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SOMMAIRE

MICROMYCÈTES PARASITES DE FRANCE

II. *ERYSIPHACEAE*

GAVRIL NEGREAN

The author presents a number of 49 *Erysiphaceae*, living on 139 plants, which were gathered in 1990 from different regions of France. Among these are 2 new species from France, 12 "matrix nova" and 20 new combinations from France.

Dans une première note, publiée dans la Revue Roumaine de Biologie, Série de biologie végétale 40(1), 1995, nous avons présenté une liste des champignons parasites récoltées en France en 1990 (*Peronosporaceae* et *Ascomycetes*, excl. *Erysiphaceae*).

Dans cette deuxième note nous présentons les *Erysiphaceae* (pour des détails voir note n° I). Les 216 exemplaires ont été récoltés dans de différentes zones de la France. Nous avons identifié 49 espèces et 139 combinaisons. Parmis celles-ci il y a aussi deux espèces (*Microsphaera vanbruntiana* et *Sphaerotheca verbenae*), nouvelles pour la France, douze combinaisons de champignons - plantes-hôtes, qui n'étaient pas citées ainsi dans la littérature consultée. (K. Hirata, Host range and geographical distribution of the powdery mildews, 1966). Il s'agit de «matrix nova» («*»). Nous avons aussi présenté dans notre note, 20 combinaisons signalées pour la première fois aux nouveaux hôtes pour la France («**»).

39. **Erysiphe aquilegiae** DC.
Sur *Aquilegia* sp.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.864).
Sur *Caltha palustris* L.- *PNV*: Grese-en Vercors, 44°54'..N, 5°34'..E, alt. circa 1400 m, 13 X 1990, GN (BUCM 117.910).

40. **Erysiphe artemisiae** Grev.
Sur *Artemisa vulgaris* L.- *HL*: Montregard, 45°09'58" N, 4°26'..E, alt. circa 1000 m, 9 X 1990, GN (BUCM 117.665); Les Estables, 44°55'..N, 4°14'..E, alt.1340 m, 4 X 1990, GN (BUCM 117.384).

41. **Erysiphe biocellata** Ehrenb.
Sur *Mentha rotundifolia* L.- *HL*: Sainte Sigolène N, 45°35'..N, 4°19'..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.357).

42. **Erysiphe cichoracearum** DC.
Sur *Achillea ptarmica* L. forma pleno (cult.) - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.823).

Sur **Aster novae-angliae* L. (cult.) - *HL*: Sainte-Sigolène, centrum, 45°13'40"N, 4°19'00"E, alt. 800 m, 21 X 1990, GN (BUCM 118.110).
Sur *Aster novi-belgii* L.(subspont.) - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.5239).
Sur *Carduus crispus* L. subsp. *crispus* - Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1600 m, 15 X 1990, GN (BUCM 118.028).
Sur **Centaurea bracteata* Scop.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1400 m, 13 X 1990, GN (BUCM 117.897).
Sur *Centaurea montana* L.- *PNV*: Gresse-en-Vercors, Pas de la Ville, 44°54'..N, 5° 34'..E, alt. 1700 m, 13 X 1990, GN (BUCM 117.9189).
Sur *Centaurea nigra* L. subsp. *nigra* - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.516).
Sur *Centaurea rhenana* Boreau subsp. *rhenana* - *HL*: Le Puy, Notre-Dame de France, 45°02'30"N, 3°55'..E, alt 630 m, 10 X 1990, GN (BUCM 117.756).
Sur **Centaurea scabiosa* L.- *PNV*: Gresse-en-Vercors, Pas de la Ville, 44°54'..N, 5° 34'..E, alt. 1700 m, 13 X 1990, GN (BUCM 117.919).
Sur *Cirsium acaule* Scop. subsp. *acaule* - Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1600 m, 15 X 1990, GN (BUCM 117.951); *PNV*: Col d'Alinas, 44° 54'..N, 5°34'..E, alt. 1500 m, 14 X 1990, GN (BUCM 117.930), Pas de la Ville, id., alt. 1700 m, 13 X 1990, GN (BUCM 117.917).
Sur *Cirsium arvense* (L.) Scop.- *HL*: Sainte-Sigolène, centrum, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.598); *AR*: Sainte Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GM (BUCM 117.498).
Sur *Cirsium eriophorum* (L.) Scop.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.829), Pas de la Ville, id., alt. 1700 m, 13 X 1990, GN (BUCM 117.913). *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.403).
Sur ***Crepis capillaris* (L.) Wallr.- *HL*: Sainte-Sigolène E, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.714) et 45°13'40"N, 4°19'02"E, alt 800 m, 21 X 1990, GN (BUCM 118.113).
Sur **Helianthus tuberosus* L.- Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 5 m, 18 X 1990, GN (BUCM 118.058).
Sur *Hieracium murorum* L. (grup) - *HL*: Les Estables, Mont Mézenc, 44°54'28"N, 4°15'25"E, alt. 1650 m, 7 X 1990, GN (BUCM 117.584).
Sur ***Lactuca viminea* (L.) J. & C. Presl subsp. *chondrilliflora* (Boreau) Bonnier - *HL*: Le Puy, Aiguilhe, 45°02'30"N, 3°55'..E, alt. 630 m, 10 X 1990, GN (BUCM 117.758).
Sur *Lapsana communis* L. - *HL*: Sainte-Sigolène, centrum, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.622).
Sur **Leontodon hispidus* L. subsp. *hispidus* - *PNV*: Gresse-en-Vercors, 44°54'..N, 5° 34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.833).
Sur *Mycelis muralis* (L.) Dum.- *HL*: La Chaise-Dieu S, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.305).
Sur *Omalotheca sylvatica* (L.) Schultz Bip.- *AR*: Ville-Vieille, Vallée de la Loire, 44°52'..N, 4°16'..E, alt. 1400 m, 4 X 1990, GN (BUCM 117.421).

Sur *Picris echioides* L. - Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 5 m, 18 X 1990, GN (BUCM 118.062).
Sur *Picris hieracioides* L. s.l.- Paris: Jardin de Tuilleries, prope Louvre 48° 50'..N, 2°20'..E, 2 X 1990, GN (BUCM 117.345).
Sur *Prenanthes purpurea* L.- Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1500 m, 15 X 1990, GN (BUCM 117.968).
Sur *Solidago virgaurea* L.s.l.- *PNV*: 44°54'..N, 5°34'..E, alt. circa 1400 m, 13 X 1990, GN (BUCM 117.900).
Sur *Sonchus asper* (L.) Hill s.l.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.830).
Sur *Sonchus oleraceus* L.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.617). Paris: Gare de Lyon, 48°50'..N, 2°20'..E, 1 X 1990, GN (BUCM 117.341).
Sur **Tanacetum parthenium* (L.) Schultz Bip. (cult.) - Alpe d'Huez, ad basilica, 44°55'..N, 5°50'..E, alt. 1800 m, 14 X 1990, GN (BUCM 118.036). *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'02"E, alt. 800 m, 21 X 1990, GN (BUCM 118.115).
Sur *Tragopogon pratensis* L. s.l.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.390).

43. **Erysiphe convolvuli** DC.
Sur *Calystegia sepium* (L.) R. Br.- Paris: Trocadero, 48°50'..N, 2°20'..E, 1 X 1990, GN (BUCM 117.348).
Sur *Convolvulus arvensis* L.- Paris: Gare de Lyon et Trocadero, 48°50'..N, 2°20'..E, 1 X 1990, GN (BUCM 117.340 et 117.342). *HL*: La Chaise-Dieu S, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.296). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.876).

44. *Erysiphe cruchetiana* Blumer
Sur **Ononis spinosa* L. subsp. *austriaca* (G. Beck) Gams - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1400 m, 13 X 1990, GN (BUCM 117.907).

45. *Erysiphe cruciferarum* Opiz ex Junell
Sur *Biscutella laevigata* L. subsp. *laevigata* - Alpe d'Heuz, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1500 m, 15 X 1990, GN (BUCM 117.991).
Sur *Capsella bursa-pastoris* (L.) Medicus - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.865).
Sur **Eschscholzia california* Cham. (cult.) - *HL*: Sainte-Sigolène, 45°35'..N, 4°19'..E, alt. circa 800 m, 29 IX 1990, GN (BUCM 117.286).
Sur *Papaver dubium* L.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.832).
Sur *Papaver somniferum* L. (cult.) - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.843).
Sur *Raphanus raphanistrum* L. subsp. *raphanistrum* - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.512).
Sur ***Rapistrum rugosum* (L.) All. subsp. *linnaeanum* Rouy & Fouc.- Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 0,5 m, 18 X 1990, GN (BUCM 118.111).
Sur *Sisymbrium irio* L.- Paris: Trocadero, 48°50'..N, 2°20'..E, 1 X 1990, GN (BUCM)

Sur *Sisymbrium officinale* (L.) Scop.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.615).

46 *Erysiphe cynoglossi* (Wallr.) U. Braun
Sur *Anchusa arvensis* L. subsp. *arvenesis* - *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.593).
Sur *Echium vulgare* L.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.511). *AR*: Sainte Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.490); Mazan l'Abbaye, 44°45'..N, 4°08'.. E, alt. 1200 m, 6 X 1990, GN (BUCM 117.478).
Sur *Myosotis sylvatica* Hoffm. subsp. *sylvatica* - *HL*: La Chaise-Dieu, 45°15'.. N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCN 117.2889); Sainte-Silogène, 45°35'..N, 4°19'..E, alt. 800 m, 3 X 1990, GN (BUCM 117.371); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.522). *PNV*: Gresse-en-Vercors, 44°54'-N, 5°34'..E, alt. circa 1400 m, 13 X 1990, GN (BUCM 117.899).
Sur *Symphytum officinale* L. s.l.-*HL*: Les Estables N, Mont d'Alambre, 44°55'40" N, 4°14'..E, alt. 1550 m, 5 X 1990, GN (BUCM 117.454).

47. **Erysiphe fischeri** Blumer
Sur *Senecio viscosus* L.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.616); Les Estables, 44°55'..N, 4°14'..E, alt 1340 m, 4 X 1990, GN (BUCM 117.388). PNV: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.845).
Sur *Senecio vulgaris* L.- Paris: cart. Villette, prope GEOL, 48°50'..N, 2°20'..E, 2 X 1990, GN (BUCM 117.349). *HL*: Sainte-Sigolène, 45°13'00"N, 4°19'..E, alt. 800 m, 9 X 1990, GN (BUCM 117.642).

48. **Erysiphe galeopsidis** DC.
Sur *Galeopsis tetrahit* L.- *HL*: La Chaise-Dieu, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.308); Montregard, 45°09'58"N, 4°26'..E, alt. 1000 m, 9 X 1990, GN (BUCM 117.672). *AR*: Mazan l'Abbaye, 44°45'..N, 4°08'..E, alt. 1200 m, 6 X 1990, GN (BUCM 117.479).
Sur *Lamium album* L.- *HL*: La Chaise-Dieu, 45°15I..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.289).
Sur *Lamium amplexicaule* L.- *HL*: Les Estables, Mont Mézenc, 44°54'20"N, 4°15'..E, alt. 1550 m, 5 X 1990, GN (BUCM 117.452).
Sur *Stachys sylvatica* L.- *HL*: Les Estables, 44°55'..N, 4°13'..E, alt. 1300 m, 5 X 1990, GN (BUCM 117.460).

49. **Erysiphe galii** Blumer var. *riedliana* (Speer) U. Braun
Sur *Galium verum* L. subsp. *verum* - HL: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.531).

50. **Erysiphe heraclei** DC.
Sur *Angelica sylvestris* L.- Alpe d'Huez: Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1500 m, 15X 1990, GN (BUCM 117.985).
Sur *Anthriscus sylvestris* (L.) Hoffm.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00" E, alt. 800 m, 8 X 1990, GN (BUCM 117.611) et 9 X 1990, GN (BUCM 117. 646).

Sur *Chaerophyllum aureum* L.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt.1340 m, 4 X 1990, GN (BUCM 117.394).
Sur *Heracleum sphondylium* L. subsp. *sphondylium* - *HL*: Sainte-Sigolène N, 45°35'..N, 4°19'..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.359), id. 45°13'..N, 4°13'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.732); Montregard, 45°09'58"N, 4°26'..E, alt. circa 1000 m, 9 X 1990, GN (BUCM 117.651). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1300 m, 13 X 1990, GN (BUCM 118.154).
Sur *Peucedanum oreoselinum* (L.) Moench - Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1600 m, 15 X 1990, GN (BUCM 117.972).
Sur *Pimpinella saxifraga* L.- *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt 800 m, 10 X 1990, GN (BUCM 117.711).

51. **Erysiphe hyperici** (Wallr.) Blumer
Sur *Hypericum maculatum* Crantz - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.407).
Sur *Hypericum perforatum* L.- Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1500 m, 15 X 1990, GN (BUCM 118.016).

52. **Erysiphe knautiae** Duby
Sur *Knautia arvensis* (L.) Coult.- *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.743); Les Estables, 44°55'..N, 4°13'..E, alt. 1300 m, 5 X 1990, GN (BUCM 117.459) et 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.398).
Sur ***Knautia arvernensis* (Briq.) Szabó - *HL*: Les Estables, Mont Mézenc, 44°54'28"N, 4°15'25"E, alt. 1650 m, 7 X 1990, GN (BUCM 117.582).

53. **Erysiphe limonii** Junell
Sur ***Limonium minutum* (L.) Fourr.- Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 0,5 m, 18 X 1990, GN (BUCM 118.053).

54. **Erysiphe mougeotii** (Lév.) De Bary
Sur *Lycium barbarum* L. - *HL*: Le Puy, Notre-Dame de France, 45°02'30" N, 3°55'..E, alt. 630 m, 10 X 1990, GN (BUCM 117.753).

55. **Erysiphe orontii** Cast.
Sur **Petunia integrifolia* (Hooker) Schinz & Thell. (cult.) - *Loire*: Firminy, 45°21'..N, 4°21'..E, alt. circa 700 m, 20 X 1990, GN (BUCM 118.136). *HL*: St. Pal-de-Mons, 45°14'..N, 4°21'..E, alt. circa 800 m, 11 X 1990, GN (BUCM 118.039); Montfaucon-en-Velay, 45°10'04"N, 4°24'..E, alt. circa 900 m, 9 X 1990, GN (BUCM 117.654).
Sur **Viola arvensis* Murray - *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.731); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.727).
Sur *Viola* x *wittrockiana* Gams (cult.) - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1250 m, 12 X 1990, GN (BUCM 117.841).

56. **Erysiphe pisi** DC.
Sur *Pisum sativum* L. (cult.) - *HL*: La Chaise-Dieu, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.292).

Sur *Vicia cracca* L. - *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.7379). *AR*: Sainte Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.484). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1250 m, 13 X 1990, GN (BUCM 117.861).

57. **Erysiphe polygoni** DC.

Sur ***Polygonum ?arenastrum* Boreau - *HL*: Montregard, 45°09'58"N, 4°26'..E, alt. 1000 m, 9 X 1990, GN (BUCM 117.667).

Sur *Polygonum aviculare* L. - *HL*: Sainte-Sigolène, ruderal, 45°13'40"N, 4°19'00" E, alt. 800 m, 8 X 1990, GN (BUCM 117.607); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.406). *AR*: Sainte-Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.488).

Sur *Rumex acetosella* L.- *HL*: Sainte-Sigolène E, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.708), St.-Sigolène N, 45°35'..N, 4°19'..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.351).

58. **Erysiphe ranunculi** Grev.

Sur *Aconitum anthora* L.- Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1500 m, 15 X 1990, GN (BUCM 118.013).

Sur *Delphinium elatum* L. subsp. *helveticum* Pawl. (cult.) - *PNV*. Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.822).

Sur *Ranunculus aconitifolius* L.- Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1500 m, 15 X 1990, GN (BUCM 117.992).

Sur *Ranunculus acris* L. subsp. *acris* - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.835).

Sur *Ranunculus repens* L.- *HL*: La Chaise-Dieu, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.290). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1400 m, 13 X 1990, GN (BUCM 117.911). Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1500 m, 15 X 1990, GN (BUCM 117.963).

59. **Erysiphe sordida** Junell

Sur *Plantago major* L. subsp. *major* - *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00" E, alt. 800 m, 8 X 1990, GN (BUCM 117.6109); Montregard, 45°09'58"N, 4°26'..E, alt. circa 1000 m, 9 X 1990, GN (BUCM 117.657). *AR*: Sainte Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.482).

60. **Erysiphe trifolii** Grev.

Sur *Anthyllis vulneraria* L.s.l.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1400 m, 13 X 1990, GN (BUCM 117.906).

Sur *Lathyrus pratensis* L.- *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.717); Montregard, 45°09'58"N, 4°26'..E, alt. circa 1000 m, 9 X 1990, GN (BUCM 117.656); Les Estables, Mont Mézenc, 44°54'20"N, 4°15'..E, alt. 1550 m, 5 X 1990, GN (BUCM 117.450). *AR*: Mazan l'Abbays, 44°45'..N, 4°08'..E, alt. 1200 m, 6 X 1990, GN (BUCM 117.480). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.837).

Sur.**Lathyrus sylvestris* L.- Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1500 m, 15 X 1990, GN (BUCM 117.989).

Sur *Lupinus* sp. (cult.) - *HL*: Sainte-Silogène, 45°35'..N, 4°19'..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.373); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m 7 X 1990, GN (BUCM 117.510).
Sur *Melilotus officinalis* (L.) Pallas - *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 14 X 1990, GN (BUCM 117.920).
Sur *Onobrychis montana* DC. subsp. *montana* - PNV: Pas de la Ville, 44°54'..N, 5°34'..E, alt. 1800 m, 13 X 1990, GN (BUCM 117.854).
Sur *Trifolium pratense* L. s.l.- *HL*: Sainte-Sigolène, 45°35'..N, 4°19..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.374), 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.592); Montregard, 45°09'58"N, 4°26'..E, alt. circa 1000 m, 9 X 1990, GN (BUCM 117.663). *AR*: Sainte Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.486). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.827).

61. **Erysiphe urticae** (Wallr.) Blumer
Sur *Urtica dioica* L.- *HL*: Sainte-Sigolène N, 45°35'..N, 4°19'..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.354), 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.603).

62. *Erysiphe verbasci* (Jacz.) Blumer
Sur *Verbascum nigrum* L. s.l.- *HL*: Sainte-Sigolène, 45°13'00"N, 4°19'..E, alt. 800 m, 9 X 1990, GN (BUCM 117.644).

63. *Leveillula taurica* (Lév.) Arn.
Sur *Ipomoea purpurea* Roth - Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 2 m, 18 X 1990, GN (BUCM 118.066).

64. *Microsphaera alphitoides* Griff. & Maubl.
Sur *Quercus petraea* (Mattuschka) Liebl.- *Ar*: Vallé de la Loire, 44°50'..N, 4°18'..E, 6 X 1990, GN (BUCM 117.468). *HL*: Sainte-Sigolène, 45°35'..N, 4°19'..E, alt. circa 800 m, 3 X 1990, GN (BUCM 117.353).
Sur *Quercus pubescens* Willd. subsp. *pubescens* - *VL*: Apt E, Caseneuve, 43°50'..N, 5°20'..E, alt. 250 m, 17 X 1990, GN (BUCM 118.0459). *VR*: Île de Porquerolle, 43°02'..N, 6°15'..E, alt.5 m, 18 X 1990, GN (BUCM 118.059).
Sur *Quercus robur* L.- *HL*: Sainte-Sigolène, 45°35'..N, 4°19'..E, alt, circa 800 m, 3 X 1990, GN (BUCM 117.358).

65. **Microsphaera baeumleri** Magn.
Sur *Vicia sylvatica* L.- Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. circa 1600 m, 15 X 1990, GN (BUCM 117.971).

66. **Microsphaera berberidis** (DC.) Lév.
Sur *Berberis vulgaris* L. (cult.) -*VL*: Avignon, hortus Curiae Papalae, 43°55'..N, 4°50'..E, alt. 100 m, 16X 1990, GN (BUCM 117.040). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 14 X 1990, GN (BUCM 117.847).
Sur *Mahonia aquifolium* (Pursh) Nutt. (cult.) - *HL*: Sainte-Sigolène, 45°13'00"N, 4°19'..E, alt. 800 m, 9 X 1990, GN (BUCM 117.641); Le Puy, 45°02'30"N, 3°30"..E, alt. 630 m, 10 X 1990, GN (BUCM 117.759).

67. **Mircrosphaera euonymi-japonici** Viennot-Bourgin
Sur *Euonymus japonicus* L. fil. (cult.) - Lyon, 45°45'..N, 5°01'..E, alt. circa 200 m, 29 IX 1990, GN (BUCM 117.282). *VR*: Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 3 m, 18 X 1990, GN (BUCM 118.050).

68. **Microsphaera hedwigii** Lév.
Sur *Viburnum lantana* L.- *PNV*. Pas de la Ville, 44°54'..N, 5°34'..E, alt. 1700 m, 13 X 1990, GN (BUCM 117.912).

69. **Microsphaera lonicerae** (DC.) Winter
Sur *Lonicera ?implexans* Aiton - *VR*: Île de Porquerolle, 43°02'..N, 6°15'..E, alt. 6 m, 18 X 1990, GN (BUCM 118.069).

70. **Microsphaera penicillata** (Wallr.: Fr.) Lév.
Sur *Alnus viridis* (Chaix) DC. - Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1600 m, 15 X 1990, GN (BUCM 117.953).

71. **Microsphaera platani** Howe
Sur **Platanus occidentalis* L. (cult.) - Paris, Jardin de Tuilleries, prope Louvre, 48°50'..N, 2°20'..E, 1 X 1990, GN (BUCM 117.339). *Gard*: Villeneuve-lés-Avignon, Place de l'Oratorie, 43°55'..N, 4°40'..E, alt. 120 m, 17 X 1990, GN (BUCM 118.042).

72. **Microsphaera vanbruntiana** Gerard
Sur **Sambucus racemosa* L.- *AR*: Ville-Vieille, 44°52'..N, 4°16'..E, alt. 1400 m., 4 X 1990, GN (BUCM 117.431); Vallée de la Loire, 44°50'..n, 4°18'..E, 6 X 1990, GN (BUCM 117.469). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.870). Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1500 m, 15 X 1990, GN (BUCM 117.955).

73. **Phyllactinia fraxini** (DC.) Fuss
Sur *Fraxinus excelsior* L.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'02"E, alt. 800 m, 20 X 1990, GN (BUCM 118.108).

74. **Phyllactinia guttata** (Wallr.: Fr.) Lév.
Sur *Carpinus betulus* L.- *HL*: Le Puy, 45°02'30"N, 3°55'..E, alt. 630 m, 10 X 1990, GN (BUCM 117.760).
Sur **Corylus maxima* Miller (cult.) - *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 21 X 1990, GN (BUCM 118.102).
Sur *Fagus sylvatica* L.- *AR*: Ville-Vieille, Vallée de la Loire, 44°52'..N, 4°16'..E, alt. 1400 m, 4 X 1990, GN (BUCM 117.415). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 13 X 1990, GN (BUCM 117.852).
Sur **Pyrus amygdaliformis* Vill.- *VL*: Apt E, Caseneuve, 43°50'..N, 5°20'..E, alt. 250 m, 17 X 1990, GN (BUCM 118.043).

75. **Phyllactinia mali** (Duby) U. Braun
Sur *Crataegus monogyna* Jacq.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'02"E, alt. 800 m, 21 X 1990, GN (BUCM 118.116).

76. **Sphaerotheca aphanis** (Wallr.) U. Braun
Sur ***Alchemilla gracillis* Opiz - *HL*: Les Estables, Mont Mézenc, 44°54'20"N, 4°15'..E, alt. 1550 m, 5 X 1990, GN (BUCM 117.447).

Sur ***Alchemilla plicatula* Gand.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1500 m, 13 X 1990, GN (BUCM 117.945)
Sur *Alchemilla xanthochlora* Rothm.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.513).
Sur *Geum urbanum* L.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.599).
Sur *Potentilla erecta* (L.) Räuschel - *HL*: Les Estables, Mont d'Alambre, 44°55'40"N, 4°14'..E, alt. 1500 m, 5 X 1990, GN (BUCM 117.443).
Sur **Potentilla fruticosa* L. (cult.) - *HL*. Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 21 X 1990, GN (BUCM 118.101).
Sur *Potentilla palustris* (L.) Scop.- *HL*: La Chaise-Dieu, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.312).

77. **Sphaerotheca epilobii** (Wallr.) Sacc.
Sur ***Epilobium collinum* C. C. Gmelin - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.527).

78. **Sphaerotheca euphorbiae** (Cast.) Salmon
Sur *Euphorbia peplus* L.- Paris: Trocadero, 48°50'..N, 2°20'..E, 1 X 1990, GN (BUCM 117.346). *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.701).

79. **Sphaerotheca ferruginea** (Schlecht.: Fr.) Junell
Sur *Sanguisorba minor* Scop. subsp. *minor* - *HL*: Sainte-Sigolène, 45°13'..N, 4°19..E, alt. 800 m, 10 X 1990, GN (BUCM 117.703).
Sur *Sanguisorba officinalis* L.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.528).

80. **Sphaerotheca fugax** Penzig & Sacc.
Sur *Geranium sylvaticum* L.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.5199). *PNV*: Gresse-en-Vercors, Pas de la Ville, 44°54'..N, 5° 34'..E, alt. 1600 m, 13 X 1990, GN (BUCM 117.904).

81. **Sphaerotheca fuliginea** (Schlecht.: Fr.) Poll.
Sur *Veronica persica* Poiret - *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.596).
Sur *Veronica spicata* L. subsp. *spicata* (cult.) - Alpe d'Huez, ad basilica, 44°55'..N, 5°50'..E, alt. 1800 m, 15 X 1990, GN (BUCM 118.014).

