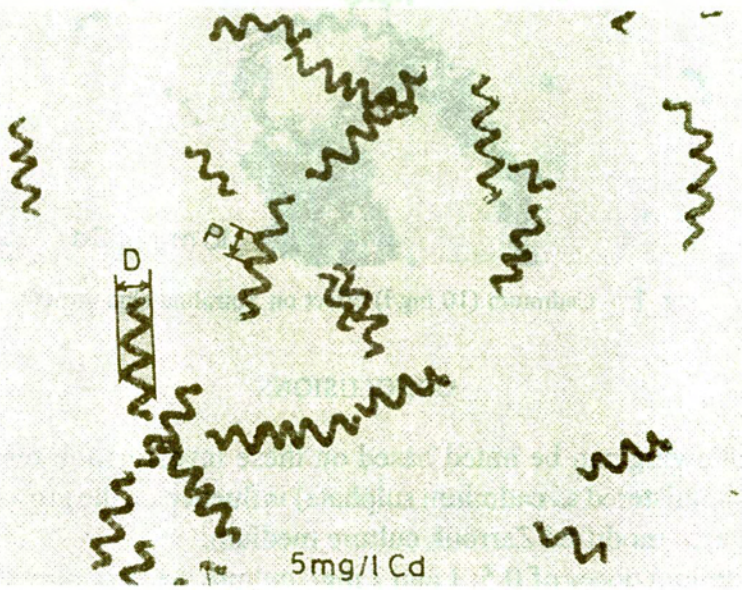

Fig. 6. – Cadmium (5 mg/l) effect on Spirulina alga growth.

A noticeable toxic effect of cadmium took place at high doses of 10 (Fig. 7) and 20 mg/l which destroyed the algal cells in the first days of the

Table 1

Cadmium effect on dry matter accumulation and protein content in Spirulina

mg/l cadmium	Protein content (N ×6.25)	Dry matter g/l/day
Control (no cadmium added)	54.1	0.141
0.5	56.3	0.148
1.0	57.9	0.158
2.0	59.9	0.167
5.0	46.6	0.094

experiment and therefore analyses of assimilatory pigments and protein nitrogen content in algal biomass were not possible.

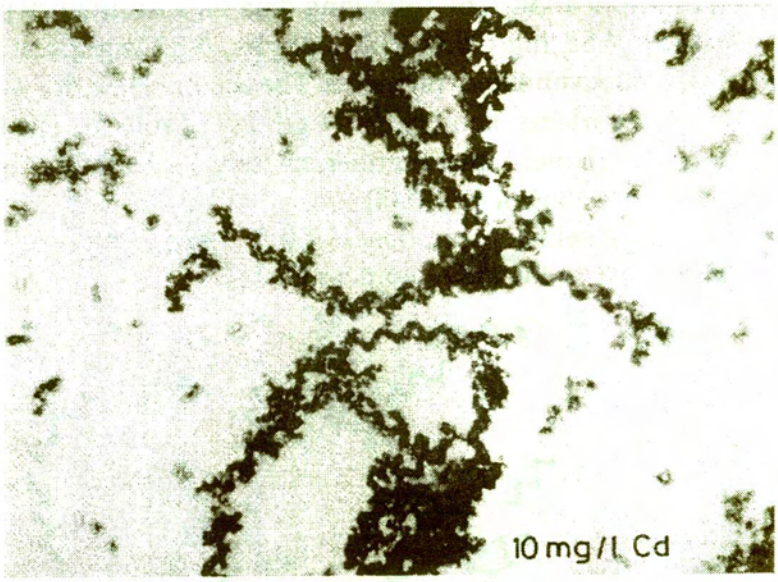

Fig. 7. – Cadmium (10 mg/l) effect on Spirulina alga growth.

CONCLUSIONS

The following can be noted based on these investigation results regarding cadmium (administered as cadmium sulphate) influence on the growth of *Spirulina platensis* alga, in modified Zarrouk culture medium:

- cadmium doses of 0.5; 1 and 2 mg/l culture medium stimulated the chlorophyll synthesis, the dry weight accumulation and the protein content increase in algal biomass. The highest values of these were obtained with 2 mg/l cadmium dose;
- dose of 5 mg/l cadmium induced the decrease both of cell multiplication and assimilatory pigment synthesis as compared to the control;
- high cadmium doses of 10 and 20 mg/l nutritive solution destroyed algal cells from the first days of the experiment;
- algal filament spire number was the highest 11.37 ± 1.73 at 2mg/l cadmium dose and the lowest 3.37 ± 1.73 at the 5 mg/l dose.

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CALLUS INDUCTION IN *MALVA PARVIFLORA* L. AND ITS USE IN THE STUDY OF ALLELOPATHY EXERTED BY *BRASSICA KABER* (D.C.) WHEELER

ARMERIA VICOL* and CRISTINA DOBROTĂ**

The conditions for callus induction and maintenance in country mallow (*Malva parviflora* L.) are described. The success in callus induction depended mostly on the type of explant used and not on the medium, but the morphology of the calli was greatly influenced by the culture media. Callus growth was inhibited by wild mustard (*Brassica kaber* (D.C.) Wheeler) water extracts. The possible use of callus cultures as bioassays for the study of allelopathic effects against this species is proposed.

Malva parviflora L. (Malvaceae) is a weed (common mallow, cheeseweed) present in both Europe and North America (Britton and Brown, 1970). It is considered unwanted on farms because it competes with the crops for water, nutrients and light, may harbor diseases and insect pests and hamper the application of various treatments needed by the crop plants (e.g., fungicides and plowing). There are various control methods to reduce weed problems (Lorenzi and Jeffery, 1987), but many are either labor-intensive, or imply the use of herbicides, which are potential hazards for man. Emphasis is growing presently for the use of the biochemical interaction between plants – allelopathy – for weed management. The most widely used bioassays in the study of allelopathy are seed germination (Wink, 1983; Lehle and Putnam, 1982; Rasmussen and Einhelling, 1979; McPherson et al., 1971; Muller et al., 1964) and radicle elongation (Leather and Einhelling, 1985; Bhowmik and Doll, 1982; Parker, 1966). Rarely, tissue cultures have also been used (Hogan and Manners, 1990; Gressel, 1984; Heller, 1953). The first two categories of bioassays can be difficult to use in the case of plants with hard-to-break seed dormancy. Cell cultures may then represent a useful alternative. An analogy between plant parts and cell cultures of different colors and ages has been proposed by Gressel (1979) and tested in the field of herbicide research (Zilkah et al., 1977; 1978; Zilkah and Gressel 1978).

This paper describes the conditions for callus induction and maintenance in *Malva parviflora* L. and the effect of wild mustard extract on its growth. Wild mustard inhibits growth of common mallow under natural conditions (S. Gliessman, personal communication) and allelopathy may be one of the mechanisms involved. Seed germination bioassays cannot be easily used with *Malva parviflora* L., as the dormancy of its seeds is very difficult to break.

MATERIAL AND METHODS

A range of explant types of *Malva parviflora* was screened for their ability to form callus on three kinds of media. Explants were collected from mature plants grown in the field, washed under running water for 10 min and surface-sterilized with 2.62% sodium hypochlorite supplemented with 2 drops/200 ml of Tween 20 for 7–15 min, depending on the explant. Then, all explants were rinsed six times with sterile, deionized water and the tissues destroyed by the sterilizing solution

were discarded. The media used for callus induction are presented in Table 1. All explants were incubated under fluorescent light (16 hrs/day), at an intensity of 2000 lux. Temperature was maintained at an average of 25° C.

Table 1

Media used for callus induction in country mallow

Medium composition	I	II	III
macronutrients MS (ml/l)	100	-	100
micronutrients MS (ml/l)	-	-	1
macronutrients B5 (ml/l)	-	100	-
micronutrients B5 (ml/l)	-	1	-
micronutrients Heller (ml/l)	1	-	-
FeEDTA (ml/l)	5	5	5
myo-inositol (mg/l)	100	100	100
pyridoxine HCl (mg/l)	1	1	0.2
thiamine HCl (mg/l)	1	2	0.4
nicotinic acid (mg/l)	1	1	0.2
naphthylacetic acid (mg/l)	1	-	-
2,4–D (mg/l)	-	5	1
benzyladenine (mg/l)	1	0.1	2.5
casein hydrolysate (mg/l)	-	2	-
sucrose (g/l)	20	20	20
agar (g/l)	7	6.5	6
pH	5.8	5.5	5.6

MS: Murashige and Skoog salts medium
B5: Gamborg's B5 salts medium
2,4–D: (2,4 dichlorophenoxy) acetic acid

The calli were maintained by transfer to fresh media of the same composition as the ones used for induction, at an interval of four weeks. For allelopathy experiments only callus cultures derived from stem internodes, and cultured on medium type I, were used. These were cream calli with green nodules.

Callus growth was compared between medium type I and the same medium, where the water needed for medium preparation was replaced by a mixture of wild mustard extract and water, 1:1 (w/w). The extract was obtained by soaking 20g of fresh, whole, 30-day-old wild mustard plants in 100 ml deionized water, for 12 hrs. It had a low osmotic concentration (only 33 m0sm) and a pH close to the one of the agar medium (pH 6.2), which made the preparation of osmotic and pH controls unnecessary. The extract was added after autoclaving, by filter-sterilization using Nalgene disposable filters with low protein binding membranes. Twenty vials (scintillation vials of 20 ml, with screw caps) were prepared per treatment, with 4 ml of medium per vial.

Preweighed pieces of callus tissue (three weeks from the last subculture) were allowed to grow under treatment conditions for a month before reweighing. Data are expressed as percent increase in gram fresh weight.

RESULTS AND DISCUSSIONS

Callus induction and morphology were compared among explant types and culture media (Table 2).

Table 2

Callus induction and morphology from different explants of country mallow

Explant type	Culture medium								
	no.+ expl.	I calli** %	callus type*	no. expl.	II calli %	callus type	no. expl.	III calli %	callus type
mature leaf blade	18	44	2	13	46	2	26	3.9	2
young leaf blade	17	20	2	20	30	3	27	7.4	3
fruit with peduncle	14	14	2	11	27	4	20	0	1
petiole	12	33	2	10	60	3–4	20	60	3
stem node	16	87	5	31	81	3–5	14	86	3
stem internode	24	92	5	30	83	3–5	15	93	3
flower bud	20	10	4	18	22	4	20	0	1

\+ No. expl. – lists the number of explants of each type;
** calli % – lists the percentage of explants that produced calli;
* Key to callus types: 1 – no callus formed;
2 – a little callus formed at the edge of the explant;
3 – beige, friable, fast-growing callus;
4 – white, nodular callus;
5 – cream callus with green nodules, friable, medium growing.

As studies on herbicide screening by using cell cultures showed that green and non-green cultures behave in a different way when they are subjected to the action of various chemicals (Zilkah and Gressel 1978; Zilkah et al. 1978), the cream calli with green nodules obtained on medium I were used as bioassays for the action of wild mustard extract. This enabled us to notice that the cream callus parts cultured on the extract medium became beige, and the green nodules were less conspicuous and numerous. The extract also inhibited callus growth (Table 3).

Table 3

The effect of wild mustard extract on country mallow callus growth*

Treatment	Increase (%) in gram fresh weight
control	190.79 [a]
wild mustard extract	116.82 [b]

*Numbers not followed by the same letter are significantly different at $p = 0.05$

The allelopathic potential demonstrated by the wild mustard extract on country mallow callus suggests that allelopathy may be a mechanism by which wild mustard inhibits country mallow in the field. Cell suspension cultures can be further obtained, and used in the study of the biochemical background of this allelopathic interaction.

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STUDIES OF LYTIC AND TEMPERATE BACTERIOPHAGES OF SOME LACTIC ACID BACTERIA

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15 strains of lactic streptococci were examined for lysogeny by treatment with ultraviolet light. Seven strains showed evidence of induced phages. The sensitivity of lactic streptococci to lytic phages present in industrial products was also tested. A brief examination of plasmid content of two strains of *S. lactis* was performed in order to determine the involvement of plasmids in the phage-defence systems.

Milk fermentations rely on the growth and acid-producing ability of lactic streptococci to impart the desired flavour, texture and preservative qualities of cheese and cultures dairy products. The process occurs in open vats under non-aseptic conditions and is, therefore, highly susceptible to bacteriophage contamination and failure due to phage-induced lysis of starter cultures. Regardless of the precautions taken to select and prepare starter cultures, the fermentation bacteria cannot be protected from phage contamination of proliferation in the cheese vat under the existing conditions of these processes. Consequently, lactic streptococci that have mechanisms to resist to bacteriophage attack and remain resistant under phage pressure imposed by the fermentation process are of enormous practical significance to the dairy industries (Klaehammer and Sanozky, 1985; Davidson et al., 1990).

Naturally occurring lactococci with a high degree of phage resistance have been shown to possess several types of phage resistance mechanisms including adsorption interference, restriction and modification and abortive infection (Bidnenko et al., 1993). Plasmid involvement in phage-defense systems present in lactococci is significant in two respects. First, genetic instability of plasmids encoding phage-resistance provides a genetic mechanism to explain the rapid accumulation of phage-sensitive variants within starter cultures (Sanders and Klaenhammer, 1981; 1983, de Vos et al., 1984; Jarvis et al., 1986; 1988). Secondly, plasmid involvement in phage-resistance mechanisms may be most advantageous in the development of phage-resistant strains, because of ease of isolation, manipulation and transmission of these characters when located on extrachromosomal elements Klaenhammer and Sanozky, 1985; Gasson, 1990).

The present study was undertaken to determine the incidence of lysogeny in 15 strains of lactococci and the presence of lytic bacteriophages in some industrial wheys. We also describe the plasmidic profile in different strains of lactococci.

MATERIAL AND METHODS

1. *Bacteria and culture conditions*. Four strains of *Streptococcus lactis* subsp. *lactis*. five strains of *S. cremoris* and six *strains* of *S. lactis* subsp. *diacetylactis* were used in our experiments. Cultures were maintained by a

biweekly transfer of 1% inoculum into sterile 11% reconstitued nonfat milk. Lactic streptococci were propagated in M17 broth at 30°C as described by Terzaghi and Sandine (1975).

