Diplotaxis muralis (L.) DC., Reg. Veg. Syst. Nat. 2: 634 (1821). — Heywood in Fl. Eur. 1: 335 (1964). — A. Franco, Nov. Fl. Port. 1: 230 (1971).

Forma **caulescens** Kitt., Taschenb. Fl. Deutschl. ed. 2: 907 (1843). — Markgraf in Hegi, Ill. Fl. Mitteleur. 4, 1: 431 (1962).

Diplotaxis muralis var. *ramosa* Neil., Fl. Wien: 498 (1846).
Diplotaxis intermedia Schur, Enum. Pl. Transsil.: 60 (1866).
Diplotaxis muralis var. *intermedia* (Schur) Nyman, Consp.: 49 (1878).
Brassica muralis var. *ramosa* (Neil.) Kuntze, Rev. Gen. Pl. 1: 18 (1891).
Diplotaxis muralis forma *biennis* Rouy & Fouc., Fl. Fr. 2: 48 (1895).

Esta espécie é nova para Portugal continental e a sua forma *caulescens* nova para o nosso país, visto que o tipo já é citado para os Açores (cf. A. Franco, loc. cit.; a avaliar pela descrição que este autor apresenta da disposição das folhas, trata-se do tipo da espécie). A forma *caulescens* distingue-se do tipo, principalmente pela maior duração (planta bienal a perenante e não anual) e pelos caules ramificados, folhosos para a parte superior (no tipo, as folhas dispõem-se em rosetas basilares).

A planta agora referida (Est. IV) foi colhida nas dunas da Praia das Maçãs já há alguns anos, encontrando-se determinada em COI como *D. viminea,* determinação que foi corrigida posteriormente para *D. catholica.* Como esta última espécie é muito diferente do exemplar em questão [*D. catholica* (L.) DC. pertence à sect. *Rhynchocarpum* Prantl emend. O. E. Schulz, enquanto *D. viminea* (L.) DC. e *D. muralis* se integram na sect. *Anocarpum* DC. emend. O. E. Schulz], limitamo-nos a mencionar no Quadro I alguns dos principais caracteres que distinguem *D. muralis* de *D. viminea,* espécies que mostram entre si bastantes analogias (entre parênteses indicam-se as medidas que registamos para a planta da Praia das Maçãs).

O exemplar de COI pertencente a *D. muralis* forma *caulescens* é o seguinte:

Praia das Maçãs, nas areias das dunas, 8-VI-1950, *A. Fernandes, R. Fernandes & J. Matos* 7226 (COI).

D. muralis distribui-se largamente pela Europa Central e do Sul (cf. Fl. Eur., loc. cit.) e pelo norte de África (Tunísia,

QUADRO I

Diplotaxis muralis	*Diplotaxis viminea*
Sépalas 3-4.5 mm longas (3.5-4 × 1 mm)	Sépalas 1.8-3 mm longas
Pétalas 5.5-8 mm longas, obovadas (5.5-6 × ± 2.25 mm)	Pétalas 3-4 mm longas, oblongo--obovadas
Estames todos férteis, os maiores c. 5 mm longos (5 mm longos)	Estames internos 3-3.5 mm longos, férteis; os externos 1.5-2 mm longos, estéreis
Anteras c. 1.5 mm longas, oblongas (c. 1.25 mm, depois da deiscência)	Anteras férteis c. 0.5 mm longas, ovadas; anteras estéreis c. 0.25 mm longas, suborbiculares
Estigma mais largo que o estilete	Estigma tão largo como o estilete
Rostro das silíquas 1-2.5 mm longo, obcónico (1-2 mm)	Rostro das silíquas 1-1.5 mm longo
Sementes 1 mm longas, 0.5-0.6 mm diâm., lisas (1 mm longas)	Sementes 1 × 0.75 × 0.5, ovóides, levemente alveoladas

Argélia e Marrocos), aparecendo como naturalizada e casual em alguns países fora da sua área. Embora possa viver, entre outros vários habitats, em lugares arenosos das margens dos rios, não temos informação de que tenha sido encontrada em areias marítimas. Por esse facto, é possível que o seu aparecimento nas areias da Praia das Maçãs tenha sido apenas ocasional. Em próxima herborização no mesmo local, procuraremos saber se a planta se mantém, o que provaria a sua possível espontaneidade no nosso país.

É interessante notar que O. E. SCHULZ (op. cit.: 175) refere um exemplar (que não vimos) de R. FRITZE, colhido em Albufeira (Algarve), à var. *pinnatifida* de *D. muralis*.

Diplotaxis tenuifolia (L.) DC., Reg. Veg. Syst. Nat. 2: 632 (1821). — Mariz in Bol. Soc. Brot. 3: 104 (1885). — Samp., Man. Fl. Portug.: 193 (1910); Fl. Portug.: 235 (1947). — Cout., Fl. Port.: 262 (1913); op. cit. ed. 2: 314 (1939). — Heywood in Fl. Eur. 1: 335 (1964).

Nem A. FRANCO (Nov. Fl. Port. 1, 1971), nem HEYWOOD (loc. cit.) referem esta espécie para o nosso país. No entanto, em COI encontra-se um exemplar colhido por WELWITSCH em Tróia (Setúbal), em V-1849, o qual pertence indubitavelmente a *D. tenuifolia*. É possível que, como para *D. erucoides*, se trate apenas de um efemerófito, pois que não voltou a ser colhida nesse local. Não deixa de ser um tanto estranho que WELWITSCH tenha encontrado em Tróia exemplares de duas espécies — *D. erucoides* e *D. tenuifolia* —, que nunca mais aí foram herborizadas, registando-se ainda o facto de a última não voltar a ser colhida em Portugal.

Diplotaxis vicentina (Cout.) Rothm. in Agron. Lusit. 2: 84 (1940). — Heywood in Fl. Eur. 1: 335 (1964). — A. Franco, Nov. Fl. Port. 1: 229 (1971).

Diplotaxis virgata sensu Mariz in Bol. Soc. Brot. 3: 105 (1885) quoad specim. a *Welwtsch* in Promont. Sacro lecto, non (Cav.) DC. (1821).

Diplotaxis virgata raça *vicentina* Samp., Man. Fl. Portug.: 194 (1910), comb. illeg.

Diplotaxis virgata subsp. *vicentina* Cout., Fl. Port.: 262 (1913).

Diplotaxis siifolia var. *vicentina* (Cout.) Cout., Supl. Fl. Port. (in Bol. Soc. Brot., Sér. 2, 10): 57 (1935); Fl. Port. ed. 2: 315 (1939). — Samp., Fl. Portug.: 236 (1947).

O tipo de *D. vicentina* é o exemplar colhido por WELWISTCH no Cabo de S. Vicente em Junho de 1847.

Este taxon, como se pode ver pela sua sinonímia, tem sido diversamente interpretado pelos investigadores, os quais o têm subordinado ou a *D. virgata* (Cav.) DC. ou a *D. siifolia* Kunze. Estes dois pontos de vista justificam-se em parte, porquanto, pelo hábito (planta, por vezes, perenante, com as folhas reunidas predominantemente na base) e pelo indumento do caule e folhas, se assemelha a *D. virgata*, enquanto que, pelas folhas penatipartidas ou penatissectas com o segmento terminal maior que os laterais e pelas dimensões das flores se assemelha a *D. siifolia*. Embora esta última seja uma planta anual, com caules mais longos e mais ramificados, cachos frutíferos relativamente mais curtos [em *D. vicentina* os cachos frutíferos são cerca do dobro (ou mais) mais longos que o caule], silíquas mais estreitas e menos

aquilhadas e com o rostro mais curto (3-5 mm longo, enquanto em *D. vicentina* o seu comportamento varia de 5 a 9 mm), comprimido e não tetragonal (como é o caso do rostro de *D. vicentina*), certos exemplares de *D. siifolia,* como os de Vila Nova de Milfontes, assemelham-se bastante, pelo aspecto geral, disposição das folhas, etc., a *D. vicentina.* Há, no entanto um carácter que permite distinguir as duas entidades, o qual consiste na forma das sementes, que são ovóides em *D. catholica* subsp. *siifolia (D. siifolia)* e globosas em *D. vicentina,* espécie em que elas são ainda um pouco maiores do que naquele taxon. Na falta de sementes maduras, a identidade de exemplares com outros caracteres duvidosos, só poderá ser esclarecido pelo exame de material do mesmo local que apresente sementes em completa maturação. Está neste caso uma colheita feita nas dunas de S. Torpes e que foi referida a *D. vicentina* (cf. Mem. Soc. Brot. **21**: 168, 1970-71), tanto mais que nas proximidades de Sines foi colhida a *D. catholica* subsp. *siifolia* (entre a ponte de Provença e o mar, *Bento Rainha* 1002, LISE).

As afinidades entre *D. vicentina* e *D. catholica* subsp. *siifolia* são ainda realçadas pela existência nesta última de uma variedade — var. *latirostris* (Br.-Bl.) Maire (= *Erucastrum latirostre* Br.-Bl) — com sementes esféricas como as de *D. vicentina.* Como pensássemos que esta entidade do litoral sudoeste marroquino, onde é bastante frequente (cf. MAIRE, Fl. Afr. Nord **12**: 283-284, 1965), se pudesse identificar com a planta algarvia, resolvemos esclarecer o caso [11]. O confronto de exemplares de *D. vicentina* com o da var. *latirostris* (tipo e alguns outros espécimes) mostrou-nos que se trata de dois taxa diferentes: a planta de Marrocos distingue-se da portuguesa, entre outros caracteres, pelo indumento do caule, formado por pêlos mais curtos (0.4-0.6 mm, contra 1 mm e mais longos em *D. vicentina*), pelas sépalas um pouco mais curtas e mais largas, pelas pétalas um pouco menores (7×3.25 mm contra $\pm 8.5 \times 3.5$ mm em *D. vicentina*), pelas silíquas mais estreitas e, sobretudo, pelo rostro quase da mesma largura da base ao cimo e comprimido, enquanto em *D. vicentina* o rostro estreita da base para o cimo e é distintamente tetragonal, com a nervura mediana formando quilha. Não há, pois, dúvida

[11] Ao Director de MPU, agradecemos o pronto envio do material da var. *latirostris* que lhe solicitámos em empréstimo.

que a planta do litoral de Marrocos é diferente de *D. vicentina,* filiando-se, pelo que se refere ao tipo do indumento e divisão do limbo foliar (além de outros caracteres), em *D. catholica* subsp. *sifolia,* conforme MAIRE o fez.

Diplotaxis virgata forma **humilis** Coss., Comp. Fl. Atl. **2**: 166 (1887)..

Diplotaxis virgata var. *platystylos* sensu Willk. in Willk. & Lange, Prodr. Fl. Hisp. **3**: 866 (1880) pro parte, et auct. plur., non var. *platystylos* (Willk.) Nyman (1878) nec *Diplotaxis platystylos* Willk. (1847).

?Diplotaxis virgata forma *saharensis* Coss., op. cit.: 165 (1887).

Brassica erucoides (L.) Kuntze β *catholica* (L.) Kuntze forma *integrifolia* Kuntze et forma *humilis* (Coss.) Kuntze, Rev. Gen. Pl. **1**: 17 (1891).