82. **Sphaerotheca fusca** (Fr.) Blumer
Sur *Calendula officinalis* L. (cult.) - *HL*: Sainte-Sigolène, 45°35'..N, 4°19'..E, alt. 800 m, 3 X 1990, GN (BUCM 117.383) et 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.626); Montfaucon-en-Velay, 45°10'04"N, 4°24'..E, alt. circa 900 m, 9 X 1990, GN (BUCM 117.650); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.535). PNV. Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. circa 1250 m, 12 X 1990, GN (BUCM 117.828).
Sur *Chamonilla suaveolens* (Pursh) Rydb.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.625); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.396). *AR*: Sainte-Cirgues-en-

Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.497). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1250 m, 14 X 1990, GN (BUCM 117.848). Alpe d'Huez, Gorge de Sarenne, 44°55'..N, 5°50'..E, alt. 1500 m, 15 X 1990, GN (BUCM 117.958).
Sur *Conyza canadensis* (L.) Cronq. - *HL*: Sainte-Sigolène, Reveyrolles, 45°14'50"N, 4°17'..E, alt. 800 m, 9 X 1990, GN (BUCM 117.700). *VR*: Île de Porquerolle, 43°02'..6°15'..E, alt. 5 m, 18 X 1990, GN (BUCM 118.052).
Sur *Cucurbita pepo* L. (cult.) - Paris: Jardin de Tuilleries, prope Louvre, 48°50'..N, 2°20'..E, 2 X 1990, GN (BUCM 117.344). *HL*. la Chaise-Dieu, 45°15'..N, 3°45'..E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.302); Les Estables, Mont Mézenc, 44°54'20"N, 4°15'..E, alt. 1550 m, 5 X 1990, GN (BUCM 117.455).
Sur *Doronicum austriacum* Jacq.- *HL*: Les Estables, Mont Mézenc, 44°55'15"N, 4°15'00"E, alt. 1600 m, 7 X 1990, GN (BUCM 117.575).
Sur **Leontodon autumnalis* L. subsp. *autumnalis* - *HL*: Sainte Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.749).
Sur **Linaria repens* (L.) Miller - *HL*: Sainte-Sigolène, 45°13'..N, 4°19'..E, alt. 800 m, 10 X 1990, GN (BUCM 117.735).
Sur *Taraxacum "officinale* Weber"- *HL*: Siante-Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.618); Montregard, 45°09'58"N, 4°26'..E, alt. 1000 m, 9 X 1990, GN (BUCM 117.659); Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.404). *AR*: Sainte-Cirgues-en-Montagne, 44°46'..N, 4°09'..E, alt. 1060 m, 6 X 1990, GN (BUCM 117.491). *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1250 m, 12 X 1990, GN (BUCM 117.890).

83. **Sphaerotheca plantaginis** (Cast.) Junell
Sur *Plantago lanceolata* L.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 7 X 1990, GN (BUCM 117.509).

84. **Sphaerotheca pannosa** (Wallr.: Fr.) Lév.
Sur **Rosa rogosa* Thunb. (cult.) - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.401).
Sur ***Rosa stylosa* Desv.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1350 m, 13 X 1990, GN (BUCM 117.947).

85. **Sphaerotheca verbenae** Săvul. & Negru
Sur **Verbena* sp. (cult.) - *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.387).

86. **Uncinula adunca** (Wallr.: Fr.) Lév.
Sur *Populus nigra* L.- *HL*: Sainte-Sigolène, 45°13'40"N, 4°19'02"E, alt. 800 m, 21 X 1990, GN (BUCM 118.106).
Sur ***Salix appendiculata* Vill.- *PNV*: Gresse-en-Vercors, 44°54'..N, 5°34'..E, alt. 1500 m, 13 X 1990, GN (BUCM 117.936).

87. **Uncinula bicornis** (Wallr.: Fr.) Lév.
Sur *Acer platanoides* L. (cult.) - Paris: Jardin de Tuileries, prope Louvre, 48°50'..N, 2°20'..E, alt. 46 m, 1 X 1990, GN (BUCM 118.157).
Sur *Acer pseudoplatanus* L.- *HL*: Les Estables, 44°55'..N, 4°14'..E, alt. 1340 m, 4 X 1990, GN (BUCM 117.391); Le Puy, Notre-Dame de France, 45°02'30"N, 3°55'..E, alt. 630 m, 10 X 1990, GN (BUCM 117.751).

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SOIL POLLUTION REMOVAL BY MAIZE ROOT ABSORPTION

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Soil residues of simazine and propanzine are often important for the rotation of different agricultural crops. To reduce the risks of herbicides for the subsequent crop, it was tried to absorb the residues by a dense root system of maize sown at high density. The experiments were conducted 2 years with 40 and 60 plants/m^2 on brown-reddish forest soil.

The soil surface sprayed prior to sowing with simazine at 3 kg/ha a.i. and in other experiment with propazine 2.5 kg/ha a.i. Every month soil samples have been collected at two depts: 0 - 10, and 10 - 20 cm, as well as maize plants and weeds, for residue analysis by TLC.

After the artificial pollution, the surface level of simazine was 2,5 - 2,2 mg/kg. After the 3 months under the influence of high density of root system and root absorption the residue decreased to 0,24 mg/kg soil at depth 0 - 10 and 0,15 mg/kg soil at depth 10 - 20. A 84,4 % reduction of soil residues was obtained as compared to 38% reduction of simazine residues in control plot without maize plants.

Propazine artificial pollution gave 2,2 mg/kg soil. After 3 months the amount of residues decrease from 2.2 - 2 mg/kg to 0,65 mg/kg at depth 0 - 10 cm and 0,38 mg/kg at depth 10 - 20 cm, under the influence of maize root system absorption, the percent reduction was about 53,18% as compared to 22,05% in the control without maize plant.

These results suggested that at a lower pollution level of soils usually observed in our country (0,1 - 0,2 mg simazine/kg soil) the proposed system (root absorption by high densities of maize plants) can reduce or avoid the risks for subsequent more sensitive crops; nevertheless, sugarbeet is highly sensitive to simazine residues.

INTRODUCTION

Usage of herbicides to control weeds from various crops enables to obtain high and quality harvest.

Application of several pesticide groups, such as triazines, urea derivatives, dinitroanilines and others are rather persistent and susceptible to raise problems to subsequent agricultural crops.

Dosage recommended for many cultures reach their upper limits, enhancing the residue risk in various soils. For example, data accumulated (Caramete,1983) showed that after maize harvest atrazine residues in different Romanian districts were rather high, namely: Ilfov -0.01- 4.08 mg/kg, Ialomița 0.02-3.7 mg/kg, Arad 0.02-3.22 mg/kg, etc.

Similar examples may also be cited for linuron, which left significant soil residues at the end of the season.

When annually applied to various vegetable crops, trifluralin accumulated in soil from year to year, reaching excessive levels. Similarly, simazine and particularly propazine can leave, at the usual rates, significant soil residues (*Ghinea et al.,1987*).

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The current solutions to avoid these challenges mainly refer, as is known, to choose cropping plants tolerant to each of the herbicides mentioned above. Another method consists in favourizing the soil microbiologic activity, in cases where these processes have a greater role in degradation of herbicides, through special soil and crop management practices.

Frequently, lowering dosage of a persistent compound and usage of less persistent mixtures are advocated.

Methods for residues absorption with active coal or other adsorbent matters are known; nevertheless, their domain of application is restricted.

In certain cases persistent compounds are replaced by some with lower persistence.

At the R.I.P.P.-Bucharest a new method has been devised to extract soil residues and to breakdown these to various extents by some selected plants which are not affected by a specific herbicide.

This method is based on the idea of choosing a specific plant for every active ingredient, having a key-element - a high plant density per surface unit, so that a very dense root system could develop in the polluted layer, each soil particle being in contact with the root system. In this manner a compound is extracted from all the arable layer. Moreover, a very dense root system enhances an abundant micro-flora, able to accelerate degradation.

A range of soil and crop management procedures, such as the use of mineral and especially organic fertilizers, as well as irrigation, can intensify microbiological activity, and consequently microbiological and chemical breakdown of a compound.

The cropped plant sown at an exaggerated density does not yield seed, but rarely, it being in fact harvested as green mass, thus removing the most part of residues. In this way the plants can free the soil previously treated with some more sensitive plants, particularly vegetables.

Starting from these ideas, several depollution methods have been developed, as follows:

Atrazine with maize at high densities and a specific system of soil management (Baicu and Caramete,1982);

Linuron with lupine at increased densities and particular soil management (Baicu and Caramete,1986);

Trifluralin with rape (Caramete et al.,1990).

The present paper followed the same goal to reduce simazine (6-chloro-N^2, N^4 - 1,3,5 - triazine - 2,4-diamine), and propazine (6-chloro-N^2, N^4 di-isopropyl - 1,3,5-triazine - 2,4 diamine) residue content, by means of maize cropped at high densities.

MATERIAL AND METHODS

Simadon 50 WP from Borzeşti with 50% simazine, and Prosinex with 80% propazine from Makhteshim-Agan, have been used in these trials.

The experiments have been performed at the R.I.P.P. - Bucharest, on a reddish-brown forest soil with the following characteristics (*Cernescu,1973*):

colloidal clay (< 0.002 mm) in the 0-20 cm horizon - 36.7%; dust (0.01-0.02 mm) - 31.5%; fine sand (0.02-0.2 mm) - 29.4%. The humus content was about 2.49%.

Deeper layers, e.g. 40-60 cm, contained more colloidal clay, (42.7%), while humus decreased to 1.56%.

The C:N ratio was 12.5-14.

This trial was carried out in 1993, on a ground of artificial pollution with 3 kg/ha a.i. simazine, and 2.5 kg/ha a.i. propazine. In that year pollution was achieved on April 29, and sowing on April 30. Thus, the pollution level of soil surface was 2.5 mg simazine/kg soil.

Artificial pollution with propazine resulted in a level of 2.2 mg/kg soil.

Field experiments were performed on 4.5 sq.m. plots in 4 replications, in a randomized layout. The simazine trial was sown on April 30, after the artificial pollution with Simadon 50 WP, on April 29.

Maize seedlings emergence took place on June, and harvest was done on August 9.

The propazine trial was sown on May 11, after pollution with Prosinex 80 WP, on May 10. Maize emergence occurred on May 23, and green mass was harvested on August 11.

In order to facilitate interpretation of results, the following variants with artificial pollution and maize densities of Pirat hybrid were tested:

V_1 - Simazine 3 kg/ha a.i. ..... 400,000 kernels/ha
V_2 - Simazine 3 kg/ha a.i. ..... 600,000 kernels/ha
V_3 - Simazine 3 kg/ha a.i. ..... without maize
V_4 - Without pollution ..... 400,000 kernels/ha
V_5 - Without pollution ..... 400,000 kernels/ha
V_6 - Without pollution ..... without maize

Similarly, the variants with propazin were as follows:

V_1 - Propazine 2.5 kg/ha a.i. ..... 400,000 kernels/ha
V_2 - Propazine 2.5 kg/ha a.i. ..... 600,000 kernels/ha
V_3 - Propazine 2.5 kg/ha a.i. ..... without maize
V_4 - Without pollution ..... 400,000 kernels/ha
V_5 - Without pollution ..... 600,000 kernels/ha
V_6 - Without pollution ..... without maize

In this manner the effect of maize crop on the evolution of soil residues, the phytotoxic effect on maize plant, and residue breakdown in the absence of maize plants, could be watched.

To establish soil simazine and propazine residue levels, and in cropped plants and weeds, thin layer chromatography (TLC) has been used (*Zweig, 1972*).

Soil samples have been collected from 0-10 and 10-20 cm layers, 2 hours after the artificial pollution, and 1, 2 and 3 months thereafter.

Plants have been harvested from a 0.5 × 0.5 m (0.25 sq.m.) area, after 1, 2 and 3 months.

In season, as drought was steady, an irrigation has been applied on July 2, using 10 l water per sq.m., and mineral fertilization (ammonium nitrate) - 200 kg/ha, on June 25.

Chemical analyses have been paralleled by glasshouse biotests, to reveal the effect of soil simazine and propazine residues. To this end, average soil samples have been collected from every plot in 4 replications, which were set in wooden boxes 0.25 × 0.25 m, and were sown with the test-plant - sugarbeet.

Sugarbeet was selected for test-plant as being highly sensitive to triazines. In other experiment, wheat was used, being more resistant.

The phytotoxicity level was scored by the number of dead plants.

RESULTS

Maize seeds germinated and seedlings emerged at 10-12°C within 15 days in simazine trial, and after 12 days in the one with propazine.

The emergence results were recorded on June 14, and are exposed in Table 1.

Table 1

Emergence of maize plants

Experiment 1	Emergence (%)	*Experiment 2*	Emergence (%)
V_1 - Simazine 3kg/ha a.i.+ 400,000 kernels/ha	89.72	V_1 - Propazine 2.5kg/ha a.i.+ 400,000 kernels/ha	89.28
V_2 - Simazine 3kg/ha a.i.+ 600,000 kernels/ha	91.66	V_2 - Propazine 2.5kg/ha a.i.+ 600,000 kernels/ha	93.97
V_4 - 400,000 kernels/ha	95.13	V_4 - 400,000 kernels/ha	93.32
V_5 - 600,000 kernels/ha	91.00	V_5 - 600,000 kernels/ha	94.53

It can be seen that maize emergence was not affected. Statistic treatment of data has shown that differences between the 4 variants were not significant.

The maize primary roots developed within 2-3 weeks and grew in a tight contact with the soil layer containing the herbicide.

The embryonary root was the first to contact the soil. This branched and generated other 3-7 primary roots, from the meristem tissue at the mesocotyl basis.

Subsequently, coronate roots were developed, these being the most important in providing nutrition and water necessary to plant, and implicit herbicide absorption. All coronate roots are located above the mesocotyl and are formed at the basis of the 3- 5 very short, underground internodes.

From each internode 4-8 permanent roots emerged and spread radially, these being ramified in multiple numbers, in their turn.

They can penetrate deeply in the arable layer; however their most part occurs at 10-50 cm depth.

As plants grow, adventive roots also appeared. They were few in these experiments, because of the restricted growth of stem, caused by the high plant density per sq.m. These roots, penetrated the upper soil layer, where they absorbed the herbicide.

The maize roots are very numerous at this plant density; they are branched and able to extract soil substances.

Photo 1 shows the roots formed in the 0-25 cm soil layer. It is obvious they are very abundant and well branched. The hairy zone, which is large and has an enormous contact surface allowing absorption, cannot be seen in this figure.

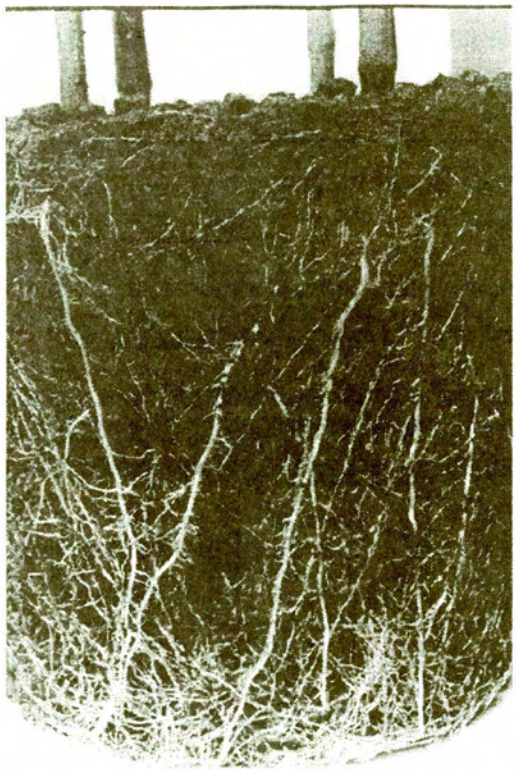

Photo 1

Stem developed normally and acquired 6-8 internodes until the end of test, formed inflorescences, but no ears.

6 to 8 leaves grew, of which 1-2 were dry at the trial conclusion

Weight growth of maize plant, matter accumulation followed a "S" curve with a 50% level after 60 days from planting. In the case of very dense crops recommended by us, this process was considerably delayed; nevertheless a 6-7 fold density increase led to the formation of a large biomass, in the early part of its vegetation period. In our experiment this level was attained in the simazine test after some 40 days (table 2), and after about 43 days with propazine (table 3).

Table 2

Evolution of green mass in the experiment with simazine (t/ha)

Variants	29.05		29.06		29.08	
	M	W	M	W	M	W
V_1 - Simazine 50 3kg/ha a.i.	45.12	7.2	276	25.4	280.6	15.4
V_2 - Simazine 3 kg/ha a.i.	57.48	7.04	211.2	26.32	256.0	20.4

M - maize; W - tolerant weeds

Table 3
Evolution of green mass in the experiment with propazine

Variants	10.06		12.07		10.08	
	M	W	M	W	M	W
V_1 - Propazine 2.500 g/ha a.i.	51.12	11.32	157.8	50.2	*260.0*	30.8
V_2 - Propazine 2.500 g/ha a.i.	52.20	16.6	204.0	40.0	*209.6*	30.4

M - maize; W - tolerant weeds

Some weeds tolerant to triazine, such as: *Echinochloa crus-galli*, *Convolvulus arvensis*, *Cirsium arvense*, whose biomass is rather low, occurred in the test plot.

Laboratory analyses for simazine (table 4) have shown that this herbicide moved in soil to10-20 cm layer.

Table 4
Simazine content of soil (mg/kg)

Date of harvest	V_1			V_2			V_3		
	Soil 0-10 cm	Soil 10-20 cm	Total	Soil 0-10 cm	Soil 10-20 cm	Total	Soil 0-10 cm	Soil 10-20 cm	Total
29.04.1993	2.50	-	2.50	2.20	-	2.20	2.50	-	2.50
29.05.1993	1.00	0.80	1.80	1.00	0.60	1.60	1.00	1.25	2.25
29.06.1993	0.65	0.45	1.10	0.56	0.82	1.38	0.80	1.40	2.20
29.07.1993	0.24	0.15	0.39	0.18	0.35	0.53	0.60	0.95	1.55

Table 5
Simazine content in maize green mass and weeds (mg/kg)

Date	V_1		V_2	
	maize	weeds	maize	weeds
29.05	0.1	0.1	0.9	0.15
29.06	0.0075	0.025	0.05	0.01
29.07	traces	0.02	0.025	0.02

Simazine content in the variant with 400,000 maize plants per hectare dropped from 2.5 mg/kg to 0.39. In the upper layer, 0-10 cm, reduction was more important, from 2.5 to 0.24 mg/kg, reaching 0.15 mg/kg in the l0-20 cm layer.

In the variant 2 with 600,000 plants/ha, reduction of simazine content was similar, from 2.2 to 0.53 mg/kg. In the 0-10 cm layer lessening was high, from 2.5 to 0.18/kg. Part of this amount migrated in the 10-20 cm layer. Reduction was slighter than in the preceding case, therefore this variant is not justified. This too high density did not favor simazine content reduction.

In the absence of maize (V_3) a lowering of simazine content was also noticed, from 2.5 to 1.55 mg/kg, and from 2.5 to 0.6 mg/kg in the upper layer (0-10 cm). A significant simazine amount migrated to the 10-20 cm layer.

Maize plants (V_1) had a relatively small simazine content: 0.1 mg/kg after one month, 0.0025 two months later, and finally only traces.

The absorption process performed along with the degradation one and, therefore, the compound accumulation was not noticed.

The same image is also shown by the tolerant weeds having a similar residue content; however, their green mass was small and did not exhibit a too high significance.

Analysis of the way the simazine content decreased (table 6) showed that the 0-10 cm layer had a very low residue content at the end of trial. This content has been reduced by 94.4%.

Table 6
Reduction of soil simazine content (%)

V_1			V_2			V_3		
0-10 cm	10-20 cm	Total	0-10 cm	10-20 cm	Total	0-10 cm	10-20 cm	Total
2.5	-	2.5	2.2	-	2.2	2.5	-	
0.24	0.15	0.39	0.18	0.35	0.53	0.6	0.95	1.55
94.4%	(6%)	84.4%	91.82%	(11.36%)	80.46%	66%	(38%)	38%

In V_1, when summing up the residue content reduction in these both layers, it was about 84.4%.

In the absence of maize, the overall residue diminution was 38%. This fact also shows the share of chemical and microbiological processes in this experiment.

As in V_1, simazine level reduction was 84.4%; then lowering of the residue content due to maize plants was 46.4%.

In V_2 lessening the simazine content by maize plants was 80.4%-38.0 %=42.4 %.

Microbiological processes cannot be separated from the chemical ones, which led to achieving this breakdown percentage (38%); seemingly, microbiology processes prevailed.

Propazine (table 7) had a similar performance; from the overall 2.2 mg/kg, its soil content decreased to 1.03 after 3 months in V_1, while for a higher number of plants (V_2), it dropped from 2.0 to 0.72 mg/kg.

When maize plants lacked the herbicide content declined from 1.95 to 1.52 mg/kg.

Table 7
Propazine content of soil (mg/kg)

Date of harvest	V_1			V_2			V_3		
	Soil 0-10 cm	Soil 10-20 cm	Total	Soil 0-10 cm	Soil 10-20 cm	Total	Soil 0-10 cm	Soil 10-20 cm	Total
10.05.1993	2.20	-	2.20	2.00	-	2.00	1.95	-	1.95
10.06.1993	1.80	0.14	1.94	1.60	0.28	1.88	1.82	0.04	1.86
10.07.1993	1.92	0.46	2.38	0.56	0.40	0.96	1.58	0.16	1.74
10.08.1993	0.65	0.38	1.03	0.40	0.32	0.72	1.20	0.32	1.52

Maize plants (table 8) development was more difficult on a background of high propazine residue content.

Table 8

Propazine content in maize green mass and weeds (mg/kg)

Date	V_1		V_2	
	maize	weeds	maize	weeds
10.06	0.05	0.08	0.1	0.08
10.07	0.16	0.12	0.2	0.24
10.08	0.028	0.02	0.06	0.05

The propazine content of maize plants fluctuated between 0.16 and 0.022 mg/kg. Likewise, weeds had relatively low residues, 0.12 to 0.02 mg/kg.

Adsorption and breakdown processes were continuous: nevertheless, data from analyses define only 3 steps of this complex performance.

Table 9 exhibits the propazine degradation process. In the absence of maize plants, breakdown merely attained 22.0%; when these were present (V_1) degradation was 53.18%. When subtracted 53.18-22.05=31.13%, thus obtaining the

Table 9

Reduction of soil propazine content (%)

V_1			V_2			V_3		
0-10 cm	10-20 cm	Total	0-10 cm	10-20 cm	Total	0-10 cm	10-20 cm	Total
2.2	-	2.2	2.0	-	2.0	1.95	-	
0.65	0.38	1.03	0.4	0.32	0.72	1.2	0.32	1.52
70.46%	(17.27%)	53.18%	80%	(16%)	64%	38.46%	(16.4%)	22.05%

degradation percentage under the influence of maize plants. A still higher number of plants (2) resulted in a more conspicuous breakdown (64%). In this case, the depollution percentage is lower. Thus, starting from a high artificial pollution, this procedure is not sufficiently efficient. Biotests with sugarbeet plants have shown that soil contained residues toxic to seedlings.

DISCUSSION

Soil application of simazine and propazine resulted in their marked adsorption. It was already stated (Hance, 1984) that, for example, simazine remained practically entirely (93%) in the upper 5 cm soil layer.

The soil can be treated from the standpoint of herbicide distribution, in solid, liquid or gaseous phase. As both simazine and propazine have low vapor pressures, the gaseous phase is not likely to be considered.

For instance, Hartley and Graham-Brice (1980) estimated that simazine percentage as liquid phase is 9.5%, and 90.5% in the solid one.

Similar data could also be assumed with propazine. Adsorption is a superficial fixation of herbicides on the soil colloids with negative charges, clays and

organic matter. It is generally admitted that adsorption on clays is temporary. In the free organic matter, the compounds released by the fresh organic matter, partially decomposed (10 to 15% of the whole organic matter), absorb high herbicide amounts.

Usually, the adsorbed pesticide accounts for biologically inactive, however adsorption is often reversible. Nevertheless, the high clay and medium organic matter contents assume a significant adsorption of these two herbicides.

Migration of simazine and propazine down to the 10-20 cm layer was noticed in our experiments, under the influence of rainfall and irrigation.

Soil samplings at 40 cm depth showed, however, that these herbicides could not be detected at this soil level.

Simazine and propazine moving at high distances by diffusion is scarce; however it could not be negligible for short distances. Their transfer by bulk water flow is by far more effective for small distances. Bulk flow is nevertheless intermittent and is performed through the large pores among the soil aggregates. Simazine and atrazine are carried towards roots by molecular diffusion and bulk transfer with the matter which descends to roots, as a result of plant transpiration.

Lavy (1968) demonstrated that atrazine accumulates around the maize roots, while simazine decreased in this area; propazine had an intermediate behavior.

The reduction processes of simazine and propazine soil contents are influenced by the maize plant, both by root absorption and by the increase of microbiological activity.

Kaufman and Kearney (1970) drew up a list enclosing 42 bacteria and fungi species able to degrade simazine, atrazine and promethrin. Some fungi, such as *Aspergillus fumigatus*, degraded simazine by N-dealkylation. Simazine can be degraded by microorganisms up to water and carbon dioxide.

Simazine and propazine are degraded in soil to their hydroxy-forms (Brown, 1978). In neutral soils breakdown is slow. It is thought that pH has a great importance in hydrolytic degradation.

The breakdown process takes place in the soil solution, but microorganisms are more abundant on or nearby the solid particles, particularly of organic matter. This means that sometimes absorption may lead to increased degradation by concentration of matter in zones with the greatest microbiological activity. Microorganisms are grouped in micro-colonies or complex, cenoses of microorganisms, at the surface of soil particles (Zviaghintsev, 1987). Likewise, there are microorganisms in the soil solution, while in capillaries or on films numerous bacteria are found, which can contribute to herbicide degradation.

In variants including maize plants, their roots favor numerous organisms, some of them, such as yeasts and bacteria adhering to roots. In this zone the number of bacteria and actinomycetes is nearly twice greater. Microbiological processes have a significant share in simazine degradation.

Triazine metabolisation by plants (Ghinea et al.,1987) and particularly maize takes place in several ways:

1) by non-enzymatic hydrolysis, sometimes catalyzed by a glycoside; generally, fertilization has a positive effect on this process.

