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Post-fire leaf structure of two seasonally dimorphic resprouters

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ABSTRACT

The post-fire ultrastructural features of leaves from recent resprouts and unburned (older) shrubs of two phryganic (East Mediterranean) species (*Phlomis fruticosa* and *Euphorbia acanthothamnos*) were examined. Mesophyll cells of resprout leaves exhibit pronounced differences from those of unburned shrubs in cell organization and organelle structure. Chloroplasts in resprouted *P. fruticosa* leaves present a highly organized system of grana thylakoids compared to the poor organization of the same organelles in the leaves of unburned plants. In *E. acanthothamnos* the main difference of the resprout leaves is the lack of the secondary metabolites which represent tremendous carbon accumulation in mesophyll and epidermal cells of unburned shrub leaves.

KEY-WORDS: Euphorbia acanthothamnos - *Fire* - *Leaf Structure* - Phlomis fruticosa - *Phrygana* - *Resprouting*.

RÉSUMÉ

Les différences structurales entre les feuilles jeunes des rejets faisant leur apparition après le passage du feu et les feuilles âgées des buissons qui n'ont pas subi l'action du feu ont été étudiées chez deux espèces de la phrygane grecque, *Phlomis fruticosa* et *Euphorbia acanthothamnos*. Les cellules du mésophylle des feuilles des rejets diffèrent profondément de celles des feuilles qui n'ont pas été soumises à l'influence du feu sur le plan de l'organisation cellulaire et de la structure des organites. Chez *P. fruticosa* les chloroplastes des feuilles des rejets montrent des thylakoïdes hautement organisés alors que chez les feuilles n'ayant pas subi l'effet du feu cette organisation est très faible. Chez *E. acanthothamnus* la différence principale porte sur l'accumulation de métabolites secondaires, qui est très faible dans les feuilles des rejets, alors que chez les feuilles des plantes qui n'ont pas subi l'effet du feu on observe au contraire une très forte accumulation des métabolites secondaires (surtout des composés polyphénoliques) dans les cellules du mésophylle et dans les cellules épidermiques.

MOTS-CLÉS : Euphorbia acanthothamnos - *Feu* - *Structure de la feuille* - Phlomis fruticosa - *Phrygane* - *Repousse*.

INTRODUCTION

It has long been considered that mediterranean type ecosystems have evolved with fire which has been a strong selective force for the dominant plant species (JEPSON, 1930; NAVEH, 1973; BISWELL, 1974; PARSONS, 1976; ARIANOUTSOU-FARAGGITAKI & MARGARIS, 1981). Post fire recovery reactions of mediterranean plants can be either obligatory resprouting by vegetative regeneration or facultative resprouting (seasonal dimorphics, in Greece: phrygana), in which fire activated seed germination also occurs.

Resprouting of burned phryganic species started soon after the first rains (October 1981, fig. 1), while unburned plants already had their large winter leaves. Leaves on resprouts are up to ten times larger and are more tender (ARIANOUTSOU-FARAGGITAKI & MARGARIS, 1981) than winter leaves. According to preliminary observations (ARIANOUTSOU, 1979) leaves of recently resprouted shrubs possess less compact mesophyll than those of the unburned ones. Chlorophyll contents were higher in resprouted than in unburned shrub winter leaves (ARIANOUTSOU-FARAGGITAKI & MARGARIS, 1981).

The following work was undertaken soon after a wild fire in June 1981. The aboveground parts of a mixed community of evergreen sclerophylls (maquis) and seasonal dimorphics (phrygana) on a mountain slope east of Athens were totally consumed by the fire. Maquis species resprouted immediately after fire, disregarding the summer drought (PSARAS *et al.*, 1984). Only after the first rains (October 1981) and just before the onset of winter, did phryganic species resprout. Among the resprouters two species (*Phlomis fruticosa* and *Euphorbia acanthothamnos*) dominating the ecosystem were selected for further study.

MATERIALS AND METHODS

Fully developed leaves from both resprouted plants and neighbouring unburned ones were collected in April 1982 (fig. 1), cut into small pieces and fixed in 2.5 % phosphate buffered glutaraldehyde at pH 7.2 for two hours at 0° C. After several buffer rinces the tissue was post-fixed for three hours in phosphate buffered 1 % osmium tetroxide. Dehydration in a series of ethanol solutions and embedding in epoxy resin (Durkupan ACM-Fluka) followed. Ultrathin sections were cut on an LKB Ultramicrotome, mounted on Formvar-coated grids and stained in uranyl acetate and lead citrate (REYNOLDS, 1963). These sections were examined with a Philips Electron Microscope 300. Semithin sections were also obtained, mounted on glass slides, stained with toluidine blue (PICKETT-HEAPS, 1969) and examined under a Leitz Light Microscope.