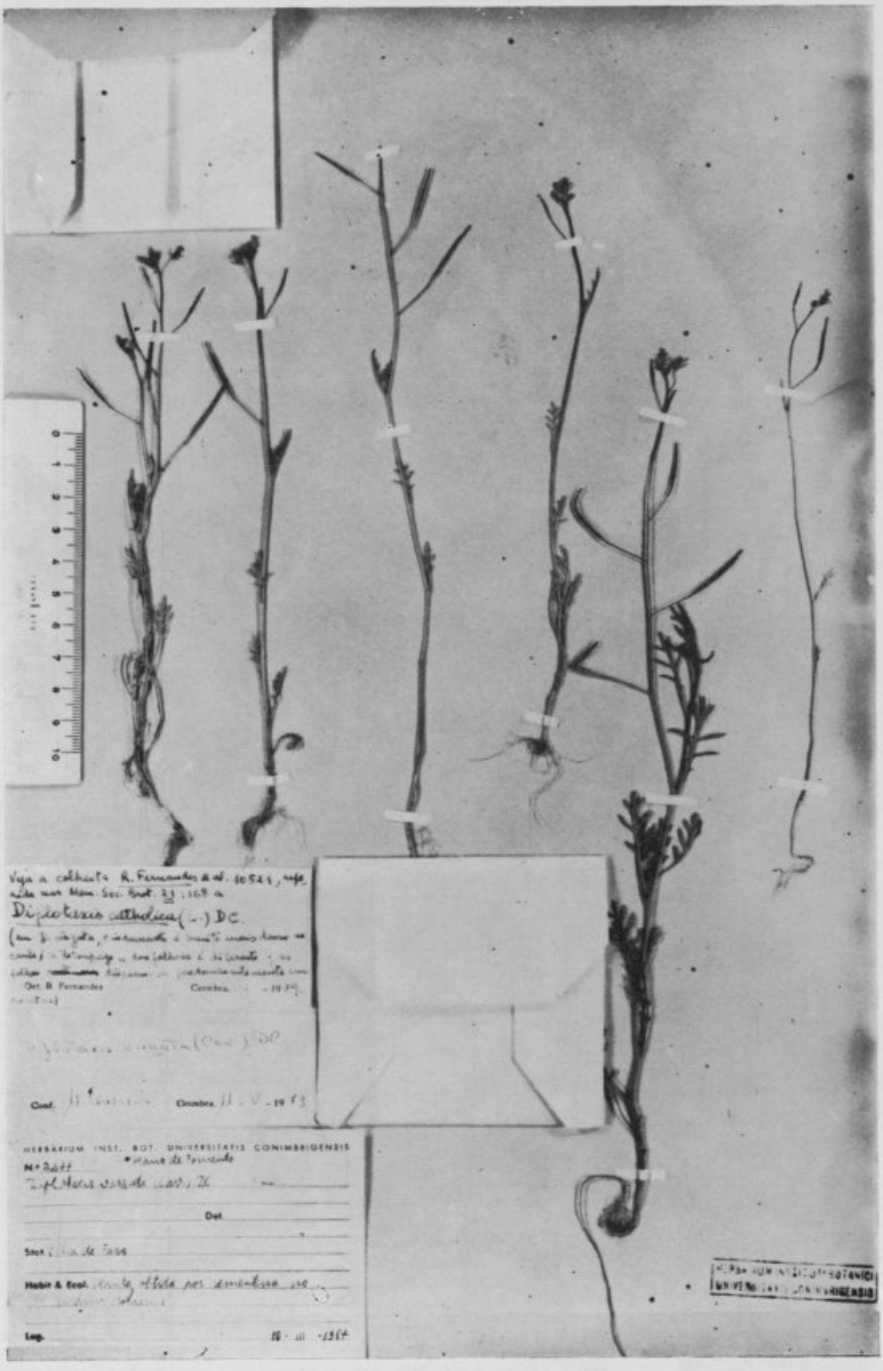
?Diplotaxis cossoniana var. *saharensis* (Coss.) Maire in Bull. Soc. Hist. Nat. Afr. Nord **30**: 330 (1939).

Como atrás dissemos, o tipo de *D. platystylos* Willk., assim como o exemplar colhido por este próximo de Albufera (Valência, Espanha) pertencem a uma forma reduzida — forma *valentina* (Pau) Maire — de *D. erucoides.* Vários autores têm referido exemplares de *D. virgata* com o rostro das silíquas curto — 1-2 mm longo — e tão largo como as valvas à var. *platystylos.* Ora, um dos espécimes que WILLKOMM, em 1880, referiu à «var. *platystylos*» é precisamente um dos síntipos da forma *humilis* Coss. Trata-se do exemplar *Bourgeau* 1564 (que designamos como lectótipo da forma), herborizado próximo de Alicante, o qual se encontra no herbário de WILLKOMM (COI) e cujo estudo nos mostrou poder incluir-se em *D. virgata,* como uma forma ou variedade.

O. E. SCHULZ, na sua monografia (in Pflanzenr. IV-105, **1**: 171, 1919), inclui a forma *humilis* na sinonímia da var. *platystylos,* taxon que coloca em *D. virgata.* Parece, no entanto, não ter visto nem o tipo dessa forma nem o desta variedade, porquanto não os refere na lista dos exemplares citados na var. *platystylos.* Esse mesmo autor menciona na var. *platystylos* um espécime colhido por LINK no Porto, cuja identificação não podemos confirmar, conforme já atrás afirmamos (cf. pág. 242), por o não termos visto [12].

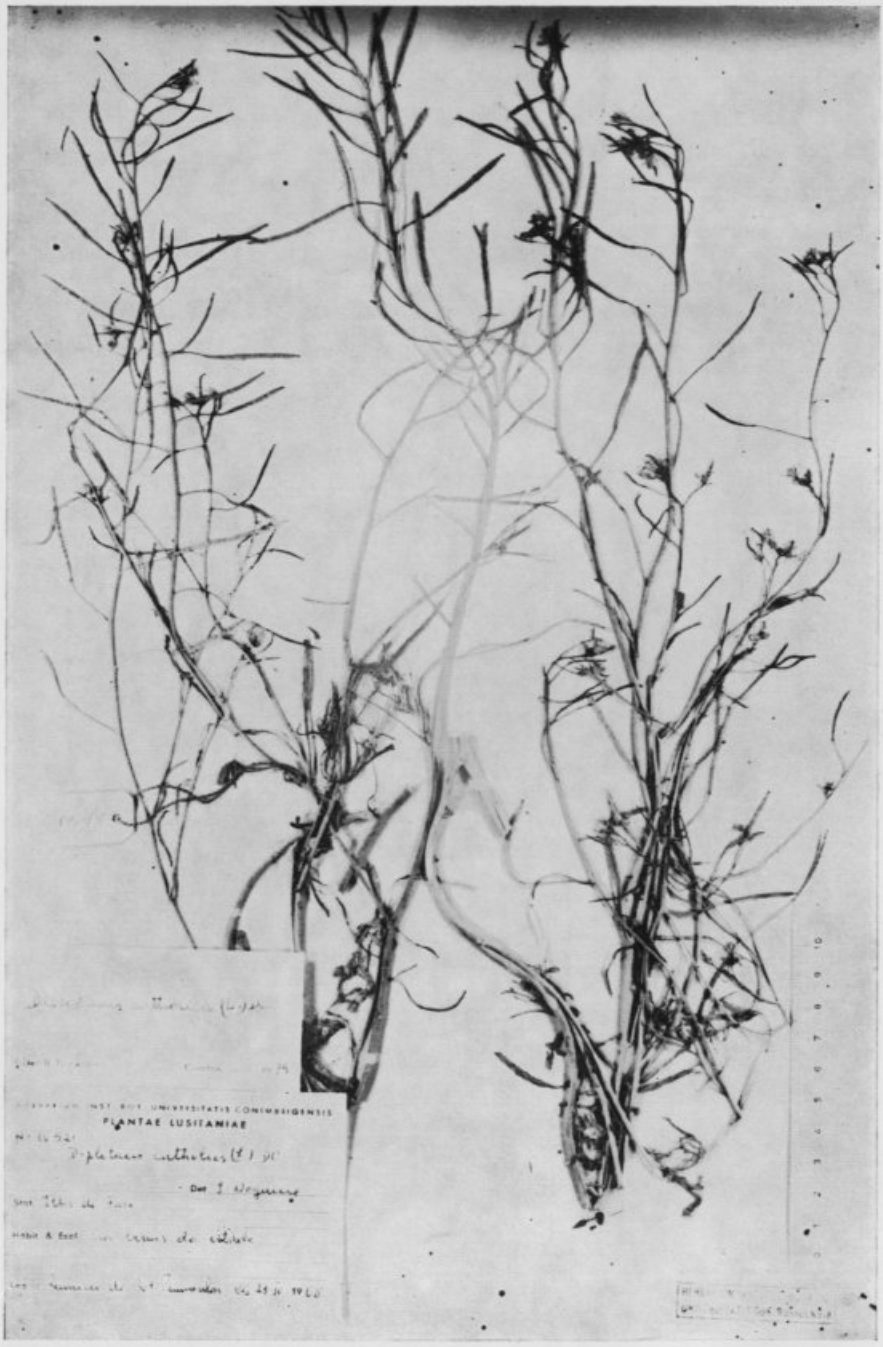
[12] Duvidamos da existência de uma forma de *D. virgata* nas margens do Douro, no Porto, ou em outro qualquer local do Douro Litoral, pois que esta espécie não tem sido encontrada nessa província pelos colectores portugueses.

A forma *humilis* encontra-se, no entanto, em Portugal, na Praia da Luz, Lagos, para onde foi referida por ROTHMALER & P. DA SILVA, em 1940 (in Agron. Lusit. 2: 83) sob *D. virgata* var. *platystylos*.



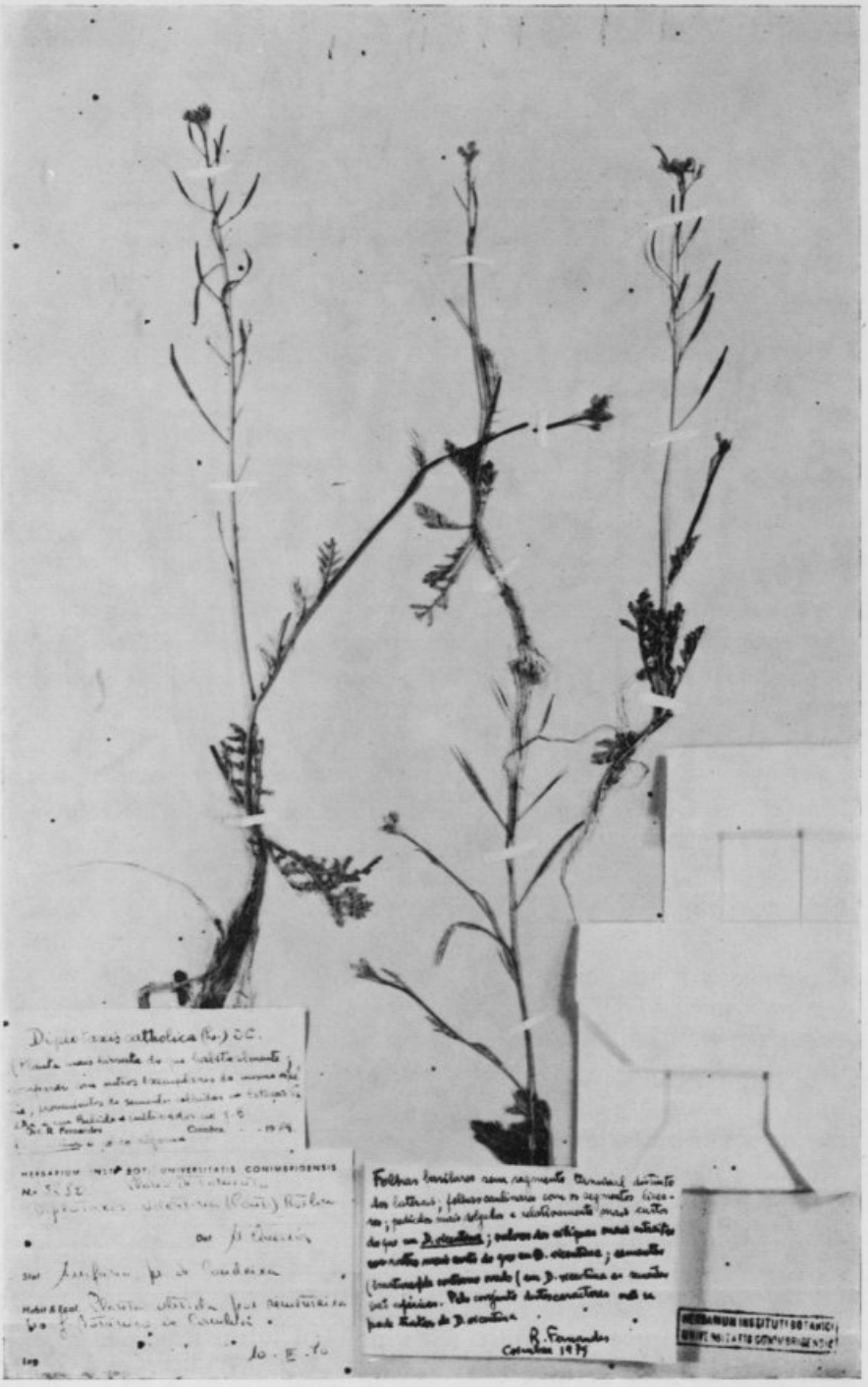
Diplotaxis catholica (L.) DC.

Planta cultivada no Jardim Botânico de Coimbra (sementeira n.º 2277) a partir de sementes colhidas na Ilha de Faro (III Reunião de Botânica Peninsular).



Diplotaxis catholica (L.) DC.

Espécime da colheita 10521 (COI) herborizado na Ilha de Faro durante a III Reunião de Botânica Peninsular, donde foram retiradas as sementes para a sementeira 2277.



Diplotaxis catholica (L.) DC.

Exemplar cultivado no Jardim Botânico de Coimbra (sementeira n.º 3280), obtido a partir de sementes colhidas em Arrifana (Condeixa).

Diplotaxis muralis (L.) DC. forma **caulescens** Kitt.

Espécime *A. Fernandes, R. Fernandes & J. Matos* 7226 (COI), colhido na Praia das Maçãs.

Bol. Soc. Brot., Sér. 2, 58: 249-257 (1985).

THE DISTRIBUTION AND ECOLOGY OF *VERBASCUM PULVERULENTUM* VILL. IN EASTERN ENGLAND

by

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Received May 22, 1985.

Vesbascum pulverulentum Vill. has a total distribution area which reaches its northern limits in eastern England southern Belgium; France; southwest Germany; southern Austria; and south east Hungary. The eastern limits are found in western Bulgaria and south-west Rumania, while the southern limits run through central Greece; Sicily; Sardinia; and central Spain and Portugal (MURBECK, 1933). The western limits have been defined by field work only in Portugal (PARKER, 1978). There are no precise details from France, although FOURNIER (1961) states that the species is mainly found in the north, north-east, and south-east of that country.