2. *Induction of phages*. Active 16h cultures were transferred as 1% inoculum into 10ml of M17 broth and incubated at 30°C for 4h, centrifuged at 5000 rpm for 10 min, resuspended in 5 ml of sterile 0.1 M $MgSO_4$, transferred to a sterile Petri dish and irradiated with UV for 10–20 sec. with constant swirling. Irradiated cells were transferred into 5ml of M17 broth and incubated at 30°C for 5h. Turbidity of cultures was measured at 600nm hourly.

3. *Bacteriophage assays*. Direct plating of induced cultures filtrates or sterile whey filtrates on potential sensitive strains was carried out as described by Terzaghi and Sandine (1975). Concentration of phage particles and electron microscopy were performed as described by Huggins and Sandine (1977).

4. *Plasmid analysis*. Plasmid purification and detection on agarose gels were done as described by Vatafu et al. (1994).

5. *Concentration of phages and electron microscopy*. The filtrates were treated with a solution of 60% PEG 6000, centrifuged and the pellet containing lytic phages was resuspended in 0.6 ml of sterile 0.1M ammonium acetate. The suspension was then dialysed in 0.1M ammonium acetate for at least 24 h at 4° C. For electron microscopy studies the direct application method was used. A drop of suspension was placed with a fine bore Pasteur pipette on a 400 mesh copper grid with a formvar carbon film. A drop of 2% acetate uranyl aqueous solution was then added on the grid. Excess fluid is removed by touching the grid content with the edge of a torn piece of filter paper, leaving the grid surface slightly moist for 3–5 min and then was air dried. The specimen analyses were made using a Tessla Electron Microscope.

RESULTS AND DISCUSSION

1. INDUCTION OF TEMPERATE PHAGES

From 15 strains of lactic streptococci, 7 showed evidence of induced phages. The strain is generally regarded as lysogenic if the turbidity stops increasing after an additional 1–4h incubation after induction and then decreases, thereby indicating lysis (Davidson et al., 1990). After UV treatment, some strains (*S. lactic* SLP, *S. lactis* subsp. *diacetylactis* SD7 and SD21) showed obvious lysis at 2 or 3h, while other strains (*S. lactis* SD19, SD64 and SL16 or *S.cremoris* SC26 and SC197) showed marked inhibition of growth (Table 1.)

Unfortunately, we were not able to identify sensitive (indicator) strains on which the induced phage is capable of forming plaques. The failure to identify an indicator strain for a temperate phage hinders structural studies on the phages because it makes bulk purification of phage particles difficult. It also complicates procedures for assaying the phage. In view of the widespread occurrence of lysogeny, the process of superinfection immunity is likely to be a major reason for the difficulty experienced in finding suitable indicator strains.

Table 1

UV irradiation effect on the growth of *S. cremoris* (SC) and *S. lactis* var. *diacetylactis* (SD) strains

Strain	OD 600 nm time (hours)					
	0	1	2	3	4	5
SC26 M	0.048	0.228	0.510	0.645	0.660	0.720
SC26 P	0.048	0.081	0.117	0.180	0.294	0.435
SC197 M	0.015	0.169	0.246	0.465	0.645	0.690
SC197 P	0.015	0.024	0.048	0.09	0.201	0.345
SD7 M	0.018	0.045	0.168	0.315	0.390	0.585
SD7 P	0.018	0.021	0.030	0.060	0.040	0.018
SD19 M	0.015	0.06	0.207	0.345	0.516	0.630
SD19 P	0.015	0.024	0.045	0.051	0.09	0.195
SD21 M	0.018	0.051	0.195	0.315	0.483	0.585
SD21 P	0.018	0.030	0.045	0.027	0.027	0.030
SD64 M	0.030	0.108	0.345	0.438	0.651	0.687
SD64 P	0.030	0.057	0.087	0.090	0.153	0.270

2. EVIDENCE OF VIRULENT BACTERIOPHAGES IN INDUSTRIAL PRODUCTS

A special attention was paid to the study of lytic bacteriophages which could perturb the normal industrial fermentations. Ten different industrial wheys were examined for the presence of lytic phages. As possible hosts for these phages we tested 4 strains of *S. lactis* var. *diacetylactis* (SD19, SD20, SD22, SD64) and 4 strains of *S. lactis* var. *lactis* (SLP, SL14, SL18 and SL28). Two ways to determine the phage contamination of wheys were used: turbidimetric assay and double-layer technique (for production of plaque). Only two wheys, designated as 2V and 2N inhibited the growth of 4 strains of lactococci (SD20, SD22, SL18 and SD64). Plaques were observed in SD20 and SD22 when the whey 2V was used, but their number was reduced (Fig. 1).

Electron micrographs of concentrated filtrates illustrated a mixture of different phage-like particles (Fig. 2), but the magnification (about × 60.000) did not permit us to specify their morphological type.

Our results suggest that the industrial products could be contaminated with different phages. The source of these phages is not very clear: they could originate from an exogenous source or could result from lysogenic strains as a consequence of spontaneous phage induction.

3. PLASMID CONTENT

Most of the lactic acid bacterial species are well known to contain several endogenous plasmids. In the case of lactococci, essential metabolic traits are often carried by these extrachromosomal elements (McKay and Baldwin, 1990; Kok, 1990; Vătafu et al., 1994). Moreover, a variety of plasmids encoding genes conferring resistance to bacteriophages have been indentified in *S. lactis*.

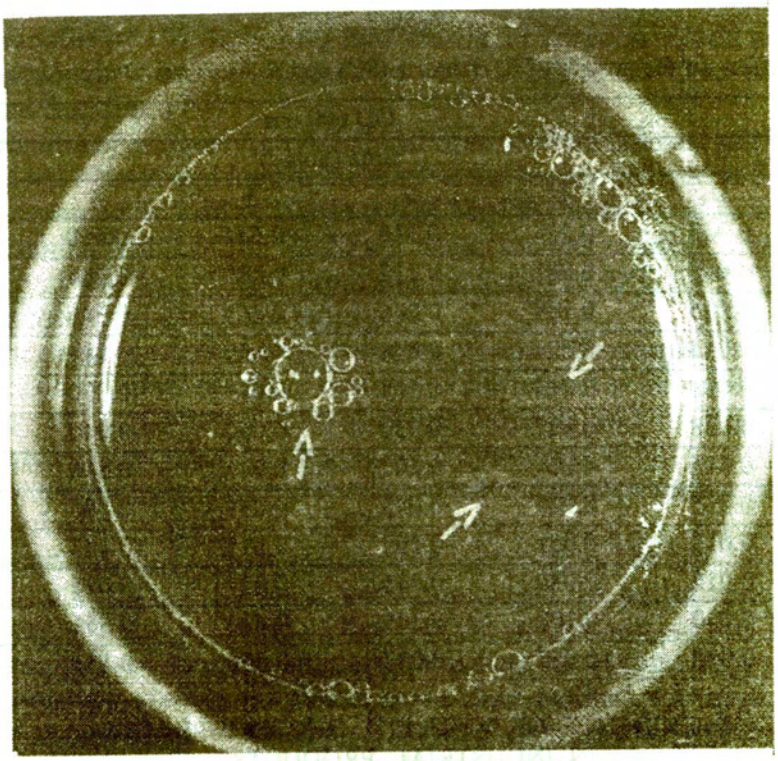

Fig. 1. –Plaques (arrows) produced by a lytic phage on *Streptococcus lactis* var. *diacetylactis* SD20.

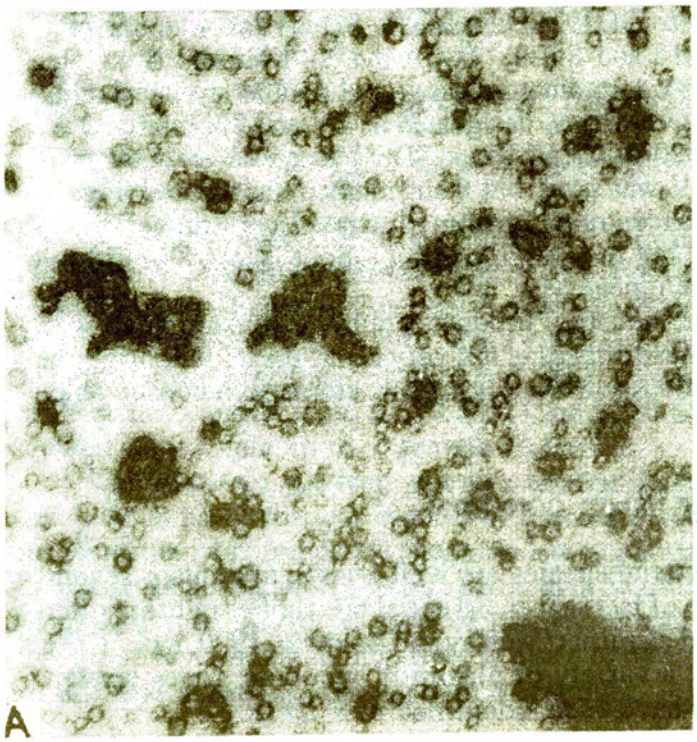

Fig. 2. – Electron photomicrographs of phage-like particles concentrated from the whey 2V (A). A detail of A plate is presented in plate B. Bar graph (B) represents 0.1μ.

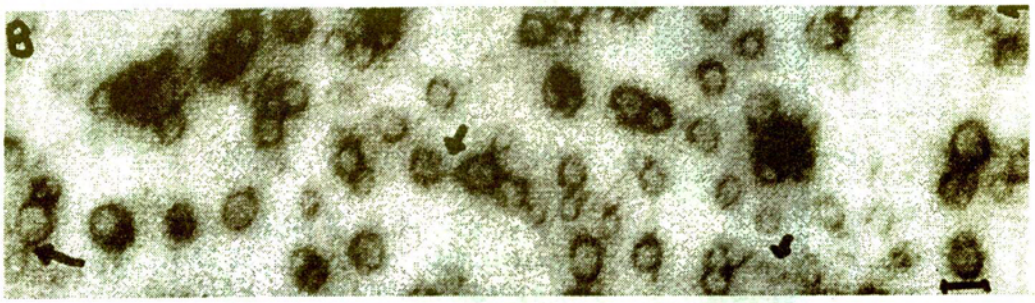

Fig. 2B.

Five different plasmids from *S. lactis* SL14 were isolated (Fig. 3). The plasmidic profile as well as the results obtained with this strain in previous experiments (Vătafu et al., 1994) were compared with the data existing in literature (Klaenhammer and Sanotzky, 1985). We presume that the strain SL14 which is not lysogenic and is resistant to lytic bacteriophages from industrial products tested contains a plasmid, similar as molecular weight (30MDa) with pTR2030 described by Klaenhammer and Sanotzky (1985) as a bacteriophage resistance plasmid. The resistance mechanism conferred by pTR2030 is double: restriction/modification and abortive infection (Moineau et al., 1994). In addition, the largest plasmid which is absent from Lac$^-$ variants of SL14 (fig. 3) could be similar with pTR1040 described by the same authors.

Several (up to 6) plasmid forms were encountered in a strain of *S. lactis* var. *diacetylactis* (SD22.1) (Fig. 4). This strain is a bacteriophage resistant variant of SD22 selected after several passages in the presence of the probe 2V (containing lytic bacteriophages). Our data were compared with the results of other authors (McKay and Baldwin, 1984; Jarvis, 1988) and we could suppose that one of the largest plasmids (with about 40MDa) is similar with pNP40 (described by Jarvis, 1988) which contains the genes encoding phage resistance. This assumption is

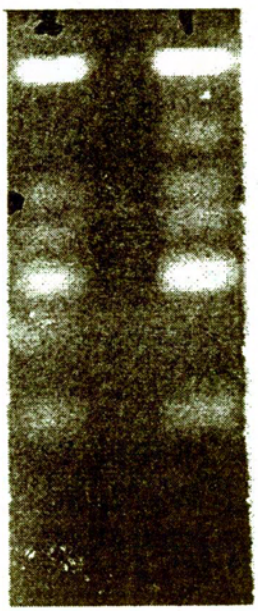

Fig. 3. – Plasmid profile of *S. lactis* SL14 Lac$^+$ (1) and a Lac$^-$ variant (2). The arrow indicates the plasmid encoding phage-resistance.

Fig. 4. – Plasmid profile of *S. lactis* var. *diacetylactis* SD22.1, a phage-resistant variant of SD22. The arrow indicates the position of a plasmid possibly involved in the bacteriophage-resistance mechanisms (chr = chromosomal DNA).

supported by the observation that incubation at 37° C of the strain SD22.1 with an enriched phage suspension led to a lowering of bacterial resistance. This result is in agreement with those reported by other scientists working with similar bacteria (Sanders and Klaenhammer, 1980; Gasson, 1990).

The data resulting from our experiments could be used in future studies concerning the selection of bacteriophage-resistant starters that are of direct value in industrial scale fermentations.

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THE ULTRASTRUCTURE MODIFICATION OF THE FOLIAR PARENCHYMA CELLS AT TOMATO SORTS UNDER THE INFLUENCE OF IONISANT RAYS

G. C. CORNEANU*, C. CRĂCIUN**, VERONICA CRĂCIUN**

The ultrastructure of the foliar palisadic parenchyma cells at the normal and mutant plants belonging to three tomato genotypes was studied. The alterations in the cell ultrastructure depend on the mutant type. They affect the nucleus, mitochondria and chloroplast ultrastructure, as well as the quantity of cellular organelles. In the nucleus there are present the corpuscles of loose nuclear body type, near nucleous, as well as the bodyguard (at the albino-chlorina mutant). Mitochondria present the dilated cristae, and at the albino chlorina mutant there is an electrodense substance in the cristae. The chloroplast ultrastructure is altered through thylakoids dilatation and grana fragmentation. These modifications are proportional to the mutant gravity.

Chlorophyllous mutants are found in low percentage both in natural state and also as a result of the action of some mutagen factors. In the specialized literature there are few studies which tackle the ultrastructural characteristics of chlorophyllous mutants. Von Wangenheim described the ultrastructural changes in the primordia of the leaves of wheat plantlets under the action of X-rays. Andersen et al. described the chloroplast ultrastructure in the various coloured tissue sector of the leaves (green, gray, yellow and white tissues) in the *ghost* mutant of *Lycopersicon esculentum*. Corneanu et al. and Crăciun et al. described the ultrastructure of palysade parenchyma cells of the leaves from many normal and mutant tomato genotypes.