For the cause of the proposed procedure, nitrogen fertilization, besides enhancement of plant growth, has also a favorable effect on the degradation process.

2) by non-enzymatic desalkylation of side-catenes, as is obviously produced in pea plants.

Hydrolysis and dealkylation processes are performed in roots.

3) by enzymatic conjugation (simazine, propazine and others) which is encountered in maize and sorghum plants, where two metabolites are formed. The process is catalyzed by glutathion-s-transferases, present in number of three in maize.

Root absorption and translocation depend on the transpiration flow or the absorptive power of roots.

The absorbing power of solution is proportional to its strength at the root surface and with the total root surface.

In the case examined, the high density of roots per c.c. of soil allows absorption of increased amounts of solution.

At the same time this absorption induces translocation or dissolved herbicide compound towards the roots by bulk water transfer, or by diffusion in the direction of concentration gradient.

In some tests (Hartley and Graham-Brice, 1980), total accumulation in plants increases linearly with the time and thus with the transpired water.

Schematically (Baicu, 1989), the application of simazine and propazine in this experiment led to a distribution, and then to a degradation, according to figure 1.

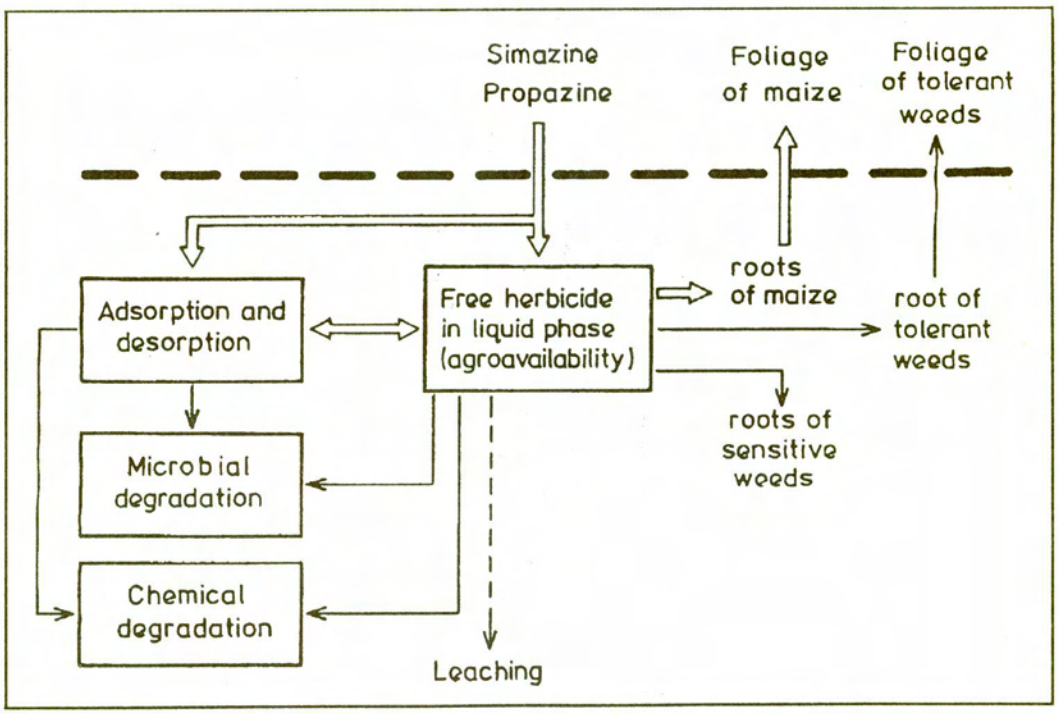

Fig. 1. – Pathway of simazine and propazine movement and soil residues removal by maize plants.

The depolluting method proposed was tested on a soil heavily contaminated with herbicide. Even under these conditions, artificially controlled pollution with simazine could be removed to a high extent.

Commonly it is estimated (Ghinea et al., 1987) that propazine persistence lasts 77 to 400 days, while that of simazine 200 to 400 days.

Simazine and propazine soil residues, after their application in some crops, are sharply lesser. Under these conditions, usage of high maize densities can free the soil much more completely from simazine residues. It can also be forecasted that in propazine this effect will be much more important.

This procedure can be applied to various grounds; however, a residue analysis should be made after 3 months from planting, to check the depollution level. Only after that a subsequent crop will be chosen, which will not be affected by the casual residue unextracted by maize plants from the soil.

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UREA AS NITROGEN SOURCE IN MODIFIED ZARROUK MEDIUM

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The results of the use of urea as nitrogen source for the growth of a *Spirulina platensis* strain on modified ($KCl+Na_2HPO_4$) Zarrouk nutritive medium are reported. Nitrate ions present in medium were partially or totally replaced by corresponding urea doses of 0.1 to 0.8 g/l nutritive solution. Urea used as the only nitrogen source in doses of 0.1-0.8 g/l (laboratory) and 0.8 g/l (pilot plant) enabled a good survival and growth of *Spirulina plantensis*: the yield of biomass accounted for 103% of the control, in laboratory and 110% in pilot plant conditions; chlorophyll content of biomass reached maximum of 124% of the control at 0.4 g/l urea, in laboratory and in pilot plant the value was 4 times higher than the laboratory value; protein biosynthesis was close to control at doses higher than 0.2 g/l; total saccharide content of biomass accounted for more than 120% of the control for low urea doses but less than 50% for doses higher than 0.2 g/l.

INTRODUCTION

The aquatic microorganism ability to use urea as nitrogen source was demonstrated by several foreign and Romanian investigators. Thus, Wafar et al. (10) found that stimulation of carbon fixation by addition of urea was, on average, as good as by NO_3 or NH_4 addition, revealing a potential for urea uptake by phytoplankton in tropical oceanic waters. Timperley et al. (8) reported that *Chlorella sp.* grew well on urea from atmospheric precipitations. Boldor et al. (1) attempted to influence the protein synthesis in *Chlorella vulgaris* and *Scenedesmus obliquus* algae by various nitrogen sources (NH_4Cl, $NaNO_3$, urea) using Arnon medium. They found urea was best assimilated by Chlorella (47.8% protein against 41.2% at control) compared to Scenedesmus. Stanca et al. (7) cultured the alga *Chlorella vulgaris* on Knop medium using 0.96 g/l alanine or 0.34 g/l urea as organic nitrogen. The alga assimilated best urea nitrogen (yield of biomass - 0.3 g dry wt./l; protein content - 40.4%).

Utilization of urea in biomass production of *Spirulina sp.* cyanobacterium gave good results as reported by Crance et al.(*2*) and Faucher et al. (4). Studies by Tredici et al. (9) with *Spirulina maxima* showed similar results in outdoor mass culture on sea water. They obtained 8.14 g. dry wt./m^2/day on classic Zarrouk medium; 7.35 g. dry wt./m^2/day on sea water with urea as single source of nitrogen; 5.20 g. dry wt./m^2/day on sea water with nitrate. Dragoş et al. (3) reached the conclusion that *Spirulina plantensis* effectively used urea doses below 0.2 g/l, using classic Zarrouk medium.

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The quantity and quality of nitrogen sources used in culture medium could influence the protein content, as well as other characteristics of *Spirulina sp.* - cyanobacterium with distinct nitrogen metabolism by which it syntheses high amounts of proteins (65-70% of dry wt.). Urea may be a valuable source of organic nitrogen for the growth of *Spirulina cyanobacterium*, as judged by the above mentioned results, obtained in different culture conditions. Therefore, we examined whether previous results coud be improved by use of a modified Zarrouk medium for the first time.

MATERIALS AND METHODS

A *Spirulina plantesis* cyanobacterium strain, cultured in the Laboratory of Plant Physiology (Institute of Biology - Bucharest) on a modified Zarrouk medium was used.

Composition of modified Zarrouk medium

Substance	$NaHCO_3$	Na_2HPO_4	KCl	$NaNO_3$	K_2SO_4
g / liter	16.8	0.55	0.43	2.5	1.0
Substance	$MgSO_4 \cdot 7H_2O$	$CaCl_2$	$FeSO_4 \cdot 7H_2O$	EDTA	$ZnSO_4 \cdot 2H_2O$
g / liter	0.2	0.04	0.01	0.08	0.2
Substance	$CuSO_4 \cdot 5H_2O$	$Co(NO_3)_2 \cdot 5H_2O$	H_3BO_3		
g / liter	0.1	0.04	3.0		

Nitrate ions were partially and totally replaced by urea as follows:

	Control	Series of experiments						
$NaNO_3$ (g / l)	2.5	2.0	1.0	0.5	–	–	–	–
urea (g / l)	–	0.1	0.2	0.4	0.1	0.2	0.4	0.8

Corresponding amounts of NaCl were added to maintain the osmotic balance in culture medium.

The experiments were carried out in the laboratory and pilot plant.

- In laboratory conditions of a culture chamber, Spirulina suspensions were incubated and continuously bubbled with air, in cylindrical glass recipients (1000 ml) at 24±1°C and 8000 lux artificial light. The culture cycle was established at 8 days. Each experimental treatment was repeated three times.

- Pilot plant culture was made in a 400 l basin and continuously air bubbled at 34°C and under natural illumination (June). The culture cycle was set at 12 days.

The Spirulina biomass quantity and quality were determined by: a) yield of fresh biomass; b) assimilatory pigment content (chlorophylls and carotenoids), Holm method (5); c) protein content (N × 6.25), Kjeldahl method; d) total saccharide content, Hagedorn-Jensen method (6).

RESULTS AND DISCUSSION

Our results are consistent with those reported by other authors (2, 3, 4, 9), namely that Spirulina species are able to assimilate well urea nitrogen.

BIOMASS YIELD (fresh wt.: mg/l/day). Generally, we obtained values around control in experiments with urea added as single nitrogen source. The biomass yield values were smaller than control ones when medium contained urea and nitrate (Table 1). Dragoş et al. (3) stated that urea in a classic Zarrouk medium might be efficiently used as a nitrogen source up to a concentration of 0.22 g/l, but we obtained an increased productivity on modified Zarrouk medium (Na_2HPO_4+KCl) with 0.4 and 0.8 g/l doses of added urea. Moreover, the 0.8 g/l dose resulted in a very high biomass yield of 96 mg/l/day in pilot plant conditions (Table 1).

Table 1

Spirulina platensis cultured in modified Zarrouk medium

Experimental series	Fresh biomass yield (mg / l / day)	(%)
LABORATORY		
urea 0.1 g / l + $NaNO_3$ 2.0g / l	43	55
urea 0.1 g / l	74	97
urea 0.2 g / l + $NaNO_3$ 1.0g / l	49	64
urea 0.2 g / l	55	72
urea 0.4 g / l + $NaNO_3$ 0.5g / l	63	82
urea 0.4 g / l	56	73
urea 0.8 g / l	80	103
control – $NaNO_3$ 2.5g / l	77	100
PILOT PLANT		
urea 0.8g / l	96	–

ASSIMILATORY PIGMENTS (mg/g fresh wt). In general, assimilatory pigment quantities were higher when urea was added as single nitrogen source, rather than in association with nitrate (Table 2). The highest values for laboratory conditions were: 124% against control chlorophyll at 0.4 g/l dose, 265 % carotenoid at 0.8 g/l and 143% total assimilatory pigments at 0.8 g/l. Assimilatory pigment

Table 2
Assimilatory pigment synthesis by *Spirulina platensis* cultured on modified Zarrouk medium

Experimental series	Chlorophylls			Carotenoids			Total assimilatory pigments		
	(mg/g fr wt)	(%)	(mg/g fr wt/day)	(mg/g fr wt)	(%)	(mg/g fr wt/day)	(mg/g fr wt)	(%)	(mg/g fr wt/day)
LABORATORY									
urea 0.1 g/l+ $NaNO_3$ 2.0 g/l	0.62	81	0.07	0.19	114	0.02	0.81	87	0.10
urea 0.1 g/l	0.73	94	0.09	0.21	126	0.02	0.94	100	0.12
urea 0.2 g/l+ $NaNO_3$ 1.0 g/l	0.47	62	0.06	0.17	105	0.02	0.65	69	0.08
urea 0.2 g/l	0.92	120	0.15	0.33	199	0.04	1.25	134	0.15
urea 0.4 g/l+ $NaNO_3$ 0.5 g/l	0.42	54	0.05	0.14	86	0.02	0.56	60	0.07
urea 0.4 g/l	0.95	124	0.12	0.32	193	0.04	1.27	136	0.16
urea 0.8 g/l	0.89	116	0.11	0.44	265	0.05	1.33	143	0.16
control - $NaNO_3$ 2.5 g/l	0.77	100	0.09	0.17	100	0.02	0.93	100	0.11
PILOT PLANT									
urea 0.8 g/l	5.55	-	0.46	4.43	-	0.36	9.98	-	0.83

biosynthesis in pilot plant conditions showed a marked increase with 0.8 g/l urea dose, compared to the same dose in laboratory (chlorophylls 4 times, carotenoids 7 times and total pigments 5 times higher) (Table 2).

PROTEIN CONTENT (N × 6.25; % against control). Protein quantity maintained at high levels except the case where the nitrogen dose in medium was under the amount according to the receipt (2.5 g/l $NaNO_3$, respectively 0.8 g/l urea (Fig. 1). Dragoş et al. (3) obtained similar results.

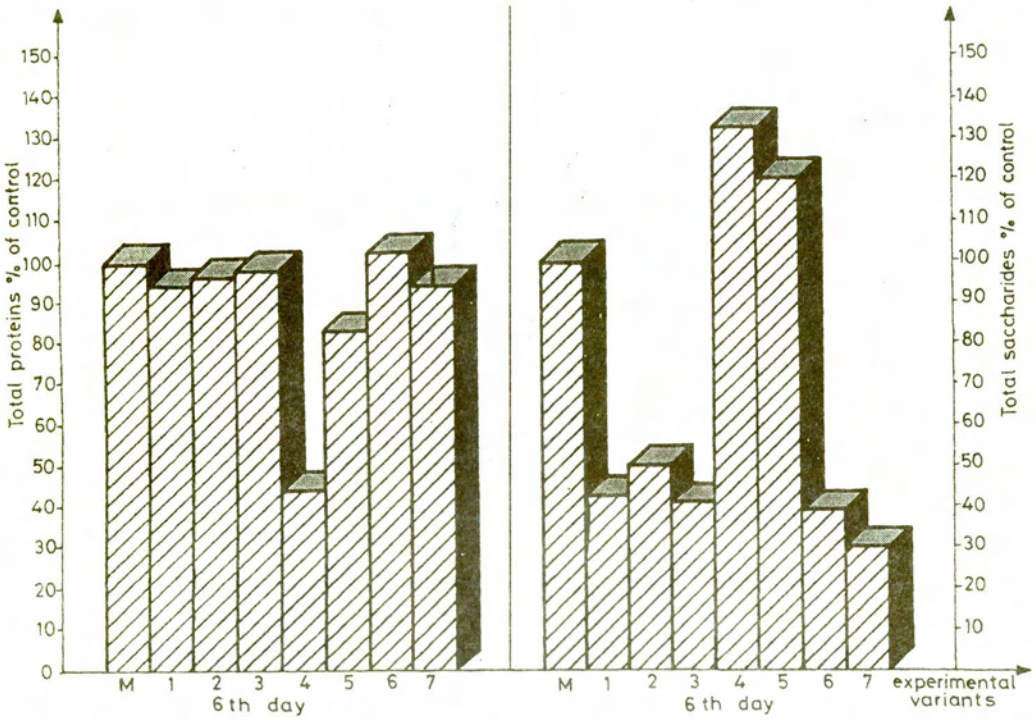

Fig. 1. – Protein and total saccharide content of *Spirulina plantensis* biomass obtained on modified Zarrouk medium: M - control - 2.5 g/l $NaNO_3$; 1-2.0 g/l $NaNO_3$ + 0.1 g/l urea; 2-1.0 g/l $NaNO_3$ + 0.2 g/l urea; 3-0.5 g/l $NaNO_3$ + 0.4 g/l urea; 4 - 0.1 g/l urea; 5 - 0.2 g/l urea; 6 - 0.4 g/l urea;7 - 0.8 g/l urea.

TOTAL SACCHARIDES (% against control). The content of the biomass total saccharides was related to doses of urea as single nitrogen source. It featured a specific pattern: values around 130% at lower urea doses sharply decreasing to about 50% for urea doses above 0.2 g/l (Fig. 1). It is interesting that Dragoş et al. (3) had similar changes of total saccharide content in *Spirulina platensis* biomass.

Our results showed that *Spirulina plantensis* developed in general well on modified Zarrouk medium when urea was added as single nitrogen source, in contrast to the conclusions drawn by Dragoş et al. (3).

As regards morphologic characteristics of *Spirulina planensis* filaments, no major significant changes of the spires number or diameter were seen as compared to control, in laboratory and pilot plant conditions (Figs. 2, 3, 4).

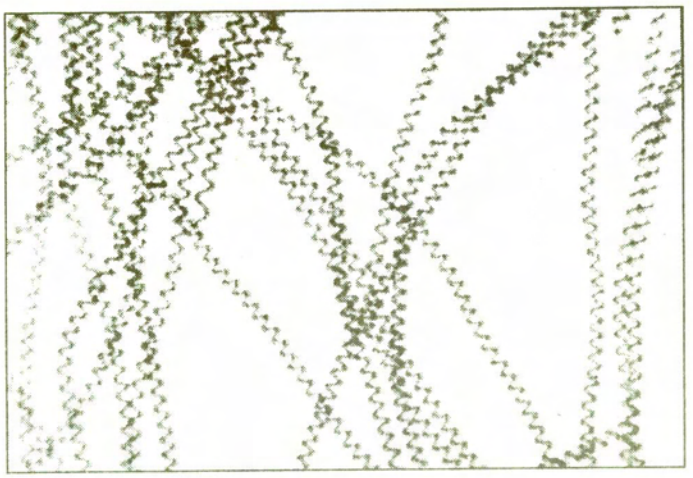

Fig. 2. – Filaments of *Spirulina plantensis* cultured on classic Zarrouk medium (2.5 g/l $NaNO_3$) in laboratory (control).

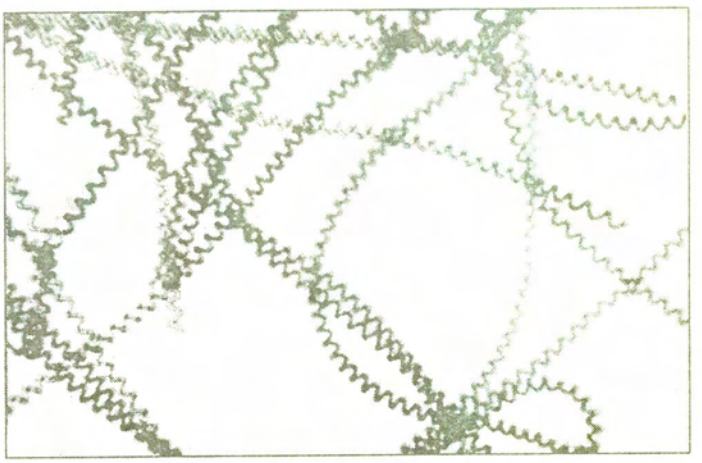

Fig. 3. – Filaments of *Spirulina plantensis* cultured on modified Zarrouk medium (0.8 g/l urea as single nitrogen source) in laboratory.

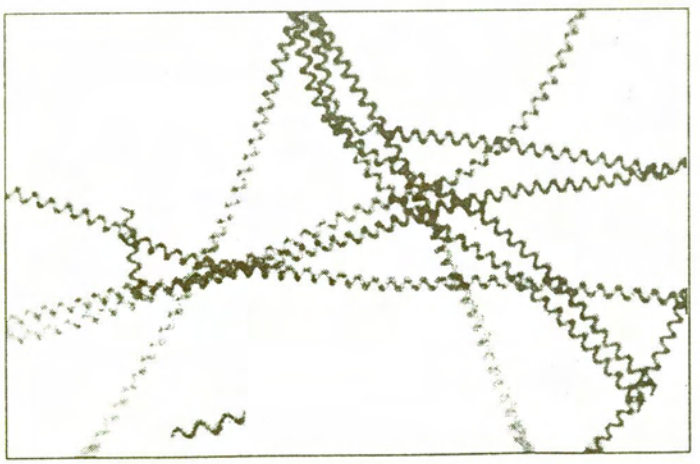

Fig. 4. – Filaments of *Spirulina plantensis* cultured on modified Zarrouk medium (0.8 g/l urea as single nitrogen source) in pilot plant.

CONCLUSIONS

Regarding utilization of urea as single nitrogen source for *Spirulina plantensis* cyanobacterium growth on modified Zarrouk medium:

1. - Our results suggest that growth is possible at doses of 0.1 - 0.8 g/l nutritive solution; 0.8 g/l dose resulted in a maximum biomass yield of cyanobacterium both in laboratory and pilot plant conditions;

2. - Maximum increase of chlorophyll biosynthesis in laboratory was obtained at 0.4 g/l dose; a several times increase was induced in pilot plant by 0.8 g/l dose;

3. - Carotenoid biosynthesis had a clear increase at 0.8 g/l urea both in laboratory and pilot plant conditions;

4. - The protein content maintained at levels around control at doses higher than 0.2 g/l;

5. - The total saccharide biosynthesis was stimulated by small urea doses (0.1-0.2 g/l) and inhibited by high ones (0.4-0.8).

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"An ecologist wants to clean up the world; an environmentalist wants to clean up your yard".
(Bill Copeland in Saratosa, Fla.)

PHYCOPHYSIOLOGICAL ANALYSES OF THE ZLATNA SURROUNDINGS

F. NAGY-TÓTH and ADRIANA BARNA

Interrelations between the plants and the environmental elements have been developed during long lasting adaptation. Likely, it was governed rather by the quantity than the quality of the elements; i.e. biochemical proportions and cycles, since the composition of the earth-crust has been ancestrally given. The original environmental conditions could be considered normal in comparison to those altered by human impact. Life is only possible if material and informational recycling can take place.

In Zlatna area the biogeochemical proportions of the elements and the environmental processes became disturbed by gold mining long time ago it continues unceasingly at the present by the Non-ferrous Processing Plant. The deteriorated conditions put topical the task to investigate the effects of the polluting, toxic compounds (SO_2, heavy metals and their residuals) discharged by the Processing Plant and spread in the surroundings (atmosphere, water, soil), and to elaborate certain appropriate proposals of recovery.

Some results of the experiments and observations on the site concerning the ecophysiological effects on the soils and waters, on the algal flora and their tolerance (a kind of biotitration of the environment) are summarized in this study.

MATERIAL AND METHODS

Soil samples were taken from a beech forest S-E of, and nearly 500 m far from the Non-ferrous Processing Plant, at about 100-150 m altitude above Zlatna ground level (samples A and B) and a little lower from a barren plot (sample C), as well as control soil sample (D) from a brown chernozem plot near the Horticultural Research Station of Cluj (H.R.S.). Water samples of Ampoi river (up- and downstream of Zlatna) and integrated polluted water descharged by the pipe line were taken. As an all control medium the Knop-Pringsheim's nutrient solution was considered.

The soil and water samples were set up in the following experimental variants:

I. soil samples (5g): A, B, C, D supplemented with 5 ml solution of:

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1. Witsch's medium compounds;
2. $Ca(NO_3)_2.4H_2O$, 1 g/l;
3. $Zn(NO_3)_2.7H_2O$, 0.1 g/l;
4. $Cd(NO_3)_2.4H_2O$, 0.05 g/l;
5. $Pb(NO_3)_2$, 0.05 g/l;
6. integrated polluted water from the pipe line;
7. Ampoi river-water from near-by Processing Plant;
8. distilled water;

as well as with:

1. EDTA, 18,6 g/l
2. urea, 3 g/l
3. Knop-Pringsheim's medium compounds;
4. distilled water;
5. Ampoi river-water up-stream Zlatna;
6. integrated polluted water from the pipe line.

II. Water samples completed with:

1. Ampoi river-water up-stream Zlatna + Knop-Pringsheim's solution;
2. Ampoi river-watr near-by Plant + Knop-Pringsheim's solution;
3. Ampoi river-water near-by Plant + Ampoi river-water up-stream Zlatna;
4. integrated polluted water + Knop-Pringsheim's solution;
5. integrated polluted water + Ampoi river-water up-stream Zlatna;
6. Ampoi river-water down-stream Zlatna (10 km) + Knop-Pringsheim's solution;
7. Ampoi river-water down-stream Zlatna (15 km) + Knop-Pringsheim's solution;

The variants thus prepared were distributed (5 repetition each) in Erlenmeyer-flasks (50 ml) or in test tubes (10 ml) and then inoculated with aliquot volume (3 or 2 ml) of algal suspension taken from a pure culture being in the exponential growth phase.

According to several pertinent studies, algae provide adequate procedures to determine trophic or toxic qualities and resilience capacities of the soils and waters. Unicellular algae (e.g. Chlorella, Scenedesmus, Ankistrodesmus) can reveal the complex effects of heavy metals or harmful residuals of the environment (B. B. Hosetti et al. 1993). Their many advantages are improved by their different specificities (W. Reichardt et al. 1993). Pure algal population grown in controlled condition in the most instances could satisfy the required "standard biomaterial ... of ecotoxicology" (S. Apostol 1994).

The biotest alga in these experiments was: Scenedesmus intermedius Chod. strain "Cazan". It was collected (June 10th, 1967) from a puddle (pH 7.5, temp. 20° C) in the Danube water-meadow at Cazane (Cazan gorge, Iron Gate),

isolated in pure cultures maintained in the "Babeş-Bolyai" University's culture Collection (F. Nagy-Tóth 1987).

The experiments were repeated twice. The results were evaluated (after 21, as well as 14 days) by cell- and optical-density measurements, and by the morphological and structural variations of the cells and coenobia. Otherwise, the procedures used in these experiments can be compared with some recently published ones (J. E. Plekhanov et al. 1990; C. D. Maxwell, N. C. McKenna 1994; H. Okamura, A. Isao 1994).