Within the area it occupies, distribution of the plant is invariably scattered and uneven. This is particularly so in England and Belgium, where it is a lowland plant which favours light calcareous soils. In Germany it occurs in the upper Rhine provinces, Alsace and Bavaria; where HEGI (1965) considers it to be adventive. In Austria the plant occurs in the lowlands of the southern Tyrol and south-east Steyermark, mainly near cities. In Switzerland it occurs in the south Jura and Waadt in the west, and south Tessin in the east. In both these areas it is found on warm sunny slopes in dry meadows and vineyards, even in these areas however, it is uncommon (HESS *et al.*, 1967), as it is in northern Italy. It is only the south and south-eastern part of its range that the species is relatively more frequent. In this region it is also found as an upland plant. It occurs up to 700 m.

in Jugoslavia, 914 m. in Greece (in the sub-alpine zone), and 1200 m. in Calabria, southern Italy (MURBECK, l. c.). Throughout most of its range, the plant is only recorded from open sunny places, occurring in associations of ruderal plants. The most common habitats are open grassland, bare and rocky hillsides, disturbed soil in hedgebanks or by roadsides, and fallow or neglected farmland.

V. pulverulentum Vill. is at the extreme north-western limit of its range in Britain, and the existing stable long term populations occur in East Anglia (Fig. 1). Elsewhere in Britain the plant only occurs as an adventive, often associated with introduced soil (WATSON, 1934; Miss M. MCCALLUM-WEBSTER pers. comm.).

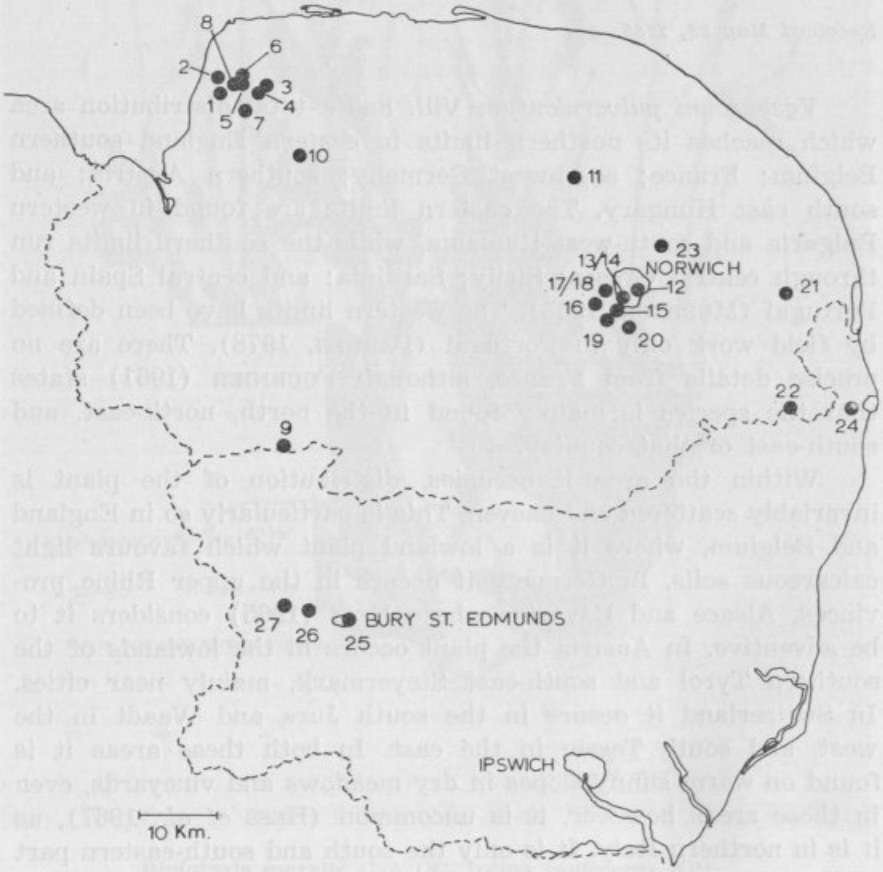

Fig. 1. — Map of distribution of *Verbascum pulverulentum* Vill. in East Anglia, England.

The present distribution of *Verbascum pulverulentum* Vill. in East Anglia is quite strongly localised. In the west at Snettisham there is a quarry probably 200 years old, used for road stone and sand, which contains a large persistent population of the species. In the east there is a cluster of populations in and around the city of Norwich, also further south, in and around Bury St. Edmunds. In each case the species has apparently colonised outwards from areas disturbed by man, probably being assisted in part by transport of seed in sand and ballast. This process has been observed by the author on a roadside verge near Snettisham, where quarry material had been used as infill.

Within East Anglia, *V. pulverulentum* Vill. is found in rough open grassland, on disturbed roadside verges, quarry spoil heaps, and railway embankments and cuttings. Because of the type of habitat it occupies, the associates of this species are varied, and include many ruderal species. The twenty-seven sites selected for study cover the main areas of distribution. For recording purposes at each site, the area on which *Verbascum* occurred was delimited, then all the species found growing within one metre of any plant in the population were recorded, their abundance being expressed on a five-class scale (TANSLEY & CHIPP, 1926). Table 1 lists species recorded from the selected sites in order of the frequency in which they occur, all biomials are as in Flora Europea. Those species with which *V. pulverulentum* L. is most commonly associated in these communities, *Arrhenatherum elatius, Dactylis glomerata, Urtica dioica, Rubus fruticosus* agg., *Plantago lanceolata,* and *Achillea millifolium,* are all frequently found in uncut and ungrazed grassland habitats.

The localities and brief descriptions of the sites recorded in this survey, along with the names of additional species occurring at low frequency, are presented in Table 2. All the sites are clearly associated with human activity, they are either still in a disturbed open state, or in areas where a rather open herbaceous vegetation has developed. The type of soil favoured by the species is quite consistent; where populations occur on railway embankments built from both sandy soils, and heavier chalky clay, as at Barrow Bottom near Bury St. Edmunds, only the sandy areas support plants of *V. pulverulentum.* The preference for light soils is apparently a common factor in Europe. In Portugal, the soils on which it grows are sandy and stony (PARKER, l. c.). In Hungary

TABLE 1. SPECIES LISTS FROM SITES CONTAINING VERBASCUM PULVERULENTUM L

SITE NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
BARE GROUND %	30	5	5	10	5	20	20	5	25	30	50	40	15	40	5	25	10	50	20	60	60	40	15	50	50	70	10
COMMON																											
Achillea millefolium	o	f	f	f	-	f	a	f	-	a	a	-	f	-	f	o	-	-	f	-	f	a	f	-	f	-	a
Arrhenatherum elatius	o	-	a	f	a	f	a	-	f	f	o	-	a	a	f	r	o	f	f	-	f	f	a	1.a	f	f	a
Dactylis glomerata	o	f	a	f	-	o	a	a	-	f	f	-	-	a	f	o	-	f	o	f	o	o	o	-	-	-	o
Plantago lanceolata	o	f	f	-	-	o	a	f	-	o	-	-	f	f	f	-	-	f	-	-	f	f	f	-	f	-	f
Rubus fruticosus	-	-	o	o	-	o	o	o	o	f	-	-	-	f	o	o	r	r	f	-	f	-	-	r	r	o	a
Urtica dioica	r	-	o	o	o	o	o	-	-	r	-	o	-	-	o	o	-	-	f	f	f	-	o	-	o	-	-
FREQUENT																											
Anthriscus sylvestris	-	-	o	f	-	o	-	f	f	o	-	o	f	f	-	-	-	-	o	f	-	-	-	f	-	-	-
Atremisia vulgaris	r	-	f	-	-	-	-	-	-	-	r	o	o	-	f	-	o	-	o	o	-	-	-	o	f	o	o
Calystegia sepium	-	-	o	-	o	o	o	-	a	-	-	-	-	-	-	o	-	-	-	-	-	-	o	-	f	-	-	a
Cirsium arvense	o	-	-	-	o	-	o	o	-	-	o	a	-	-	o	f	o	-	f	f	-	-	o	-	o	-	f
Glechoma hederacea	o	-	-	-	f	-	o	o	-	o	-	-	-	-	-	-	-	-	f	-	o	o	-	-	-	-	-
Heracleum sphondylium	-	o	-	-	-	-	-	o	r	o	-	-	-	-	-	-	-	-	f	-	r	-	-	-	-	-	o
Hieracium pilosella	o	-	-	-	-	o	-	-	a	-	-	-	-	-	f	-	o	-	-	-	a	-	f	f	-	-	-
Holcus lanatus	-	-	f	o	-	-	-	-	-	-	f	-	-	-	f	-	f	-	o	-	-	f	-	-	-	-	-
Hypericum perforatum	-	-	-	-	-	-	-	-	-	-	-	-	o	-	f	f	-	-	-	-	f	a	r	-	-	-	a
Knautia arvensis	o	-	o	-	-	-	f	-	f	o	o	-	f	o	-	-	-	-	-	o	-	-	o	-	-	-	f
Lamium album	r	-	-	-	o	o	-	-	a	o	-	-	-	-	-	-	-	r	-	o	o	-	-	-	o	o	-
Lamium purpureum	o	-	o	-	-	a	-	-	-	-	-	o	r	-	f	-	-	-	f	-	o	-	-	-	-	-	-
Lolium perenne	-	f	-	-	-	-	a	o	-	-	f	-	-	-	-	-	-	-	-	-	-	o	f	-	f	-	-
Plantago media	o	f	o	-	-	o	a	f	-	-	f	-	-	-	f	-	-	f	-	f	-	-	-	-	-	-	-
Poa annua	f	-	-	-	-	-	a	-	-	-	f	-	-	-	-	-	-	f	-	f	-	f	-	f	-	-	-

Poa annua	f	-	-	-	-	-	a	-	-	-	f	-	-	-	-	-	-	f	-	f	-	f	-	f	-	-	-	-	-
Poa pratensis	f	o	f	-	-	-	-	-	-	-	-	-	-	-	-	f	f	-	-	-	-	-	f	-	f	-	-		
Potentilla reptans	-	-	f	-	-	-	f	f	-	o	-	-	f	-	-	-	-	-	f	o	-	-	-	-	f	-	-		
Rumex crispus	o	-	-	-	o	-	-	-	-	-	o	-	o	-	-	-	-	-	-	o	-	-	-	-	r	-	-		
Senecio jacobaea	o	o	o	-	-	-	-	-	-	-	o	f	a	-	-	-	-	o	o	-	o	o	f	-	-	-	-		
Silene alba	r	o	f	-	-	-	-	f	-	-	o	-	-	-	-	o	o	r	-	o	-	-	-	-	-	-	o		
Trifolium repens	o	-	-	f	-	f	-	f	-	-	a	-	-	-	a	-	-	o	o	f	-	a	-	-	-	-	-		
OCCASIONAL																													
Agrostis stolonifera	-	o	-	-	-	-	o	-	-	-	-	-	-	-	-	-	a	-	-	-	-	-	a	-	-	-	-		
Crepis capillaris	-	-	-	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	o	o	-	f	-	-		
Daucus carota	-	-	-	-	-	-	-	-	-	-	f	-	f	o	o	o	-	o	-	-	-	-	-	-	-	-	-		
Elymus repens	-	-	o	-	-	-	r	-	-	-	-	-	-	-	a	-	o	-	-	-	-	o	-	-	-	-	-		
Epilobium angustifolium	-	-	-	-	-	-	-	-	f	-	-	f	-	-	-	-	o	-	-	-	-	-	-	-	o	-	-		
Galium aparine	o	-	-	f	r	-	-	-	-	-	-	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	o		
Galium verum	-	-	f	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	o	-	-	-	a		
Matricaria maritima	-	-	-	-	-	-	-	-	-	-	o	-	-	-	f	o	-	-	-	f	o	f	-	-	-	-	-		
Subsp. *inodora*																													
Reseda lutea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	f	-	f	-	-	-	-	-	-	-	f	f	a		
Reseda luteola	o	-	o	-	-	-	f	-	-	-	-	o	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Rumex obtusifolius	-	-	o	-	-	-	o	-	-	-	-	-	-	-	-	-	-	o	-	-	o	-	-	-	-	-	-		
Taraxacum officinale	o	-	-	-	-	f	-	f	-	-	-	-	-	-	-	-	o	o	-	-	-	-	-	-	-	-	-		
Trisetum flavescens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	f	-	o	o	o	-	-	-	-		

Abundance recorded on a five class scale. (Tansley & Chipp 1926). All quadrats 1 M^2

va = very abundant;
a = abundant;
f = frequent;
o = occasional;
r = rare.