In this paper, the ultrastructure features of some tomato genotypes (normal and mutants) are described.

MATERIAL AND METHODS

The dry seeds belonging to three tomato genotypes (Eurovite, Nemavite and Sonato hybrids) were irradiated with X-rays (dose flow 2 Gy/min, the doses between 0 – 350 Gy). In the X_1 generation the following chlorophyllous mutants were found out: *viridis* mutant (Sonato 250 Gy and Nemavite 300 Gy), *normal - viridis sector* mutant (Eurovite 250 Gy) and *albino-chlorina* mutant (Nemavite 350 Gy).

The analysis of the main ultrastructural features of the foliar parenchyma cells was made at the mature plants (at flowering). The pieces of leaves about 1 mm^3 were prefixed in glutaraldehyde 3% (2 h), postfixed in Milloning fixing solution 1% (1 1/2 h) and included in vestopal W. Seriated sections of about 800 - 900 Å thickness were contrasted with uranyl acetate and lead citrate and analyzed at EM TESLA BS – 613 (Biology Department, Cluj-Napoca University).

RESULTS AND DISCUSSIONS

The *normal plants* of the three analyzed genotypes presented a similar ultrastructure. The cells of foliar palisadic parenchyma are big, with a rich cytoplasm, with numerous cellular organites. The mature chloroplasts, of

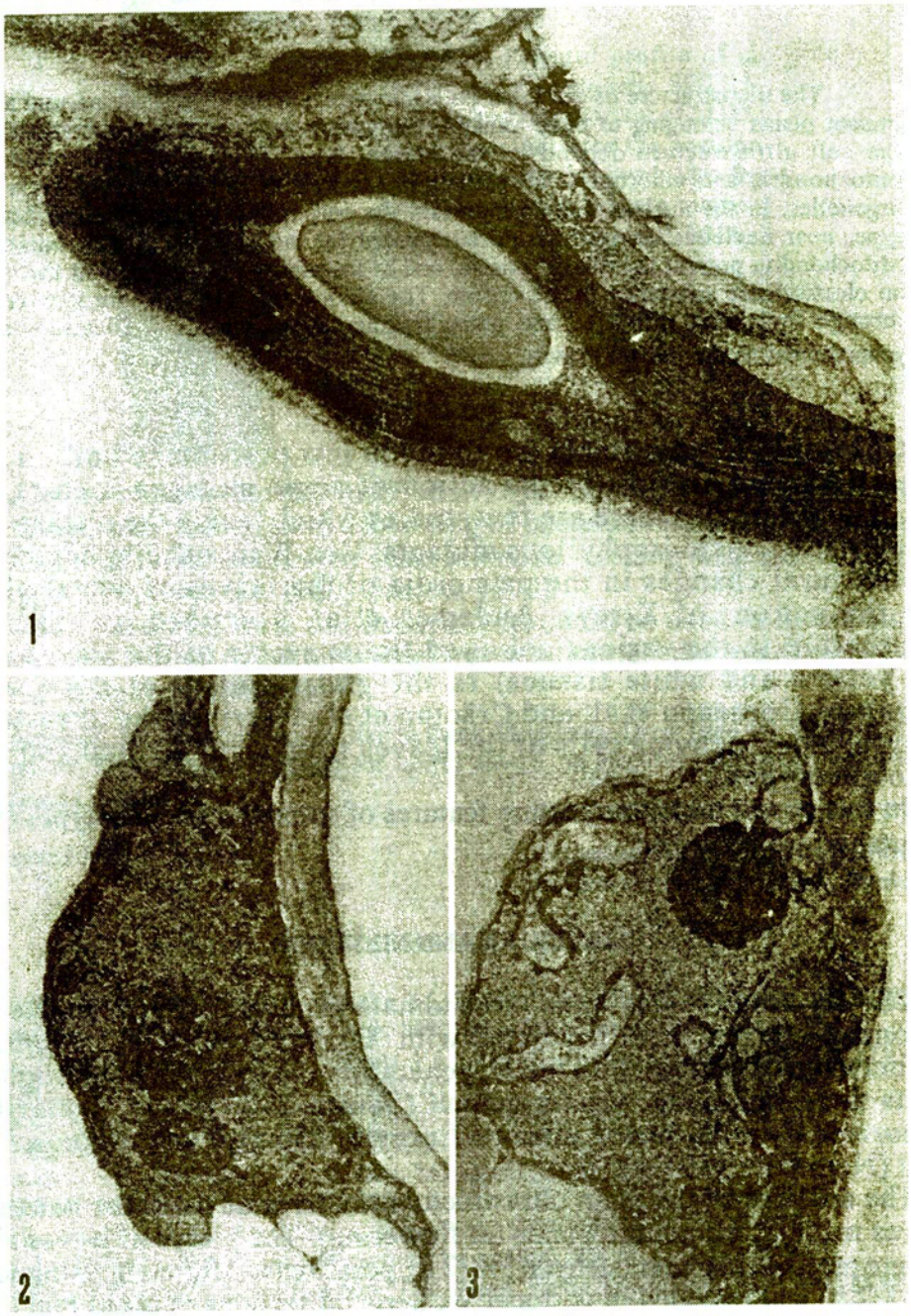


Fig. 1. Eurovite – control. Chloroplast with granal system well developed, starch granule and rare plastoglobules. × 23,000
Fig. 2. Sonato – control. Normal aspect of nucleus. × 11,000
Fig. 3. Santato – viridis mutant. nucleus with invaginations and losse nuclear body. × 5,000

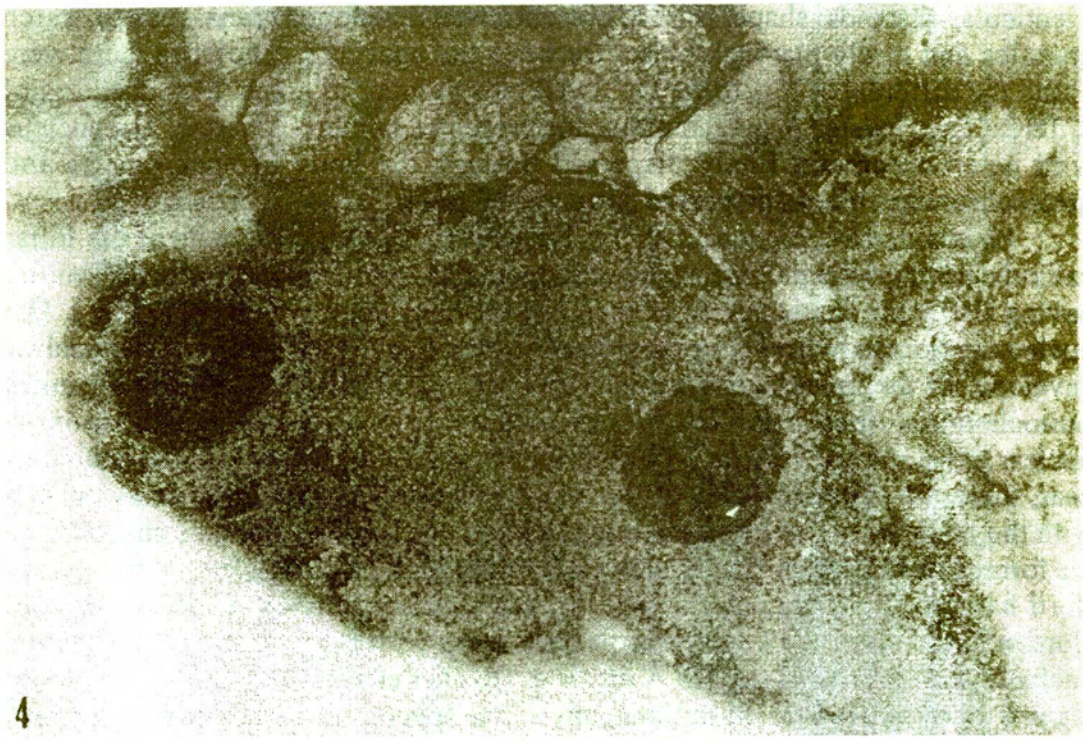
a lenticular-discoidal shape, (Fig. 2) present a well developed granal system, starch granules and rarely plastoglobules. Between the analyzed genotypes there were found the differences regarding the thylakoids number in grana. Mitochondria of different form existed in great number and presented numerous cristae. The nucleus with heterochromatin finely dispersed through the body of euchromatin and heterochromatin blocks dispersed parietally (Fig. 1).

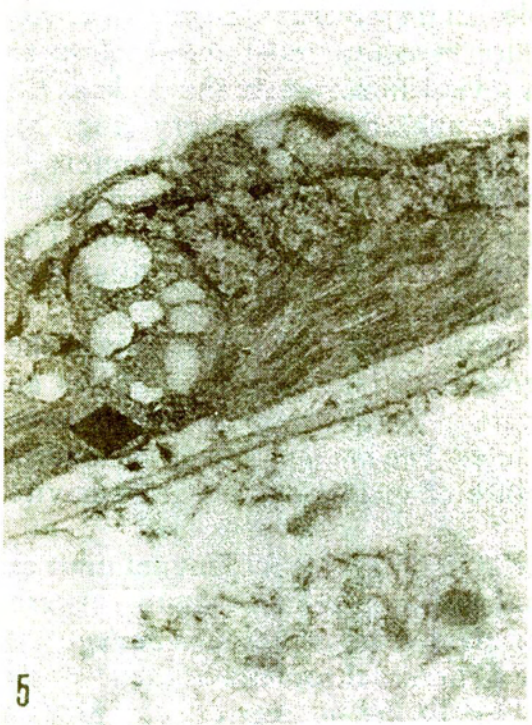
Viridis mutant (Sonato 250 Gy; Nemavite 300 Gy). The cells of the foliar palisadic parenchyma present an altered ultrastructure in comparison with the control, having also some characteristics depending on the genotype (Figs. 3, 4, 5, 6,). The nucleus in Sonato hybrid (Fig. 3) presents numerous invaginations of the nuclear envelope. In the invagination regions there is cytoplasm with cellular organites (mitochondria, ribosomes a.o.). In Nemavite sort (Fig. 4), the invaginations of the nuclear envelope are very rare. In the nucleus, at both genotypes, in the nucleolus near, there are the corpuscles of the loose nuclear body type, free or associated with the nucleolus. The loose nuclear body has a spheric or elongate shape. They are formed of fibrils dispersed relatively parallel in an electron-clear matrix. Every fibril has 24-30 nm diameter and is formed of many fibrils with 16 Å diameter (Jordan; Crăciun et al.) Their structure is similar with those of the nucleolus, the nucleolonema fibrils having 15 Å diameter (Recher et al.). Corpuscles of the loose nuclear body type were found at many plant species of different origin (fern, gymnospermae, angiospermae). It is probable that their presence is correlated with an intense metabolic activity. They exist both in the normal cells (for example in the meristematic cells), and in the cells with a viral infection. In the nucleus, the heterochromatin region is found at the nucleolus periphery (Fig. 3).

The chloroplasts present rare starch granules, but numerous plastoglobules (Figs. 5, 6). They sometimes have the disorganized grana, because of the thylakoids dilatations and grana fragmentation. The mitochondria present generally few cristae, they being slightly dilated as well as electron-clear zones in the matrix (Fig. 4).

Normal-viridis sector mutant (Eurovite 250 Gy). The analysis of the ultrastructural features of the cells from both leaf sectors indicates an alteration, bigger in the viridis sector (Fig. 7-9). The chloroplasts have many plastoglobules and rare starch granules. The nucleus and mitochondria ultrastructure is similar with the structure of viridis mutant at Nemavite hybrid.

Albino-chlorina mutant (Nemavite, 350 Gy). This mutant determines the plant death at flowering (sublethal action), because of the severe disorganisation of the cell (Fig. 10). In nucleus there is present a *bodyguard* (Fig. 11). This is formed of a thick zone of heterochromatin at the nucleus periphery, situated on the inner membrane of the nuclear envelope. It is formed probably of the constitutive heterochromatin and DNA repetitive, having the protective role of the active genes from euchromatin against the stress agents (ionizing radiations, viral infections, chemical agents a.o.; Hsu; Corneanu et al.).