Specific histochemical reactions (REEVE, 1959; MACE, 1963; MACE & HOWELL, 1974) were employed in free hand sections of fresh leaves to detect the presence and distribution of phenolic tannin precursors and condensed polyphenolics. These compounds were present in both species. In *P. fruticosa* only tannin precursors were scattered in some cells but in *E. acanthothamnos* tannins were abundant in all tissues.

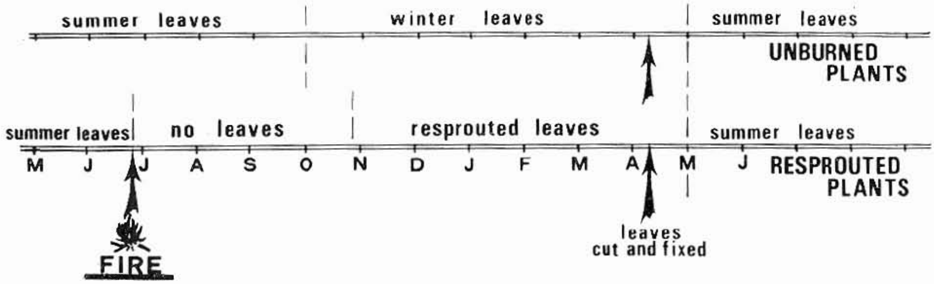

FIG. 1. — Seasonal alternation and leaf sampling design of unburned and resprouted plants.

RESULTS AND DISCUSSION

Light microscopic examination comparing cross sections of resprouted and unburned *P. fruticosa* leaves reveals pronounced differences between them. The compactness of the mesophyll, particularly that of the spongy parenchyma, the vascular and mechanical tissue and the density of the hair cover were all more developed in the leaves of unburned shrubs whereas the leaves of the resprouted ones tend to be flatter (cf. fig. 2 *a* to fig. 2 *b*).

In *E. acanthothamnos* pronounced differences are observed in epidermal cells. In unburned leaves they have thick walls with peculiar projections and large amounts of polyphenolic compounds (fig. 3 *a*). These secondary metabolites, representing a tremendous carbon accumulation in a relative disusable form, are stored in epidermal and mesophyll cells of unburned shrub leaves. On the contrary, epidermal cells in resprout leaves seem to have thinner cell walls and their vacuole contains only granules of polyphenolic compounds (fig. 3 *b*). They also have reduced palisade to spongy tissue ratio (cf. fig. 3 *a* to fig. 3 *b*) while differences in the structure of vascular and mechanical tissue were not observed.

Electron microscopy of both species provided more confirmation of the above mentioned structural differences. Moreover, great differences were observed in chloroplast structure between unburned and resprouted shrub leaves of *P. fruticosa*. In the former chloroplasts possess several stroma but hardly developed grana thylakoids, while lipid droplets are abundant (fig. 2 *c*). Chloroplasts with starch grains and a membrane bound protein body can also be observed (fig. 2 *d*). This could be a result of plant dessication (Kirk & Tilney-Basset, 1978). Resprout leaves on the contrary present chloroplasts with strongly developed grana thylakoids, some osmiophilic globuli and traces of starch accumulation, an overall structure indicating a powerful photosynthetic unit (fig. 2 *e*).

In *E. acanthothamnos* differences between unburned and resprouted shrub leaves are limited to the vacuole. The cells in unburned plants accumulate large quantities of polyphenolics in their expanded vacuole which restricts the cytoplasm close to the inner cell wall (fig. 3 *c*). A large vacuole can be observed in epidermal cells of resprout leaves but only a few granules of polyphenolic compounds are accumulated in it (fig. 3 *d*). The number of mesophyll cells containing polyphenolics and the degree of condensation of these compounds is greater in unburned than in resprouted shrub leaves.

Careful examination of chloroplasts in both types of leaves does not reveal any major difference between them. Chloroplasts in unburned shrub leaves (fig. 3 *e*) possess more lipid droplets and denser stroma than in resprouted ones (fig. 3 *f*).

Post fire reactions differ in these two species. From evaluation of both light and electron microscopy data we can assume that what is common in resprouted shrub leaves is their tendency to be less xerophytic. Reduction of palisade to spongy parenchyma ratio, reduced cell wall thickness, less mesophyll compactness and mechanical tissue development as well as secondary metabolite accumulation which drastically reduced in resprout leaves support this conclusion. These changes in xeromorphy, the cost of an immediate restoration of the above ground parts, leave the plant weakly protected against the unpredictable unfavorable periods of the mediterranean climate.