TABLE 2

Localities and habitats of the *Verbascum pulverulentum* Vill. populations recorded, plus additional species of low frequency

Locality	Habitat	Site no.
Snettisham	Carr stone quarry (sandstone). Plants discontinuously distributed over central spoil heaps and surronding cliff tops. Soil pH 7.9. [*Veronica chamaedrys* (o), *V. arvensis* (r)].	1
Heacham	Along the grass verge of the A. 149. Soil pH 7.9.	2
Docking	Roadside verge, on S.W. facing slope. Soil pH 7.8. [*Silene vulgaris* (o), *Tragopogon pratensis* (o)].	3
Fring	Hollow area in steep roadside bank. Soil pH 8.1. [*Geranium pratensis* (r), *Silene vulgaris* (o)].	4
Sedgeford	Glovers farm, along field edge, and roadside ditch. Soil pH 7.7. [*Veronica chamaedrys* (f), *Scabiosa columbaria* (f)].	5
Sedgeford	Verge of A.1067 by Peddars Way, S.W. aspect. Soil pH 8.0. [*Malva sylvestris* (r), *Medicago sativa* (o)].	6
Shernborne	Farm track off the Peddars Way. Soil pH 7.2. [*Pastinaca sativa* (r), *Veronica arvensis* (f)].	7
Sedgeford	West Hall Farm, road verge, and steep slope in pasture. Soil pH 7.8. [*Leontodon autumnalis* (o), *Medicago sativa* (o), *Tragopogon pratensis* (o), *Vicia sativa* ssp. *nigra* (o)].	8
Brandon	S.E. side of railway embankment just north of bridge over Little Ouse river. Soil pH 7.9. [*Equisetum arvense* (f), *Oenothera erythrosepala* (o)].	9
Harpley Dams	Verges of A.148. Soil pH 7.9. [*Geranium molle* (f), *Malva sylvestris* (o)].	10
Cawston	Disturbed soil at the juction of B.1149 and B.1145. Soil pH 7.9. [*Scabiosa columbaria* (f)].	11
Norwich	Car park and soil tip, Lawrence Scott and Electromotors Ltd. [*Medicago lupulina* (o), *Oenothera erythrosepala* (f), *Urtica urens* (o)].	12

TABLE 2 *(Contd.)*

Locality	Habitat	Site no.
Norwich	Waste ground by Harford cattle market. Soil pH 8.0. [*Medicago lupulina* (o), *Fragaria vesca* (o)].	13
Norwich	South facing slope of railway cutting. Soil pH 7.7.	14
Norwich	Neglected grassland facing south, Marston Lane Soil pH 7.6. [*Chenopodium album* (o), *Malva sylvestris* (o), *Papaver rhoeas* (o), *Silene vulgaris* (f)].	15
Norwich	Disturbed soil and neglected grassland, Colney Lane. Soil pH 7.6. [*Bulderdykia convulvulus* (o), *Rumex acetosa* (f), *Teucrium scorodonia* (o).]	16
Norwich	Neglected grassland on south facing slopes of Yare valley, Eaton. Soil pH 7.6. [*Medicago lupulina* (f)].	17
Norwich	Gravel area by Park church, Eaton. Soil pH 7.5. [*Hirschfeldia incana* (f), *Medicago* × *varia* (f)].	18
Keswick	Trackway and hedge bank by chalk quarries. Soil pH 7.9.	19
Arminghal	South facing slope of sand pit. Soil pH 8.1. [*Carduus nutans* (f), *Chenopodium album* (o), *Urtica urens* (f)].	20
Acle	Railway bridge embankment, very loose disturbed soil. Soil pH 7.9. [*Papaver rhoeas* (o), *Pastinaca sativa* (f)].	21
Aldeby	Abandoned railway sidings by river Waveney. Soil pH 7.8. [*Equisetum arvense* (f), *Geranium molle* (o), *Oenothera erythrosepala* (a), *Rumex acetosa* (f)].	22
Rackheath	Neglected grassland on south facing slope by church. Soil pH 6.6. [*Agrostis tenuis* (f), *Teucrium scorodonia* (f)].	23

TABLE 2 *(Contd.)*

Locality	Habitat	Site no.
Oulton Broad	Gravel area by boat yard, Commodore Road. Soil pH 7.7. [*Equisetum arvense* (r), *Rumex acetosa* (o)].	24
Bury St. Edmonds	Goods yard by railway station. Soil pH 7.9.	25
Saxham	By station on south side of railway embankment. Soil pH 7.9.	26
Barrow Bottom	South facing slope of very high railway embankment, on areas of light soil. Soil pH 7.8. [*Carduus nutans* (f), *Papaver rhoeas* (o), *Thymus pulegoides* (f), *Verbascum nigrum* (r)].	27

the plant occurs on the more basic, dry, warm, loose, sandy soils (Soo, 1968), and in Belgium only on light calcareous soils derived from the chalk (MULLENDERS, 1967). In Britain, few such habitats are found. Only on certain alkaline sandy soils in East Anglia is the semi-natural vegetation open enough to allow germination of seed, and establishment of persistent populations of this species. Even here, the degree of competition from associated herbaceous species is enough to lengthen the rosette stage of the plant to considerably more than two years (PARKER in prep.). It is probable that the species came in with man after the deforestation of eastern England, surviving in only the most favourable places since that time.

ACKNOWLEDGEMENTS

I would like to record my appreciation of the travel grant provided by the University of Leicester Research Board.

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NUEVO MODELO DE REPRESENTACIÓN DE LOS DATOS FENOLÓGICOS DE LA ESCALA DE ELLEMBERG

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Recibido el 25 Mayo, 1985.

RESUMEN

La representación gráfica de los datos fenológicos de la escala de Ellemberg presenta dificultades cuando se emiten más de dos renuevos foliares al año. Se propone para estos casos difíciles una nueva representación matricial escalonada donde las columnas corresponden a los meses del año y las filas a los renuevos foliares. Se amplía la metodología propuesta a casos especiales, como los terófitos con más de una generación anual o las plantas heterófilas.

ABSTRACT

New model for phenological data representation of Ellemberg's scale.

The phenological data graphic representation of Ellemberg's scale present difficulties when more than two leaf shoots are emited annually. For these difficult cases, a new spreaded out matricial representation is proposed, where the columnes correspond to the year months and the files to the leaf shoots. The proposed methodology is enlarged to special cases like the terophytes with more than one annual generation or the heterophylle plants.

INTRODUCCIÓN

DURANTE los últimos años se han realizado en la botánica española diversos trabajos destinados a establecer las relaciones entre el desarrollo fenológico de las especies vegetales y las variables ecológicas que lo determinan. En algunos de ellos, como el de MORENO RODRIGUEZ (1982), se utiliza la escala fenológica de Ellemberg para la toma de datos. Esta escala se basa

en una aproximación subjetiva al desarrollo de los órganos aéreos de la planta, especialmente en lo que se refiere a vástagos y hojas.

Cuando una planta emite un sólo renuevo foliar al año, como ocurre en la mayoría de terófitos, geófitos bulbosos y fanerófitos caducifolios, la representación gráfica de los datos es muy sencilla. En el caso de que sean dos los renuevos foliares emitidos al año puede representarse sólamente uno de ellos, tal y como observa la metodología de Balatová-Diercke (MORENO RODRIGUEZ, op. cit.).

Los casos anteriores agrupan a la mayoría de las fanerógamas atlánticas y continentales, donde las condiciones climáticas no suelen permitir la emisión de más de 2 renuevos foliares por planta al año. Sin embargo, en áreas mediterraneas cálidas los inviernos suaves pueden permitir la emisión de renuevos foliares en los meses de Noviembre y Diciembre, con lo que algunas fanerógamas presentan al año siguiente hasta 3 y 4 renuevos foliares superpuestos. Para estos casos las representaciones gráficas clásicas se hacen inoperantes.

A lo largo de un estudio fenológico de la flora del Barranco Real — Sierra del Caballón, Valencia, UTM 30S XJ 95 e YJ 05 — encontramos numerosos casos de táxones que emitían más de dos renuevos foliares al año. El seguimiento se realizó durante el año 1983, en el que las condiciones meteorológicas fueron muy favorables. En la tabla nº 1 se presentan los datos climáticos medios mensuales para el área de estudio. Puede apreciarse la suavidad de las temperaturas invernales a principios de 1983. También destacan las altas temperaturas del mes de Octubre, típicas de los veranos de tipo «desplazado» (DAGET, 1977*a* y *b*) del área valenciana. Estas temperaturas cálidas de otoño, junto a las fuertes lluvias, permiten la emisión de renuevos anómalos en numerosos táxones, tal y como habíamos observado en años anteriores al del presente estudio. También se dieron con frecuencia las floraciones múltiples.

Nosotros proponemos una nueva metodología de representación de los datos de la escala de Ellemberg para estos casos difíciles, que exponemos a continuación.

METODOLOGIA PROPUESTA

La representación que proponemos se basa en la disposición matricial escalonada de los datos de la escala fenológica, donde las filas equivalen a los renuevos foliares y las columnas a los

TABLA Nº 1

	E	F	M	A	M	J	J	A	S	O	N	D
Datos térmicos												
Temperaturas en ºC												
Máxima absoluta	27.4	28.0	29.2	32.4	32.0	39.4	38.6	38.2	37.0	36.6	20.6	16.4
Media de las máximas	21.3	18.2	23.2	25.1	28.3	30.6	34.1	33.7	33.8	29.1	17.7	13.6
Media	15.2	13.1	16.8	19.2	22.5	25.1	28.6	28.3	27.7	22.7	14.3	8.5
Media de las mínimas	6.3	8.0	12.4	13.2	16.7	19.6	23.0	22.9	21.6	16.3	10.8	3.4
Mínima absoluta	1.8	1.6	3.6	6.6	10.0	15.4	18.0	16.6	15.8	10.0	4.0	-1.2
Datos hídricos												
Precipitaciones en mm.	0.0	14.7	8.6	2.3	0.0	14.6	3.3	104.0	0.0	23.6	299.6	10.4

Climatología del área de estudio durante 1983. Datos extraídos de las estaciones de la Red Meteorológica Nacional más cercanas al área de estudio.

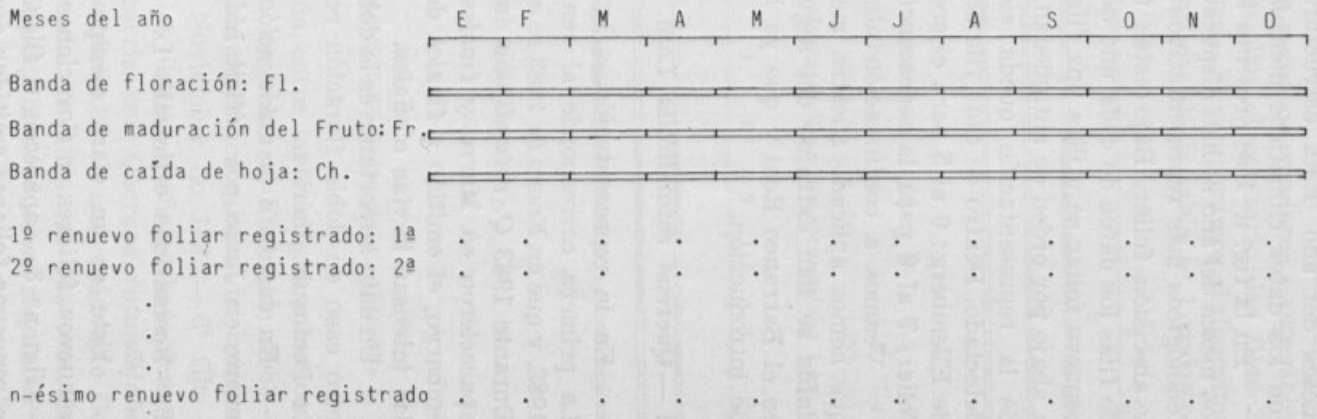


Fig. n.º 1. — Matriz en blanco de la nueva representación propuesta para los datos fenológicos de la escala de Ellemberg.

meses del año. Esta estructura permite comparaciones fáciles con los datos climáticos mensuales.

En la fig. nº 1 se presenta la estructura de esta matriz. Bajo los meses del año se han dispuesto tres bandas para los fenómenos fenológicos más representativos: floración, maduración de frutos y abscisión foliar. Bajo estas tres bandas se disponen a modo de filas los datos de cada uno de los renuevos foliares. Para cada renuevo foliar se utiliza una fila y las filas se ordenan de arriba a abajo por orden de antigüedad, de modo que en la parte inferior de la representación queda siempre el último renuevo foliar detectado. Dentro de cada fila se disponen los valores de la escala de Ellemberg: 0 al 5 para el crecimiento foliar, 6 para el óptimo foliar, 7 al 9 para la senescencia y 10 para la abscisión.

Veamos a continuación algunos ejemplos ilustrativos a los que hemos aplicado nuestra metodología de representación. Los datos se han extraído del seguimiento que realizamos en 1983 en el Barranco Real y que ya ha sido comentado en el capítulo de introducción.

1 — **Quercus rotundifolia** Lam.

En la representación — fig. nº 2 — son observables 4 filas. La primera corresponde al renuevo foliar emitido en otoño de 1982, y que en Enero de 1983 se encontraba en el óptimo vegetativo. Durante 1983 *Q. rotundifolia* emitió 3 renuevos foliares. Los que aparecieron en Marzo y finales de Junio fueron regulares. Sin embargo, el emitido a finales de Noviembre fue una respuesta a las intensas lluvias otoñales.