RESULTS AND DISCUSSIONS

In the vicinity of the Non-ferrous Processing Plant of Zlatna no algae could be found in the Ampoi river or in soils. From the biotopes of the surroundings 49 taxa could be identified, from which 24 were diatoms, 10 blue-greens, 15 greens (in majority). Most of them are ubiquitous common species, which can tolerate rigurous environmental conditions and arhythmic fluctuations of injurious physical and chemical factors: e.g. Gloeocapsa montana Kütz., Gomphonema ventricosum Greg., Cymbella affinis Kütz., Synedra ulna (Nitzche) Ehr., Hormidium flaccidum A. Br., Gloeotila protogenita Kütz., Chlorococcum humicolum (Naegeli) Rabenh. However, beside these common species some noteworthy (both taxonomically and arealgeographically) rheocrene, halotolerant north-alpine taxa could be remarked: e.g. Achnannes minutissima Kütz., Nitzchia linearis W. Smith, Eunotia faba (Ehr.) Grun., Pinnularia divergentissima (Grun.) Cleve, Characium ornithocephalum A. Br., Ch. sieboldii A. Br. (A. Barna, F. Nagy-Tóth 1993). The poorish algal flora, its reduced diversity is not surprising since the amounts of heavy metals, sulphates (1429.1 mg/l) and other noxious substances were higher than the standard values (S. R. Smith 1991; D. Báthory et al. 1992; A. Barna, F. Nagy-Tóth 1993).

Algal growth might be hardly registered on/in soils or waters near the Processing Plant.

Thus,

1. Soils of the beech forest (A, B) and that of the barren plot (C) moistened with:
   a) Ampoi river-water up-stream Zlatna, or
   b) integrated polluted water,
   were toxic for Scenedesmus intermedius. These soils moistened with,
   c) distilled water,

inhibited strongly (in a rate of 88-96 %) the survival of the alga (fig. 1-2). The soil of H.R.S. (D) moistened with clean river-water or distilled water supported the growth and multiplication in a rate of 25-30%, but moistened with integrated polluted water, the inhibition achieved 92-98%. This drastic effect can indicate, among others, that the protective chelatizing humic

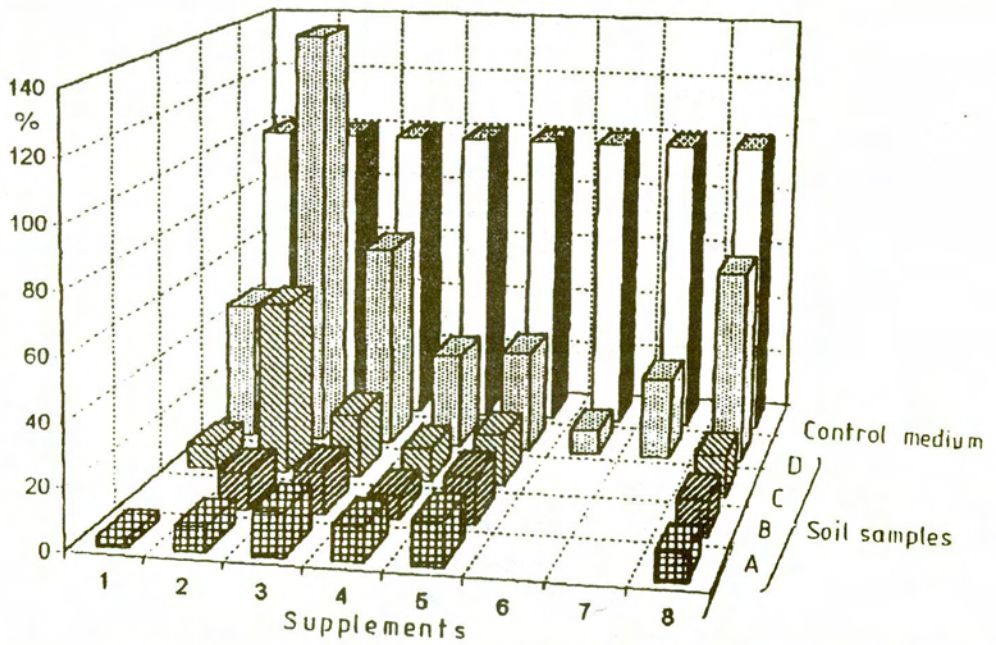

Fig. 1. – Effects of soils (A-C) and waters from Zlatna area and its supplemented variations with different compounds (1-5) on the alga Scenedesmus intermedius Chod. A-B: soil samples from beech forest, and C: from barren plot, at different altitudes; D: brown chernozem control soil; 1: Witsch's nutrient solution: $Ca(NO_3)_2.4H_2O$; 3: $Zn(NO_3)_2.7H_2O$; 4: $Cd(NO_3)_2.4H_2O$; 5: $Pb(NO_3)_2$; 6: Integrated waste water 7: Ampoi river water up-stream Zlatna; 8: Distilled water.

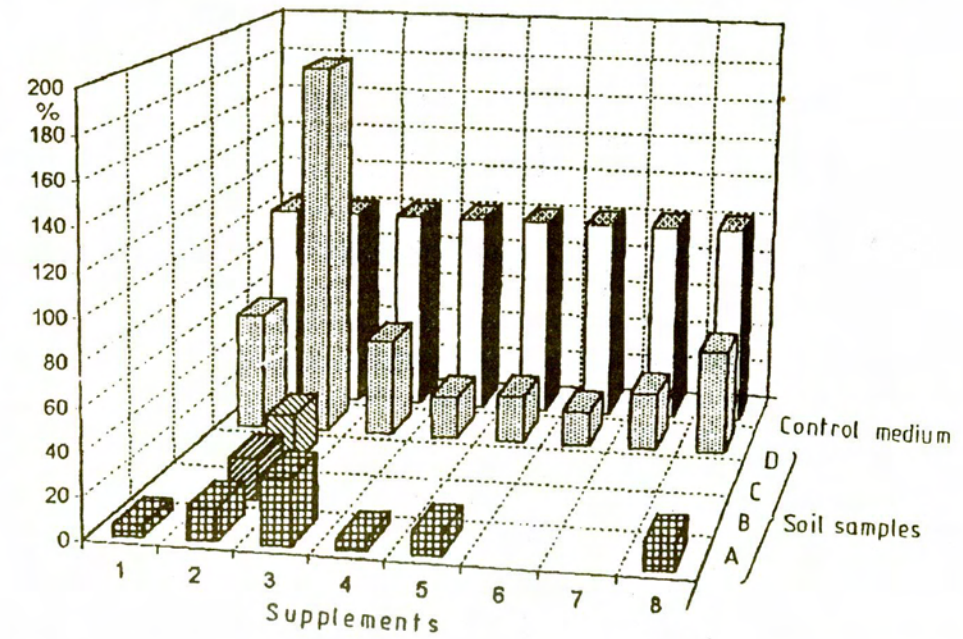

Fig. 2. – Phycophysiological potential of soils and waters of Zlatna surroundings revealed by *Scenedesmus intermedius* Chod. (expl. Fig. 1).

substances existing in "normal" soils (N. Senesi 1992; Fung-Jou Lu et al. 1994) were not enough to neutralize the toxic residuals of the polluted water discharged by the Plant.

2. Surprising results were produced by addition to the polluted soils of artificial nutrient solutions, or necessary as well as unnecessary compounds only. Such as:

a) When Knop-Pringsheim's medium (its total concentration 1.472 g/l) was added to the polluted soils (A, B, C), a complete toxicity appeared, whereas with H.R.S. soil (D) a considerable inhibition (in a rate of 40%) resulted; due probably to the ionic dis-balance of the experimental micro-environments, even if it was caused by necessary nutrient elements.

b) Witsch's medium (its total concentration being 0.181 g/l) addition lessened the harmful effects of the polluted soils (A, B, C), so the alga could grow in a rate of 5-20%. But if it was added to the "normal" soil (D), the algal growth was favourized in comparison with the Knop-Pringsheim's control solution (Figs. 1-2); reminding of the most polyvalent, balanced seminatural biphasic algal medium (F.R. Stein 1973).

c) In order to moderate, maybe to reduce the aggressivity of polluted soils and waters, within a set of experiments, the effects of EDTA were followed. The results obtained seem to be especially instructive. While it optimized unexpectedly the H.R.S. brown chernozem soil, its beneficial properties hardly tamed the injuriousness of the polluted soils and waters (Fig. 3). Though the cells remained alive, seeming integer, the chloroplasts were badly damaged. The total inhibition rate achieved 85-95%.

d) Supplied with urea, the polluted soils and waters also inhibited strongly the growth of the alga (in a rate of 95-98%). Its effect was significantly less in the H.R.S. soil, but higher in clean river-water than that of EDTA. The differences warn that N-supply only does not improve trophically damaged soils and waters; however, it can elicit eutrophicity in clean waters (Fig. 3).

e) The effects of $Ca(NO_3)_2.4H_2O$ were somewhat similar to that of urea concerning the polluted soils and waters (the inhibition rates varied between 75% and 85%), whereas in the H.R.S. soil it induced stimulation (40% and 85% respectively).

f) Supplying the soils and waters with $Zn(NO_3)_2.7H_2O$), $Cd(NO_3)_2.4H_2O$, and $Pb(NO_3)_2$, their unpropitious conditions did not change considerably. In all cases they inhibited the algal growth (in a rate of 80-90%) nearly similarly to the other compounds; whereas in unpolluted soil the inhibition was little less due probably to the chelating effects of the humic compounds. Surprisingly, Zn, which is a well known indispensable microelement (C. K. Shrotri et al. 1983; J. S. Kuwabara 1985) had not any favourable influence in the polluted soils; however in the unpolluted one its effect was significantly high. The series of

efficiency in all sets were: Cd > Pb > Zn which could demonstrate their stronger physiological influence than the buffering capacity of the soils (Figs. 1-2). Thus, the data obtained did not reveal any correlative effects of these definite compounds with the uncontrolled residuals spread in Zlatna's environment (containing the same heavy metals, too). It could be resumed, in agreement with other published data (T.A. Evdokimova et al. 1988; J. B. Mott, D. A. Zuberer 1991), that the recovery of the damaged soils is a difficult task, a long lasting process.

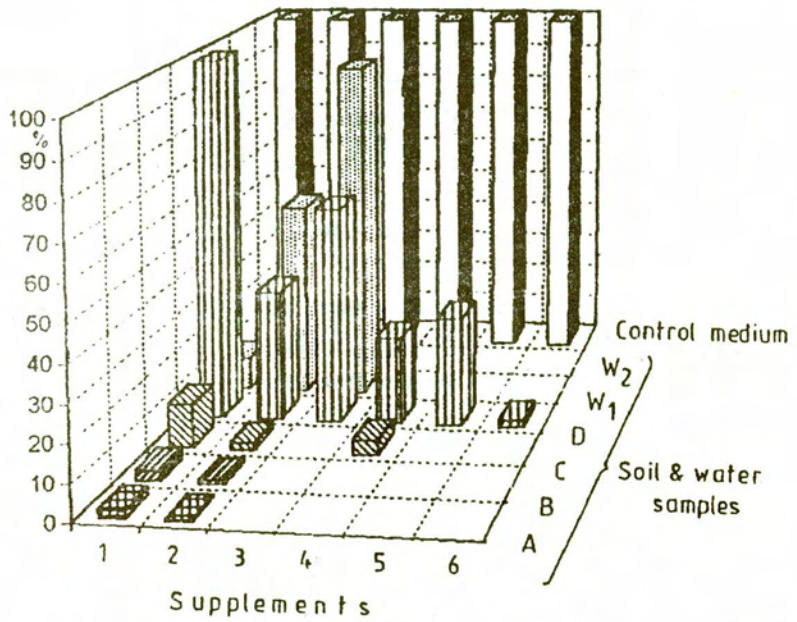

Fig.3. – Influence of different trophic materials on the phycophysiological potential of the soils and waters of Zlatna area. A-C: polluted; D: unpolluted soil; W_1: clean river water; W_2: Integrated waste water; 1: EDTA; 2: urea; 3: Knop-Pringsheim's solution; 4: Distilled water; 5: clean river water; 6: Integrated waste water.

3. Polluted water discharged by the Non-ferrous Processing Plant and that of Ampoi river were mixed (diluted) with clean river-water or nutrient solution, as well as supplemented with absolutely necessary nutrients, trying in this way to model some possible measures of softening their aggressiveness (fig. 4-5). Data gathered proffer to conclude:

a) Integrated residual water discharged, mixed with clean river-water in a proportion of 49+1 strongly inhibits the algal growth (in a rate 80-90%); whereas mixed in the same proportion with Knop-Pringsheim's nutrient solution the inhibition drops to 50%.

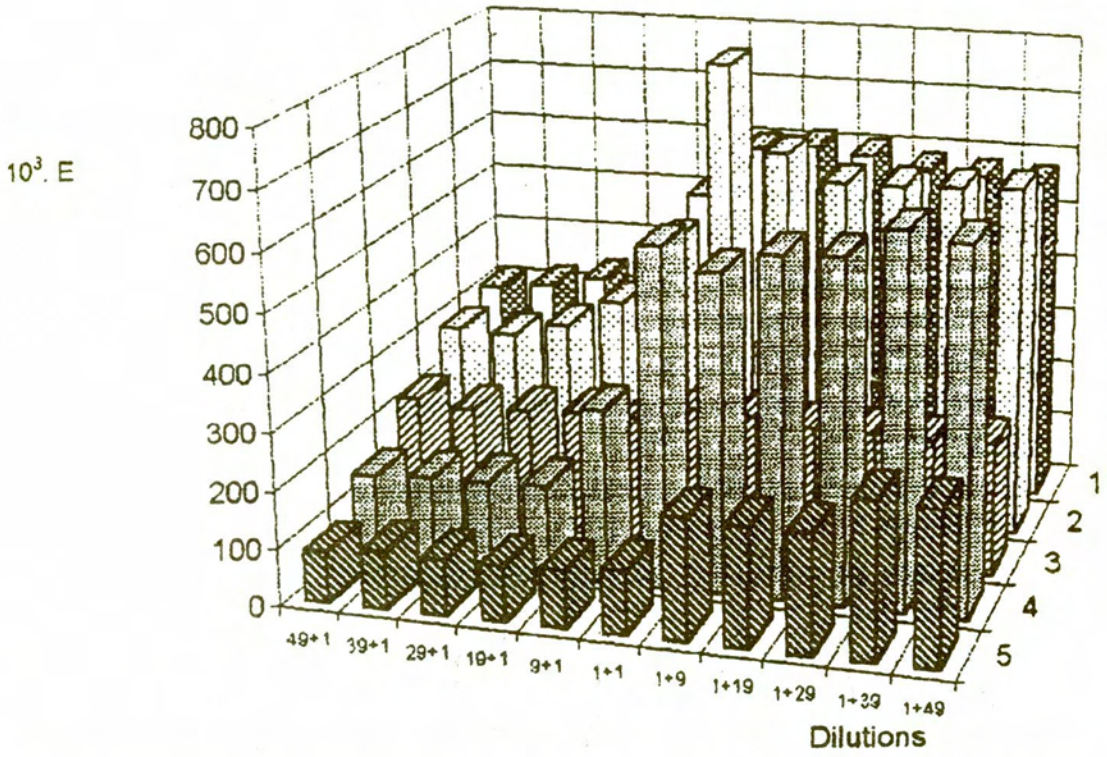

Fig. 4. – Survivability of Scenedesmus intemedius Chod. in media prepared from waste waters of Zlatna area mixing with: 1: Ampoi river waste up-stream Zlatna + Knop Pringsheim's solution; 2: Ampoi river waste near-by Processing Plant + Knop Pringsheim's solution; 3; Ampoi river water near-by Processing Plant + Ampoi river water up-stream Zlatna; 4: Integrated waste water + Knop Pringsheim's solution; 5: Integrated waste water + Ampoi river water up-stream Zlatna; 6: Ampoi river water down-stream (10 km) Zlatna + Knop Pringsheim's solution; 7: Ampoi river water down-stream (15 km) Zlatna + Knop Pringsheim's solution.

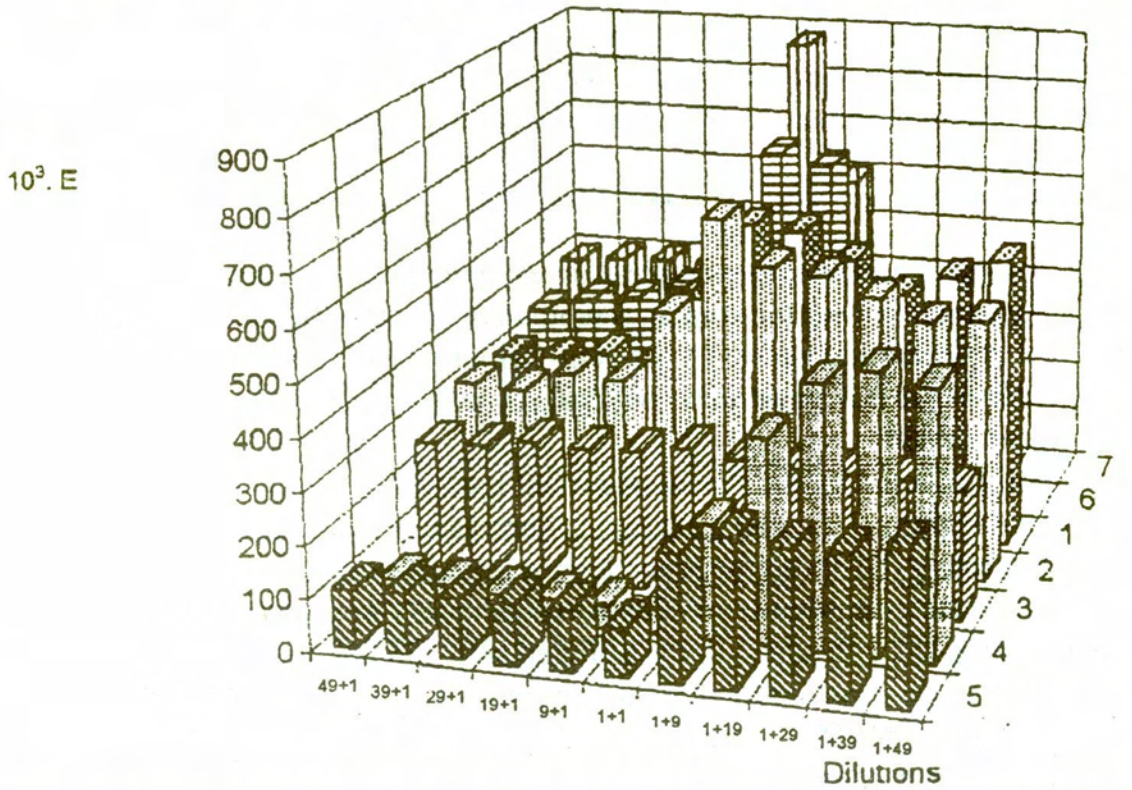

Fig. 5. – Changing the toxicity of the waters from Zlatna area by mutual mixing or supplementing with different nutrients (expl. Fig. 4).

b) Polluted river-water near-by the Processing Plant, mixed with clean river-water (in proportions between 49 +1 and 1+1) inhibited algal growth in a rate of 60%; while mixed with Knop-Pringsheim's solution the inhibition dropped to 50% and 15%, respectively. In a higher proportion the mixture became stimulant (Figs. 4/2, 5/2).

c) River-water down-stream of Zlatna (at 10 and 15 km) completed in a less proportion with Knop-Pringsheim's solution (49+1) still inhibits (30-60%), but in higher ones (19 + 1 – 1 + 19) stimulates the growth of Scenedesmus intermedius (Figs. 4/6-7, 5/6-7).

The experiments carried out can demonstrate, in agreement with previous studies (F. Nagy-Tóth, A. Barna 1982; A. Barna, F. Nagy-Tóth 1993), that there are certain possibilities to improve (neutralize, balance) trophically the polluted waters.

4. Morphoses induced by environmental factors are usual in Chlorococcales algae. They are peculiary characteristic among coenobial Scenedesmus species, and Scenedesmus intermedius implicitly (F. Nagy-Tóth 1987). During these experiments it appeared in coccoid, oocystoid and chodatelloid shape, as well as in mono-, di- and tetradesmoidic status, and in variable terratological mono-, bi-, tri-, tetra- and multispined or in apo-spined forms as well (fig. 6). Monodesmoidic coccoid, aberrated coenobia were frequent especially in the cultures grown with EDTA and urea with polluted soils.

It is interesting and even delightful to observe microscopically pleomorphic algal populations. However, in spite of numerous studies (F. Nagy-Tóth 1987), unfortunately there were not established yet precisely the cause ↔ effect correlations between a certain morphosis induced and the elicitor factor.

CONCLUSIONS

Algological and ecophysiological investigations of soils and waters of the Zlatna environment aimed to reveal the cumulative effects of the polluting residuals discharged in the air and waters by the Non-ferrous Processing Plant and based on experimental results to suggest some proposals of recovery. From the results recorded it may be concluded:

1. Soils affected by the Processing Plant moistened with Ampoi river-water (up- or down-stream of Zlatna) were toxic, whereas moistened with distilled water strongly inhibiting (88-96%) for Scenedesmus intermedius.

2. Nutrient compounds of Knop-Pringsheim's complete solution (total concentration 1.472 g/l) added to the polluted soils did not abolish their toxicity, but that of Witsch's medium (total concentration 0.181 g/l) commuted it into a strong inhibition (75-95%). Addition of EDTA, urea or $Ca(NO_3)_2.4H_2O$ to these soils tamed also their aggressivity.

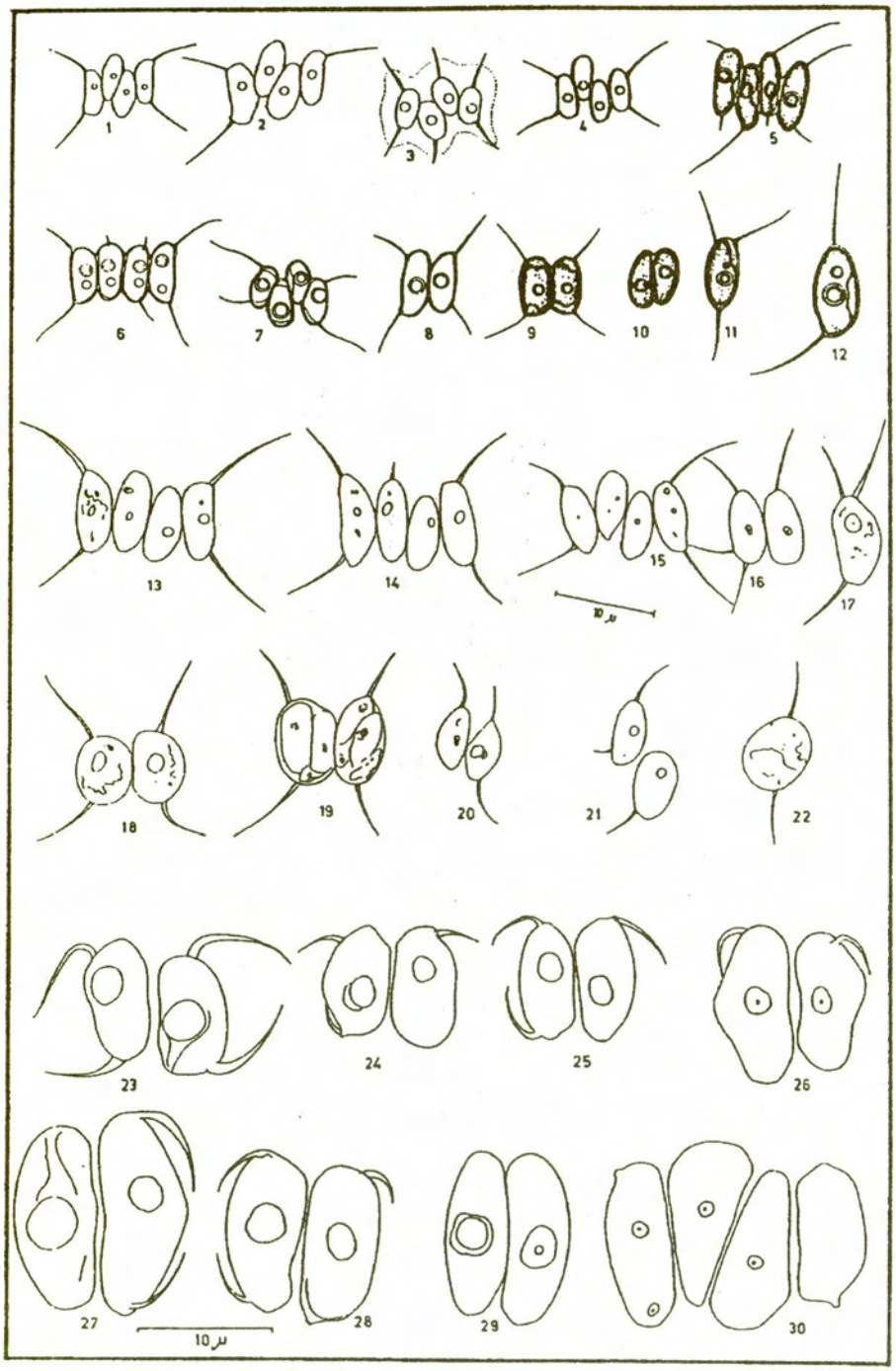

Fig. 6. – Pleomorphic and terratologic stages of *Scenedesmus intermedius* Chod. induced by environmental factors compared with some resembling taxa. 1-5: typical forms; 6: *Sc. intemedius* var. *balantonicus Hortob.* 7. *Sc. intemedius* f. *heterocaudatus Hortob.*; 8: *Sc. opoliensis* P. Richt. var. *mononensis* Chod.; 9: *Sc. ellipsoideus* Chod.; 10: *Sc. intemedius* var. *acaudatus* Hortob.; 11: *Chodatella balatonica* Scherff.; 12: *Ch. symmetrica* Hortob.; 13-14, 19: *Sc. intemedius* Chod. grown in intensive batch culture in Kuhl-Lorenzen's solution; and 15-18, 20-22: in its mixtures with waste water from Leather Factory; 23-26, 30: in static cultures in Knop-Pringsheim's; 27-28: in Benecke's agarized media; and 29: in Knop-Pringsheim-Felföldy's solution.