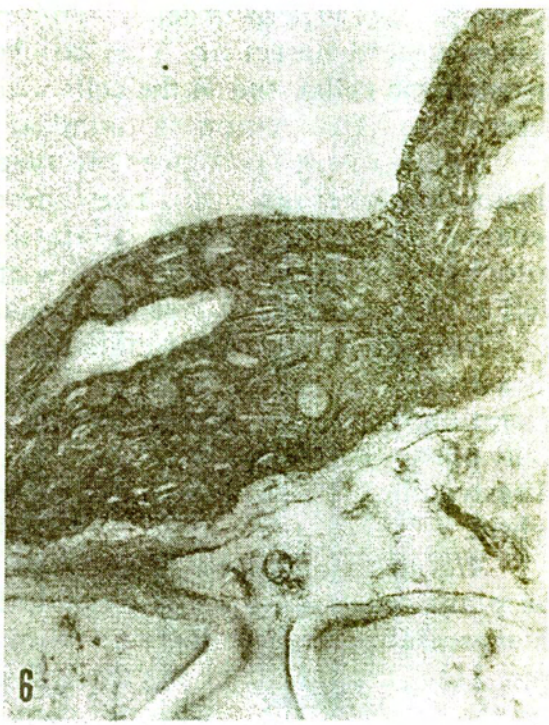


Fig. 4. Nemavite – viridis mutant. Nucleus without invaginations. × 16,500
Fig. 5. Nemavite – viridis mutant. Chloroplast with slightly dilated thylakoids and peroxisome. × 11,000
Fig. 6. Sonato – viridis mutant. Chloroplast with disorganized grana, slightly dilated thylakoids and many plastoglobules. × 15,000

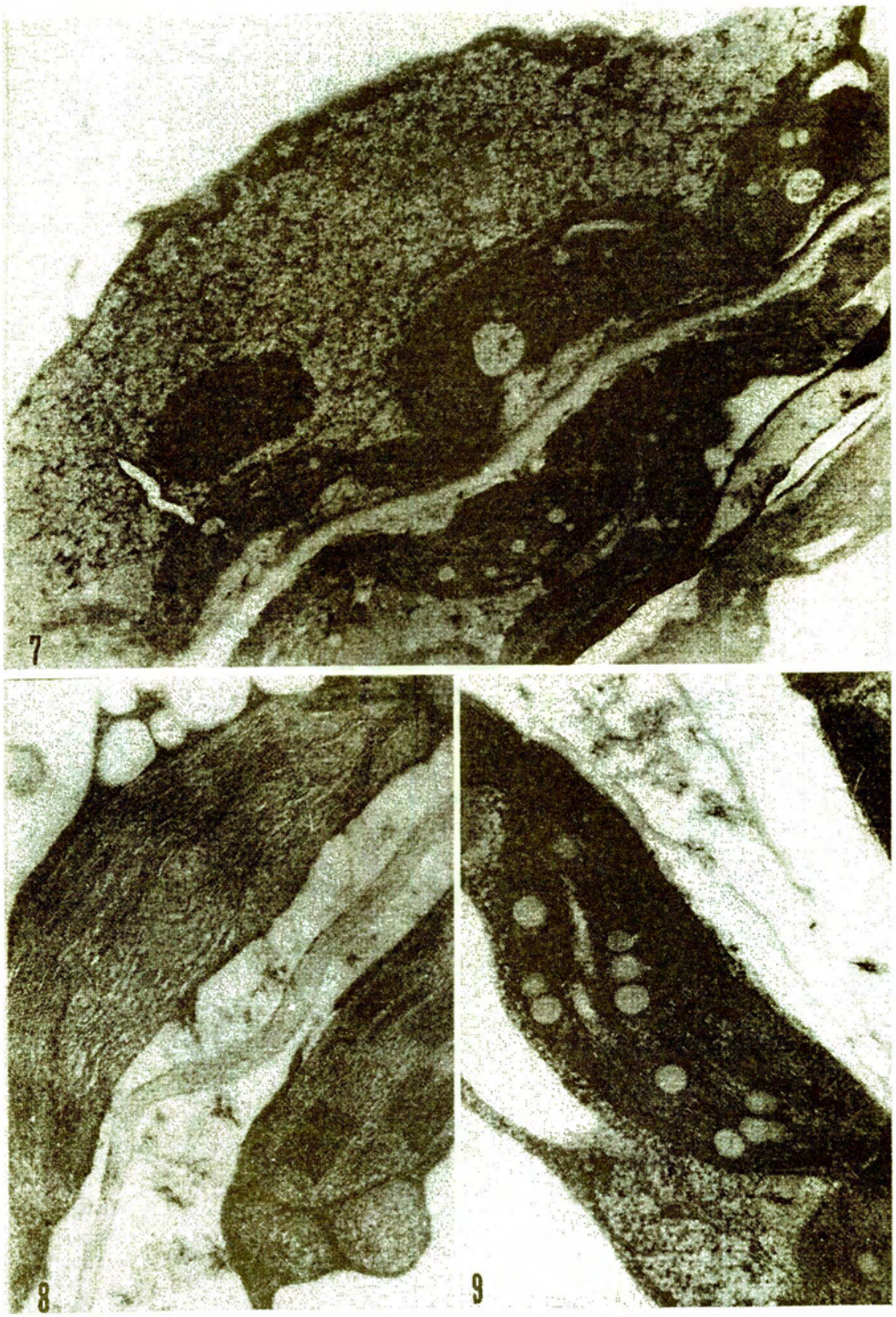
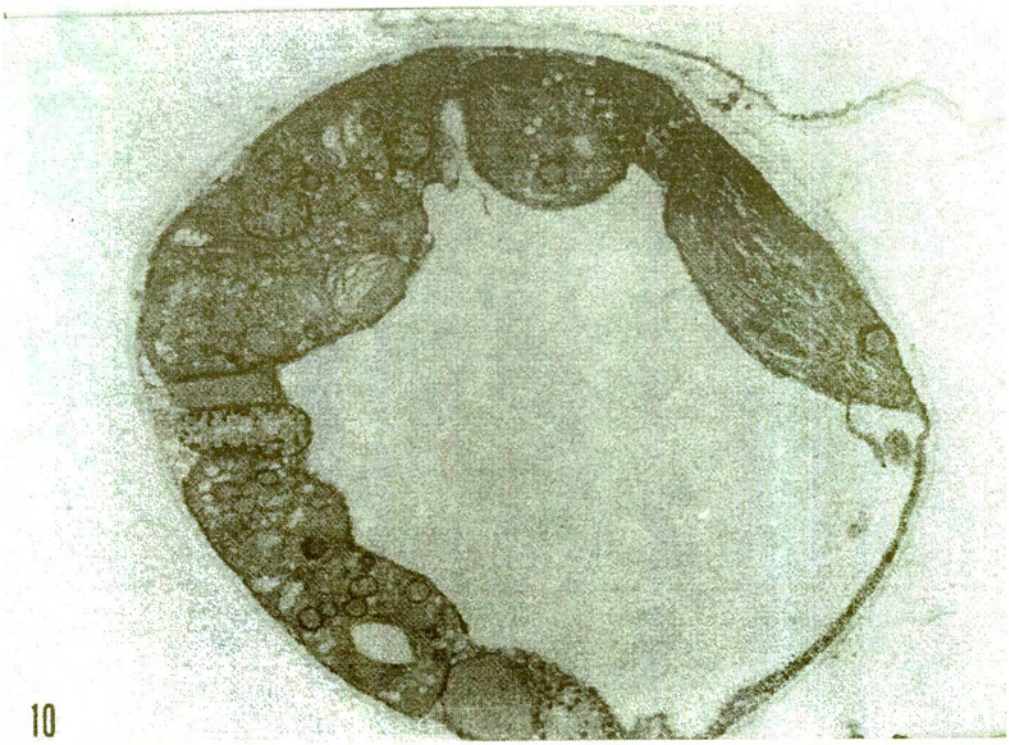
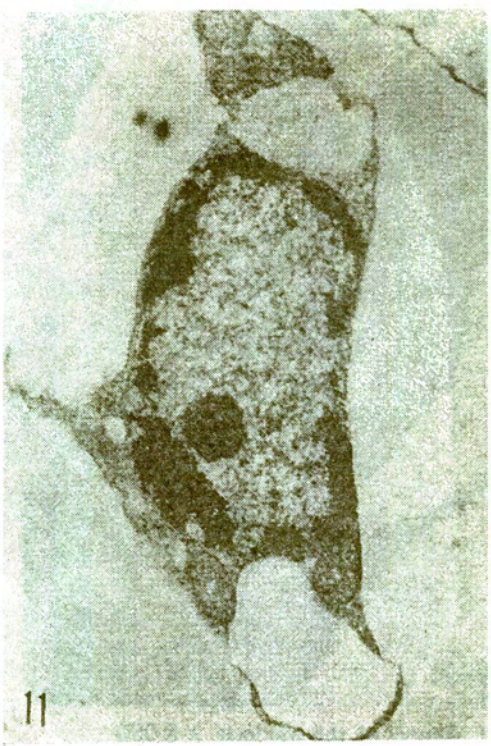


Fig. 7. Eurovite – normal-viridis sector mutant. Aspect of the nucleus. × 8,000
Fig. 8. Eurovite – normal-viridis sector mutant. Chloroplasts with dilated thylakoids. × 21,000
Fig. 9. Eurovite – normal-viridis sector mutant. Chloroplasts with many plastoglobules and dilated thylakoids. × 14,500





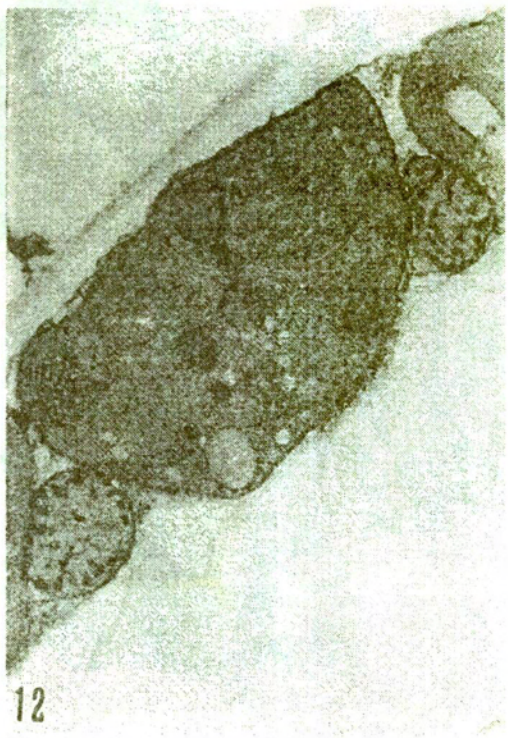


Fig. 10. Nemavite – albino-chlorina mutant. General aspect of the cell. × 8,000
Fig. 11. Nemavite – albino-chlorina mutant. Nucleus with bodyguard. × 11,000
Fig. 12. Nemavite – albino-chlorina mutant. Chloroplast and mitochondria with disorganized ultrastructure. × 15,000

Chloroplasts present a strong disorganized structure (Fig. 12). The grana thylakoids and stroma thylakoids are dilated, resulting the electron-clear spaces which join and cross the grana. In some chloroplasts existing invaginations in which cytoplasm with cellular organites (mitochondria, ribosomes a.o.) penetrate. A similar aspect was described in *Hordeum vulgare* by Knoth.

Mitochondria present cristae in the small number in which there is an electrodense substance (Fig. 12).

CONCLUSIONS

1. The chlorophyllous mutants from some tomato genotypes present the ultrastructure of strongly affected parenchymatous cells.

2. There are ultrastructural modifications of the nucleus, mitochondria and chloroplasts, which lead to the cell function alteration.

3. The ultrastructural modifications are proportional to the lesions gravity induced by X-rays and similar with those produced by other stress factors (viral infection, thermal shocks, action of recessive homozygote genes, a.o.)

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INTERACTIVE EFFECT OF TEMPERATURE AND SALINITY ON THE GROWTH OF SOME FUNGI ISOLATED FROM THE TECHIRGHIOL LAKE

IOANA GOMOIU*, G. ZARNEA**

Ten fungal strains isolated from the Techirghiol lake have a wide range of tolerance for NaCl. As they grow on a medium with 150 g/l NaCl we consider all of them as halotolerant fungi.
These studies have demonstrated a high salt requirement for growth at a high temperature in case of soil fungi which is similar to what has been mentioned from marine fungi.

Water molds have long been considered as freshwater species. They were isolated by Te Strake (10) from minimally saline regions of estuaries or by Padgett from estuarine sampling sites where salinity reaches 12 g‰ (4,7). Andrews's (1) and Wheelea's (13) preliminary studies indicated that fungal growth is stimulated by NaCl although there was no obligate requirement.

The optimum salinity for growth in some marine fungi shifts upwards with the increasing incubation temperature. The interaction between temperature and salinity is known as the "Phoma" pattern because it was found for the first time with a marine species of "Phoma" (9) but it was also found with other fungal species (2, 3, 11).

This paper reports the responses to various salinity and temperature values of ten fungi isolated from the Techirghiol lake.

MATERIAL AND METHODS

Stemphylium sp., *Penicillium sp.* 15, *Penicillium sp.* 5a–1, *Penicillium sp.* 5a–2, *Aspergillus sp.*1, *Aspergillus sp.*10a–2, *Alternaria sp.*1, *Alternaria sp.* 2, *Oospora variabilis sp.*1, *Oospora variabilis sp.* 2, were isolated from the mud of Techirghiol lake. They are maintained on YPG medium (yeast extract lg, peptone and dextrose lg agar 1,8 g prepared with water from Techirghiol lake – 100 ml – containing 65–68 g/l NaCl. Inocula for growth experiments were obtained from cultures grown on enriched corn meal agar (corn meal agar Difco 17 g, glucose 2 g, yeast extract lg) prepared with water from Techirghiol lake. After 7 days of incubation at 25°C, plugs (1 mm diameter) were cut aseptically from the grown margin of the colony and were transferred to the YPG medium.

Growth experiments have been done on the same medium with different salinities (10, 30, 50, 70, 90, 110, 130, 150 g/l NaCl). The mean colony diameter was calculated from a set of triplicate plates by two measurements (mm) taken of each colony at right angles to each other.

The mean growth rate was measured daily and was obtained for each triplicate set of Petri dishes.

The effects of temperature on the growth were observed by incubating

cultures at the same salinities and 10, 20, 30, 40°C. The mean diameter of colony and rate of growth were measured in the same way.

RESULTS AND DISCUSSION

Fungal tolerance for NaCl has a wide range. All strains can grow to 130–150 g/l NaCl (Figs. 1–4)

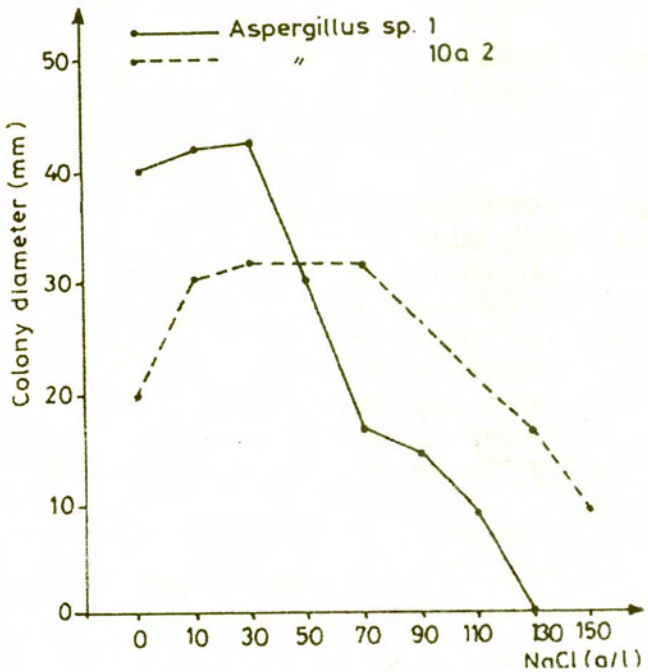

Fig. 1. – Effect of salinity on the growth of *Aspergillus* strains.