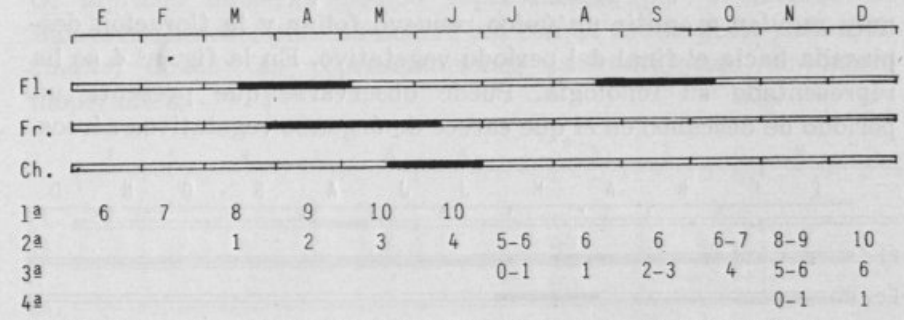
Un dato importante es la doble floración. No conocemos ningún otro caso de doble floración registrado en *Q. rotundifolia* para la Península Ibérica.

En cuanto a la maduración del fruto, ésta se frenó bruscamente en Junio por déficit hídrico.

2 — **Rosmarinus officinalis** L.

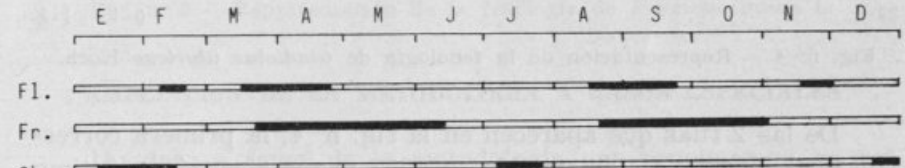
Este es un claro ejemplo de emisión supernumeraria de renuevos foliares y floraciones. En la representación matricial — fig. nº 3 — aparecen 6 filas. Las dos primeras corresponden a renuevos foliares emitidos durante 1982. Las 4 últimas corres-

ponden a los renuevos emitidos durante 1983. Concretamente, las filas 4ª y 5ª corresponden a los renuevos regulares o esperables de primavera y finales de verano. Por el contrario, las filas 3ª y 6ª corresponden a renuevos «extra». Si comparamos el diagrama de la fig. nº 3 con los datos climáticos de la tabla nº 1 podemos



1ª	6	7	8	9	10	10						
2ª			1	2	3	4	5-6	6	6	6-7	8-9	10
3ª							0-1	1	2-3	4	5-6	6
4ª											0-1	1

Fig. nº 2. — Representación de la fenología de *Quercus rotundifolia* Lam.



1ª	9-10	10										
2ª	5	6	6	6-7	7	8-9	10					
3ª	0-1	1	2	3	4	4-5	6	6	7	8	9	10
4ª					1	2	3	4-5	6	6	6-7	8
5ª								0-1	2	3	4	5
6ª											0-1	1

Fig. nº 3. — Representación de la fenología de *Rosmarinus officinalis* L.

ver que existe una fuerte relación entre la fenología y las condiciones ambientales; el renuevo emitido en Enero de 1983 — 3.ª fila — se dio aprovechando las condiciones cálidas del invierno 1982-83. El renuevo emitido en Noviembre do 1983 — 6ª fila — corresponde a una respuesta a las lluvias otoñales bajo temperaturas suaves, al igual que ocurría con *Quercus rotundifolia*.

Destaca la existencia en *R. officinalis* de una cuádruple floración para la población muestreada. De ellas, las dos centrales — Marzo a Abril y Agosto a Septiembre — fueron regulares y de gran potencia. Por el contrario, las de Febrero y Noviembre

fueron poco potentes y de corta duración, viéndose frenadas por el descenso de temperaturas, que impidió la formación de frutos.

3 — **Gladiolus illyricus** Koch.

Este geófito, al igual que los terófitos presenta un tendencia muy regular a emitir un único renuevo foliar y la floración desplazada hacia el final del período vegetativo. En la fig. nº 4 se ha representado su fenología. Puede observarse que presenta un período de descanso en el que carece de órganos vegetativos aéreos.

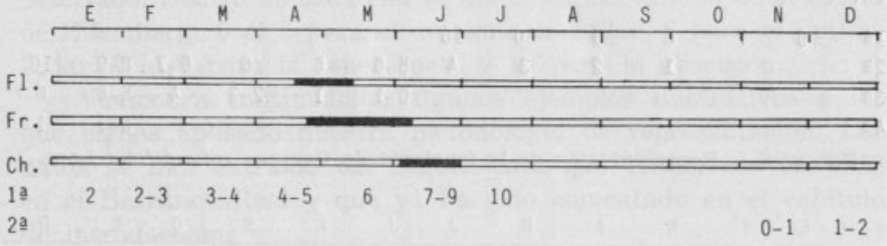


Fig. nº 4. — Representación de la fenología de *Gladiolus illyricus* Koch.

De las 2 filas que aparecen en la fig. nº 4, la primera corresponde al renuevo foliar emitido en 1982 y que alcanzó su óptimo en la primavera de 1983. La 2ª corresponde al emitido en 1983, que alcanzaría su óptimo en la primavera de 1984, no representada en el diagrama.

Reseñamos aquí que los geófitos mediterraneos desarrollan sus formas aéreas en invierno y primavera como respuesta a las condiciones hidrotérmicas, de modo que permanecen en estado de reposo durante la época calurosa del verano. Ello lo diferencia totalemente de la fenología de hemicriptófitos y fanerófitos caducifolios, que presentan formas de descanso en la época invernal. Es característico de los geófitos la aceleración de la senescencia foliar, lo que demuestra el alto consumo de energía que les provoca la floración y la maduración del fruto.

4 — **Fraxinus ornus** L.

Tenemos aquí el caso opuesto a la fenología de los geófitos. *F. ornus,* árbol caducifolio característico de las serranías valencianas y alicantinas, presenta su óptimo vegetativo en la época

estival. Por ello, en nuestro diagrama — fig. nº 5 — aparece representado por una única fila en la matriz de datos.

Al contrario que en los geófitos, *F. ornus* presenta la floración al principio del período vegetativo. No existe aceleración de la senescencia foliar. Reseñamos que tanto este caso como el de *G. illyricus* hubieran podido representarse por el método de Balatová-Diercke, pero no ocurre así con *Q. rotundifolia* o *R. officinalis,* dónde las representaciones convencionales se hacen inoperantes.

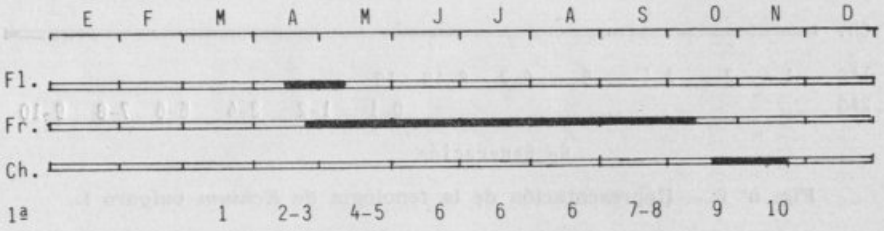


Fig. nº 5. — Representación de la fenología de *Fraxinus ornus* L.

AMPLIACIÓN DE LA METODOLOGÍA A CASOS ESPECIALES

Un dato a favor de la metodología que proponemos es que permite representar casos regulares de fenologías difícilmente abordables por los métodos convencionales. Nos referimos aquí a casos que, aun siendo normales incluso en climas continentales, necesitan de representaciones múltiples. Destacamos entre ellos los siguientes:

1 — Terófitos con más de una generación al año. Fig. nº 6

En algunos casos, las condiciones climáticas permiten el acoplamiento de más de una generación de terófitos en el lapso de un año. Presentamos aquí el de *Echium vulgare* L., cuyo diagrama aparece en la fig. nº 6. En nuestra zona de estudio, el ciclo de vida de *E. vulgare* fue de aproximadamente medio año de duración, permitiendo el solapamiento de 2 generaciones: una entre Enero y Julio y otra entre Julio y Diciembre. Cada generación se ha identificado con la letra G.

2 — **Plantas heterófilas.** Fig. nº 7

En las plantas heterófilas es frecuente que los 2 tipos de hoja presenten distinta fenología. Este caso es muy significativo en *Hedera helix* L., que se ha representado en la fig. nº 7. Aquí

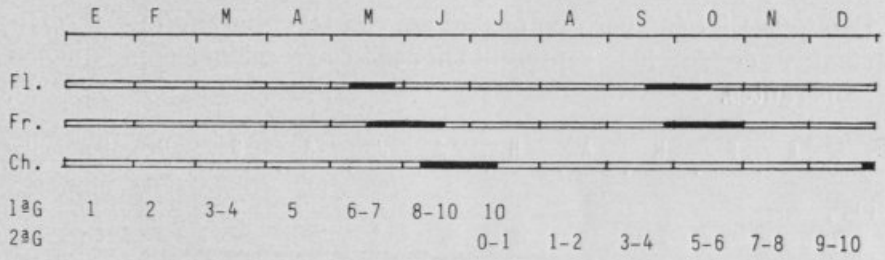


Fig. nº 6. — Representación de la fenología de *Echium vulgare* L.

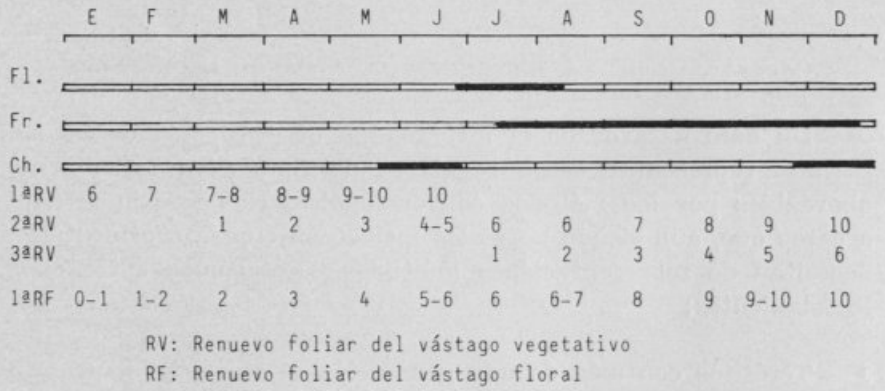


Fig. nº 7. — Representación de la fenología de *Hedera helix* L.

los renuevos del vástago vegetativo — RV — siguen distinta fenología que los del vástago floral — RF —, de morfología y funcionalidad marcadamente distinta. Puede apreciarse que durante 1983 *H. helix* emitió en el área de estudio un único renuevo del vástago floral, concretamente en el mes de Enero. Durante el mismo año presentó 3 renuevos foliares del vástago vegetativo: uno emitido en 1982 — fila 1ª — y dos emitidos en 1983 — filas 2ª y 3ª.

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EFFECTS OF PENICILLINS ON PLANT TISSUE CULTURES:

ULTRASTRUCTURAL AND BIOCHEMICAL ASPECTS

by

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Received December 15, 1985.

SUMMARY

A stablished callus culture derived from leaf mesophyll of *Sedum Telephium* L. was grown for 14 days on solid Gamborg's B-5 medium with 10 mg/dm³ of benzylaminopurine plus 0.1 mg/dm³ of naphtalene acetic acid as control, and on the same medium to which Penicillin G or Penicillin V (100 μg/cm³) were added through filter sterilization.

Morphometric determinations showed that treated cells were different from control ones, and also different among themselves. Considering a somewhat differentiated type of cell endoplasmic reticulum Sv values were determined as 1.48 for control, 1.78 for Penicillin G treated and 1.64 for Penicillin V treated tissues.

Following the same order, values for mitochondrial Vv were found as 0.30, 0.33 and 0.39; for thylakoid membranes Sv values of 1.92, 2.58 and 2.73 were determined and for microbodies Vv 0.041, 0.062 and 0.063. Starch grains were only observed in control and Penicillin G treated calli whereas they were never seen in Penicillin V treated cells. Penicillin G-treatment induced formation of vacuoles, often of polygonal shap in mitochondria, organelles which significantly increased by treatment with Penicillin V.

Penicillin G induced an increase of 33.8 % in chlorophylls and Penicillin V of 47.8 %. Soluble proteins showed the highest level on callus treated with Penicillin G (more 7.24 % than control) and the increase promoted by Penicillin V was of the order of 1.97 %. Growth ratio was only significantly increased by Penicillin V treatment (24 %). Values for water contents were determined as 96.9 % for control, 97.3 % for Penicillin G treated and 97.07 % for Panicillin V treated cells.

The results produced by these two antibiotics of quite similar chemical structure are discussed in comparision with work done by others.

INTRODUCTION

THE technique of plant tissue culture is nowadays currently used to tackle down various problems in areas as far apart as plant physiology, plant breeding and genetics, production of secondary plant metabolites and a plethora of biotechnology processes and practical applications. The wast experience gained through the application of this methodology revealed that the use of even the most stringent asseptic conditions, is sometimes unable to prevent microbial infections. This has prompted the addition of antibiotics to culture media, a stratagem giving mixed results. Indeed, if in short-term cultures some antibiotics can be successfully used, their action is not completely innocuous in longer treatments (SALEMA, 1984; SALEMA & SANTOS, 1984; SANTOS & SALEMA, 1985).