3. Waters in the surroundings of the Processing Plant are harmful for algae. Integrated residual water discharged into Ampoi river was toxic, but after dilution with clean river-water (50%) became inhibiting for Scenedesmus intermedius. It had a strong inhibition (92-98%) in brown chernozem soil, too. Polluted river-water near-by the Processing Plant inhibits also the algal growth, but depending on the distance from the polluting source, i.e. on dilution and enrichment during the course, it becomes more and more favourable and even stimulative (eutrophicated) for the algae.

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THE INFLUENCE OF THE MICROWAVES TREATMENT ON SOME PHENOTYPICAL PARAMETERS AT *SECALE CEREALE* L.

DORINA CREANGĂ*, ION I. BĂRA**, MARIA CERNEA+, FL. M. TUFESCU*

A study of the microwave treatment on some phenotypical parameter like: photosynthetical pigments contents, height and fresh substance mass at *Secale cereale* L. individuals was carried out. A positive influence of this treatment on the young rye plants as well as its amplification to the samples watered with an aqueous solution of KNO_3 irradiated with visible range radiations ($\lambda = 546$ nm) for 10 seconds was noticed.

INTRODUCTION

In the latest decades the influence of a wide range of electromagnetic waves on the living beings as well as on the cell tissues cultures has been more and more studied; both general and specifical actions of low intensity microwaves flows from natural and artificial sources on the animal and on the vegetable organisms [1] were analysed and the sensitivity of living beings to this type of irradiation was underlined [2, 3, 4, 5]. The stimulative action of red and blue light short exposure on the photosynthetical organisms was explained by Blinks and Emerson [6] on the basis of molecular absorption spectra of vegetable pigments. Certain papers reported the optical waves range effect on the enzymatic reactions, on the interactions of water with irradiated molecules, on the selective assimilation of abiotic factors in plants [7, 8, 9]. The overlapping of two different energy flows actions represents, perhaps, one of the major coordinates of the interpretation of radiation biological effects, if we think only about the permanent wide range frequency natural radiations which influence the human body directly or indirectly by means of food, air and water.

MATERIALS AND METHODS

Selected individuals of *Secale cereale* L., genetically uniform, were cultivated in Petri dishes (50 plants in every dish) in constant environmental conditions (24°C temperature, 98% humidity, 12 hours artificial light and 12 hours darkness). At the beginning of the experiment the seeds were already germinated. Equal numbers of individuals were studied in the presence of the microwaves treatment and in its absence; when microwaves were used, seeds exposure for two hours, before germination, was performed. Every sample (a Petri dish) was arranged at a distance of about 25 cm under the horn antenna of the microwaves generator on a dielectric support. The generator work frequency was of 10.75 GHz while its power was of 120 mW, that means 0.9 $mWcm^{-2}$ at the seed level. The support dielectric constant ($\varepsilon_r = 1$) was unfavourable to the appearance of reflected waves.

After germination the samples were watered every two hours during the artificial light half day with 10 ml portions of a 2 ‰ KNO_3 aqueous solution. The crystalline powder of KNO_3 was irradiated with a 500 l × beam ($\lambda = 546$ nm) for different time durations (suggested by the results reported previously [10, 11]: t=0; 5; 7; 10; 13 seconds). The plants heights and the fresh substance mass for every dish were measured daily during the eight days of experimental observations. The chlorophyll a, chlorophyll b and carotene like pigments contents were evaluated spectrophotometrically on the eighth day, taking into account the fact that the carotene-like pigments are synthesized in plants beginning with the sixth day. To study the plant height dynamics, a graphical method suitable to investigate and compare data points series distributions, both for large and relatively small series [12, 13, 14], was used. So, any type of distribution is transformed into a "box", characterised by the next parameters.

– Q_1, the box bottom, corresponding to a cumulated relative frequency of values appearance of 25%;

– Q_3, the box top, corresponding to a cumulated frequency of 75%;

– M, the mediane, corresponding to a cumulated frequency of 50%;

– A_1, the down tail, given by $Q_1 - 1.5*(Q_3 - Q_1)$;

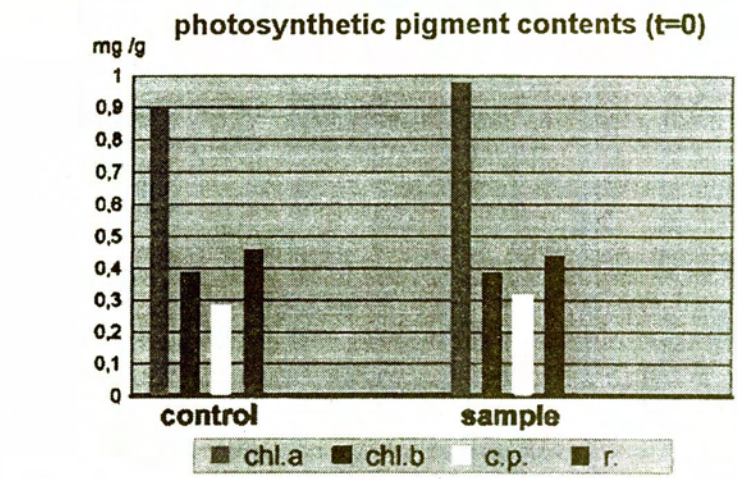
– A_3, the up tail, given by $Q_3 + 1.5*(Q_3 - Q_1)$;

– two types of outliers are revealed insight and outsight of the intervals defined by Q_1 and $Q_1 - 3*(Q_3 - Q_1)$ and Q_3 and $Q_3 + 3*(Q_3 - Q_1)$, Figure 1, The asymmetry tendency can be also easily underlined for the studied data points series.

Fig. 1. – The box graph parameters.

EXPERIMENTAL RESULTS AND DISCUSSION

In Figure 2 one can see the microwaves treatment effect on the photosynthetical pigments contents in samples watered with KNO_3 solution containing KNO_3 irradiated ($\lambda = 546$ nm) for the above time durations. The values of pigments contents are generally larger in the case of treatment with microwaves. It is also remarkable the amplitude of these values for $t = 10$ seconds.



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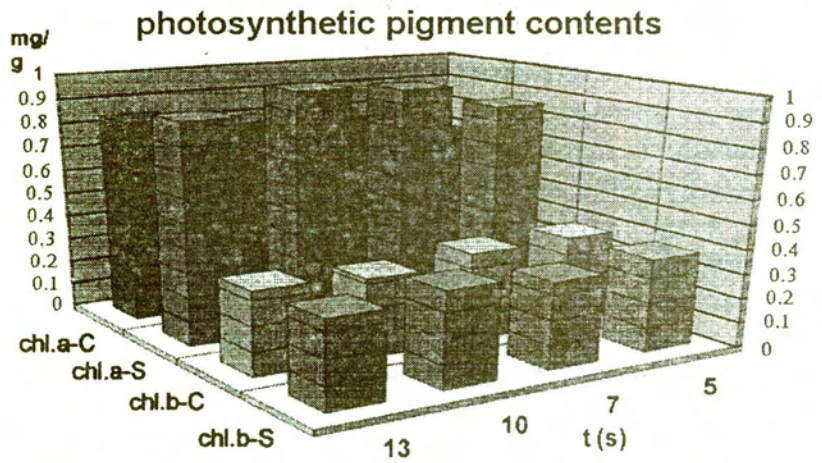

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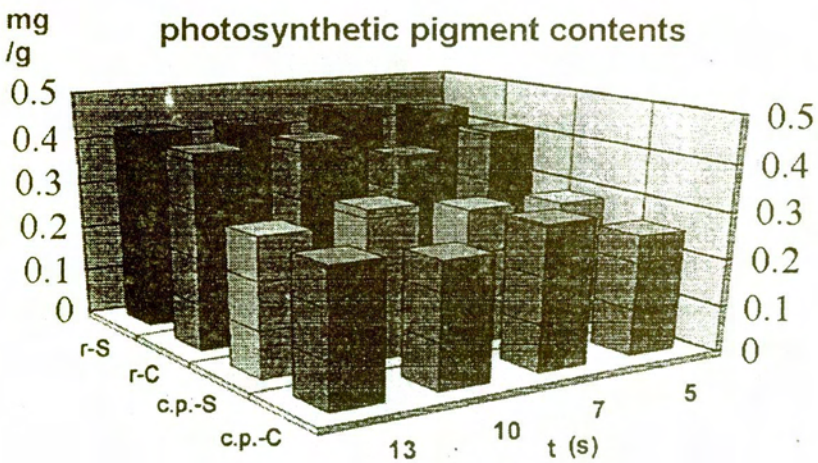


c)

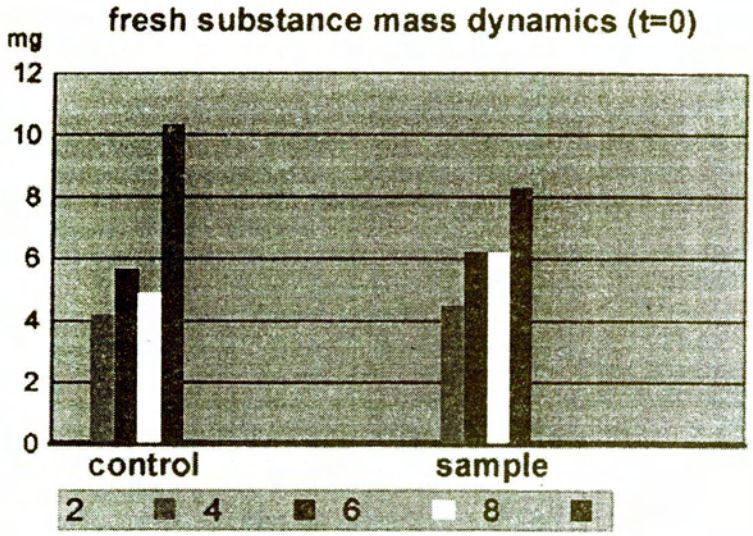
Fig. 2. – The microwaves influence on the photosynthetical pigments contents: a. – Controls and plants obtained from seeds treated with microwaves (c and s) for which $t = 0$; b. – Chlorophyll a and Chlorophyll b in controls and in plants obtained from seeds treated with microwaves (c and s) and $t = 5; 7; 10; 13$ seconds; c. – Carotene like pigments and the rate r in controls and in plants obtained from seeds treated with microwaves (c an s) and $t = 5; 7; 10; 13$ seconds.

In Figure 3 the fresh substance mass dynamics is represented by means of the values recorded on the second, the fourth, the sixth and the eighth days. The microwaves exposure leads to a relative maximum appearance on the sixth day for the same irradiation time of 10 seconds; however, a legical dependence of the studied phenotypical parameters on t could not be revealed. The positive influence of the microwaves treatment can be assigned to the energy accumulation in the form of a dielectric relaxation energy at the level of the water molecules as well as in other organic polar molecules from the seeds composition. The seeds average molar refraction in correlation with the microwaves frequency could lead to wavelength values comparable to the sizes of seeds, so it is possible that the seeds behave as resonance cavities insight of which the energy presents relative amplification areas [15, 16]. The microwaves effect amplification for $t = 10$ seconds might suggest that the direct seeds exposure to microwaves could interact in a constructive way with the indirect irradiation of the plants by means of KNO_3 ($\lambda = 546$ nm) only in the situation when the corresponding time duration is of 10 seconds; in order to explain the plants receptivity to such short time action of optical range waves, some hypotheses involving excitons, solitons, resonance type phenomena have been previously formulated [7, 3].

Analysing the plants heights dynamics, using also the values recorded on the second, the fourth, the sixth and the eighth days (Figure 4), one can notice the shaping of a relative maximum for the mediane M, in the case of microwaves treatment and for $t = 10$ seconds on the sixth observation day, when, possibly, the starting of the carotene-like pigments synthesis determines a new qualitative aspect in the growth process evolution. In the same conditions it is to remark the larger values of the box bottom, Q_1 and the concentration of the Q_1Q_3 interval to larger values too, as a consequence of low intensive microwaves involving. Generally speaking, all over the experiment observation time the microwaves determine the increase of the box parameter values together with the installation of the right side asymmetry (the increase of larger values weight).

CONCLUSIONS

We think that the low intensity microwaves treatment influence on the *Secale cereale* L. individuals could be correlated to the increase of the thermal movement energy of water molecules, but also of other organical substances existing in the seeds, both by means of the dieletric relaxation phenomena and of the local energy amplification process in the resonance cavities of seeds. It is not exclusive either the existence of a specific effect generated by the low intensity microwaves in plants and amplified when plants are watered with aqueous solution of KNO_3 irradiated ($\lambda = 546$ nm) for 10 seconds though this process mechanism could not be specified.



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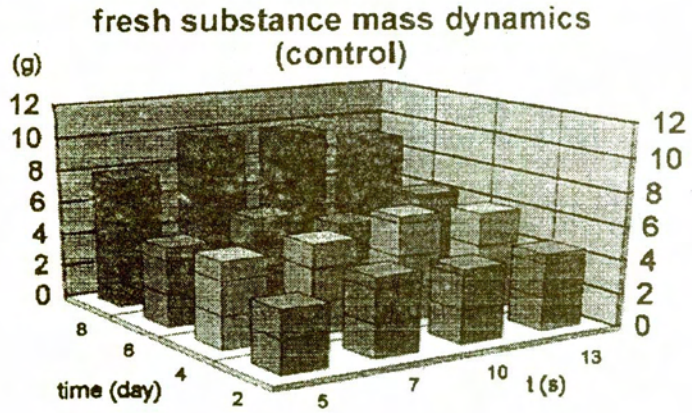

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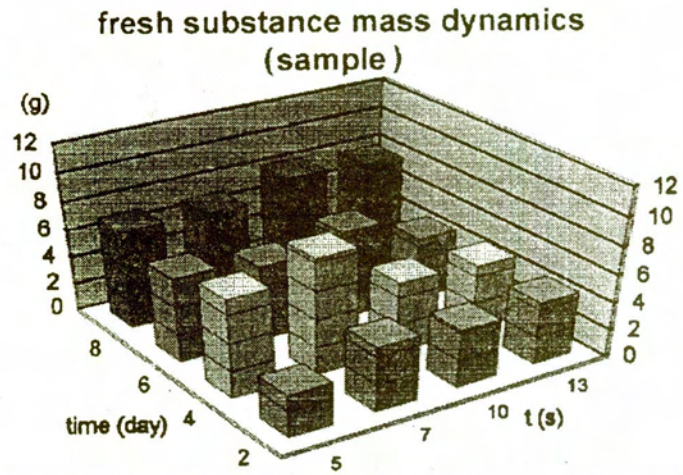

c)

Fig. 3. – The fresh substance mass dynamics: a. – Controls and plants obtained from seeds treated with microwaves (control and sample) for $t = 0$; b. – Controls; $t = 5$; 7; 10; 13 seconds; c. – Plants obtained from seeds treated with microwaves (sample); $t = 5$; 7; 10; 13 seconds.

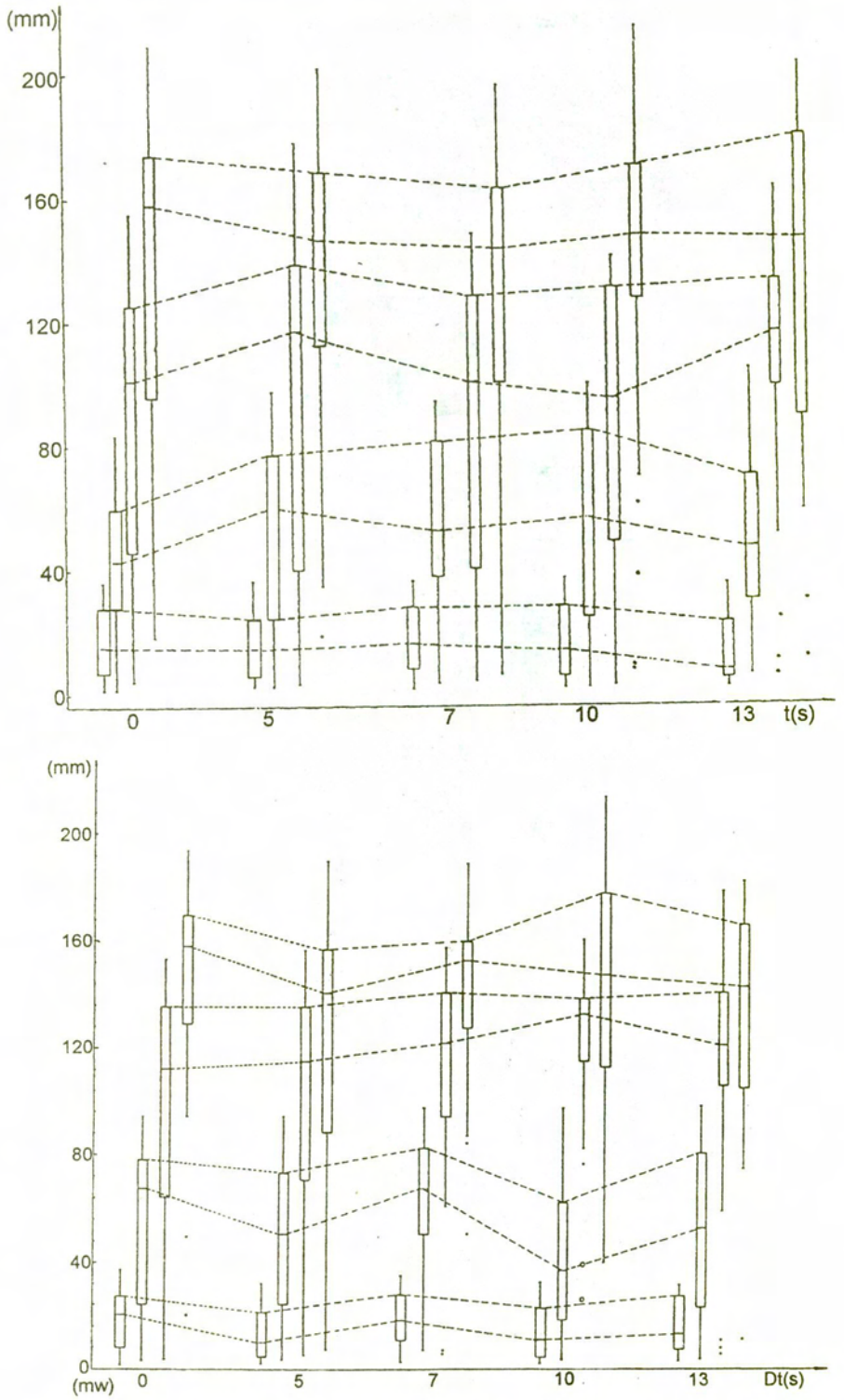

Fig. 4. – The plants height dynamics: 4a. – Controls; 4b. – Plants obtained from seeds treated with microwaves. Every group of four boxes represents the distribution curves on the second, the fourth, the sixth and the eight observation day.

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THE EFFECT OF BIOCOMPATIBLE MAGNETIC FLUIDS ON *IN VITRO* CULTURE *IN MAMMILLARIA DUWEI (CACTACEAE)* IN THE CONDITIONS OF A NEAR - NULL GEOMAGNETIC FIELD

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The presence of a magnetic fluid in culture media at *in vitro* plant culture determines different effects depending on the carrier liquid of the magnetic fluids, the concentration of magnetite particles in culture medium, analyzed features and genotype, a.o. The magnetic fluids, as well as a nearly-null geomagnetic field, alterate the growth processes (caulogenesis and rhysogenesis), division spindle orientation, a.o.
Key words: magnetic fluids, geomagnetic field, *in vitro* culture

1. INTRODUCTION

Biocompatible magnetic fluids effects on *in vitro* plant culture were studied beginning with 1992 [1, 2, 3] at different species and it was observed that in certain concentrations for each species and explant type improved the development of the plants, caulogenesis and rooting processes.

Researches about the microgravity effect on *in vitro* development of the plants were made in the U.S.A., Germany, Italy, France, Russia and Japan, and they permitted the detection of some cell organelles with role in the perception of gravity [4]. The aim of this paper is to relieve the effect of interaction between biocompatible magnetic fluids from culture media and near-null geomagnetic field on the development of plant-tissue culture.

2. MATERIAL AND METHOD

As biological material were used *in vitro* neoformed plantlets, two months age, of *Mammillaria duwei* (*Cactaceae*). The explants were inoculated on MS basal medium with growth hormones (1.8 mg/l IAA and 0.011 mg/l KIN) - control medium, corresponding to 610 variant, or supplemented with biocompatible magnetic fluids with water as carrier liquid (LMW) at different concentrations of magnetite particles ($\Phi = 12 \cdot 10^{-6}$; $24 \cdot 10^{-6}$; $36 \cdot 10^{-6}$ cm^3 Fe_3O_4 / cm^3 culture medium, corresponding to the variants 613, 614 and 615), or on a control medium supplemented with a magnetic fluid with petroleum as carrier liquid (LMP) in the same concentrations of magnetite particles (the 616, 617 and 618 variants). Half of inoculated plantlets of each medium variant were maintained 111 hours in a

near-null geomagnetic field (0-GMF), at dark and 15°-17°C, and the other half was maintained in normal geomagnetic field (NGMF), the same conditions. Then, until 60 days the plantlets were grown in normal conditions for *in vitro* cultures. There were made observations about growth rhythm and rooting process time of 60 days after inoculation.

3. RESULTS AND DISCUSSIONS

The growth in diameter was initially inhibited on control medium in NGMF (0%) in comparison with the results recorded on the same medium in 0-GMF (3% at 14 days; 18.2% at 45 days). At 60 days (Fig. 1) there was observed that the best values for the growth in diameter with NGMF were recorded on medium supplied with $36 \cdot 10^{-6}$ cm^3 Fe_3O_4/ cm^3 medium LMW (22.2%) in comparison with control (8.2%) and on medium supplied with LMP, also at the highest concentration (40%).

In 0-GMF, LMW partially (at $\Phi = 12 \cdot 10^{-6}$ cm^3 Fe_3O_4 / cm^3 medium) or totally (at the highest concentration) affected the growth in diameter of plants. By contrary,

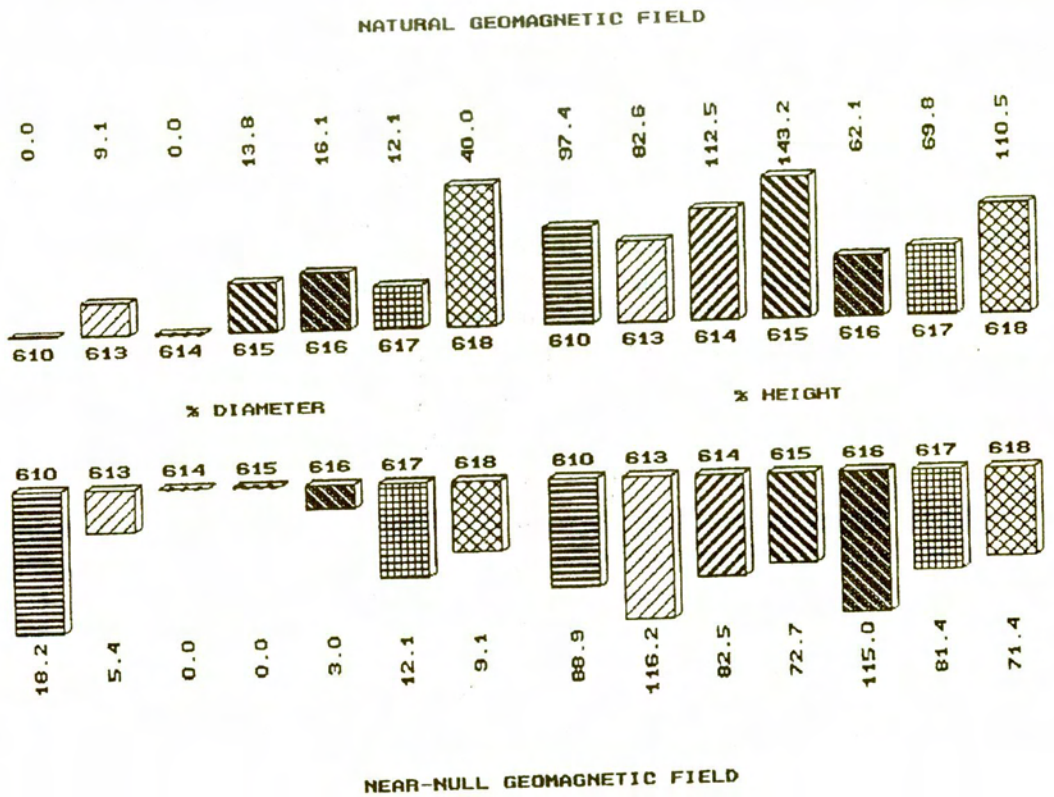

Fig. 1 – The effect of the magnetic fluids (LMW - 613, 614, 615 variants; LMP - 616, 617, 618 variants; control - 610 variant) and of the geomagnetic field (natural and near - null) on the *Mammillaria duwei* plant development, 60 days after inoculation (*in vitro* culture).

LMP stimulated the process, the results obtained at a big concentration being superior 21.2% to the control in 0-GMF (18.2%) as well as in NGMF (8.6%). These phenomena suggest that the magnetic fluids from culture media and/or the near-null geomagnetic field affected the orientation of the cell division spindle from the meristematic tissues (Fig.1). For the growth in height of the plant there was recorded the stimulatory effect of the presence of LMW and LMP in culture media in NGMF, as well as in 0-GMF, but if in the first case the best results were obtained at the highest concentration, in the second case they were recorded at the lowest concentration in magnetic fluids. 60 days after inoculation, the growth in height was 97.4% in NGMF and 88.9 % in 0-GMF at 610 variant. The presence of LMW in culture media improved the growth in height of the plants at a concentration of $36 \cdot 10^{-6}$ cm^3 Fe_3O_4 / cm^3 medium (143.2%) in NGMF. By contrary, in 0-GMF the best results were obtained at 12 $\cdot 10^{-6}$ cm^3 Fe_3O_4 / cm^3 medium (116.2%). Similar results were obtained on media supplied with LMP, but at lower values (Fig. 1).