In both species unburned and resprouted shrub leaves have something in common. They are all about to be replaced by summer leaves (seasonal dimorphism,

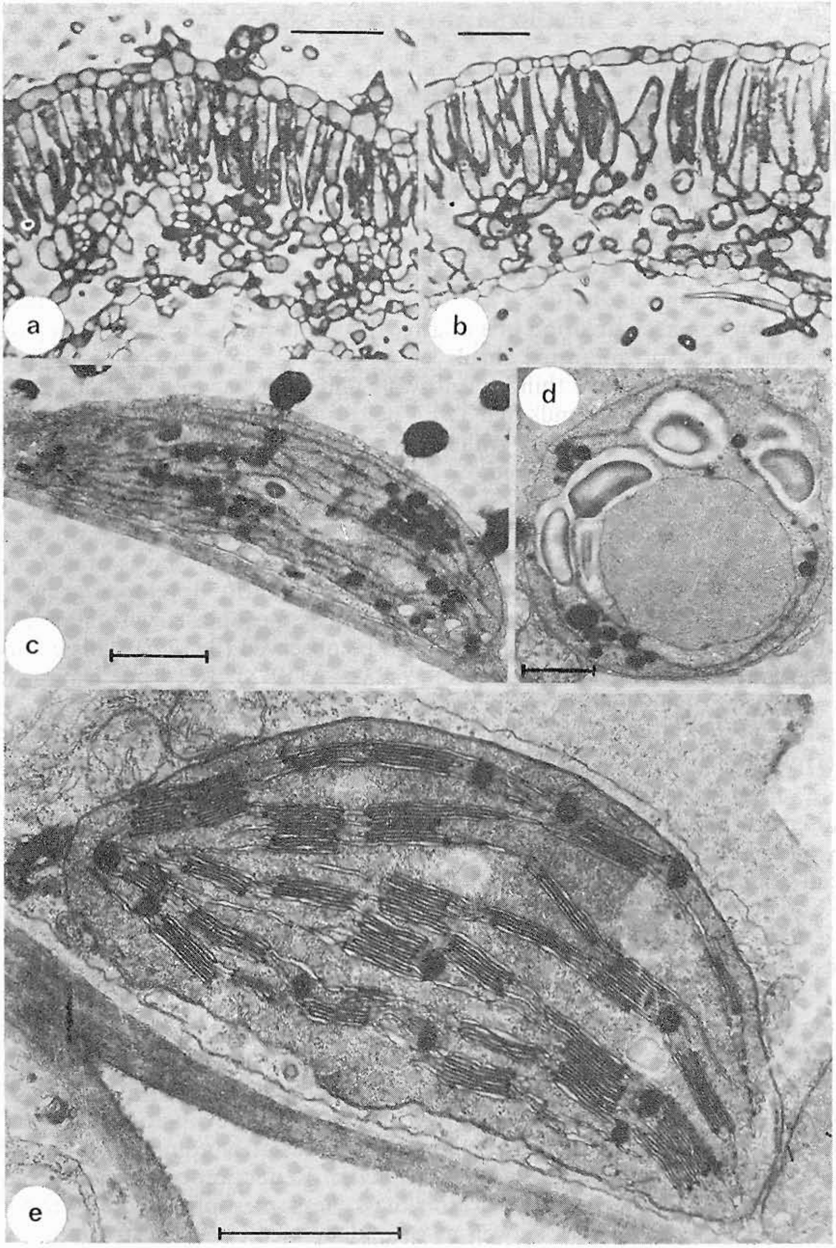

FIG. 2. — Micrographs from *Phlomis fruticosa* leaves.

a, Cross section of unburned shrub leaf. *b*, Cross section of resprout leaf. *c*, Chloroplast from an unburned shrub leaf. *d*, Plastid with a membrane bound protein body from an unburned shrub leaf. *e*, Typical chloroplast from a resprout leaf. Bars in light micrographs (*a*, *b*) represent 100 μm; in electron micrographs (*c*, *e*) bars represent 1 μm.