Previous work along this line, done by our group, revealed that Penicillins, besides their high level of effectiveness against bacteria, notably Gram negative (GALE *et al.*, 1972), could even be beneficial to the cultured plant tissues (SALEMA, 1984; SALEMA & SANTOS, 1984; SANTOS & SALEMA, 1985). These findings were not completely unexpected, since reports were known of favourable effects of Penicillins on higher plant cells (MURKHERJI & BISWAS, 1981; 1984) and also on short-term plant tissue cultures (YOUNG *et al.*, 1984). In higher plants it was shown that various metabolic pathways were influenced by Penicillin treatment, including, for instance, regulation of chlorophyll formation, Hill activity, α-amilase biosynthesis, formation of ribonuclease and levels of endogeneous gibberelins and cytokinins (MUKHERJI & BISWAS, 1979; 1981; MUKHERJI & WAREING, 1983).

Stimulation of cell division in cultured explants of Jerusalem artichoke (PHILLIPS *et al.*, 1981) and of growth ratio of cultured mesophyll protoplasts of haploid *Nicotiana plumbaginifolia* (POLLOCK *et al.*, 1983) was reported, in either case only for short time treatments.

Data obtained by our group, as well as results reported elsewhere, showed that many aspects were still not known, and deserved a deeper study.

This paper reports on the general cytology changes observed at the ultrastructural level on plant tissue cultures treated with Penicillin G or Penicillin V, as well as the effects of these

antibiotics on some aspects of the biochemical composition of the tissues, when treated for periods as long as a fortnight.

MATERIAL AND METHODS

Plant material used for this study was taken from stablished callus culture derived from leaf mesophyll of *Sedum telephium* L. Innocula about 5 g in weight were transferred to plastic petri dishes onto Gamborg's B-5 medium (GAMBORG *et al.*, 1968) enriched with 10 mg/dm³ of benzylaminopurine plus 0.1 mg/dm³ of naphtalene acetic acid, solidified with 0.6 % agar, either containing the antibiotic under study or without it as control.

Sodium salts of Penicillins G and V (100 μg/cm³) were dissolved in liquid culture medium; the antibiotic solutions were sterilized by filtration through a 0.45 μm mesh Millipore membrane and added to the already autoclaved medium, after cooling it till about 45° C.

Callus tissue was grown in a culture cabinet at 27° C in a 12 h light period (10 W m^{-2}) for 14 days; collected samples were fixed in 2.5 % glutaraldehyde followed by 2 % osmium tetroxide using Na-PIPES buffer (SALEMA & BRANDÃO, 1973), dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were cut with a diamond knife using a LKB ultrotome III, mounted on 400 mesh uncoated grids or on collodium covered one hole grids, contrasted with uranyl acetate and lead citrate and viewed in a Siemens Elmiskop IA or 102.

Callus growth was estimated by calculating the ratio callus weight after 14 days/initial callus weight ($n = 9$).

Chlorophyll contents were determined in samples, similarly collected at the end of the treatment, according to STRAIN *et al.* (1971) and soluble proteins present in aqueous homogenates of the tissue was determined spectrophotometrically with the Folin reagent (LOWRY *et al.*, 1963).

In order to obtain enough data for statistics analysis of the referred biochemical determinations, 9 petri dishes were used for control and for each drug assayed, samples collected from each one of them, individually homogeneized and aliquots of every sample used for readings in a Shimadzu 240 Graphicord spectrophotometer. In addition to that all experiments were run thrice.

Stereological analysis was done according to WEIBEL (1973), WILLIAMS (1977) and TOTH (1982). For each situation under study 5 blocks were made from each one of the nine initial petri dishes, giving a total of 45 blocks; to assure randomness 5 blocks were chosen by lottery, thin sections cut from them and mounted on one hole grids. A total of 30 random photographs were taken for each of the above referred situations and printed to give final magnifications of 10.000 × and 30.000 ×. Statistical significance was estimated through Student's t test, considering a level of 95 %.

RESULTS

Samples of callus tissue collected after 14 days of culture were basically composed of two types of cells, easily recognized under the electron microscope. One type appears less differentiated, more meristem-like, whereas the other had a huge central vacuole, differentiated chloroplasts, somehow resembling chlorenquyma cells.

The first type had abundant ribosomes and polysomes, mitochondria with normal aspect, plastids of the proplastid type, with just few thylakoid membranes, round regular nucleus, frequent endoplasmic reticulum profiles of long cisternae with rough and smooth regions, dictyosomes showing some degree of associated vesicles, few scattered microbodies, and tiny to small vacuoles (Fig. 1 and 2, Plate I). The other type of more differentiated cells (Fig. 3 and 4, Plate II) differed mostly by the development of their vacuoles, generally only a large one by cell, and by the presence of more thylakoids with organized grana in their plastids; endoplasmic reticulum and microbodies were not so conspicuous as in the first referred type of cell. Noteworthy, the plastids in both kind of cells showed starch grains, small ones in the proplastids, very large, sometimes two or three in the chloroplasts, which not infrequently showed a very reduced stroma.

Considering a mean cell of the meristematic type and a mean cell of the chlorenquyma type values for fractional volume (Vv) of their vacuoles were almost reversed, since the first cell type had only 36.5 % of their protoplasm volume occupied by vacuoles, whereas the other type of cell had 68 % of vacuole volume.

Material treated with the antibiotics was not only different from the untreated one but, curiously enough, differences could

be seen between cells grown in the presence of Penicillin G and Penicillin V albeit the molecules of the two drugs are quite similar (PELCZAR *et al.*, 1980).

In general, and considering only the differentiated type, treated cells, in comparision to control cultures, showed more endoplasmic reticulum, more mitochondria, more thylakoid membranes in their plastids (Fig. 5 and 6, Plate II). These differences were analysed morphometrically and the results presented in Table I.

TABLE I

Morphometric data of material grown for 14 days under different conditions

	Control	Penicillin G	Penicillin V
ER Sv (ER, Cyt.) $\mu m^2/\mu m^3$	1.48 ± 0.102	1.78 ± 0.098	1.64 ± 0.090
Mitochondria Vv (Mito., Cyt.)	0.30 ± 0.022	0.33 ± 0.027	0.39 ± 0.025
Thylakoids Sv (Thyl., Plast.) $\mu m^2/\mu m^3$	1.92 ± 0.120	2.58 ± 0.102	2.73 ± 0.110
Microbodies Vv (Micr., Cyt.)	0.041 ± 0.0037	0.062 ± 0.0064	0.063 ± 0.0071

Values represent means $\pm$ SE (Standard Error) attached to 95 % confidence limits ($n = 30$).

The action of either Penicillin G or Penicillin V on the cultured tissues seems to promote a healthy state in the cells, as could be evaluated through the calculated growth ratios and assessed by electron microscopy of the tissues. Considering the less developed, meristem-like cells, their general ultrastructure is very similar to the observed in control material.

Cytoplasm with numerous ribosomes and polysomes, well developed rough endoplasmic reticulum with cisternae frequently running parallel to the cell periphery, dictyosomes quite often with four to eight saccules, proplastids with some thylakoid membranes,

abundant, well developed mitochondria, a round nucleus with some masses of condensed chromatin, are features commonly observed and shown in Fig. 7 and 8 (Plate III).

When comparing cells of this undifferentiated type, grown in medium containing Penicillin G, with those grown in medium containing Penicillin V, some differences clearly stood out, a situation which continues in the differentiated type of cells. Starch grains were only found in plastids of material treated with Penicillin G whereas no accumulated starch whatsoever was observed in the other material, although several hundreds of organelle profiles were scanned (Fig. 9 and 10, Plate IV). Also changes were observed in mitochondria of the same material which showed no comparable alterations in tissues treated with Penicillin V. Indeed, treatment with Penicillin G induced a remarkable enlargement of one or two, very rarely more, cristae per mitochondrion (Fig. 11, Plate IV), initially producing a vesiculation of the crista involved, later progressing to a vacuole-like structure (Fig. 11, Plate IV). Worth pointing, the fact that this vacuole-like formation, which occupies sometimes as much as 50 % of the organelle, more often than not shows a polygonal outline instead of a round shape (Fig. 11, Plate IV). In opposition to that, mitochondria of tissues treated with Penicillin V show a regular aspect with abundant short, somewhat vesiculated cristae (Fig. 12, Plate IV). This tissue shows more mitochondria per unit volume ($Vv = 0.39$) than either the control ($Vv = 0.30$) or the treated with Penicillin G ($Vv = 0.33$), these two latter showing no statistical diffrerence between them.

Biochemical determinations done on untreated and treated callus tissue gave results summarized in Table II. As Table II shows both Penicillins promoted an increase in chlorophylls contents approaching 34 % in Penicillin G treated tissues and 48 % in Penicillin V treated material in relation to control. However Penicillin G was more effective in raising the contents of soluble proteins than Penicillin V, which, in opposition to this, showed a higher growth ratio, attaining some 24 % more weight at the end of the 14 days treatment.

TABLE II

Values of water contents, chloropylls (a + b), proteins and growth ratio in untreated and treated calli, determined after 14 days

	Control	Penicillin G	Penicillin V
Water percentage	96.9	97.3	97.07
*Chloropylls	11.23 ± 1.05	15.03 ± 1.04 (33.8 %)	16.60 ± 1.16 (47.8 %)
*Proteins	3.326 ± 0.095	3.567 ± 0.090 (7.24 %)	3.448 ± 0.080 (1.97 %)
*Growth ratio	2.39 ± 0.129	2.37 ± 0.11	2.7 ± 0.21

* Values represent means ± SE (Standard Error) attached to 95 % confidence limits. Chloropylls expressed as μg/g f. w. Soluble proteins expressed as mg/g f. w. Figures between brackets represent percentage of increase in relation to control, considering means values.

DISCUSSION

The stimulating effect of Penicillins, either on plants, seedlings or in tissue cultures, has been found to have an intriguing interference with various metabolic pathaways (see Introduction), although a general picture of this variety of actions is by no means clear. Callus tissue treated as described above shows differences from the control material when the ultrastructure of their cells is compared, and also differences among themselves, changes which are better noticed when quantified stereologically.

The area of endoplasmic reticulum membranes per unit of cell volume appears increased, and more so in the callus tissue which grew in medium containing Penicillin G. This morphological feature is in accordance with the higher level of soluble proteins found in the same tissue.

Concerning the increase in surface of thylakoid membranes a parallelism was found in the increased amount of chlorophylls from control to treated tissues. Here the parallelism is further stressed by the quite close figures found for the amount of photosynthetic membranes in tissues treated with either Peni-

cillin G or Penicillin V, and the amounts of chlorophylls measured in either of them. It is well established that Penicillin stimulates chlorophyll synthesis (MUKHERJI & BISWAS, 1979; BIWAS & MUKHERJI, 1978), reports which agree quite well with our present results.

Stereological calculations showed also that Penicillin-treated tissues were richer in microbody material than the control callus tissue, but here no significative difference was found to be produced by one type of treatment or the other.

On the other hand fractional volume of mitochondria was considerable higher in Penicillin V than in Penicillin G treated cells (more 18 %) a situation possibly related to the faster growth ratio displayed by the same tissue (more 24 %).

Interestingly enough, starch grains appeared in plastids of the control and also in tissues treated with Penicillin G, but they were never found in cells grown in presence of Penicillin V.

Penicillins are antibiotics of widespread use, acting on the transpeptidase which promotes crosslinking of peptide chains in bacterial cell wall peptidoglican molecule (QUESNEL & RUSSEL, 1983). Since there is no similar counterpart in higher plant cell walls the interference of such drugs has been admitted to be elsewhere.

Penicillin is thought to increase water content of treated tissues (MUKHERJI & BISWAS, 1984) possibly because of some action on higher plant membranes, since it bounds to lipoproteins (COOPER, 1956). Protein synthesis is stimulated by increased water contents (HSIAO, 1970), condition which is admitted to explain increased protein synthesis in seedlings treated with this antibiotic (STURANI, 1968). In our experiments we also found a slight increase in water contents as well as increased proteins synthesis in the treated tissues, results which accord to the above cited. These increased synthesis of proteins can be considered an adittional indication of vitality of the cells (YAMAZAKI, 1983).

The action of Penicillin has also been ascribed to the alteration of S-S/S-H ratios in the cell, as a result of donation of S-H groups from the cystein residue of the antibiotic molecule, which is considered of great importance in both cell enlargement and cell division (MUKHERJI & BISWAS, 1984), possibly by interacting with

cell wall hydroxyproline-rich glicoproteins (HRGP) (ROBERTS *et al.*, 1985).

When comparing the value of growth ratios in the treated and control tissues one can see that tissues treated with Penicillin G grew in a similar way as control tissues, whereas callus tissue placed on medium containing Penicillin V attained 24 % more weight. This could be the result of bigger cells (cell enlargement), or more cells (cell division) or both processes acting simultaneously. Stereological determinations done on light microscope photographs of semi-thin sections of callus fragments revealed that cells treated with Penicillin V were larger (ca. 12 %) and less numerous per unit volume (ca. 11 %) than control ones, results which coupled to the values determined for water contents in both tissues led to the conclusion that the mass of callus tissue treated with Penicillin V grew bigger and heavier, most likely because of enhanced cell enlargement.