The rooting process (Fig. 2) was inhibited in 0-GMF, rooting percent being 50% on control medium in comparison with 100% in NGMF. The presence of magnetic fluids stimulated rooting in 0-GMF. Therefore, rooting percent grows up

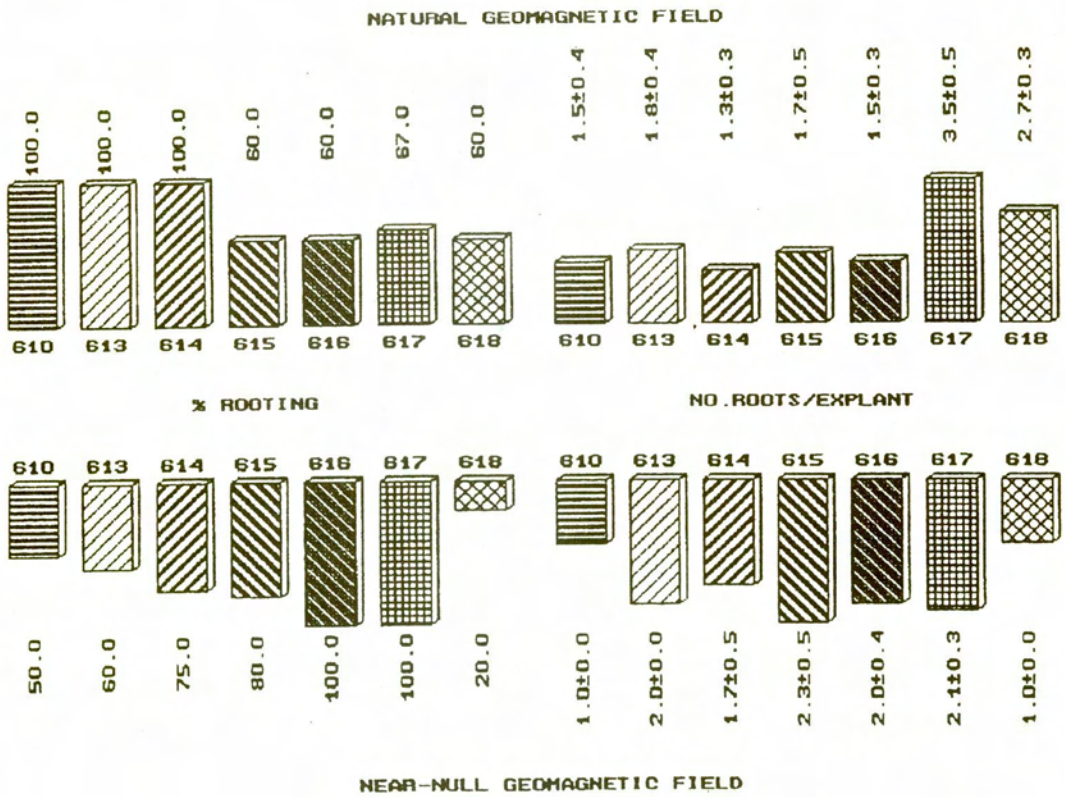

Fig. 2 – The effect of the magnetic fluids (LMW - 613, 614, 615 variants; LMP - 616, 617, 618 variants; control - 610 variant) and of the geomagnetic field (natural and near - null) on the rooting process *in Mammillaria duwei,* 60 days after inoculation (*in vitro* culture).

with a concentration in LMW from 60 % at the lowest concentration to 80% at the highest concentration. LMP in a concentration of $12 \cdot 10^{-6} - 24 \cdot 10^{-6}$ cm^3 Fe_3O_4 / cm^3 medium produced a 100% rooting process, while the highest concentration had an inhibitory effect (20% rooting).

4. CONCLUSIONS

In NGMF there was observed a positive correlation between the growth rhythm and the concentration in LMW, by contrary with the situation with 0-GMF where the correlation is negative.

The use of LMP revealed a positive correlation between the growth in diameter and the concentration in LMP in culture medium, in NGMF as well as in 0-GMF.

The growth in height of the plantlets inoculated on culture media supplied with LMP presented a positive correlation with the concentration in LMP in NGMF, and a negative one in 0-GMF.

The rooting process was inhibited in 0-GMF, being reduced at 50%, but this problem can be solved by supplying the culture media with LMP ($\Phi = 12 \cdot 10^{-6}$; $24 \cdot 10^{-6}$ cm^3 Fe_3O_4 / cm^3.

The magnetic fluids from culture media and/or a near-null geomagnetic field affected the orientation of the cell division spindle from the meristematic tissues. This determines the modification of the rate between the plant diameter and height.

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CYTOLOGICAL PECULIARITIES OF THE TUMORAL TISSUE CELLS INDUCED BY WILD AND GENETIC MODIFIED STRAINS (KANAMYCIN RESISTANT) OF *AGROBACTERIUM TUMEFACIENS*

AURELIA BREZEANU, ALEXANDRINA CĂLIN, PETRUȚA CORNEA, MARGARETA COSTACHE-IORDAN

Tumoral tissue cells induced by infection of *Solanum tuberosum*, *Populus sp.*, *Vitis vinifera* and *Nicotiana tabacum* plants with wild and kanamycin resistant strains of *Agrobacterium tumefaciens* were analysed by histological and electronomicroscopical techniques in order to characterize the wounding response as well as the possible differences of the explants cells reaction after infection with bacterial strains. The infection with *Agrobacterium* strains induced pronounced histological modifications within few days, including extensive cell proliferations, "meristemoids" differentiation as well as numerous nodules with core of tracheids surrounded by cambial like layers. At the marginal area caulinary buds appeared frequently. The C_{58} strain was able to induce rooty tumours at *V. vinifera*, a tmr mutation which affects the gene for the biosynthesis of the cytokinin precursor, and A_6 strain induced shooty tumors at potets Desiree cultivar. At the cellular level a nucleus with numerous nucleoli and an extremely lobated aspect are common. These is followed by nuclear fragmentation which finally could determine the epigenetic reversible modifications. At the same time, numerous membranary formations differentiated from external membrane of nuclear envelope which we explained as a stress effect of the bacterial infection. Nuclear peculiarities of the tumor cells are similar with those described for plant habituated cells and animal cancer cells.

The phytopathogenic soil bacterium *Agrobacterium tumefaciens* has the capacity to transfer a specific DNA segment (T-DNA) of its tumor inducing (Ti) plasmid to plant cells (dicotiledonous especially) during infection of fresh wounded tissue. Within these cells the T-DNA is integrated in chromosomal DNA and expressed. This natural vector system has been successfully applied to a broad range of dicotyledonous species and some monocotyledons.

The DNA integration into plant genome influences greatly the pattern of plant cell evolution and causes crown-gall tumour formation by transformation of healthy cells into tumour ones. A cytological analysis of the main steps of this complex process would be of interest in order to characterise the wound response and the possible differences of the explant cells reaction after infection with *Agrobacterium* strains. These analyses could also indicate the nature of the possible changes at the cellular level in the transgenic plants.

Studies in this respect are scarce and they are focused on the tumoral tissue induced by wild *Agrobacterium* strains, especially (6), (9), (10).

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The histological and electronomicroscopical peculiarities of the cells from tumour tissue (crown-gall tissue) induced by the wild and transformed (kanamicin resistance) *Agrabacterium* strains in comparison with wounded callus cells and habituated callus culture are presented in the paper.

MATERIALS AND METHODS

Three strains of *Agrobaterium tumefaciens* were used in our experiment[1]: a nopalin strain *A. tumefaciens* C_{58} with plasmid $pTiC_{58}$ (nos^+tm^+); the octopine strain *A. tumefaciens* A_6 containing the plasmid $pTiA_6$ (ocs^+tm^+) and a kanamycin resistant strain derived from A_6 (a tumorigenous strain, containing Tn5 from pJB4JIP integrated in T-DNA region of pTi A_6-K_1).

The bacterial strains were grown in LB medium containing 2% agar and bacterial suspension for infection in LB broth for 48h at 28°C.

The tumors were induced on *Vitis vinifera* L. (Fetească albă cv.), *Solanum tuberosum* (Desiree cv), *Nicotiana tabacum* L. (Xanthi cv.), and *Populus tremula* species. The infection of the plant tissues was performed *"in vitro"* conditions in three ways:

– by inoculation of bacterial suspension at a wounding site on the midrib of the leaf;

– by cocultivation of the leaves discs and stem explants with bacterial suspension (8-10 min);

– by wounding of the stem followed by infection.

Plant tissues used for bacterial infection represented by leaf, stem or minitubers tissue provided from the "in vitro" regenerated plants by meristem culture or axillary buds culture.

The infected material was placed initially on a basal Murashige-Skoog nutrient medium (MS) without hormones. After 48 hours it was transferred on the same medium but supplemented with carbenicillin (500 mg/L) or a mixture of carbenicillin+ampicillin (500 mg/L+500 mg/L) for bacteria elimination. After three subcultures the tumours were transferred on MS medium without antibiotics. In the case of the tumors obtained after infection with *A. tumefaciens* K_1, they were placed on MS medium containing kanamycin (100 mg/L).

Histological analyses were performed according to standard techniques, namely a fixation in Navashin Brown solution, paraffine embeding, sectioning at 8-10 μm and Erlich hematoxylin staining.

For electronomicroscopical analyses, small pieces of tumors 1-2 mm thick were prefixed overnight at 4°C in 3% glutaraldehyde solution in phosphate buffer 0.2M and fixed for 2 h at room temperature in 2% OsO_4 in the same buffer. After embedding in Epon 812 resin, specimens were sectioned at the Tessla ultramicrotome, stained by Reynold's methods and visualised at the Phylips and Tesla BS 600 electronmicroscope of the electronous acceleration of 80 KV.

[1] – Nopaline strain is a gift from the Institute of Plant Genetics, Slovak Republic;
– Octopine strain A_6 belongs to the collection of our laboratory;
– Kanamycin resistant strain was obtained in our laboratory by insertional mutagenesis (*13*).

RESULTS

The infection of different plant tissues with *A. tumefaciens* strains, wild as well as transformed ones (kanamycin resistant), induced pronounced histological and cytological modifications on wounded site tissue within a few days.

HISTOLOGICAL ANALYSIS

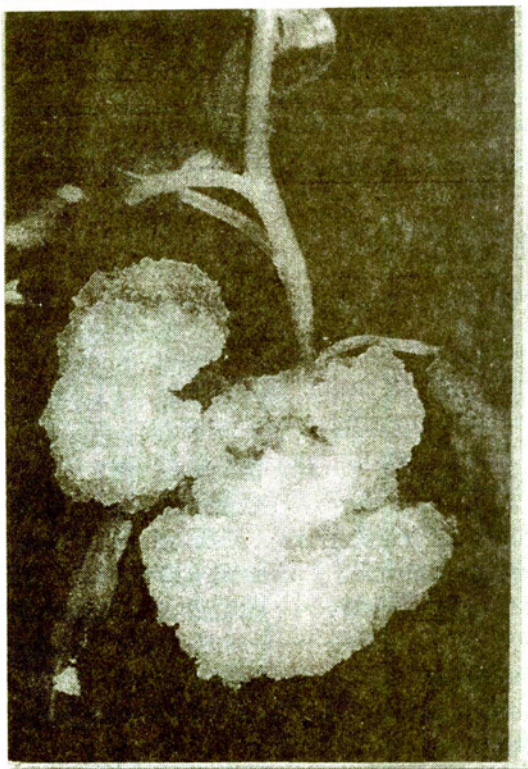
At 8-10 days after tissue infection, a callus with a specific morphological aspect developed (Plate 1). This tissue presents a continuous, anomalous and autonomous growth as an effect of molecular changes in the biosynthesis of auxin and cytokinines. Morphologically appeared like a aggregates of unorganised cells as a result of extensive cells proliferation. The C_{58} strain was able to induce rooty tumors at *Vitis vinifera* L., a tmr mutation which affects the gene for the biosynthesis of the cytokinin precursor and A_6 strain induced shooty tumors at potato Desiree cultivar (Plate I) (4), (5). Generally periclinal cell divisions with any further evolution are observed in the initial stages of the development. "Meristemoids" differentiation and numerous nodules with core of tracheids surrounded by cambial like-layers, in cortical as well in central area of the tissue are observed (Plate II). As a result, a mosaic of cellular types is a characteristic of the tumoral tissue. At the marginal area caullinary buds appeared frequently (Plate I) (3).

ELECTRONOMISCROSCOPE OBSERVATIONS

Ultrastructural observations of tumour tissue cells revealed various submicroscopical aspects including undifferentiated cells as well as cells in different stages of cytodifferentiation (phloematic or/and xylematic cells), senescent ones, in many respects similar to normal callus cells. At the same time numerous peculiarities appeared.

The most obvious and noticeable cytological aspect observed in tumor cells involved the nucleus and concerned: the nuclear shape, number of nucleoli, nuclear fine structure, nuclear anvelope aspect.

Commonly in tumoral cells the nucleus is elongated irregularly in outline and presents a very lobated aspect (Plate III, fig. 1-2) with deep invaginations of both membranes of the nuclear envelope. This is frequently followed by nuclear fragmentations and the appearence of some polynucleated cells which could indicate that bacterial infection transforms differentiated cells of the plant tissue into active meristematic ones. Finally this could determine the epigenetic reversible modifications of the cells. In some situations many perturbations of the nuclear envelope appeared (Plate IV, fig.1,4). Increasing of the perinuclear spaces, an emission of the small vesicles like bodies with heavy core delimited by a simple membrane, lying between the two membranes of the nuclear envelope are present (Plate IV). The role of the vesicle like bodies in the genetical transformation process is



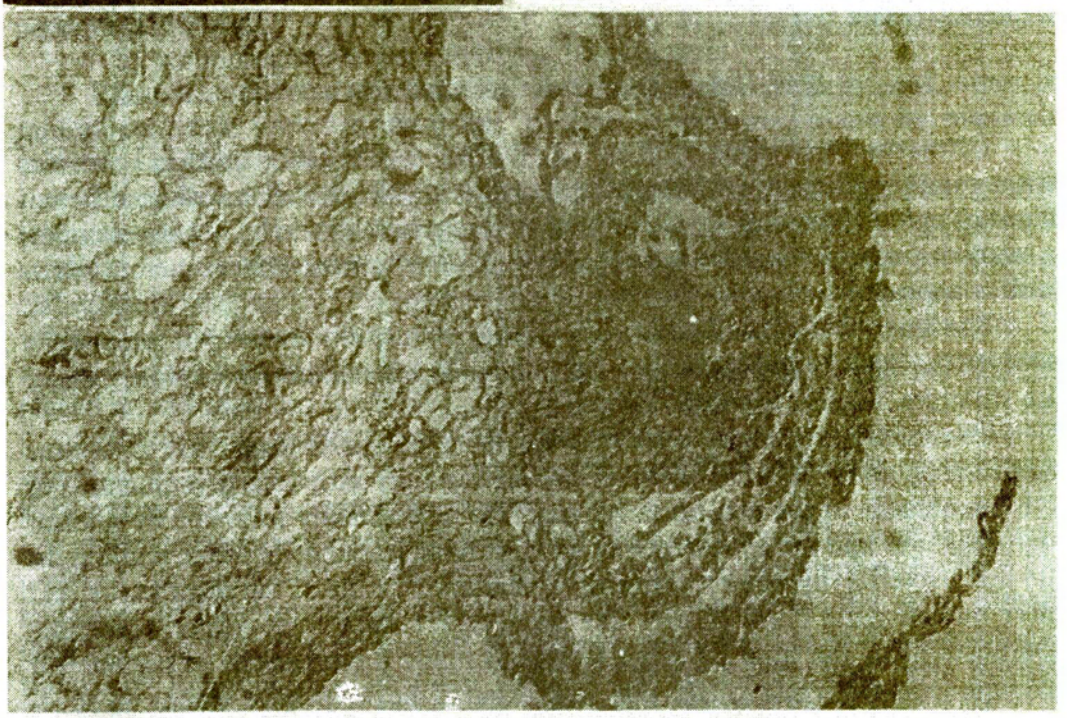


PLATE I. – Morphological aspect of the tumor tissue induced after infection with *Agrobacterium tumefaciens;*
– Rooty tumor on the stem of *Vitis vinifera* (Fetească albă cv.) after infection with nopaline C_{58} strain of *Agrobacterium tumefaciens*;
– Histological aspect of caulinary buds differentiation on the marginal areas of the tumor.

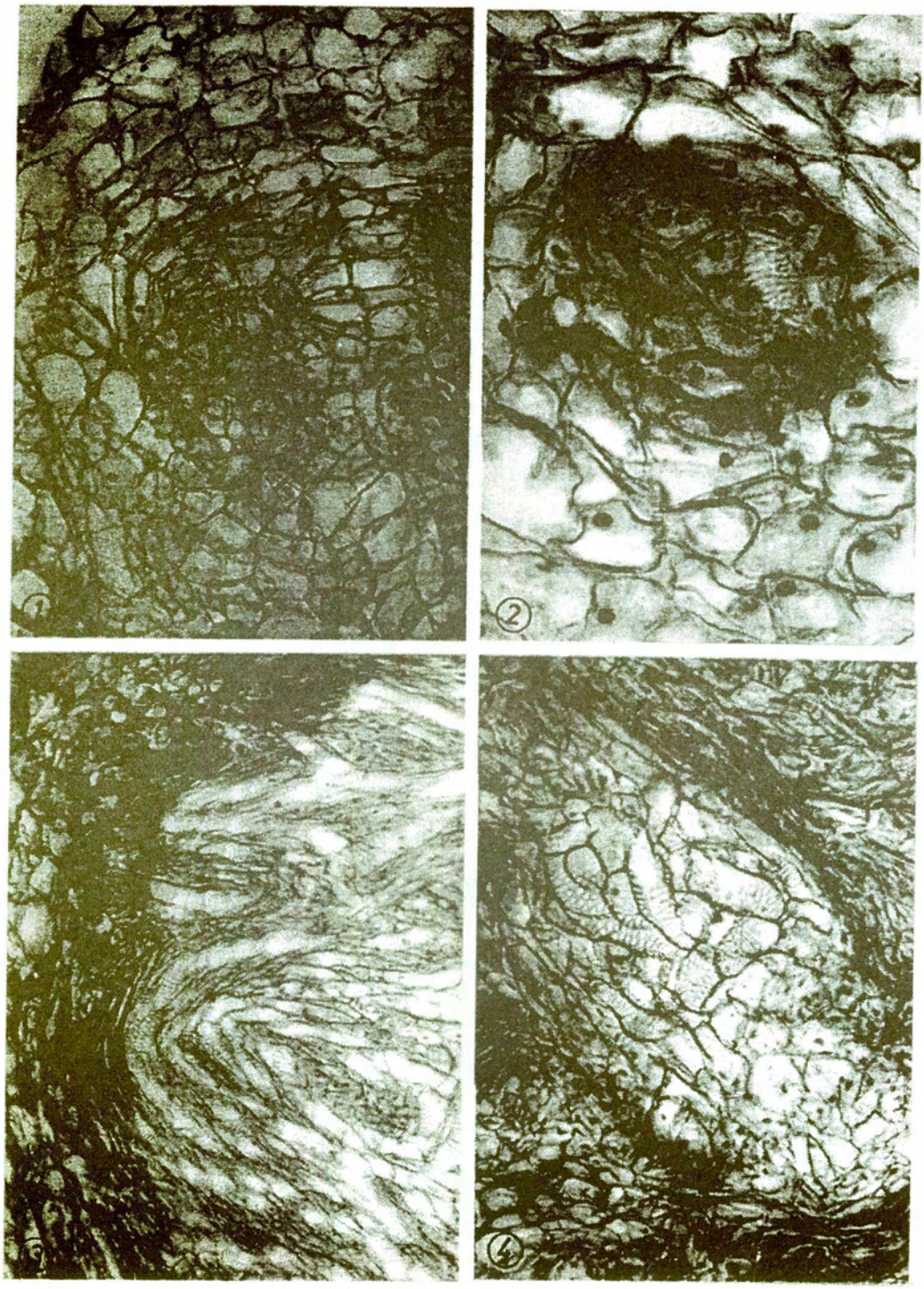


PLATE II: Histological aspect of tumor tissue. Successive stages of the characteristic tracheary elements differentiation from "meristemoids" randomly distributed in the tissue (Fig. 1, 3 - 100×; Fig. 2, 4 - 200×).

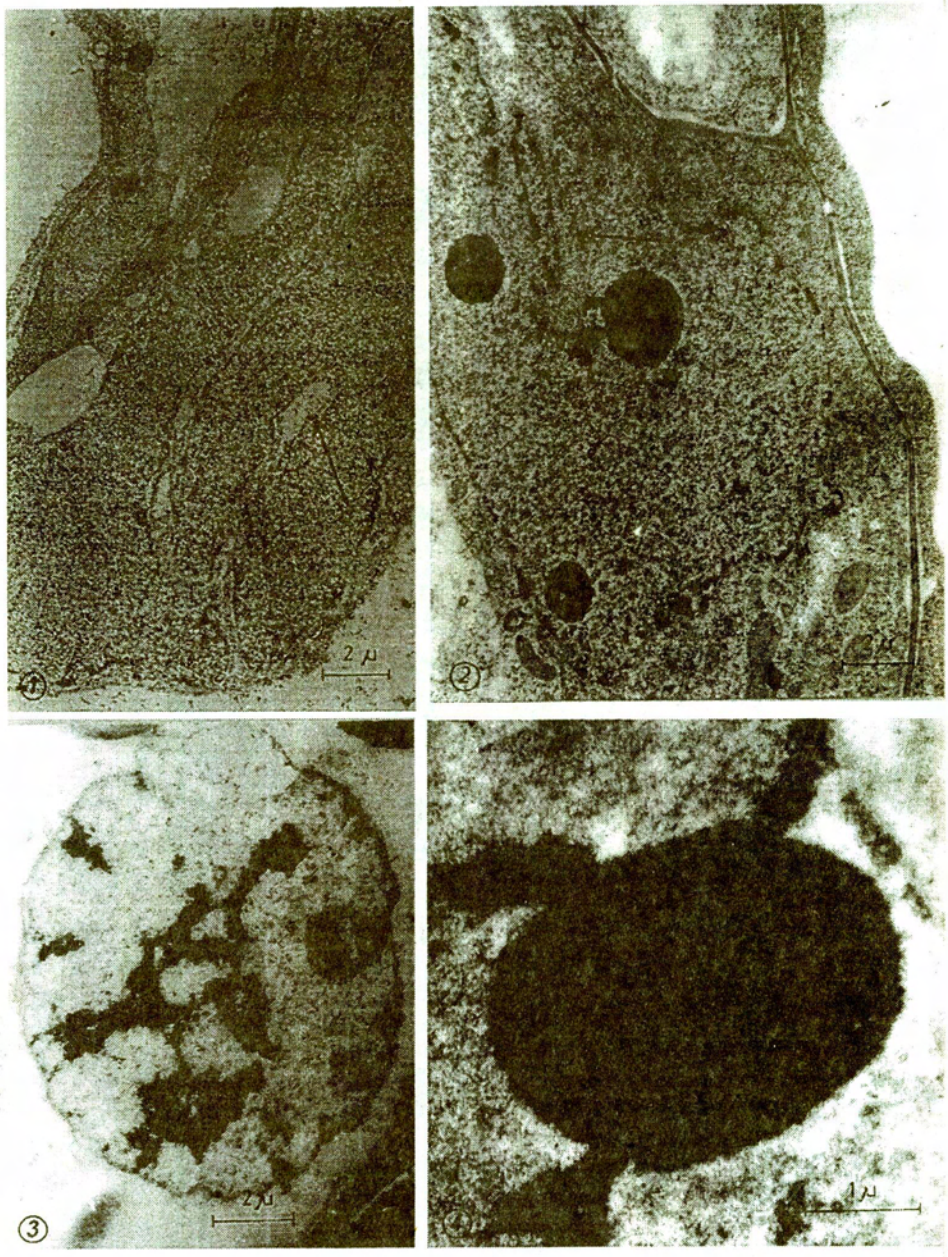


PLATE III. – Ultrastructural peculiarities of the tumor cells nucleus. The extremely irregular outline of the nucleus (Fig. 1, 2 - see arrows) and the presence of several nucleoli is noticed (Fig. 2). A nucleolus with three nucleolar organisers is evident (Fig. 4).