ORSHAN, 1964; MARGARIS, 1975). For *P. fruticosa* resprouted individuals this fact means that the plant has expended reserves to establish a photosynthetic apparatus

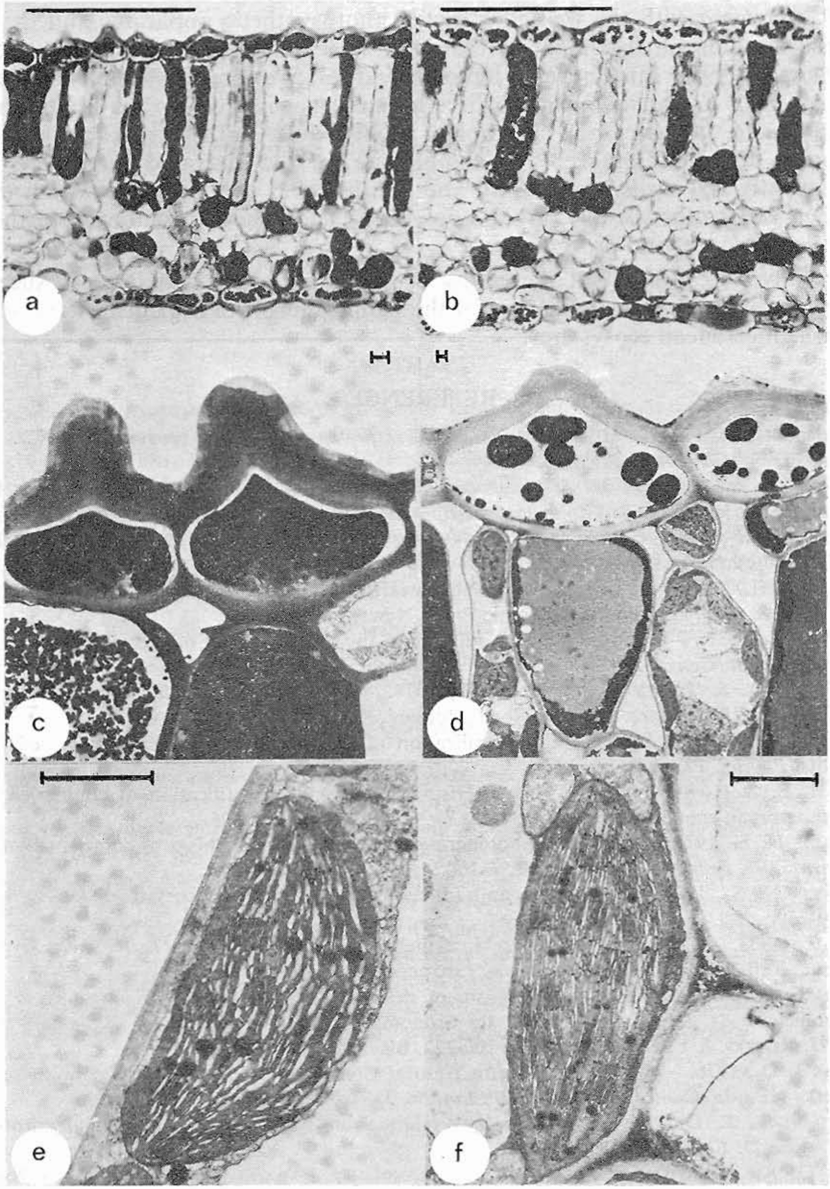

FIG. 3. — Micrographs from *Euphorbia acanthothamnos* leaves.

a, *Cross section of unburned shrub leaf*. *b*, Cross section of resprout leaf. *c*, Epidermall cells of an unburned shrub leaf. *d*, Epidermal cells of resprut leaf. *e*, Chloroplast typical of the unburned shrub leaves. *f*, Chloroplast typical of resprout leaves. Bars in *a*, *b*, represent 100 μm; in the rest micrographs bars represent 1 μm.

which will be replaced although still active. This procedure indicates that alternation of winter and summer leaves does not depend on leaf age and senescence but is triggered rather by environmental factors (MARGARIS, 1976).

P. fructicosa with the well constructed photosynthetic apparatus and the great increase in chlorophyll content of the resprout leaves (ARIANOUTSOU-FARAGGITAKI & MARGARIS, 1981) must have an increased carbon fixation. However the same does not happen in *E. acanthothamnos* where no emphasis is given to the photosynthetic apparatus and the increase in chlorophyll is only slight. In this species the shifting of metabolite accumulation seems to compensate for the increased carbon demands. Consequently cell walls remain thin and considerable quantities of carbon do not find their way to the vacuole through polyphenolic compound synthesis but they are channeled to more vital procedures.

Finally we can say that the ability of these species for a quick and successful restoration after fire is an indication for the significant role of fire during the evolution of the mediterranean ecosystems.

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