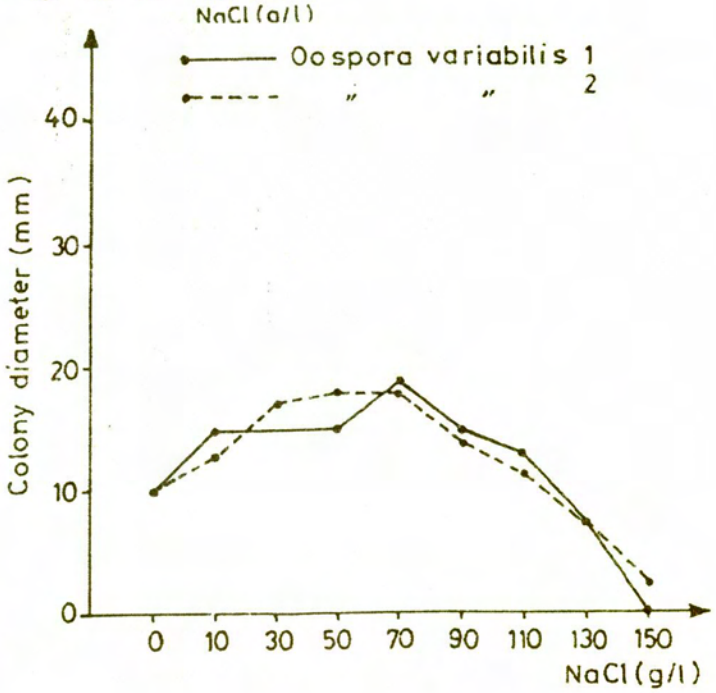

Fig. 2. – Effect of salinity on the growth of *Oospora variabilis*.

Aspergillus sp. 1 has a good growth in a medium containing 0–30 g/l NaCl and *Aspergillus sp.* 10a–2 has a poor growth in a medium without NaCl but a good one in a medium with 30–70 g/l NaCl (Fig. 1). The same situation was in case of *Oospora variabilis* 1 and *Oospora variabilis* 2 but the limit for NaCl tolerance was 110 g/l (Fig. 2). All strains of *Penicillium* had a good growth in a medium containing 30–90 g/l NaCl (Figs. 3–4). The best growth of *Alternaria sp.* 1 and *Alternaria sp.* 2 was on media without NaCl.

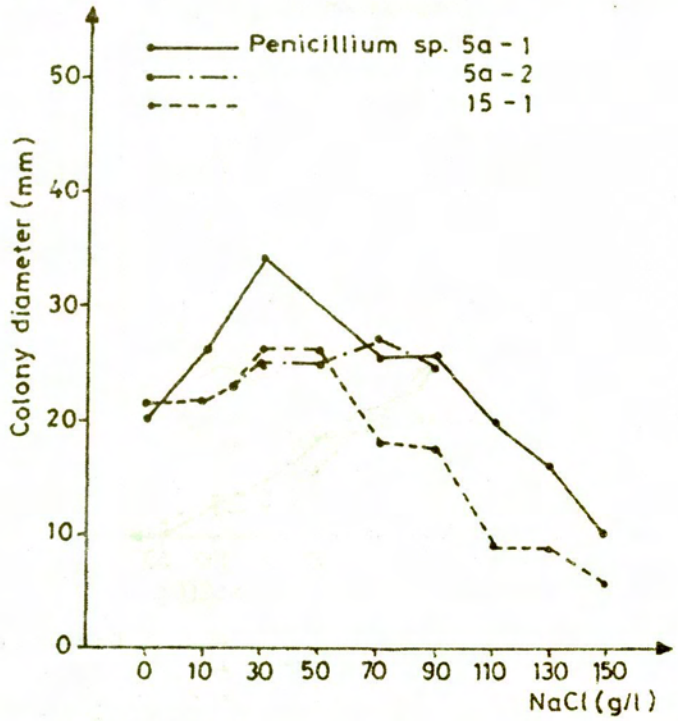

Fig. 3. – Effect of salinity on the growth of *Penicillium* strains.

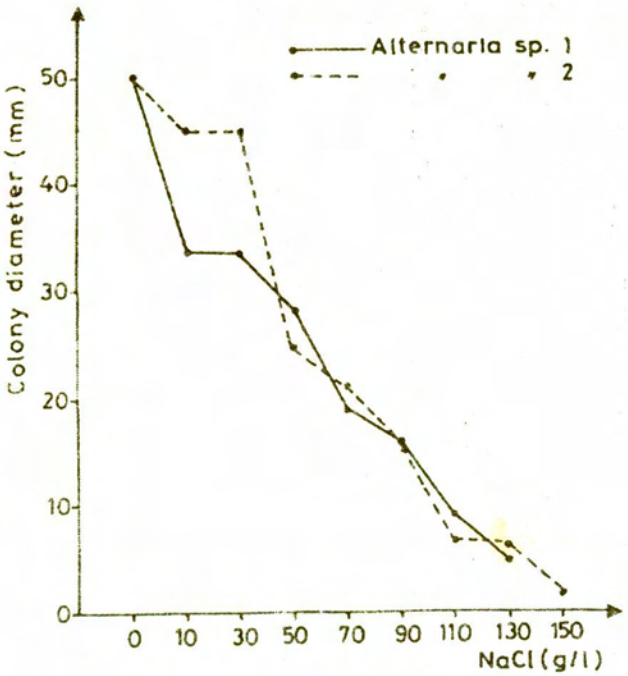

Fig. 4. – Effect of salinity on the growth of *Alternaria* strains.

The rate of growth was low for all strains except for *Alternaria sp.* 1 and *Alternaria sp.* 2 in a medium without NaCl (Figs. 5–6). The highest rate of growth was at 70 g/l NaCl for *Penicillium*, *Oospora*, and *Aspergillus strains* (Fig. 7–8). Growing on a medium with 150 g/l NaCl we consider all of them halotolerant fungi.

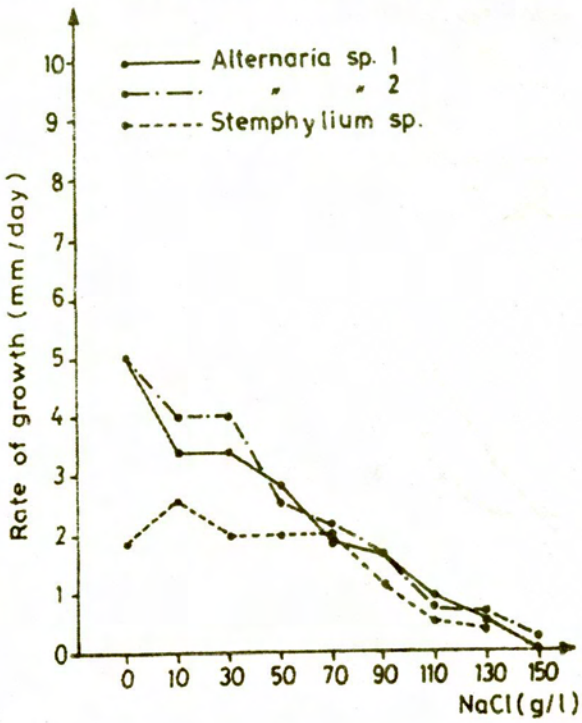

Fig. 5. – Effect of salinity on the radial growth rate of *Alternaria* and *Stemphylium* strains.

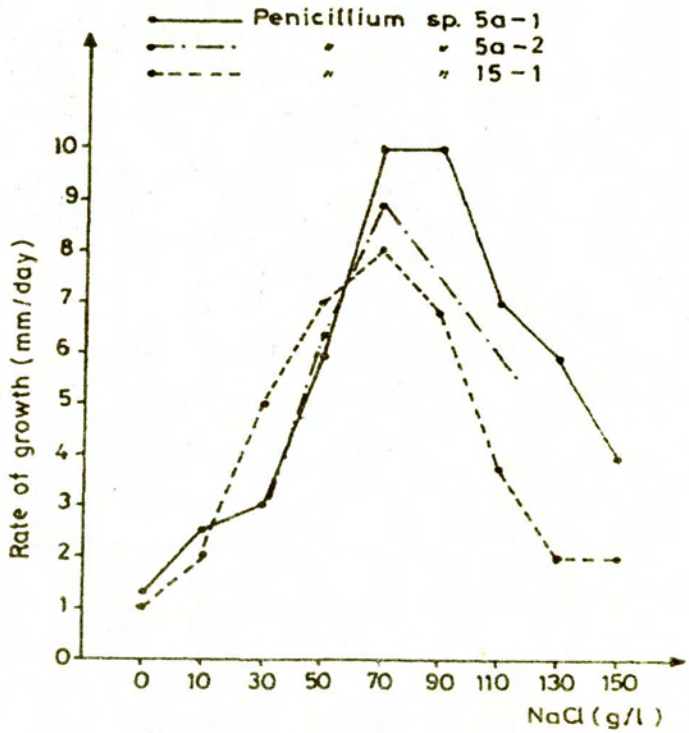

Fig. 6. – Effect of salinity on the radial growth rate of *Penicillium* strains.

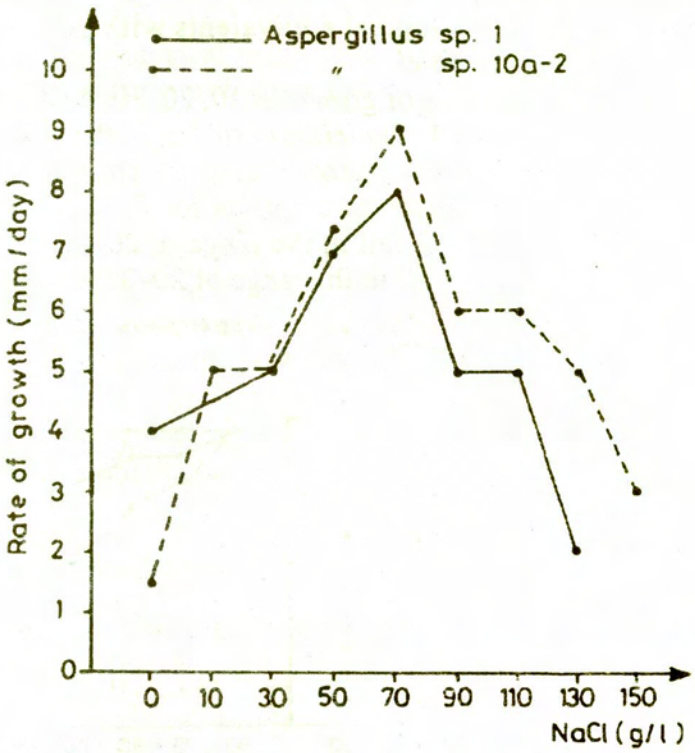

Fig. 7. – Effect of salinity on the radial growth rate of *Aspergillus* strains.

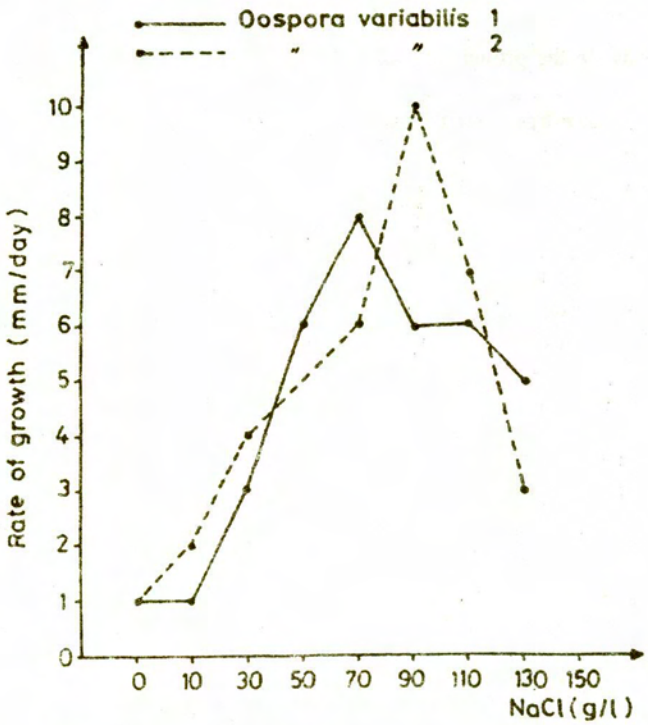

Fig. 8. – Effect of salinity on the radial growth rate of *Oospora variabilis*.

This wide range of tolerance to salinity suggests that these fungi could possibly be found over a wide range of salinities in nature both in soil or in marine environment. The ranges of these fungi in marine environment could overlap if

they behave as ecological equivalents with potentially overlapped niche they becoming competitive.

The occurrence of growth at 10, 20, 30, 40°C suggests that *Alternaria sp.* 1, *Oospora variabilis* 1, *Penicillium sp.* 5a–2, *Penicillium sp.* 15–1 and *Aspergillus sp.* 10a–2 (fig. 9,10) can grow during the entire year and could be important decomposers. The temperature optima for *Penicillium sp.* 5a–2, *Alternaria sp.* 1, *Aspergillus sp.* 10a–2 fall in the range of 20–40°C and for *Penicillium sp.* 15–1 and *Oospora variabilis* in the range of 20–30°C.