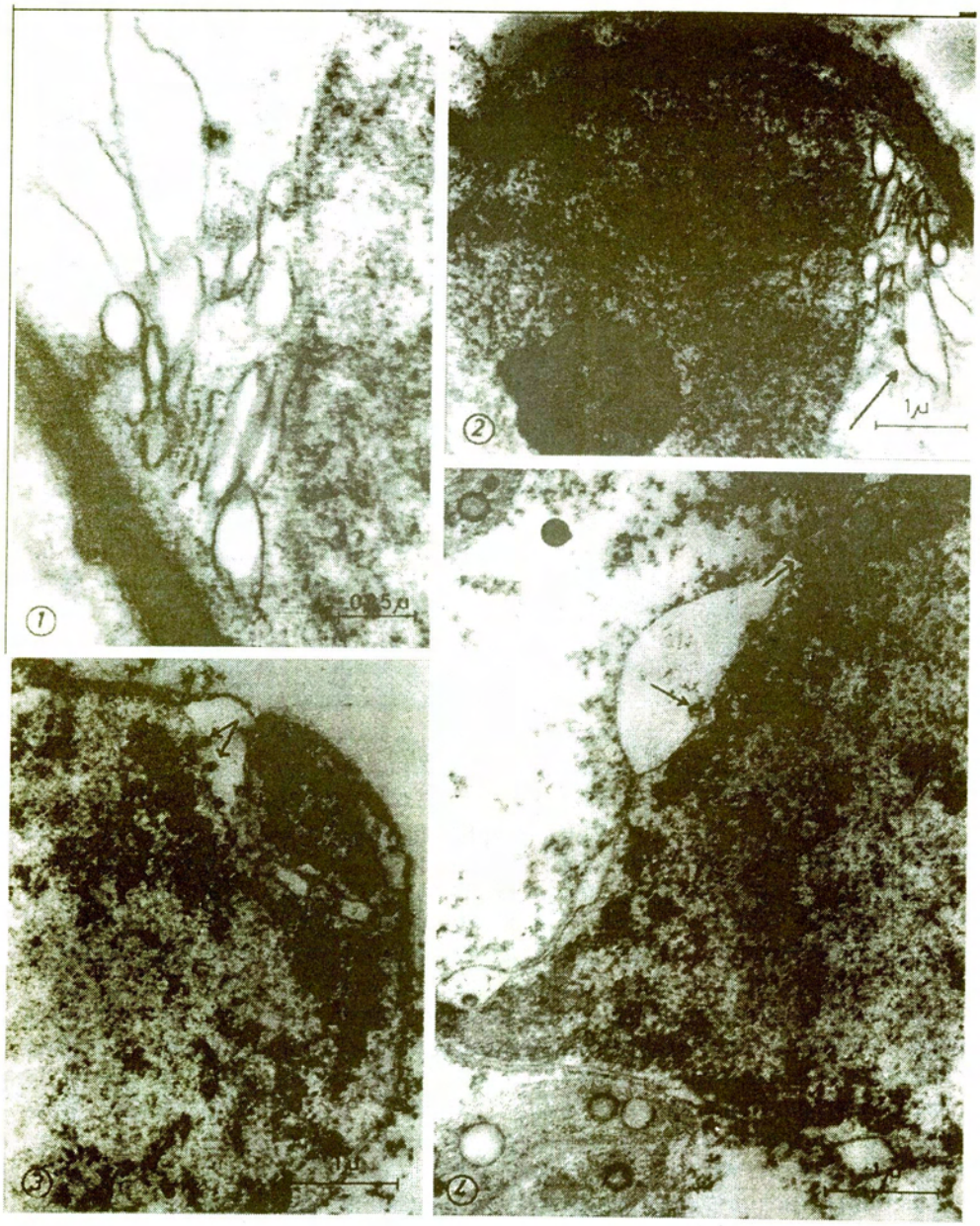


PLATE IV. – Specific modifications of the nuclear envelope (Fig. 1/fig.2 - see arrows) and an emission of small vesicles like bodies with heavy core delimited by a simple membrane lying between the two membranes of the nuclear envelope (Fig. 3-4, see arrows).

not established so far. The older papers speculated the possibility of these vesicles to carry the bacterial DNA in the plant cell nucleus (9). Anyway their involvement in the material exchanging between the nucleus and the cytoplasm would be possible. At the same time numerous membranary figures originated from the outer membrane of the nuclear envelope are observed. The functional significance of these formations is still unknown but could be, also, involved in an active exchange between the nuclear material and the cytoplasm, by specific mechanisms.

The number of nucleoli varied, generally more than two nucleoles being observed (Plate III, fig. 2). It could be an effect of the nucleolar fragmentations or may be related to the number of nucleolar organisers observed in the cells. This aspect also indicates the possibility of appearance of some polyploid mitoses in relation with "meristemoids" organisation and cytodifferentiation initiation.

An increase in the number of nucleoli was also reported by Ghadially (7) as an effect of the malignancy and to the aneuploidy of cancer cells in mammalia.

The chromatin appeared like euchromatin or as masses of dense chromatin inside of the nucleoplasm or attached to the inner membrane of the nuclear envelope (Plate V).

The presence of numerous microbodies spread throughout the cytoplasm is another characteristic of the tumor cells (Plate VI).

Each microbody consists of a voluminous crystal core in the middle and it is surrounded by a single membrane. In our experiment they are not in relationship to other organelles as in normal plant tissue (Plate VI).

Their physiological role in tumor cells is still unclear but the possible involvement in some specific proteins with high molecular weight accumulation is discussed (6), (10).

Plastids appeared like elongated organelles with an electronodense stroma and a poor lamellar system (Plate VI).

These cytological aspects were presented in tumour cells of all species studied which conduct us to idea that these are a general characteristic of transformed cells by *Agrobacterium* strains.

Some peculiarities of the nuclear apparatus of the crown-gall tissue as well as of the genetic modified tumor tissue as nuclear morphology (a large lobated outline, an increased number of nucleoli) are common in habituated cells, which are also auxin and cytokinin-autonomous (6), (8), (12).

Intensive and continuous proliferative activity of the habituated cell, on a culture medium on which normal cells are unable to grow, suggests that habituated cells might be considered as tumor cells (6). This idea is supported by Hagege's experiments regarding (unpublished data) analyses of nuclear DNA content of the habituated cell that indicated a high level of polyploid and aneuploid cells with about 67% of nuclei with a DNA content higher than 5C.

Biondi et al. (1), by polyamines level studies in tumours of higher plants and habituated tissue in correlation with nucleus and nucleoli peculiarities, and by analogy with similar phenomena which appeared in neoplasmic mammalian cells, revealed an interesting idea, that evolved polyamine levels could be associated with neoplasmic growth and considered that studies on plant tumors as well as habituated tissue make it possible to identify oncogenes in plants.

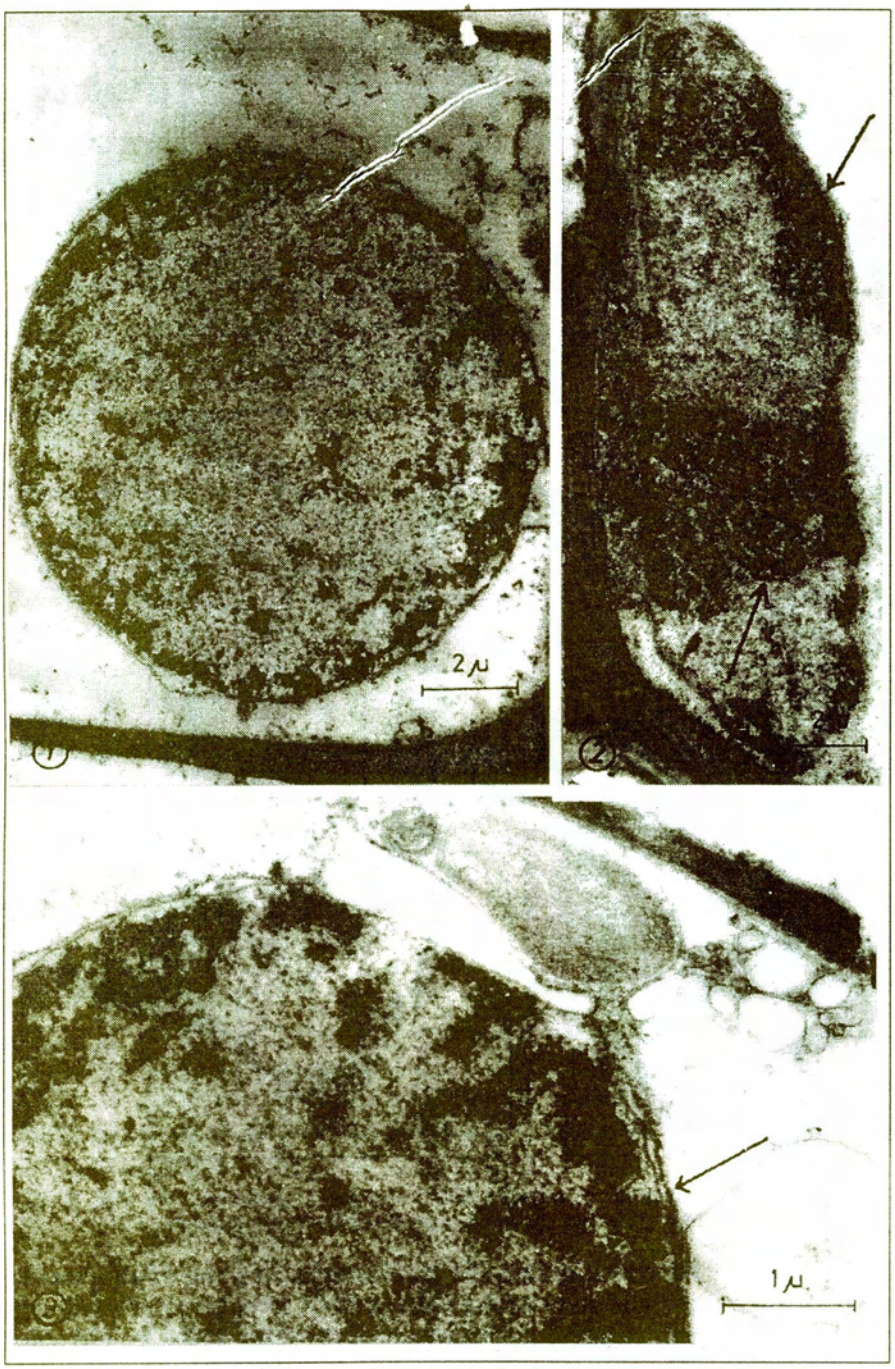


PLATE V. – The characteristics of the chromatin distribution in the nucleus of tumor cells: finely dispersed (Fig. 1) or dense masses of chromatin attached to the inner membrane of the nuclear envelope (Fig. 2, 3 - see arrows).

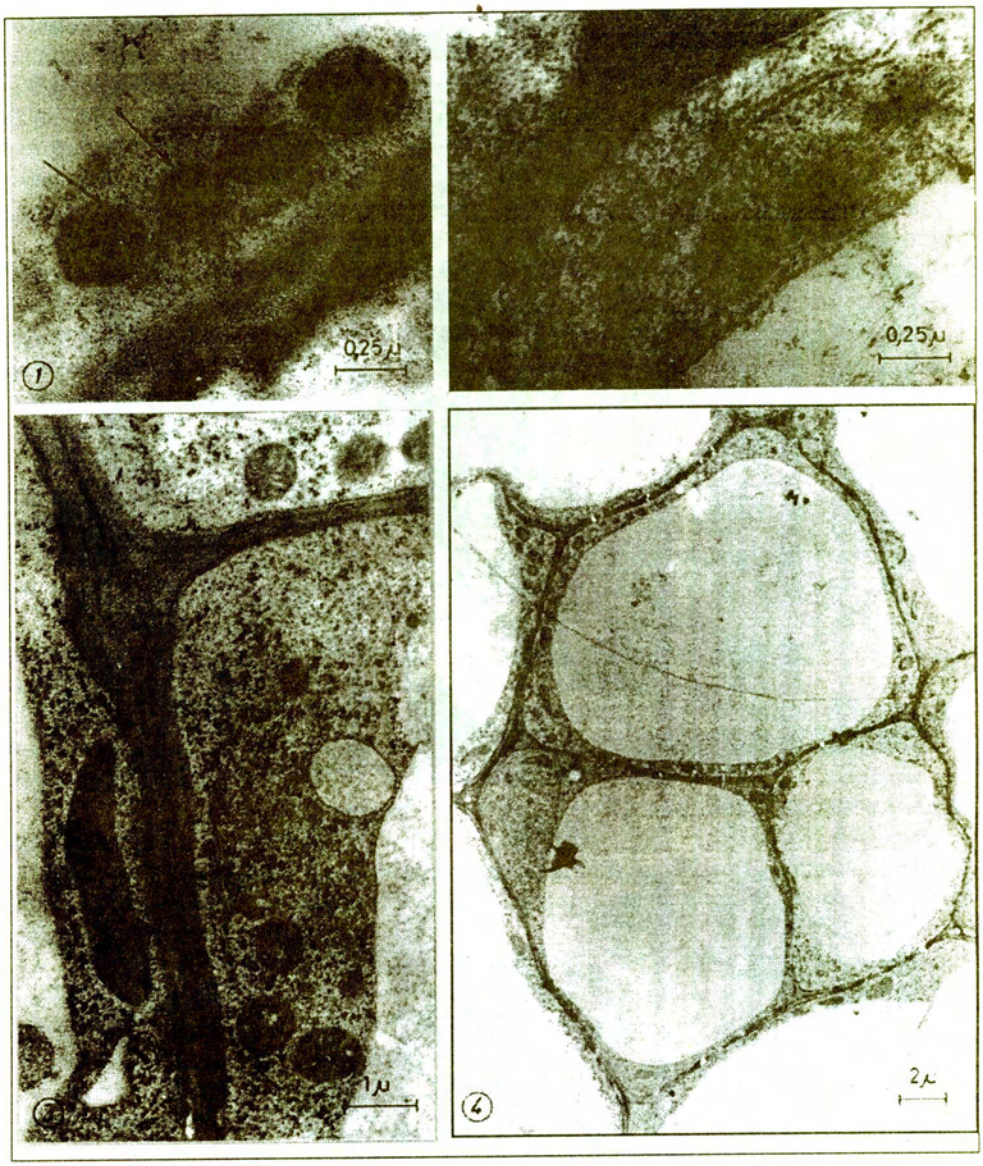


PLATE VI. – The peculiarities of microbodies with voluminous crystalline core (Fig. 1, 2) and plastid (Fig. 3) inside the tumor undifferentiated cells (Fig. 4).

In this respect we consider that the studies regarding the cytological and molecular aspect of the main steps of the foreign DNA integration in plant cells, by *Agrobacterium* strains system, are interesting and useful. These would clarify many fundamental problems concerning the molecular bases of the transformation process as well as other ones unknown yet like possible identification of oncogenes in plants.

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IDENTIFICATION OF A CONDITIONAL DOMINANT LETHAL ALLELE OF CIN8 - A *SACCHAROMYCES CEREVISIAE* GENE INVOLVED IN MICROTUBULE BASED MOTILITY

LUMINIȚA PARAOAN and ION ANGHEL

CIN8 and KIP1 genes from *Saccharomyces cerevisiae* encode force-generating molecules related to kinesin that are essential for yeast spindle morphogenesis and function. Here we describe a series of genetic assays by which a conditional dominant lethal allele of CIN8 gene was identified and isolated. The conditional dominance was shown both over CIN8 and KIP1 wild-type genes, the non-permissive temperature being 33°C. These findings proved for the first time the existence and the possibility to distinguish such mutants in the case of CIN8 gene. Conditional dominant lethal mutants are particularly useful in the study of both the relationship between Cin8p structure and its function and the interactions *in vivo* between different gene products.

The mitotic segregation of chromosomes is accomplished by the spindle, a microtubule-based structure that coordinates spatially and temporally controlled motility events. While the basic structural properties of the spindle have been observed and characterized by cell biological studies, the knowledge of the molecular basis of its function has been limited for a long period due to the complexity and accentuated dynamic nature of this structure. In recent years, however, significant progress has been made towards the identification and characterization of many of the molecules important for spindle function. The yeast *Saccharomyces cerevisiae* proved to be particularly suited to genetic and cell biological analysis.

Consequently, studies of this experimental organism have contributed significantly to our understanding of mitosis in general and to the molecular understanding of the chromosome segregation process in particular.

Two *Saccharomyces cerevisiae* genes, CIN8 and KIP1, firstly identified by their requirement for normal chromosome segregation (Hoyt et al.,1992), were then characterized as related to the microtubule-based force generating protein kinesin and found to be essential for yeast spindle morphogenesis and function; the Cin8p/Kipl function is required for the assembly of the bipolar mitotic spindle at the stage when the duplicated spindle poles separate and, also, their continued action is required to maintain the structural integrity of the pre-anaphase spindle (Saunders and Hoyt, 1992). This activity exerts an outward pushing force on the poles and, consequently, the two proteins that perform an essential but redundant function are said to belong to the group of plus-end directed mitotic motors. The

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CIN8/KIP1 gene products were the first microtubule-based force generating proteins for which a defined role in mitotic spindle was ascribed.

In order to test the way in which the kinesin-related protein Cin8p interacts with the microtubules, an extensive mutagenesis analysis of the CIN8 gene has been performed. Different screening procedures have been carried out and different classes of mutants have been isolated and characterized. A variety of genetic assays have been used with the view to characterizing the relationship between Cin8p structure and its function. In this paper we report the identification and isolation of the first conditional dominant lethal allele of CIN8 gene.

MATERIALS AND METHOD

The *Saccharomyces cerevisiae* strains used in these experiments belong to MAY collection (M.A. Hoyt Laboratory, The Johns Hopkins University, Baltimore, USA) and their relevant genotypes are presented in the text. The plasmid pMA1189 (CIN8 LYS2 CEN) derived from pRS317 vector.

Rich (YEPD) and minimal (SD and SDC) media were as described by Sherman (1991).

Plasmidial DNA was isolated from *Escherichia coli* strains using the method described by Holmes and Quigley (1981). The transformations of yeast cells were carried out in the presence of polyethyleneglycol after inducing the competence of cells with lithium acetate (Ito et al.,1983).

RESULTS

Prior to the assays described in this paper, 22 individual mutant alleles of CIN8 gene cloned in the plasmid pMA1189 had been isolated and characterized as temperature-sensitive (non-permissive temperature 33°C). All these 22 mutagenized plasmids (pMA1189*) were subjected to the following assays with the view to testing possible interactions between mutant and wild-type forms of Cin8p. The *Sacchromyces cerevisiae* strain chosen as experimental system for these tests was MAY2275 [(a cin8::URA3 kip1::HIS3 leu2-3112 lys2-801 cyh2^R (pMAl208-CIN8 LEU2 CYH2 CEN)]. Despite the double null genomic mutation for CIN8 and KIP1 genes, this strain is viable due to the plasmidial CIN8 gene.

Plasmidial DNA (pMA1189*) corresponding to all 22 mutant cin8 alleles was isolated from *Escherichia coli* strains in which it was amplified. The boiling method for isolation of plasmidial DNA yielded 2-5μg DNA when starting from 1,5 ml freshly saturated bacterial cultures. The competence of yeast cells (MAY2275) was induced with lithium acetate and the yeast transformations were carried out with 1μg plasmid DNA. Following the initial selection on SDC-Lys medium, the yeast transformants were plated out on SDC-(Lys, Leu) medium, thus creating the selective conditions for maintaining both the constitutive plasmid pMA1208 with wild-type CIN8 and the newly transformed plasmid

pMA1189* carrying the respective mutant alleles cin8. All transformants were tested for temperature sensitivity at temperatures ranging from 26°C to 37°C. All yeast transformants viable under these conditions at any given temperature were expressing both the wild-type protein Cin8p and the mutant form cin8p.

The choice of control yeast strains proved to be very important for the assessment and interpretation of results, i.e. MAY2275; MAY2275+pMA1189(CIN8); MAY2275+pRS317. Each selective plate had a replica plate on rich (YEPD) medium on which only one of the alleles (CIN8) was maintained. With only one exception, all mutant transformants grew well on selective medium, similarly with the control strains at all temperatures tested so in all these cases the mutant form cin8p could not be phenotypically noticed in the presence of the wild-type Cin8p; all these mutant alleles were thus recessive. The exception was the mutant allele cin8-5c; the maintaining through selection of the plasmid carrying this allele alongside with pMA1208 (CIN8) determined the inability of yeast cells to grow at temperatures higher than 33°C (Figure 1 a); at 33°C the growth was poor, while at 35°C-37°C the yeast cells failed completely to grow. This effect was undoubtedly due to the presence of the mutant allele cin8-5c, as the same yeast strain with the plasmid carrying the wild-type CIN8 (MAY2275+pMA1189) grew normally at all temperatures tested and had an identical phenotype with that of MAY2275+pRS317. Also, on rich medium (Figure 1b), all transformants had a wild-type-like phenotype (pMA1189* had been lost, as the selection pressure ceased). Consequently, it was concluded that the observed phenotype was due to the mutant allele cin8-5c and it was characterized as a *conditional dominant lethal effect*. This was the first such mutant allele of the gene encoding the kinesin-encoded Cin8p to be isolated.

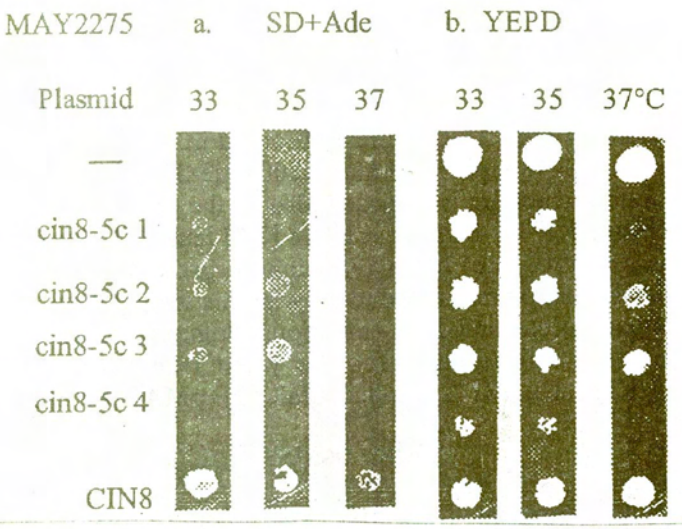

Fig. 1. – Conditional lethal dominance of cin8-5c allele in the presence of wild-type multicopy CIN8. The transformant plasmid is pMA1189 (CIN8). On minimal media the constitutive plasmid (pMA 1208-CIN8) of MAY2275 strain is also selected.

Naturally, the following 3 questions were asked following this finding:

i) Does the mutant allele cin8-5c have the same conditional dominant lethal effect on genomic wild-type CIN8?

ii) Is this effect abolished/diminished in the presence of the functionally redundant gene KIP1?

iii) Is there any effect of this mutant allele over KIP1 ?

These questions were addressed by transforming the respective mutagenized plasmid DNA into appropriate yeast strains. The results (Figure 2a, b) could be summarized as follows:

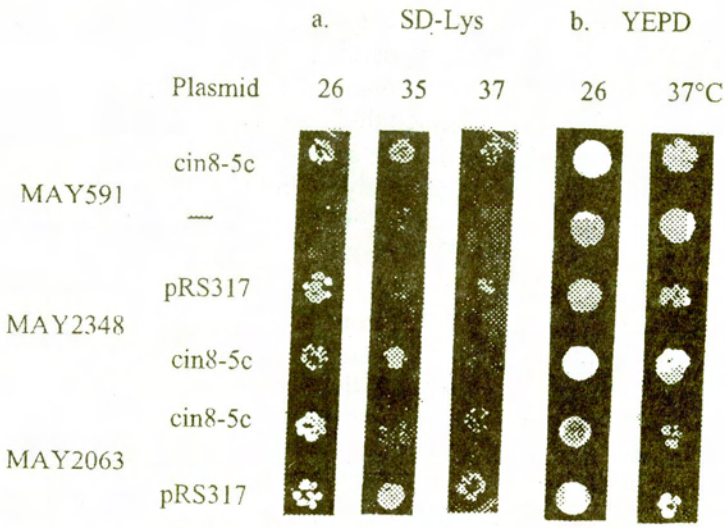

Fig. 2.– Lethal dominance of the ts cin8-5c allele over wild-type CIN8 and KIP1.

1) The mutant allele cin8-5c is dominant lethal in the presence of wild-type CIN8 at temperatures over 33°C - effect observed in *Saccharomyces cerevisiae* strain MAY2348 (a CIN8 kipl::HIS3 his3-Δ200 leu2-3119 lys2-801).

2) The same phenotype is imposed also in the presence of both genomic wild-types CIN8 and KIP1 (MAY59l: ∝ CIN8 KIP1 leu2-3112 lys2-801). A slight difference was observed, i.e. MAY591+cin8-5c grew poorly at 37°C, while MAY2348+cin8-5c completely failed to grow at this temperature.

3) The mutant allele cin8-5c is conditional dominant lethal over KIPl, too; the non-permissive temperature for the yeast transformants MAY2063 (a cin8::URA3 KIP1 leu2-3112 lys-801 ura3-52)+pMA1189-cin8-5c is 33°C.

CONCLUSIONS

These results represented the first experimental proof of an interaction between a mutant allele cin8 and wild-type genes CIN8 and KIP1 leading to a conditional dominant lethal phenotype and suggested the existence of an *in vivo*

interaction between the respective gene products. Besides the usefulness of such an isolation for further studies, these findings showed the existence and the possibility to distinguish dominant lethal mutants of CIN8 gene.

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GENETIC TRANSFER OF SHUTTLE VECTORS TO *SACCHAROMYCES CEREVISIAE* STRAINS THROUGH ELECTROPORATION

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During the last five years electroporation has become a most promising alternative to genetic transfer techniques used in molecular biology experiments. Unfortunately, the optimal parameters for highest transformation frequencies and efficiencies are to be determined empirically, for each strain. In the present study we aimed to determine the optimal conditions for the electrotransfer of the *E.coli/S.cerevisiae* shuttle vector YEp352 into two strains of *S. cerevisiae*: SC589 and Ts5. In the experiments on SC589 we varied the pulse train length between 1 and 20 msec (maintaining E=3 kV/cm), while on Ts5 we varied the electric field intensity between 1 and 3 kV/cm. Our results revealed that 3 kV/cm is a too high intensity and gives lower transformation frequencies. The highest transformation frequencies were found at 2 and 2.25 kV/cm at 4 msec, the values being twice higher than the frequencies given by the "classical" LiCl-transformation.

INTRODUCTION

During the last decade cloning technologies in microorganisms have enormously evolved. One of the problems that has to be continuously overcome is the efficiency of genetic material transfer from one species to another. There is already a set of "classical" techniques, like protoplast transformation, $CaCl_2$- or LiCl-induced whole cell transformation [6]. Each of these methods are time and labor consuming, and yet they offer a relative low efficiency of DNA transfer (10^3-10^4 transformed cells/μg plasmid DNA) [11].

In the last five years a new technique for introducing nucleic acids into living cells – electrotransformation – has gained a lot of success [24, 25]. Applying electric field on the transformation mixture, a local and reversible disorganization of the cell wall and the plasmatic membrane appears, allowing the penetration of the exogenous DNA into the cell [8]. The molecular mechanism of this phenomenon has not yet been elucidated [2].