The energy required for this higher growth ratio would be easily produced for by the numerous mitochondria this treated tissue had — more 30 % than control tissues, 18 % more than Penicillin G treated material. Also this line of reasoning could explain the absence of starch in plastids of this tissue, since growth of cells would pose a high demand for ATP, which could be obtained through starch degradation. High ATPase values have been reported in Penicillin treated seedlings (MUKHERJI & BISWAS, 1984), which adds some support to this interpretation. The putative interpretation is further complicated by the effects that Penicillin has on plant hormones. Indeed, these antibiotics can induce gibbereline (GA_3) biosynthesis in embryoless rice endosperm (BISWAS & MUKHERJI, 1979), and in mung bean seedlings (MUKHERJI & WAREING, 1983), whereas no large increase in GA_3 was found in rice seedlings by Mukherji's group (MUKHERJI & BISWAS, 1984). An alteration of the endogenous level of auxin by Penicillin treatment was reported by the same researches (MUKHERJI & BISWAS, 1984) on rice seedlings, who detected increased levels of IAA-oxidase, as well a enhanced IAA transportation. In spite of the absence of data in our material concerning hormone levels or hormone-degrading enzymes ,admiting that type of interference in the process studied is certainly not a too daring assumption.

Some results remain however somewhat puzzling. Material treated with Penicillin G have slight more soluble proteins, although they grew less than tissues treated with Penicillin V. This last aspect might possibly be a consequence of the better stability displayed by Penicillin V in acid medium (PELCZAR *et al.*, 1980). In addition to that it seems also important to keep in mind that mitochondria of Penicillin G treated tissue are not normal, at least in what concerns morphology, possibly also functionally dificient, unable to satisfy all demands of energy for growth.

Clearly the action of Penicillin in plant tissues is very intriguing and calls for further experimentation, work which is under way in this laboratory.

ACKNOWLEDGEMENTS

Grants from University of Porto, INIC — Portugal, and Stiftung Wolkswagenwerk — Federal Republic of Germany are gratefully acknowledged.

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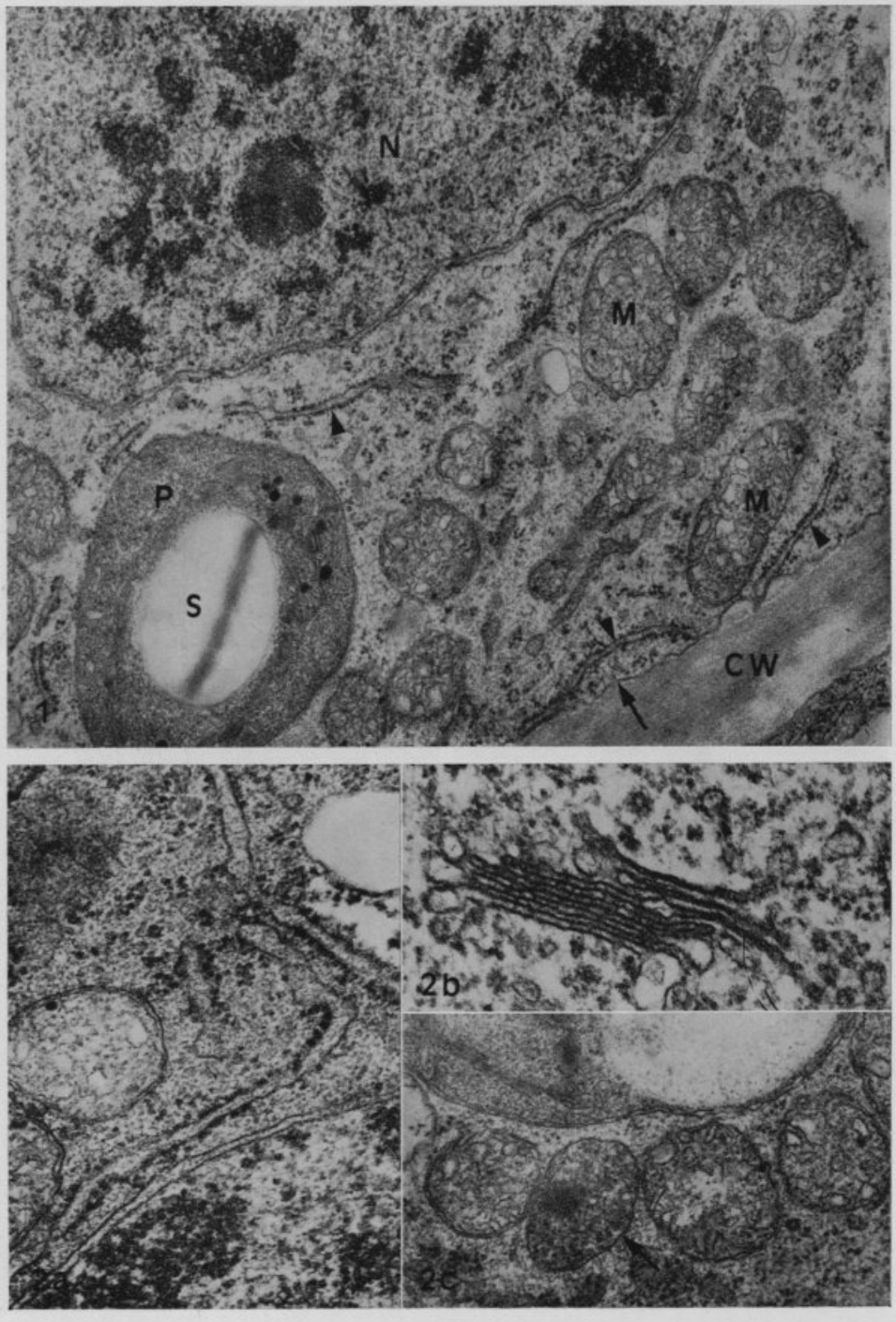
PLATES

PLATE I

Fig. 1. — Untreated material; general view of a meristem-like cell region, showing well developed nucleus (N), plastid (P) with starch (S), various mitochondria (M) of normal aspects, profiles of endoplasmic reticulum (arrow heads), plasmalemma (arrows), cell wall (CW) and other current cytoplasmic structures. 22,000 ×.

Fig. 2. — Untreated material; details of meristem-like cells. 2a. — Endoplasmic reticulum cisternae showing regions with membranes studded with ribosomes, and other areas with smooth membranes. 44,800 ×. 2b. — Golgi stack with associated vesicles. 58,300 ×. 2c. — Microbody (arrow) in close spatial association with mitochondria. 33,600 ×.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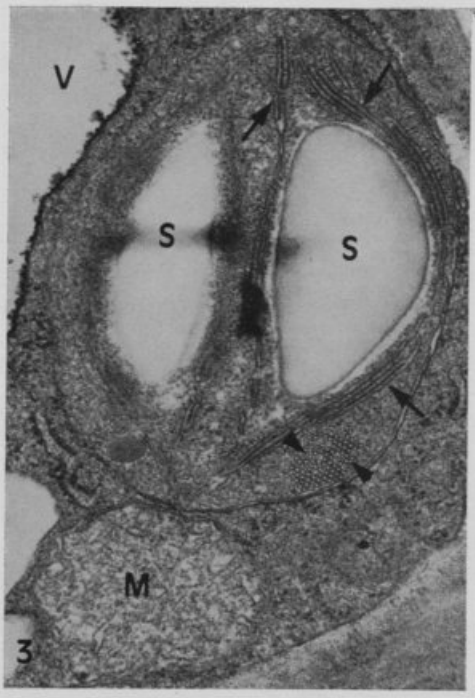
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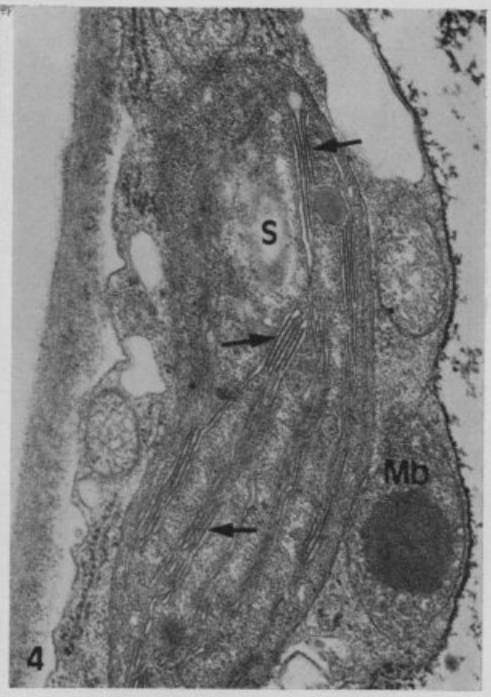
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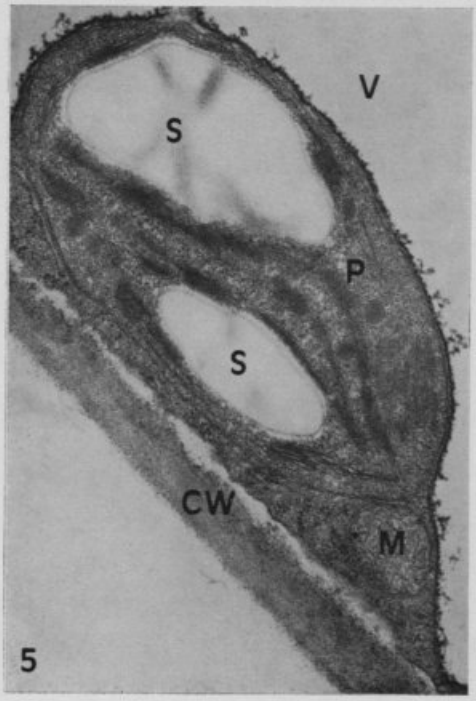
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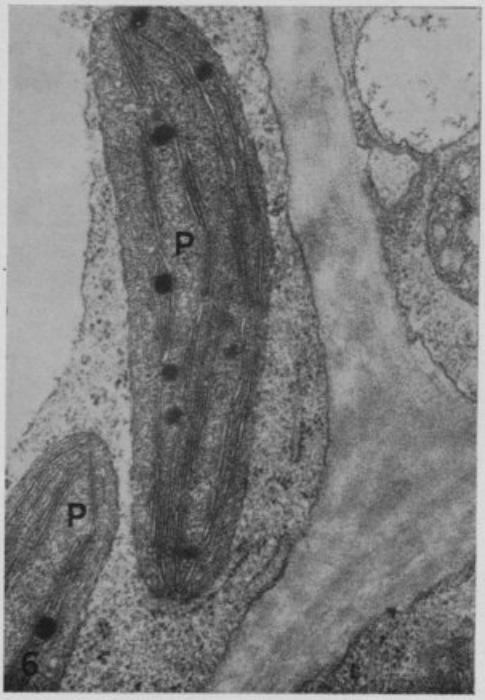
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PLATE II

Fig. 3. — Untreated material; aspect of detail of a differentiated-type cell, showing a large vacuole (V), mitochondria (M), plastid with thylakoids (arrows), starch grains (S) and also a transection of tubular protein inclusion (arrowheads) characteristic of some CAM plants and some calli derived from them. 32,200 ×.

Fig. 4. — Untreated material ;same type of cell as previous figure, showing plastid with thylakoids (arrows) and starch grains, microbody (Mb) with nucleoide. 26,600 ×.

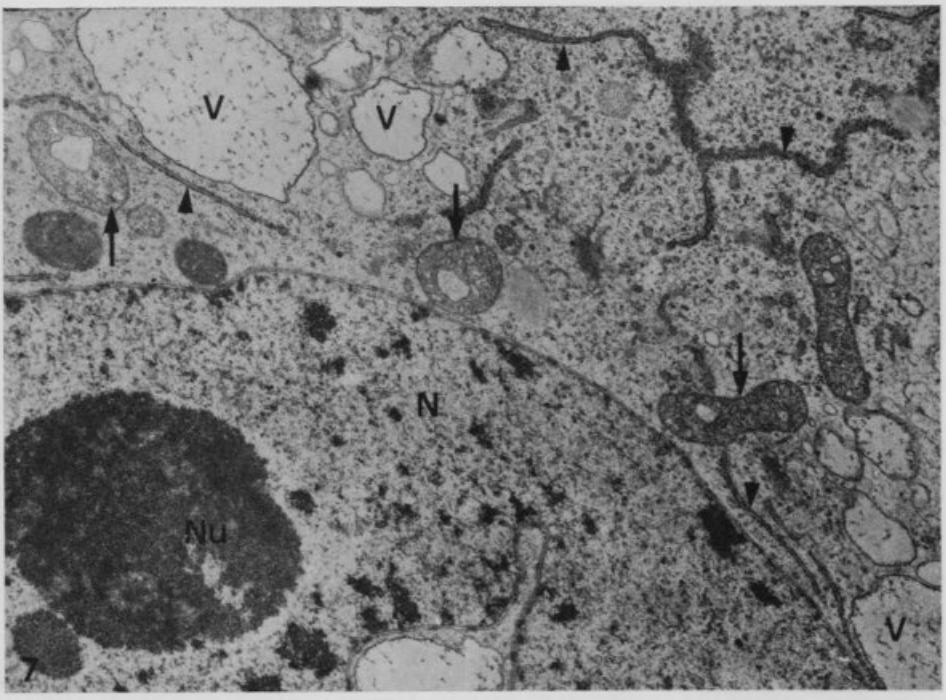
Fig. 5. — Penicillin G treatment; differentiated-type of cell showing plastid (P) with grana and stroma lamellae and starch grains (S); also visible vacuole (V), mitochondrion (M) and cell wall (CW). 21,000 ×.