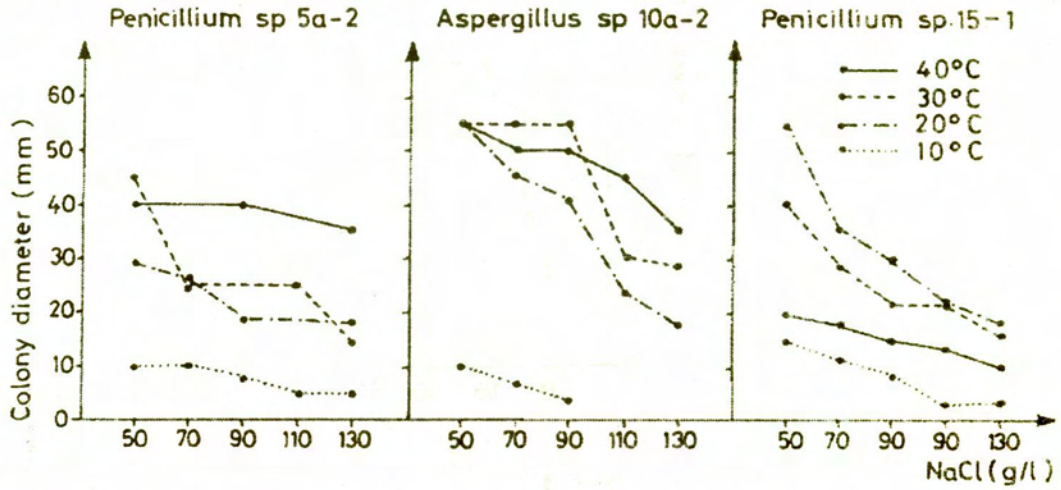

Fig. 9. – Effect of temperature and salinity on the growth of *Penicillium sp.* and *Aspergillus sp.* strains.

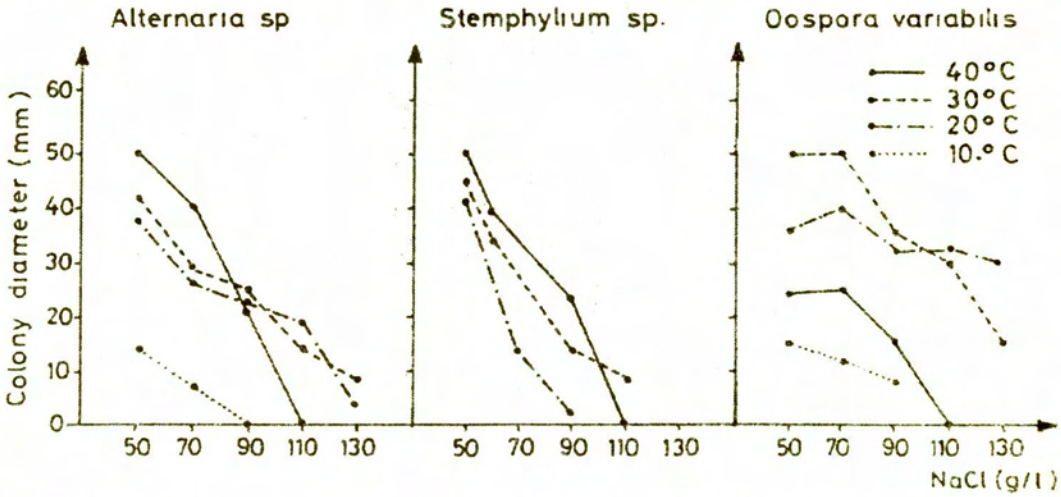

Fig. 10. – Effect of temperature and salinity on the growth of *Alternaria sp.*, *Stemphylium sp.* and *Oospora variabilis* strains.

In addition dark mycelia produced by *Alternaria sp.* on *Stemphylium sp.* at 10–30°C can absorb more radiant energy than off-colour mycelia formed at 40°C.

These studies have demonstrated a high salt requirement for growth at a high temperature in case of soil fungi which is similar to what has been mentioned for marine fungi. This could be due to an osmotic requirement or to a

requirement for specific ions.

These fungi appear to have physiological capabilities to withstand the environmental and nutritional variations which occur both in soil and marine environment.

CONCLUSIONS

1. All strains studied have wide tolerances to salinity and temperature.
2. The salinity optimum is changed as function of temperature.
3. Fungal strains studied could be sources of inoculum from terrestrial source but they may have established themselves as an ecological active invader of the Techirghiol lake.
4. All strains could be considered as halotolerant fungi and growing during the entire year they could be important decomposers.

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NUCLEOID IN PROTOPLASTS OF *BACILLUS SUBTILIS*. ULTRASTRUCTURAL ASPECTS

GR. MIHĂESCU, L. GAVRILĂ, GH. MENCINICOPSCHI

Bacillus subtilis protoplasts incubated in liquid medium containing denatured collagen preserve their viability, but do not regenerate and do not divide. As a consequence, because DNA replication continues in a sequence of cycles without division, the protoplasts with a high degree of ploidy result from it. Protoplasts polyploid genophore has an obvious tendency of segregation of its genomic units. Our results suggest that bacterial genophore, similar to eucaryotic cell chromosomes, is organized in chromosomal domains.

In bacterial cell, DNA replication process is coordinated with the rate of cell division, suggesting a reciprocal and compulsory conditioning of the two events (Ryter, 1968, Bonhoeffer, 1969, Rowbury, 1972).

The role of mesosomal membranes in chromosome replication and in segregation of the new structures into the two daughter cells is generally accepted.

In the protoplast, the nucleoid detaches from its binding situs to cytoplasmic membrane, as the mesosomal structures are extruded during the cell wall digestion (Fitz–James, 1964). In protoplasts cultured in adequate media, the mesosomal membranes are, probably, integrated into the original cytoplasmic membrane.

In the nutritive medium with denatured collagen, that gives osmotic protection, *B. subtilis* protoplasts preced the biosynthetic processes and, as a consequence, their volume increases several times, compared to the initial one. Because the liquid medium does not ensure a firm support, the division processes are missing. The protoplast volume increase is balanced, that means the quantitative ratio of different cellular components is maintained as in the original cell.

In this paper, several structural and functional peculiarities of the nucleoid in *B. subtilis* protoplasts incubated in the denatured collagen-liquid medium are pointed out. The images suggest the development of repeated processes of nucleoid replication, without cell division, as well as segregation processes of the polyploid genophore into independent genomic units.

MATERIALS AND METHODS

In this experiment, young cultures of *B. subtilis*, IFC–1.65 line, grown on nutritive broth, under stirring conditions, were used. The cells were separated by centrifugation and washed twice in 0.2 M phosphate buffer, supplemented with 0.3 M sucrose at a density of 10^6–10^7 cells/ml and treated with lysozyme in a concentration of 5 mg/ml for 20 min, at 37°C to release protoplasts. The lysozyme was removed through the protoplast suspension washing twice in sucrose buffer.

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The protoplasts were incubated for 16 hours at 30°C in a liquid medium with composition:

Sucrose solution 0.3 M, in 0.2 M phosphate buffer, pH 7.2, 90 ml
collagen denatured by acid hydrolysis, adjusted to pH 7.2, 10 ml
glucose 2 g
$MgCl_2$ 0.02 g

After centrifugation, the protoplast sediment was prefixed in the buffered glutaraldehyde solution, for two hours and 1% osmium tetraoxide for 4 hours at 4°C, in 0.15 M phosphate buffer, pH 7.2.

RESULTS AND DISCUSSIONS

Liquid medium with denatured collagen is optimum for maintaining *B. subtilis* protoplasts viability, but we did not detect images suggesting wall regeneration or division processes.

It is well-known that in Gram positive bacteria, cell division mechanism involves the formation of a division septum, that is promoted at the level of mureinic wall. The protoplasts incubated in liquid medium with denatured collagen do not divide. The prominent trait is their significant volume increase, firstly due to cell syntheses in progress and secondly due to the slow membrane turning permeable for small molecules. Electronoptical analysis points out that the nucleoid (genophore) is a very dynamic structure and it gets over a wide range of morpho-functional changes.

From the physical viewpoint, in the protoplast, as well as in the cell, the nucleoid shows a relaxation-condensation alternating state. Nucleoid dispersion is typical for protoplasts soon after cell wall division and it corresponds to a "relaxed" state of the chromosome, unprepared for replication. The intermediate condensation state is characterized by an obvious crowding of DNA fibrils in the central zone of the protoplast (Fig. 1). Nucleoid condensation, with the orderly paralleled distribution of DNA fibrils (Fig 2, 3) suggests the preparation of this structure for a new replication cycle.

B. subtilis protoplasts increase their volume several times, and the nucleoid volume increases at the same ratio as well. With no physical restraints of the rigid cellular wall and in the absence of starting division signals, the nucleoid replicates several times, without cell division. Thus, genophore acquires gradually a high degree of ploidy, as a direct consequence of its replication, in successive cycles, without cytoplasm division. All the replicas of the chromosome form a voluminous and densely packed nucleoid.. The circular configuration maintenance of the genophore, even at a high degree of ploidy, is remarkable (Fig. 8, 9).

The condensed state of the nucleoid shows its readiness for replication (Fig. 2). Highly condensed nucleoids (Figs 3,4) are associated with peculiar structures. Indefinite structure forms, being obviously attached to the nucleoid, connect directly the nucleoid structure to the plasmatic membrane. We can not specify the nature and not at all the physiological role of these structures, but it is possible they may ensure the connection of the condensed nucleoid, prepared for getting over the replication process to the plasma membrane.

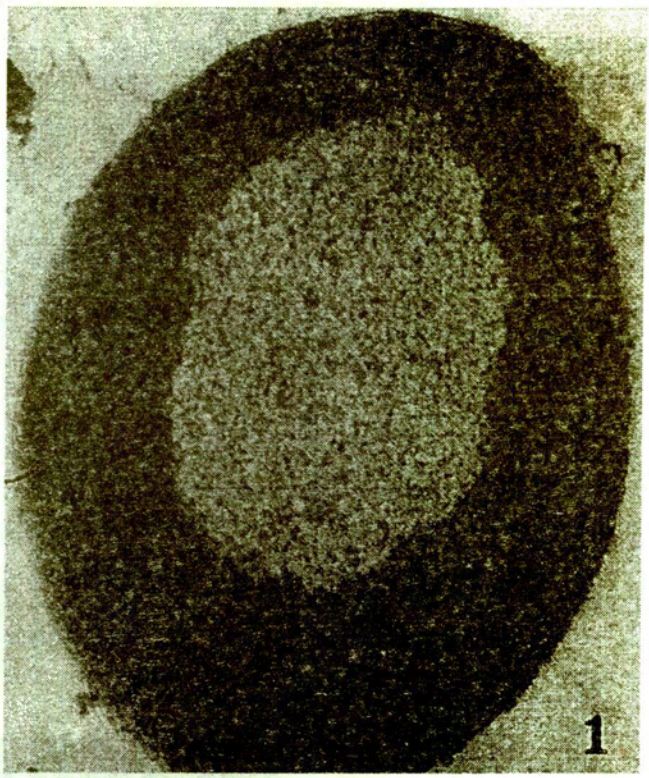

Fig. 1. – Intermediate state of polyploid nucleoid condensation, × 74000.

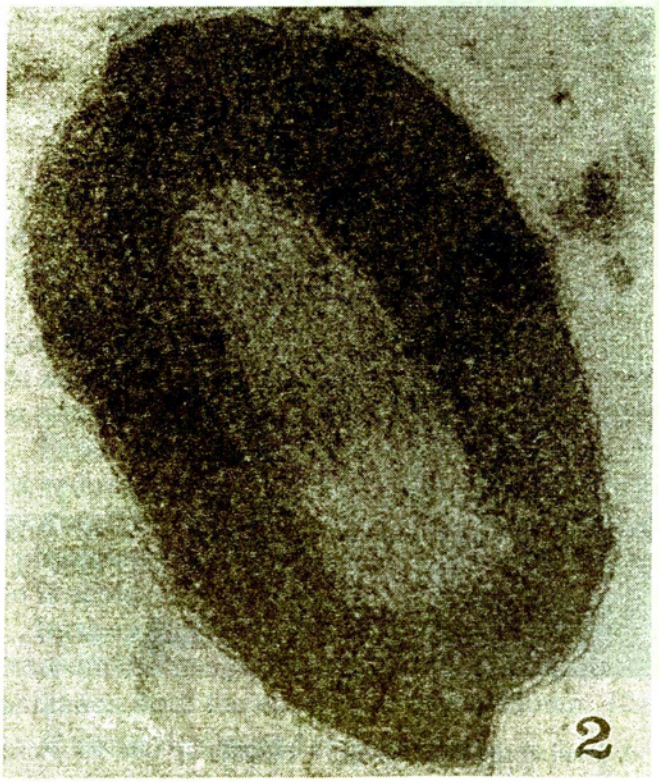

Fig. 2. – Condensed nucleoid DNA fibrils are disposed parallelly, having a bipolar orientation, × 74000.

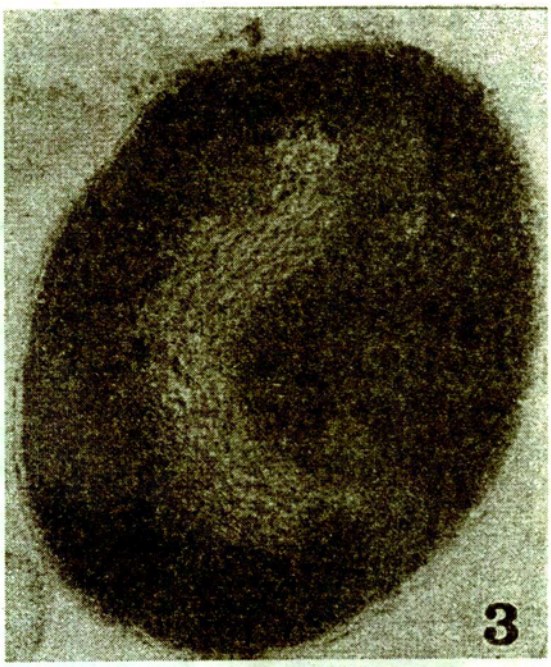

Fig. 3. – Unknown structures, probably of protein nature, are closely associated at the condensed nucleoid ends, × 74000.