The main advantages of this method are: simplicity, less reagents needed, less time consuming, higher frequency and efficiency of transformation.

In the present study we aim to determine the optimal conditions of electroporation (intensity of electric field, duration of impulse, chemical composition of the transformation solution) of some *Saccharomyces cerevisiae* strains using a set of *E.coli-S.cerevisiae* shuttle vectors.

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MATERIALS AND METHODS

Yeast strains: *Saccharomyces cerevisiae* SC589 (a ura3 leu2 his3 ade2), *S.cerevisiae* Ts5 (a ura3 leu2).

Media: Cells were cultured in YEPD broth at 30°C for 18h. They were harvested through centrifugation at 4500 rpm 10 min.

Vectors: YEp352 is an *E.coli/S.cerevisiae* shuttle vector (Fig. 1) [1,2]. It has two origins of replication: one derived from pMB1 (ColEl replicon-type), allowing replication in *E.coli*, and one from the 2 μm plasmid for replication in *S.cerevisiae*. It also has two sets of genetic markers: Ap^r that allows screening of bacterial transformants and LacZ' that permits fast histochemical identification and selection of recombinant bacterial cells, and ura3 that allows selection of $ura3^+$ yeast transformants.

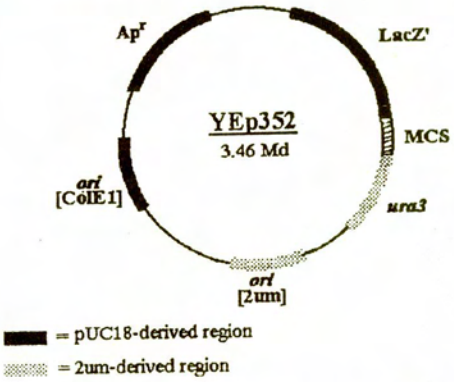

Fig. 1. – YEp352 vector, an *E.coli/S. cerevisiae* shuttle vector. It is an Yeast Episomal plasmid that has 5181 bp (3.46 Md). It has regions derived from two plasmids: pUC18/19 from *E. coli* and 2μm from *S. cerevisiae*. It has two replication origins: ori-[ColE1] for replication in *E. coli* and ori-[2μm] for replication in *S. cerevisiae*. Ap^r represents a genetic marker for screening of *E. coli* transformants. LacZ' allows histochemical identification and selection of *E. coli* recombinant transformants. MCS=MultiCloning Site. ura3 represents a genetic marker for selection of *S. cerevisiae* transformants.

Plasmid DNA preparation: From *Escherichia coli* DH5α, plasmid DNA was isolated using an alkaline lysis technique [20]. Purification was performed with one step of phenol extraction, followed by two steps of chloroform/isoamylic alcohol (24:1) extractions. DNA was then precipitated with isopropanol and dissolved in TE pH 8.0. From yeast transformants DNA was extracted through a method (adapted from T. Ausubel 1994) [2,18] that uses β-mercaptoethanol and zymolyase (for disorganizing the cell wall). The following steps are similar to the extraction of plasmid DNA from bacterial sources.

Electrotransformation: Harvested cells from both yeast strains (*S.cerevisiae* SC589 and Ts5) were suspended in 20% glycerol. Samples of 150 μl cell suspension (5.1×10^9 cells/ml) were incubated with 200 ng vector DNA [3,5,28]. Electrotransformation was performed using a 2 mm^3 cuvette. For the experiments on *S.cerevisiae* SC589, a constant 3kV/cm electric field was used, with a unipolar impulse of 10 μsec; the pulse train length varied from l to 20 msec. For the experiments on *S. cerevisiae* Ts5 the pulse train length was maintained constant, while the intensity of the electric field was varied between 1 and 3 kV/cm.

Lithium Chloride transformation: Beside the electrotransformation we also performed a "classical" yeast transformation using a LiCl-based technique, adapted after D.M. Becker and L. Guarente 1991 [14] [see also 21,22].

Selection of transformants: After both types of transformations cells were plated on minimal media (0.67% Yeast Nitrogen Base, 0.5% ammonium sulphate 2% glucose, 2% agar) supplied with 1 mM leucine for *S. cerevisiae* Ts5 and, respectively, 1 mM leucine + 1 mM histidine + 1 mM adenine for *S. cerevisiae* SC589 [7,13].

Plasmid DNA electrophoresis: Plasmid DNA, extracted and purified from *E.coli* and *S.cerevisiae* cells, was electrophoresed through 0.8% agarose gel in standard conditions [2,20]. DNA was stained with EtBr and visualized in UV light.

RESULTS AND DISCUSSION

The electrotransformation experiments cited until now in the literature revealed that each strain has its own optimal conditions for electroporation [9,10,12,15,23,26,30]. There seems to be no general rule and the parameters are empirically determined through successive trials. In this context, we performed plasmid DNA transformation on two strains of *Saccharomyces cerevisiae* (SG589 and Ts5) by electroporation.

The experiments were conducted in different conditions, varying two parameters of the electric field: electric intensity and pulse train length. For the strain 5C589 we varied the length of the pulse train between 1 and 20 msec. As seen in Table 1, the number of CFU decreased with the field length.

Table 1
Number of transformed cells and transformation frequencies for *S. cerevisiae* SC589 at E=3kV/cm and T=1-20 ms

Sample No.	Train length (ms)	No. of Transformed CELLS (CFU / ml)	Transformation frequencies
1	1	2.5×10^3	1.66×10^{-4}
2	2	3.8×10^3	2.53×10^{-4}
3	3	2.1×10^3	1.4×10^{-4}
4	4	0.8×10^3	0.53×10^{-4}
5	5	1.5×10^3	1×10^{-4}
6	6	1.5×10^3	1×10^{-4}
7	7	0.4×10^3	0.26×10^{-4}

This result could have been determined by the high intensity of the electric field (3kV/cm), correlated with the increase of the train length. For these reasons, in the experiment on the other strain (Ts5) we maintained constant the pulse train length (4 msec) while varying the electric intensity (1-3kV/cm). In this case at 2kV/ml we obtained a maximum of CFU value (6.08×10^3/ml), corresponding to a transformation frequency of 1.192×10^{-3} (Table 2).

Table 2
Number of transformants (in CFU/ml) and transformation frequencies for *S. cerevisiae* Ts5 at $T=4$ ms and $E=0.5$-3 kV/cm

Sample number	Field intensity (kV / cm)	No. Transformed CELLS (CFU / ml)	Transformation frequency
1	0.5	1×10^3	0.196×10^{-3}
2	1	2.47×10^3	0.484×10^{-3}
3	1.5	2.6×10^3	0.509×10^{-3}
4	1.75	2.65×10^3	0.519×10^{-3}
5	2	6.08×10^3	1.192×10^{-3}
6	2.25	3.76×10^3	0.735×10^{-3}
7	2.5	2.59×10^3	0.507×10^{-3}
8	3	2.5×10^3	0.490×10^{-3}

To relieve the advantages of the electrotransformation upon the "classical" technique, we also conduct a LiCl-mediated transformation. The control cells number was 2×10^8 CFU/ml and the average transformation frequency was 0.51×10^{-3} (Table 3).

Table 3
Results of LiCl transformation on *S. cerevisiae* Ts5 using Yep352

Sample number	No. of Transformed cells (CFU / ml)	Transformation frequency
1	920	0.46×10^{-3}
2	975	0.467×10^{-3}
3	901	0.46×10^{-3}
4	1140	0.57×10^{-3}

In order to confirm the electrotransformation at the molecular level, we also performed the isolation, purification and visualization of the transforming plasmid DNA. As seen in Fig. 2, the electrotransformed cells of *S. cerevisiae* presented a single band corresponding to the CCC form of YEp352 (migrated at the same

distance as YEp352 isolated from the donor strain *E.coli* DH5α). In a second gel (Fig. 3) it was also confirmed the presence of YEp352 in *S.cerevisiae* Ts5 eloctrotransformed cells.

We also establish the existence of the transforming DNA (YEp352) in LiCl-transformed cells (Fig.4).

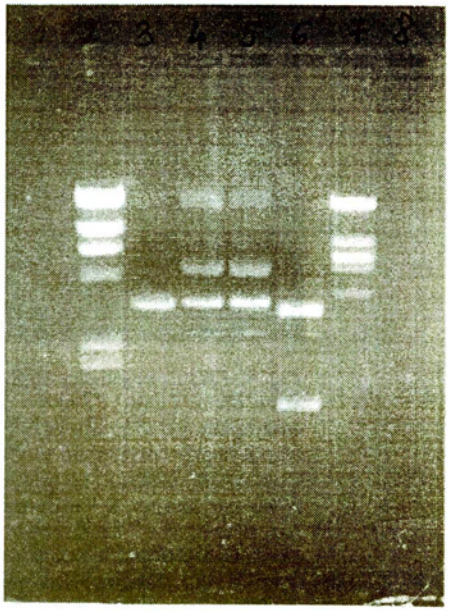

Fig. 2. – Agarose gel electrophoresis of YEp352 DNA isolated from electrotransformed cells of *S. cerevisiae* SC589. 1 – negative control of plasmid DNA from untransformed *S. cerevisiae* SC589 cells; 2 – λDNA digested with HindIII; 3 – Yep352 from electrotransformed cells of *S. cerevisiae* SC589; 4 – and 5 – Yep352 from the donor strain *E. coli* DH5α (the 3 bands correspond to the 3 molecular forms CO, L, CCC); 6 – Yep352 and pBluescript SK+ from *E. coli* DH5α; 7 – λDNA digested with EcoRI.

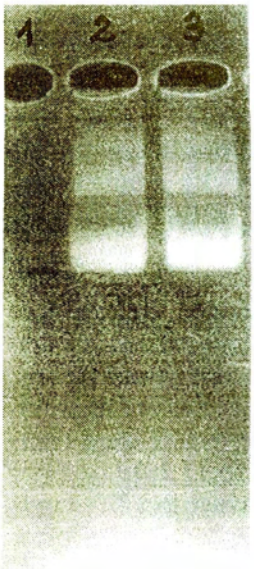

Fig. 3. – Agarose gel electrophoresis of YEp352 from electrotransformed cells of *S. cerevisiae* Lanes: 1 – negative control of plasmid DNA untransformed *S. cerevisiae* Ts5 cells; 2 – YEp352 from electrotransformants cells of *S. cerevisiae* Ts5 (the 3 bands correspond to the 3 molecular forms CO, L, CCC); 3 – Yep352 from the donor *E. coli* DH5α (positive control).

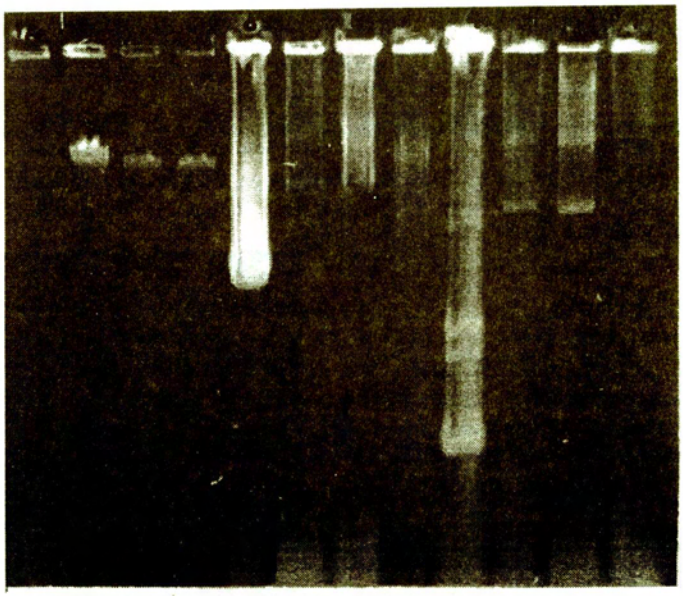

Fig. 4. – Agarose gel electrophoresis of YEp 352 isolated from different sources. Lanes: 1 and 4 – different natural plasmids isolated from enteropathogenic strains of *E. coli*; 2 and 3 – pUCl8 from *E. coli* DH5α (the 2 bands correspond to CO+L and CCC); 5 – pBluescript SK+ from *E. coli* DH5αF'; 6 and 7 – different natural plasmids from *Bacillus ssp.* strains; 8 – different natural plasmids from *Pseudomonas ssp.* strains; 9 – YEp352 from electrotransformed *S. cerevisiae* Ts5; 10 – YEp352 from LiCl-transformants of *S. cerevisiae* Ts5; 11 – YEp352 from *E. coli* DH5α; 12 – negative control for plasmid DNA of untransformed *E. cerevisiae* Ts5 cells.

CONCLUSION

In this experiment we aimed to determine the optimal conditions for the electrotransformation of some yeast strains with *E.coli/S.cerevisiae* shuttle episomal vectors and to compare the results with those obtained in LiCl technique. From our results we can conclude:

1. In the electrotransformation experiment (on *S. cerevisiae* Ts5) in which we varied the electric field intensity (1-3 kV/cm) and maintained the exposure time constant (4 msec) we obtained higher frequencies of transformation at 2 and 2.25 kV/cm. So, this might be the optimal range for electroporating yeast strains.

2. It is also remarkable that the above mentioned frequencies are approximately two times higher than our LiCl-frequencies. Similar results were compared with other studies on classical yeast transformation [l4,16,17,19].

3. Comparing the two electroporating experiments, we conclude that 3kV/cm is a too high electric field intensity that even at shorter times of exposure gives lower transformation frequencies [27,29].

It is obvious that electrotransformation provides a more efficient alternative to the "classical" lithium chloride technique. Beside higher frequencies of transformation, this method has also the advantages of using lower amounts of transforming DNA and of being simpler and less time consuming.

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THE BACTERIOPHAGES IN *METHYLOMONAS* sp. M_{14-1}

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ANCA LEPĂDATU*

This study was made in order to identify the presence of bacteriophages in the culture of *Methylomonas* sp. M_{14-1} (an obligate methylotroph) utilized in industrial production of single cell proteins (SCP). The phage genome was revealed in both the culture liquid and cells after the UV lytic induction. Our results indicate that the phage is a temperate one and its genome is represented by DNA molecule. Under normal conditions the phage genome is inserted in the host cell chromosome.

In recent years very intensive researches have been developed concerning working out of the methods for the obtaining of fodder proteins with the help of microorganisms, among which, the methylotroph bacteria. In the case of methanooxidizing bacteria, in the experiments on industrial installations, it has been pointed out that the bacteriophages can affect the technological processes for the obtaining of the fodder proteins. These phages can derive either from the environment or they can exist in the bacterial culture, being released as mature phages by the lysogene bacteria. The ability to produce lytic phage without an exogene infection is very important. The study of the bacteriophages in methylotroph bacteria as well as their relations with the host cell concerning the possibility to use the methylotrophs to industrially produce fodder proteins will play a basic role in the future investigations.

MATERIALS AND METHODS

It has been used a *Methylomonas sp.* M_{14-1} culture, an obligated methylotroph, cultured on a medium established by Bontaş et al., 1986 (2).

Due to the lack of some indicator strains with whom we could point out the existence of bacteriophages and eventually, with the aid of which to study the morphology of the isolated bacteriophages, we have approached a method with which to outline the genome of the phage existing in the *Methylomonas sp.* M_{14-1} culture.

By using the method previously established for the lambda phage DNA isolation (6) as well as the method of Maniatis et al. (3) in order to point out the plasmid DNA in bacterial cell from the *Methylomonas sp.* M_{14-1} culture untreated by inducing agent there were revealed by gel-electrophoresis but the DNA spots corresponding to chromosomal and plasmid DNA of the bacterial cell. In order to outline the lysogene state of bacterial cells in the *Methylomonas sp.* M_{14-1} culture and thus the existence of phage genome in prophage form we have subjected the culture to an UV induction (254 nm) for 5 min., the UV source being placed 20 cm away from Petri plate. The irradiation was performed in liquid medium (the growth medium of *Methylomonas sp.* M_{14-1} strain), at the end of steady phase of growth (after 48 h cultivation).

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After treatment with inducing agent the culture liquid and the bacterial cells have been separated by a centrifuge. The latter have been resuspended in chloroform for 25 min, at room temperature, to destroy the cell wall and phage capsid, in case mature phage particles have already been formed. After chloroform removal the bacterial cells were processed by Maniatis et al. (3) method in order to reveal plasmid DNA and phage genome.

To point out the phage within the culture liquid, the cells have been cultivated for 18 h (the late log phase), then UV irradiated under the same conditions and cultivated further for 24 h. After cells removal by centrifuge, the culture liquid was sterilized by filtration (bacterial filter) and utilized for phage genome purification using a method published earlier (6).

Before performing the gel-electrophoresis a treatment was applied by DN-ase and RN-ase respectively, to identify the nucleic acid type corresponding to the phage genome separated by electrophoresis.

'The electrophoresis was carried out in agarose gel 1% in TBE buffer at an 2mA\tube intensity. The gels were colored by ethidium bromide and the DNA spots have been revealed and photographed in UV light.

In order to identify the spot separated on agarose gels corresponding to phage genome there have been compared the separate spots by gel electrophoresis from the preparations derived from untreated culture by inducing agents and after lytic induction by UV utilization.

RESULTS AND DISCUSSIONS

Using a method previously established for revealing the lambda phage DNA after UV induction, on agarose gels it was revealed a spot localized away from the migration origin at a bigger distance as compared to the region where it is localized the corresponding plasmid DNA spot purified from bacterial cells untreated by inducing agent (Fig. 2, lane 2 and 3; Fig. 3, lane 1 and 2).

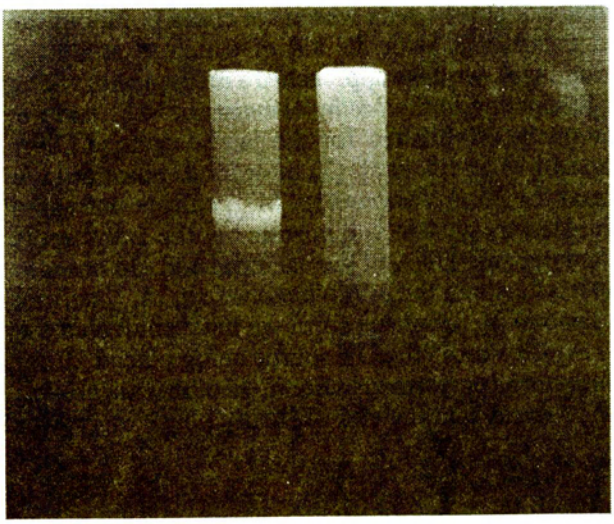

Fig. 1. – Agarose gel-electrophoresis 1% of chromosomal (lane 1) and phage genome (lane 2) from *Methylomonas sp.* M_{14-1}.

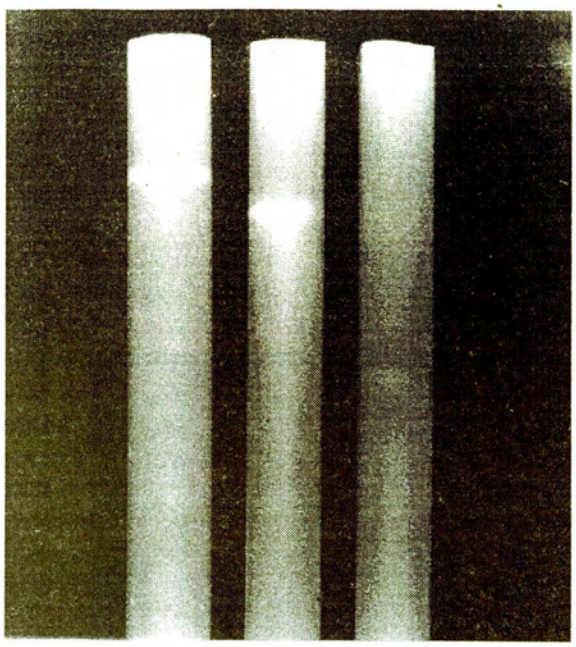

Fig. 2. – Agarose gel-electrophoresis 1% of chromosomal DNA (lane 1), plasmid DNA (lane 2) and phage genome (lane 3) from *Methylomonas sp.* M_{14-1}.

By using the techniques for revealing the plasmid DNA in bacterial cells (4) following the treatment with inducing agents (UV 254 nm) on agarose gels two spots have appeared: the spot characteristic to plasmid DNA and a spot localizated in the same region as the spot revealed following the processing of culture liquid according to the technique for lambda phage genome isolation (Fig. 3, lane 3).

Since after RN-ase treatment this spot appears on the agarose gel, but it is not revealed anymore following the DN-ase digestion, we can consider that the phage genome revealed by the two methods is represented by a DNA molecule. Considering the fact that the phage genome has been revealed only after the lytic induction, one may estimate that in the *Methylomonas sp.* M_{14-1} culture [on a previously established medium (2)] there is bacteriophage in the prophage form integrated in the bacterial chromosome, replicating at the same time with it under the above mentioned culture conditions. The bacterial cells are in a relatively stable lysogene state. Therefore, one may say that the bacteriophage, the presence of which has been demonstrated by revealing its genome, is a temperate phage which under normal conditions does not determine the host cell lysis.

Literature data concerning the lytic induction of obligate methylotrophs are very limited. Thus, the first data concerning lysogene state in methylotroph bacteria have been published by Tikhonenko et al., 1982 (5), nevertheless before 1982 it has been reported the identification of virulent phages in this bacterial group. For instance, in 1977 there have been identified some bacteriophages in methane-assimilating obligate bacteria such as the two strains GB2 and GB4 of *Methylosinus* (7). Tikhonenko et al. (5) have revealed bacteriophages only following the use of inducing agents – UV and mitomycin – in *Methylobacter bovis* 89, *Methylosinus trichosporium* GB2, *Methylosinus trichosporium* GB4 cultures (obligate methylotrophs) and in the facultative methylotroph *Flavobacterium gasotypicum* MF.

Taking into account the intensity of the spots appeared on agarose gels following the phage DNA electrophoresis (Fig. 1-3) and the insignificant decrease in the *Methylomonas sp.* M_{14-1} culture density, we can estimate the presence of the bacteriophages in a relative low concentration. One may say that 25 min. after UV induction nevertheless the amount of phage DNA excised from bacterial chromosome is relatively small. This could be explained by the fact that the bacteriophage whose genome we have revealed could be a transposon phage, like the type fT from the facultative methylotroph *Pseudomonas aeruginosa* and *Mu* phage. According to data obtained by Akhverdian et al. (*1*), D3112 phage of *Pseudomonas aeruginosa* replicates itself by a process in which the replication is coupled to

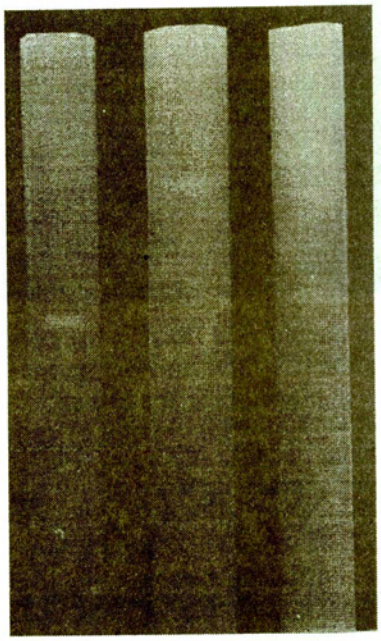

Fig. 3. – Agarose gel-electrophoresis 1% of phage genome (lane 1); plasmid DNA (lane 2); purified phage genome and plasmid DNA from *Methylomonas sp.* M_{14-1} after UV irradiation (lane 3).

transposition (the so-called replicative transposition process), new DNA phage copies being inserted at sites in bacterial chromosome, and after prophage induction, this is excised only in late stages from bacterial chromosome. The correlation of these events could be explained by the fact that in the case of D 3112 bacteriophage from *P. aeruginosa* the specific product of *c* gene in phage genome is necessary for transposition and this gene has also pleiotropical effect on phage development, controlling both the phage DNA excision process and bacterial cell lysis as well as the mature phage production. The same case has to be signalled in other transposable bacteriophage (B 39) in *P. aeruginosa* (3). Since in *P. aeruginosa* facultative methylotroph it was identified the presence of transposon bacteriophages possessing genes with pleiotropical effect on phage development, the genes controlling the phage genome insertion in bacterial chromosome and the frequency of stable lysogeny in infected bacteria, as well as the phage genome excision and the phage maturation same investigations would be necessary to be done in obligate

methylotrophs in order to demonstrate whether such mechanisms influencing the stable lysogene state of bacterial cells are functioning. Also, studies concerning the bacteriophage revealing the bacteriophage – cell host relation and the bacteriophage cycle must be carried out for each bacterial strain, because according to data reported since 1977 by Wunsche et al. (7), the bacteriophages revealed in obligate methylotrophs, namely in the two strains of *Methylosinus*, show a tight genic specificity, they being virulent only in bacterial strain from which they have been isolated (showing no reaction against other numerous methylotrophs of *Methylosinus*, *Methylomonas*, *Methylocistis* and *Methylobacter* genera). We mention the fact that the genome of the phages revealed by Wunsche et al. (7) is represented by double strand DNA.

Our results also point to the fact that the revealed phage genome is represented by a DNA molecule, but further investigations are required to establish whether this DNA is single strand or double strand. Certain is the fact that in *Methylomonas sp.* M_{14-1} cultured on the medium established by Bontaş et al. (2) there are bacteriophages, and these are in prophage form. The bacteriophage revealed by us is a temperate phage. If its lytic evolution occurs, it seems that this is produced at a very low frequency under the conditions in which the strain has been cultivated, because the phage genome has not been revealed either in culture liquid, or in the bacterial cells without a treatment with inducing agents.

CONCLUSIONS

1. Due to the relative low concentration of phages, one may estimate a stable lysogenic state, with a lytic evolution only under conditions of major changes in culture physiology, permitting the industrial utilization of *Methylomonas sp.* M_{14-1} strain.

2. It has been identified the presence of bacteriophages in *Methylomonas sp.* M_{14-1} by an indirect method of revealing the phage genome.

3. The genome of the identified phage is represented by a DNA molecule.

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