Fig. 6. — Penicillin V treatment; differentiated-type of cell showing plastid (P) with well developed thylakoids and other cell constituents. Note absence of starch. 23,000 ×.

PLATE III

Fig. 7. — Penicillin G treatment; general view of part of a meristem-like cell with large nucleus (N) with nucleolus (Nu), small vacuoles (V), endoplasmic reticulum profiles (arrowheads), mitochondria with various degrees of cristae dilation (arrows), and other cytoplasmic structures. 11,400 ×.

Fig. 8. — Penicillin V treatment; region of a meristem-like cell with abundant endoplasmic reticulum profiles (arrowheads), vacuoles (V), mitochondria (M), plastid (P). 23,000 ×.



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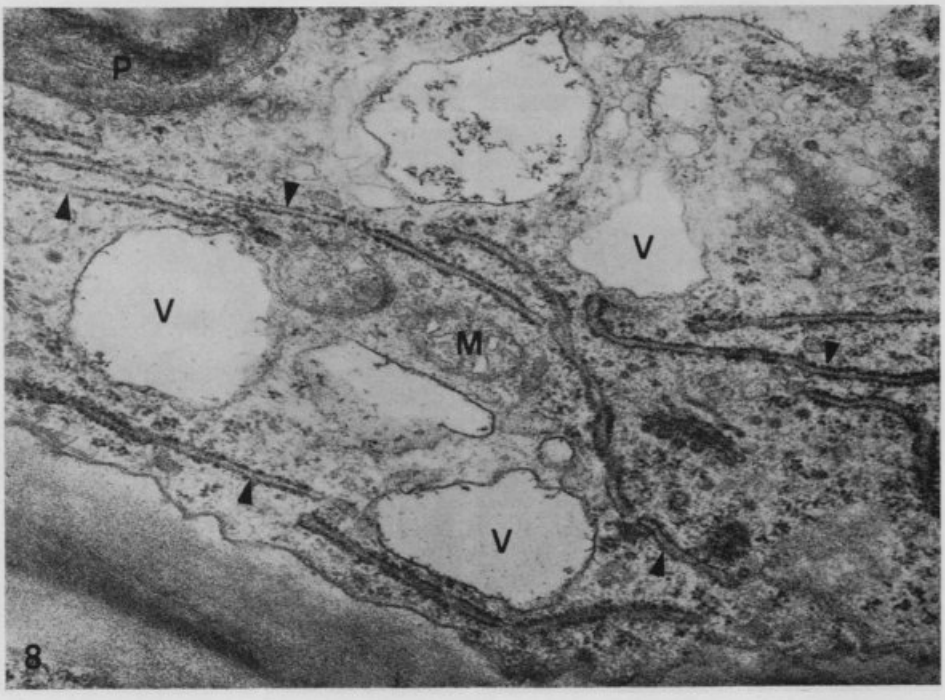

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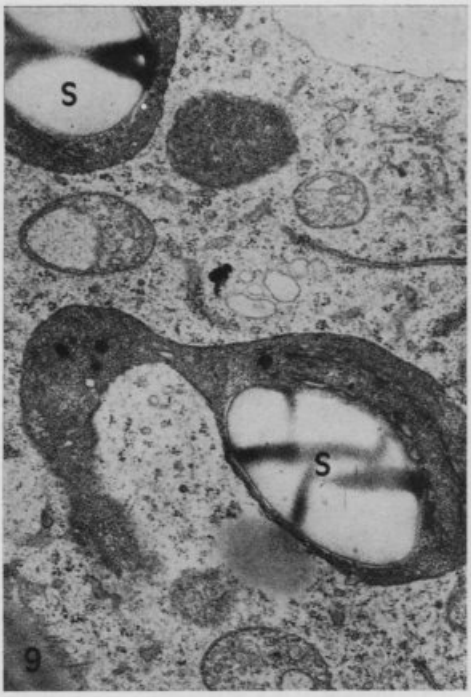
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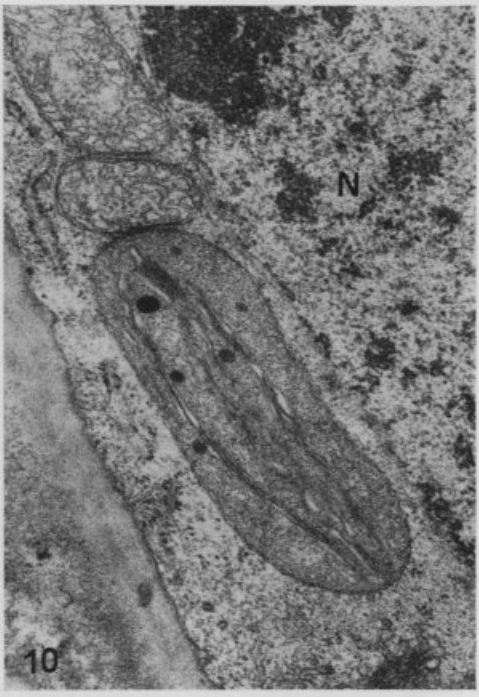
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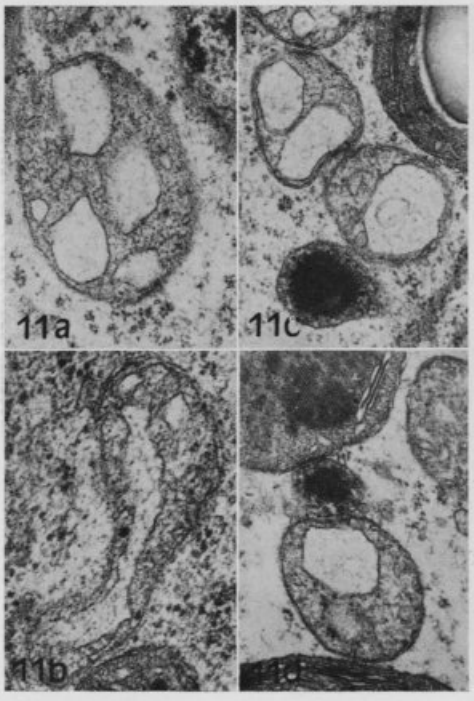
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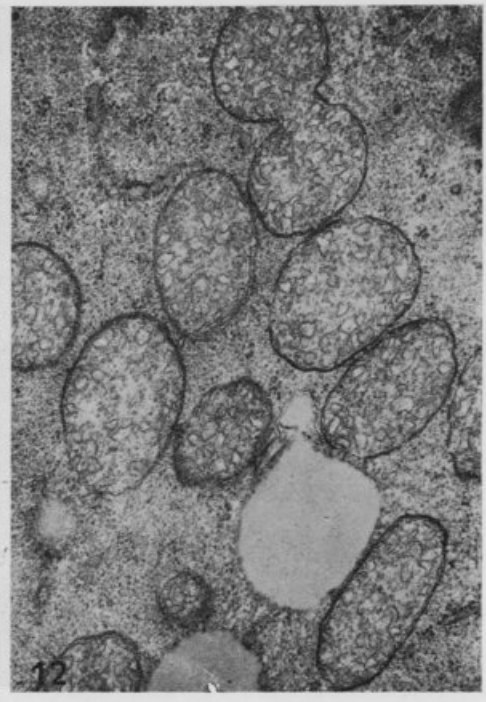
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PLATE IV

Fig. 9. — Penicillin G treatment; region of a meristem-like cell showing plastids with accumulated starch (S), mitochondria with dilated cristae, and other cytoplasmic structures. 15,000 ×.

Fig. 10. — Penicillin V treatment; area showing part of a nucleus (N), mitochondria, plastid without accumulated starch, and other current cytoplasmic features. 22,100 ×.

Fig. 11. — Penicillin G treatment. 11a. — Mitochondrion with greatly enlarged crista. 20,000 ×. 11b. — Longitudinal section through an enlarged crista. 40,000 ×. 11c. — Mitochondria with reduced matrix due to enlarged cristae. 20,000 ×. 11d. — Mitochondrion with dilated cristae with polygonal outline. 22,500 ×.

Fig. 12. — Penicillin V treatment; cell region with various mitochondrial profiles showing abundance of vesiculate cristae. 21,600 ×.

ÍNDICE

MEDALHAS COMEMORATIVAS
DO
CENTENARIO DA SOCIEDADE BROTERIANA

Possuindo ainda algumas destas Medalhas, a Direcção da Sociedade comunica aos Sócios e às pessoas interessadas que as pode fornecer aos preços de 600$00 para os Sócios e de 1000$00 para os não Sócios. A aquisição poderá ser feita directamente na Secretaria da Sociedade Broteriana, Instituto Botânico, Arcos do Jardim, 3049 Coimbra, ou mediante pedido feito ao Secretário-tesoureiro, caso em que será debitado o custo do envio pelo correio.

INSTRUÇOES AOS COLABORADORES

1. O *Boletim da Sociedade Broteriana* é uma revista destinada à publicação de artigos originais em todos os domínios da Botânica. No entanto, artigos muito extensos sobre florística, fitogeografia e fitossociologia são publicados geralmente nas *Memórias*, enquanto que os trabalhos de divulgação científica e os referentes à história da Botânica são reservados para o *Anuário* — as duas outras revistas da Sociedade.

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3. Os originais entregues para publicação devem ser dactilografados a dois espaços e possuir uma margem da largura habitual. Poderão ser redigidos em português, inglês, francês, alemão, italiano ou espanhol. O nome do autor (ou autores) deverá figurar na primeira página, bem como o endereço da Instituição em que trabalha. Um resumo não excedendo aproximadamente 300 palavras, preferivelmente em inglês, deverá iniciar o artigo.

4. Os nomes latinos dos géneros, espécies e categorias infraspecíficas que figurarem no texto devem ser sublinhados uma só vez, enquanto que os nomes dos autores, quando não escritos em maiúsculas, devem ser sublinhados com um traço ondulado. As palavras em negro devem ser sublinhadas duas vezes. Os nomes dos autores citados no texto devem ser seguidos pela data da publicação entre parênteses.

5. No que respeita à ordenação e disposição da bibliografia, seguir as normas utilizadas em um dos volumes recentes desta publicação.

6. As figuras a intercalar no texto, geralmente reproduzidas em zincogravura, não deverão exceder a mancha tipográfica. As estampas *hors-texte* (em regra fotogravuras) serão impressas em papel *couché* e não deverão ultrapassar 13 × 18 cm. Sempre que as figuras sejam de pequenas dimensões, aconselha-se a sua reunião em estampas com as dimensões acima indicadas.

7. Cada autor (ou grupo de autores) receberá 50 separatas grátis, sendo as excedentes que pretender fornecidas ao preço do custo e pagas directamente à Tipografia.

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1. Le *Boletim da Sociedade Broteriana* est un périodique destiné à la publication d'articles originaux concernant tous les domaines de la Botanique. Cependant, des articles très longs sur floristique, phytogéographie et phytosociologie sont en général publiés dans les *Memórias*, tandis que les travaux de divulgation scientifique et ceux concernant l'histoire de la Botanique sont réservés au *Anuário* — les deux autres revues de la Société.

2. Ayant particulièrement pour but la publication des articles élaborés par le personnel scientifique de l'Institut Botanique de Coimbra, ce périodique publie aussi les travaux des membres de la Société, ainsi que ceux d'autres botanistes, soit portugais, soit de quelque autre nationalité. Toutefois, la publication des articles est sous la dépendance de l'avis de la Commission de Rédaction.

3. Les manuscrits doivent être dactylographiés à deux espaces et avoir une marge. Ils peuvent être rédigés en portugais, anglais, français, allemand, italien ou espagnol. Le nom de l'auteur (ou des auteurs) devra figurer à la première page après le titre du travail, ainsi que l'adresse de l'Institution où il travaille. Un résumé ne dépassant pas 300 mots, de préférence en anglais, devra ouvrir l'article.

4. Les noms latins des genres, des espèces et des catégories infraspécifiques devront être soulignés une fois, tandis que les noms des auteurs, quand non dactylographiés en lettres majuscules, doivent être soulignés par une ligne ondulée. Les noms des auteurs cités dans le texte doivent être suivis de la date de la publication mise entre parenthèses.

5. En ce qui concerne la bibliographie, voir un des volumes récents du *Boletim*.

6. Les figures du texte, en général des dessins à l'encre de Chine, ne doivent pas, avec les légendes, dépasser 10,5 × 18 cm. Les planches hors-texte ne devront pas dépasser 13 × 18 cm. Les figures à petites dimensions doivent être réunies dans des planches aux dimensions ci-dessus mentionnées.

7. Chaque auteur (ou groupe d'auteurs) recevra 50 tirages à part gratuits, tandis que ceux excédant ce nombre lui seront fournis au prix du coût et devront être payés par l'auteur directement à l'Imprimerie.

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