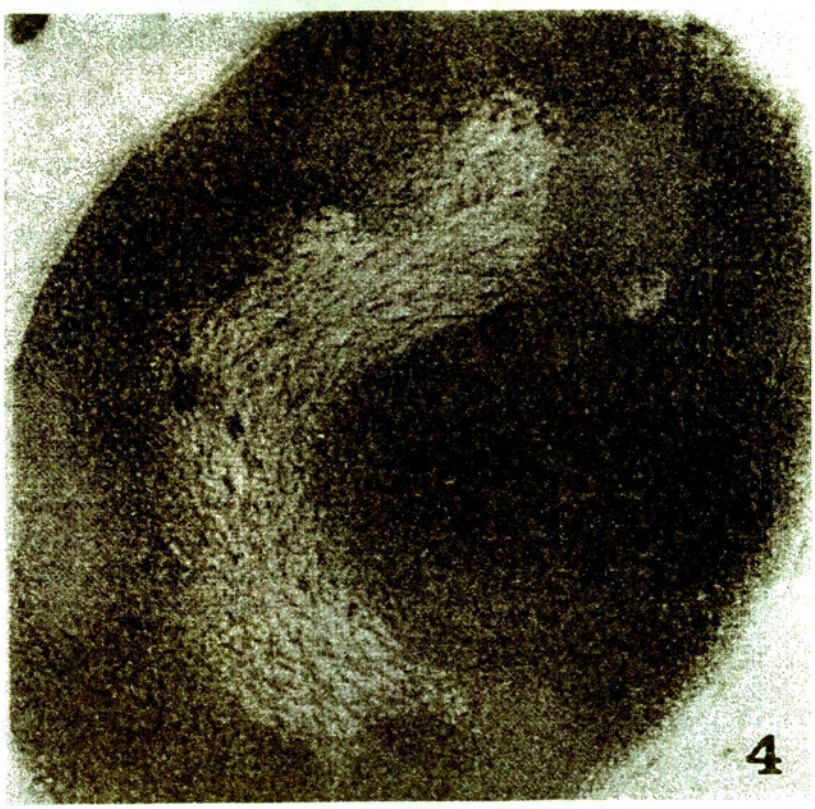

Fig. 4. – A detail of the same image, × 116000.

Nucleoids having a high degree of ploidy (Fig. 5) show a remarkable polymorphism, but it is a characteristic and obvious tendency of "genomic segregation" (Fig. 6, 7, 8, 9), i.e. a tendency of spatial separation of haploid nucleoid structures.

Fig. 5. – The nucleoid having a high degree of ploidy is homogeneous, condensed and in cross section it has a granular texture, × 74000.

The genomic segregation phenomenon noticed with *B. subtilis* protoplasts is often found in hyperploid eucaryotic cells, in which a usual mitotic cycle does not take place.

Genomic segregation or the nucleoid segregation process in its component units is very well pointed out in figures *10* and *11*. Images having a similar functional significance were noticed in eucaryotic cells that reach a high degree of ploidy, by the action of statmokinetic agents (Gavrilă, unpublished data).

These investigations enabled us to point out a remarkable fact, less commented in the reference literature, related to the conformation dynamics of the nucleoid, suggesting the possibility of the topological changes of this structure, related to the cell physiological state. The chance of topological changes increases obviously when is achieved a certain degree of ploidy.

Images suggest another fact, that the genophore is organized as real chromosomal domains (Fig. 12), thus providing an evident similarity of its functional organization with the eucaryotic chromosome. They can function as individual units of transcription and replication.

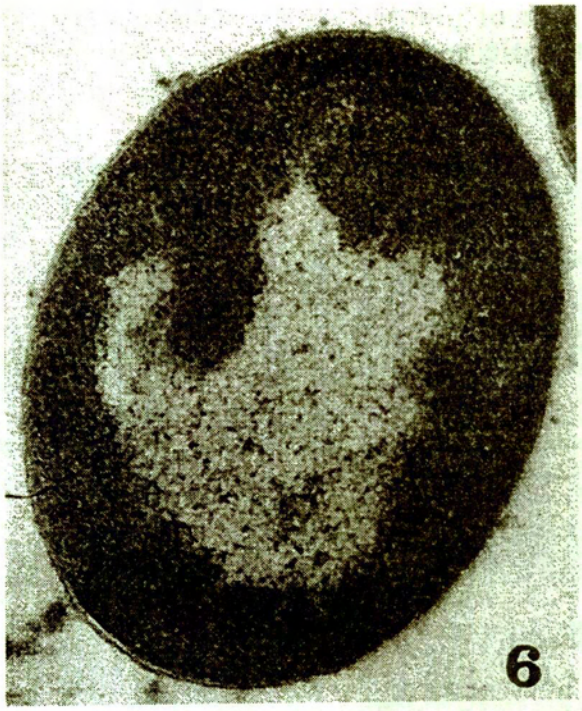

Fig. 6, 7, 8, 9. – Successive stages of the genomic segregation process. Figs 7, 8 and 9 suggest the circular configuration of bacterial chromosomes, × 74000, 74000, 116000 and 74000 respectively.

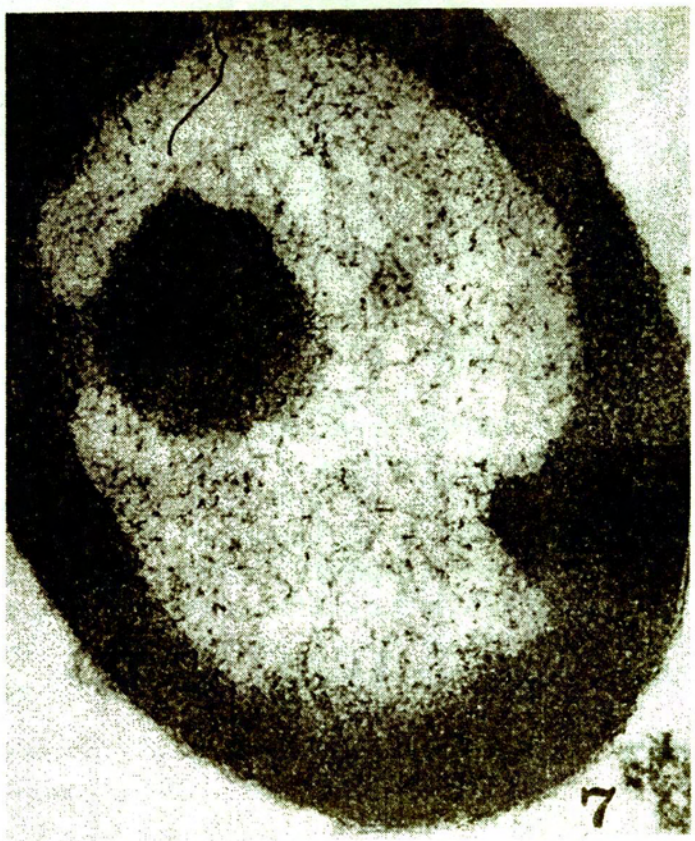

Fig. 7.

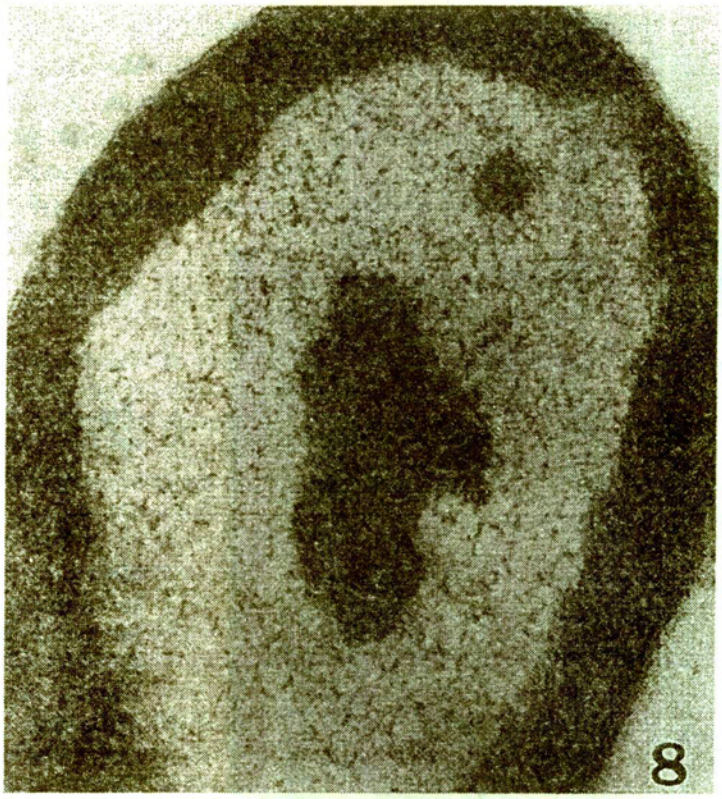

Fig. 8.

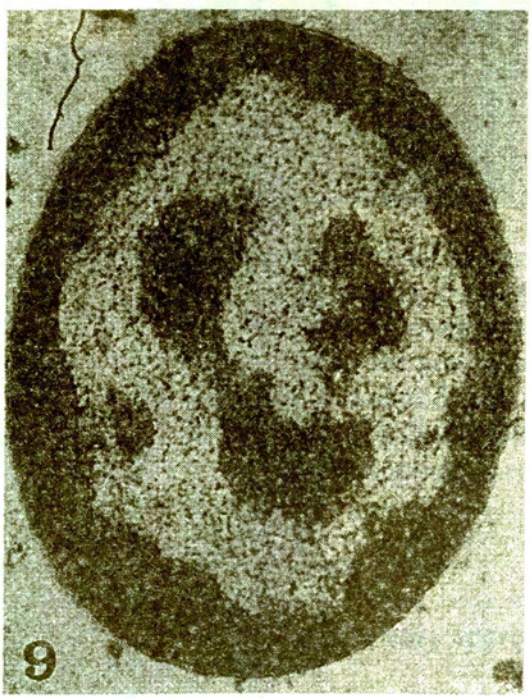

Fig. 9.

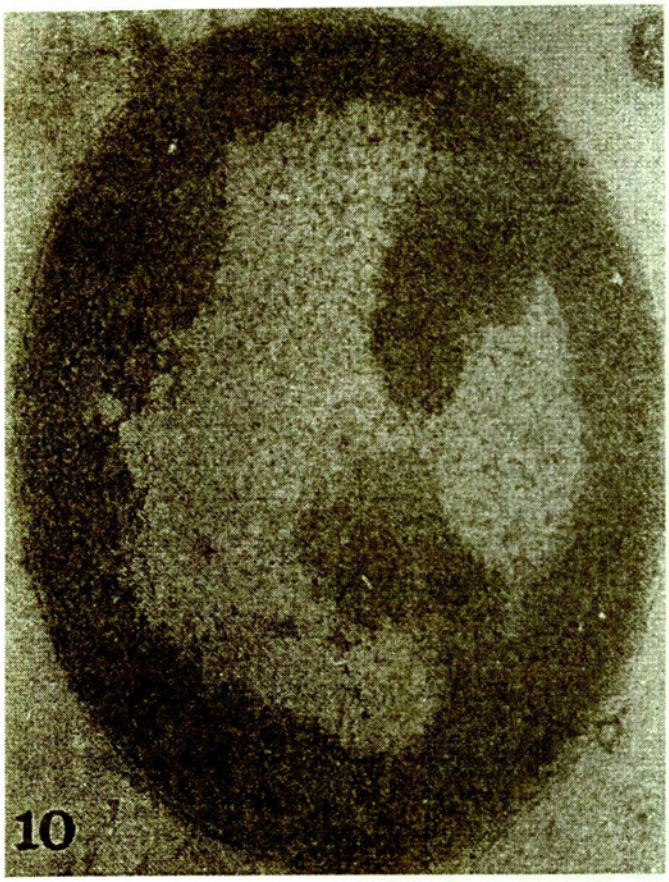

Fig. 10. – An advanced stage of the genomic segregation process, × 74000.

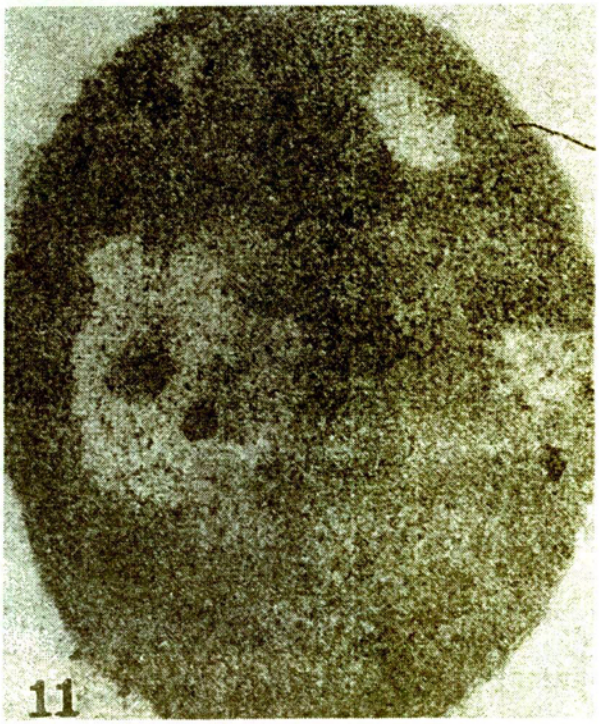

Fig. 11. – Several nucleoids appear in the protoplast as a result of the genomic segregation process, × 74000.

It is well known nowadays that interphasic chromosomes in eucaryotic cells are specifically organized in lawns (Fig. 12), having specific location and position one to another. DNA and chromatin organization of the eucaryotic cells, as topological independent loops and lawns, joined to the specific sites with nonhistonic nuclear skeleton is generally accepted now (Nagl, 1992; Hilliker and Appels, 1989; Manuelidis, 1990; Haaf and Schmid. 1991; Heslop-Harrison and Bennett, 1990).

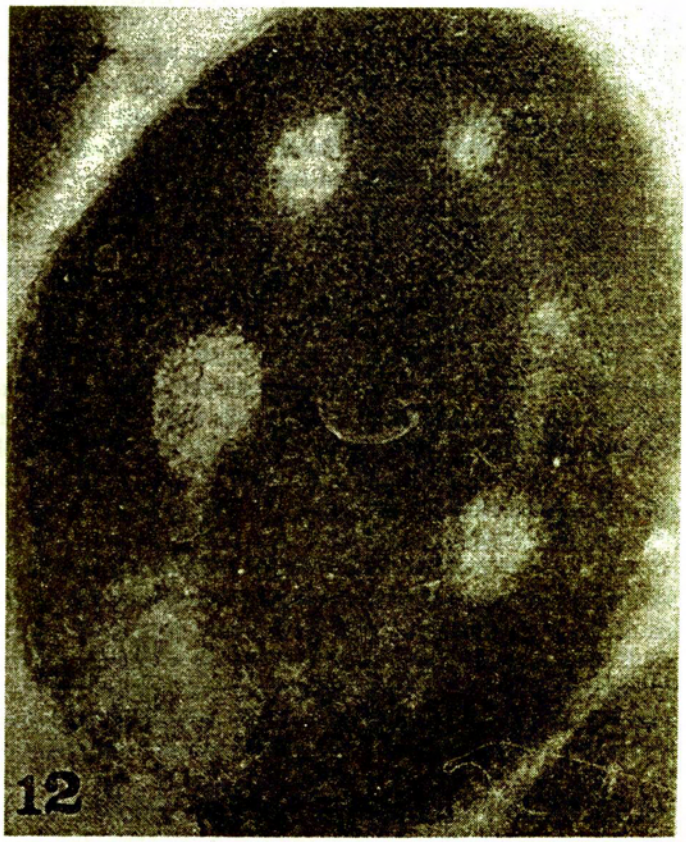

Fig. 12. – A possible organization as domains of the hyperploid bacterial chromosome. The domains belong to a unitary circular structure and seem to function as independent replication units, × 74000.

It is possible that these domains of hyperploid bacterial chromosome may signify independent replication units, but they belong to a unitary circular structure. Asynchronies of different domains replication processes may appear within this structure as a consequence of the competition for the precursors. Thus, the different sizes of these globular structures can be explained and they appear as real "puffs" within a unique gigantic circular structure (Fig. 12), that is joined itself to a complex structure, that enables the connection with the cytoplasmic membrane.

CONCLUSIONS

1. Nutritive liquid medium with collagen is very appropriate to maintain the viability and growth of *B. subtilis* protoplasts.

2. Division processes development is not observed. The genome replicates without cytokinesis; thus protoplasts having different ploidy degrees appear frequently.

3. Images suggest the development of a nucleoid segregation process into component genomic units.

4. Polyploid nucleoid is organized similarly to eucaryotic cell chromosomes, from domains that seem to signify independent replication units.

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TAXONOMICAL COMBINATIONS

The taxonomical combinations which do not respect the norms and recommendations of the International Code for Botanical Nomenclature are considered as illegitimate and thus are not taken into consideration. It is the case of many such combinations published in *The Illustrated Flora of Romania* 1 (1988), 2 (1990) by V. Ciocârlan which did not observe completely article 33.

The combinations published in the above-mentioned work can be characterized as *comb. et stat. illeq.* because they do not mention the author and the character of the combination. Some of them do not have the basionymum, others are even quaint (see *Stud. Cerc. Biol. – Biol. Veget.*, 1995). Most of them (especially in *Rubus*) are inspired from the systematization of *Flora Europaea*, without further research.

We have therefore tried to correct almost all those combinations even if some of them have been used by the author in his recent paper *The Flora of the Danube Delta* (1994), because there too they are illegitimate. It is possible that in our short note too, some mistakes may appear due to the lack of information, that is some combinations may become "redundant".

The Code also recommends that the new or modified taxons should be published in a magazine with large circulation so as to be easily spread and to avoid thus subsequent synonyms. This is the procedure used by the authors of *Flora Europaea*.

We did not remake the combinations from *The Illustrated Flora of România* for the following taxons: *Agropyron cristatum* (L.) Gaertn. subsp. *sabulosum* Lavr., *Alchemilla incisa* Buser, *Astragalus pseudoglaucus* Klok., *Hieracium irriguum* (Fries) Dahlst., *Potentilla bornmuelleri* Borb., *Taraxacum fulvum* Raunk., *Thymus tosevii* Velen., *Tragopogon brevirostris* DC., *T. floccosus* W. & K., *Veronica tenella* All., *Zannichellia prodanii* I. Serb., which either do not have a clear taxonomical position, or are kept by different authors as independent or synonymous species.

Basionymum is abbreviated (Bas.).

1. *Achillea clypeolata* Sibth. et Sm. var. *alexandri-borzae* (Prod.) Dihoru, *comb. et stat. nov.*
Bas. – *Achillea alexadri-borzae* Prod. 1931, Achill. Rom.: 26, tab. 19–20.

2. *Aconitum napellus* L. subsp. *hunyadense* (Deg.) Dihoru, *comb. et stat. nov.*
Bas. – *Aconitum hunyadense* Deg. 1906, Magyar Bot. Lapok 5: 196.

3. *Aconitum vulparia* Reichenb. subsp. *dasytrichum* (Deg. ex Gayer) Dihoru, *comb. et stat. nov.*
Bas. – *Aconitum platanifolium* Deg. ex Gayer b. *dasytrichum* Deg. ex Gayer 1907, Magyar Bot. Lapok 6: 119.

4. *Aconitum vulparia* Reichenb. subsp. *lasianthum* (Reichenb.) Dihoru, *comb. et stat. nov.*
Bas. – *Aconitum lasianthum* (Reichenb.) Simonk. 1886, Enum. Fl. Transs.: 61.

5. *Agropyron elongatum* (Host) Beauv. subsp. *ponticum* (Podp.) Dihoru, *comb. nov.*
Bas. – *Triticum ponticum* Podp. 1902, Verh. Zool.-Bot. Ges. Wien 52: 681.
Note: Some authors (1) consider it synonymous with *A. elongatum.*

6. *Agropyron junceum* (L.) Beauv. subsp. *bessarabicum* (Săvul. et Rayss) Dihoru, *stat. nov.*
Bas. – *Agropyron bessarabicum* Săvul. et Rayss 1923, Bull. Sect. Sci. Acad. Roum. 8: 282, fig. 1.

7. *Anchusa officinalis* L. subsp. *procera* (Bess.) Dihoru, *comb. et stat. nov.*
Bas. – *Anchusa procera* Bess. 1822, Enum. Pl. Volhyn.: 8.

8. *Cuscuta cesatiana* Bertol. subsp. *basarabica* (Buia) Dihoru, *comb. et stat. nov.*
Bas. – *Cuscuta basarabica* Buia 1938, Contrib. Cusc. Rom.: 35.

9. *Cytisus ciliatus* Wahlenb. subsp. *falcatus* (W. et K.) Dihoru, *comb. et stat. nov.*
Bas. – *Cytisus falcatus* W. et K. 1812, Pl. Rar. Hung. 3: 264, tab. 238.
10. *Dianthus petraeus* W. et K. var. *petraeiformis* (Péterfi) Dihoru, *comb. et stat. nov.*
Bas. – *Dianthus spiculifolius* Schur. f. *petraeiformis* Péterfi 1916, Magyar Bot. Lapok 15: 10.
11. *Draba stellata* Jacq. subsp. *simonkaiana* Dihoru, *comb. et stat. nov.*
Bas. – *Draba simonkaiana* Jáv. 1910, Bot. Kozl. 9: 281.
12. *Euphrasia stricta* Wolff subsp. *brevipila* (Burn. et Gremli) Dihoru, *comb. nov.*
Bas. – *Euphrasia brevipila* Burn. et Gremli ap. Towns 1884 Journ. Bot. 22: 167.
13. *Euphrasia stricta* Wolff subsp. *pectinata* (Ten.) Dihoru, *comb. et stat. nov.*
Bas. – *Euphrasia pectinata* Ten. 1811, Fl. Nap. 1, Prodr.: 36.
14. *Festuca beckeri* (Hack.) Trautv. subsp. *arenicola* (Prod.) Dihoru, *comb. nov.*
Bas. – *Festuca pallens* Host subsp. *arenicola* Prod. 1935, Bul. Acad. Agron. Cluj 5: 193, fig. 1.
15. *Hieracium bifidum* Kit. ex Hornem. subsp. *ammobium* (P.D. Sell et C. Vest) Dihoru, *comb. et stat. nov.*
Bas. – *Hieracium ammobium* P.D. Sell et C. Vest 1976, Bot. Jour. Linn. Soc. 71: 262.
16. *Hieracium sparsum* Friv. subsp. *borbasii* (Uechtr.) Zahn var. *fagarasense* (Nyár. et Zahn ex Zahn) Dihoru, *comb. et stat. nov.*
Bas. – *Hieracium sparsum* Friv. subsp. *fagarasense* Nyár. et Zahn ex Zahn 1928, Bul. Grad. Bot. Cluj 8: 66.
17. *Hieracium sparsum* Friv. subsp. *borbasii* (Uechtr.) Zahn var. *tomisae* (Nyár. et Zahn ex Zahn) Dihoru, *comb. et stat. nov.*
Bas. – *Hieracium sparsum* Friv. subsp. *tomisae* Nyár. et Zahn ex Zahn 1928, Bul. Grad. Bot. Cluj 8: 68.
18. *Hieracium sparsum* Friv. subsp. *borbasii* (Uechtr.) Zahn var. *tubulare* (Zahn) Dihoru, *stat. nov.*
Bas. – *Hieracium sparsum* Friv. subsp. *tubulare* Zahn 1938, in Asch. et Gr., Synopsis 12: 653.
19. *Hypericum richeri* Vill. subsp. *transsilvanicum* (Čelak.) Dihoru, *comb. et stat. nov.*
Bas. – *Hypericum transsilvanicum* Čelak. 1874, Osterr. Bot Zeitschr. 24: 138.
20. *Myosotis scorpioides* L. subsp. *nemorosa* (Bess.) Dihoru, *comb. nov.*
Bas. – *Myosotis nemorosa* Bess. 1822, Enum. Pl. Volhyn.: 52.
21. *Nonea pulla* (L.) DC. subsp. *atra* (Griseb.) Dihoru, *comb. et stat. nov.*
Bas. – *Nonea atra* Griseb. 1844, Spicil. Fl. Rumel. 2: 94.
22. *Plantago subulata* L. subsp. *holosteum* (Scop.) Dihoru, *comb. et stat. nov.*
Bas. – *Plantago holosteum* Scop. 1771, Fl. Carn. ed. 2, 1: 108, pl. 34. fig. 6.
23. *Polygala major* Jacq. subsp. *anatolica* (Boiss. et Heldr.) Dihoru, *comb. et stat. nov.*
Bas. – *Polygala anatolica* Boiss. et Heldr. 1853, Diagn. Pl. Or. Nov. 3: 57.
Note: Some authors (1) keep it as species, *P. anatolica.*
24. *Ranunculus montanus* Willd. subsp. *pseudomontanus* (Schur) Dihoru, *comb. et stat. nov.*
Bas. – *Ranunculus pseudomontanus* Schur 1877, Verh. Naturforsch. Ver. Brunn 15(2): 42.
25. *Rubus adscitus* Genev. subsp. *tenuispinosus* (Nyár.) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus tenuispinosus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 911, Add.
26. *Rubus bifrons* Vest ex Tratt. subsp. *banaticus* (Nyár.) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus banaticus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 890, Add.
27. *Rubus bifrons* Vest ex Tratt. subsp. *cuspidifer* (P.J. Müller et Lef.) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus cuspidifer* P. J. Müller et Lef. 1859, Pollichia 16–17: 89.
28. *Rubus bifrons* Vest ex Tratt. subsp. *geniculatus* (Kalt.) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus geniculatus* Kalt. 1844, Fl. Aachen. Beck. 2: 267.
29. *Rubus colemannii* Bloxam. subsp. *doftanensis* (Nyár.) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus doftanensis* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 913, Add.
30. *Rubus hirtus* W. et K. subsp. *romanicus* (Nyár.) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus romanicus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 929, Add.
31. *Rubus koehleri* Weihe subsp. *hebecarpos* (P. J. Müller) Dihoru, *comb. et stat. nov.*
Bas. – *Rubus hebecarpos* P. J. Müller 1861, Bonplandia 9: 282.
32. *Rubus macrostachys* P. J. Müller subsp. *lipovensis* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus lipovensis* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 911, Add.

33. *Rubus melanoxylon* P. J. Müller et Wirtg. subsp. *fagetanus* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus fagetanus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 919, Add.

34. *Rubus melanoxylon* P. J. Müller et Wirtg. subsp. *omalus* (Sudre) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus omalus* Sudre 1905, Bull. Soc. Bot. Fr. 52: 324.

35. *Rubus melanoxylon* P. J. Müller et Wirtg. subsp. *rubristamineus* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus rubristamineus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 918, Add.

36. *Rubus montanus* Libert ex Lej. subsp. *arduenensis* (Libert) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus arduenensis* Libert 1873, in Lej., Fl. Spa 2: 317.

37. *Rubus montanus* Libert ex Lej. subsp. *drautensis* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus drautensis* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 893, Add.

38. *Rubus montanus* Libert ex Lej. subsp. *petnicensis* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus petnicensis* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 897, Add.

39. *Rubus montanus* Libert ex Lej. subsp. *subvillosus* (Sudre) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus subvillosus* Sudre 1902, Bull. Assoc. Fr. Bot. 5: 127.

40. *Rubus muelleri* Lef. subsp. *laetecoloratus* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus laetecoloratus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 911, Add.

41. *Rubus myricae* Focke subsp. *moldavicus* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus moldavicus* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 899, Add.

42. *Rubus serpens* Weihe ex Lej. subsp. *niveoserpens* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus niveoserpens* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 925, Add.

43. *Rubus serpens* Weihe ex Lej. subsp. *rivularis* (Wirtg. et P. J. Müller) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus rivularis* Wirtg. et P. J. Müller 1859, Flora (Regensb.) 42: 237.

44. *Rubus vallisparsus* Sudre subsp. *persanimontis* (Nyár.) Dihoru, *comb. et stat. nov.*

Bas. – *Rubus persanimontis* Nyár. 1956, in Săvul., Fl. Rep. Pop. Române 4: 920, Add.

45. *Saxifraga rotundifolia* L. subsp. *heucherifolia* (Gris. et Schenk) Dihoru, *comb. et stat. nov.*

Bas. – *Saxifraga heucherifolia* Gris. et Schenk 1852, Arch. Naturgesch. (Berlin) 18: 317.

46. *Thymus longicaulis* C. Presl subsp. *illyricus* (Ronn.) Dihoru, *comb. et stat. nov.*

Bas. – *Thymus illyricus* Ronn. 1931, in Hayek, Prodr. Fl. Pen. Balc. 2: 378.

Note: The names of the authors have been abbreviated according to the recommendations of the International Code for Botanical Nomenclature.

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