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## Influence of the substratum on the morphology of *Endophragmiella boewei* and *Beltrania rhombica*

### Abstract

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The morphological variation of two Dematiaceous Hyphomycetes, *Endophragmiella boewei* (Crane) Hughes and *Beltrania rhombica* Penzig, isolated from litter of Mediterranean vegetation is investigated. The strains were inoculated on sterilized leaf litter of the different species of the mediterranean vegetation to investigate the variation of their morphological characters. The data show a certain degree of stability for *E. boewei* and great variation for *B. rhombica*.

### Introduction

In previous works we examined the fungal microflora associated with the litter of *Pistacia lentiscus* L. (Mulas & al. 1990, 1991), and a possible relation between fungal colonization and this matrix (Rambelli 1991). The researches have been carried out on Mediterranean vegetation in different places of Sardinia. From these investigations it emerged a small group of microfungi which seem to be part of the "stress-tolerant" category (Pugh 1980). According to Rambelli and Pasqualetti (1991) these fungi, limitedly to the summer period, can be considered saprotrofs specialized on the litter of *P. lentiscus*. In order to give a further contribution to the knowledge of this small group of fungi, as regards the intraspecific variation, we proceeded to their isolation in pure culture and to the reinoculation on some matrices which had been sterilized with a previously tried technique (Kabi Ouanyou & Rambelli 1990).

In this first stage of work two species have been considered: *Endophragmiella boewei* (Crane) Hughes and *Beltrania rhombica* Penzig. From the results of previous researches (Rambelli & Pasqualetti 1991) it seems probable that these two fungi colonize the *P. lentiscus* litter in succession; *E. boewei*, as "primary colonizer", could be responsible for the hydrolysis of the cellulose held in the dead leaves of the host and it could prepare, in this way, the suitable environment for the germination of the *B. rhombica* spores, species notoriously bound to damper environments.

### Materials and methods

*E. boewei* and *B. rhombica* were isolated from leaf litter of *Pistacia lentiscus* L. The former was identified on natural medium by comparison with the holotype obtained from

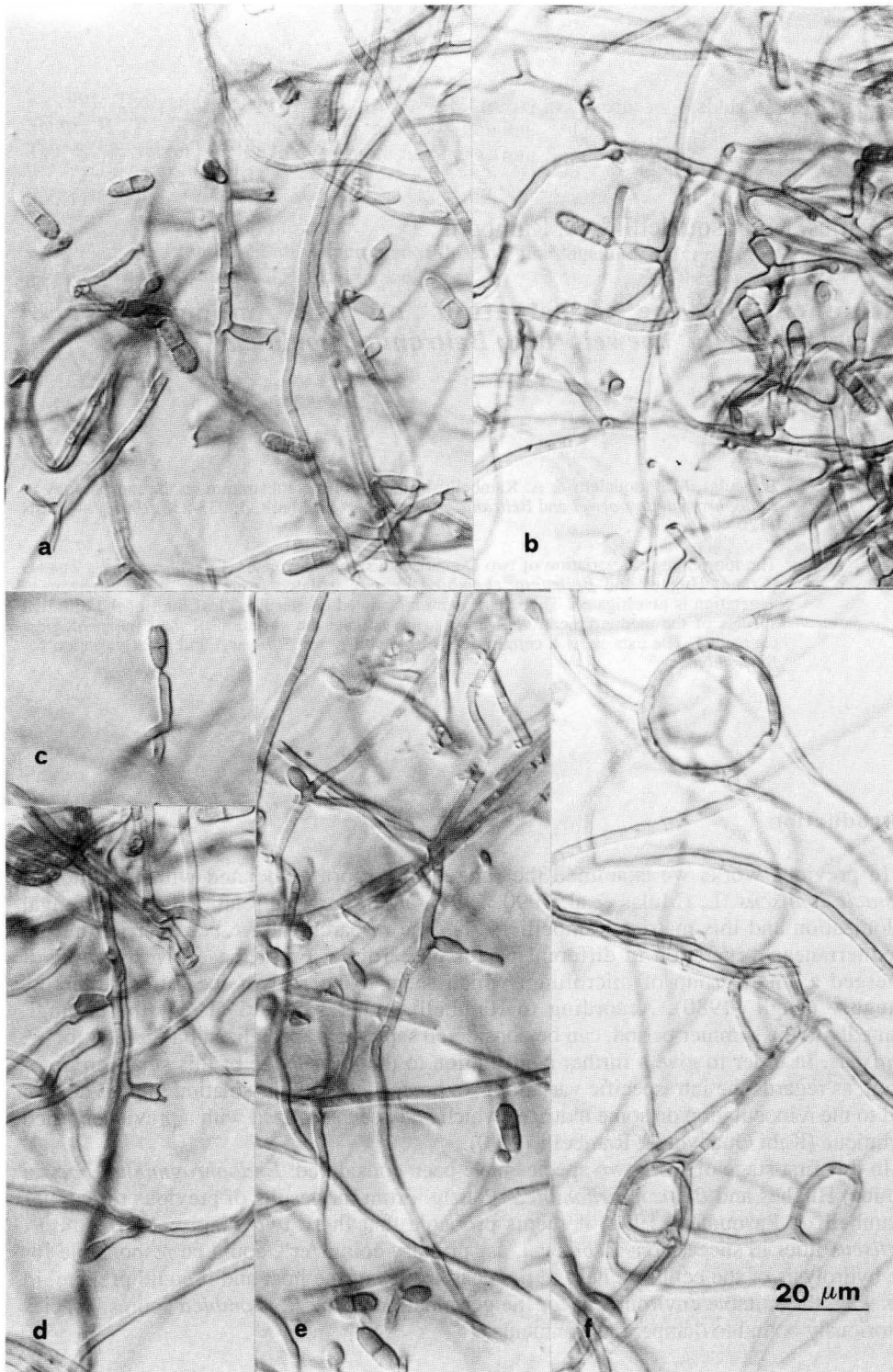


Fig. 1. *Endophragmiella boewei* (Crane) Hughes — **a**: Conidiogenous cells and conidia on *P. latifolia*; **b**: Conidiophores, conidiogenous cells and conidia on *P. lentiscus*; **c**: Conidiogenous cell and conidium on *C. humilis*; **d**: Monoblastic and polyblastic conidiogenous cells on *P. angustifolia*; **e**: Conidiogenous cells and conidia on *R. officinalis*; **f**: Hyphal windings on *P. latifolia*.

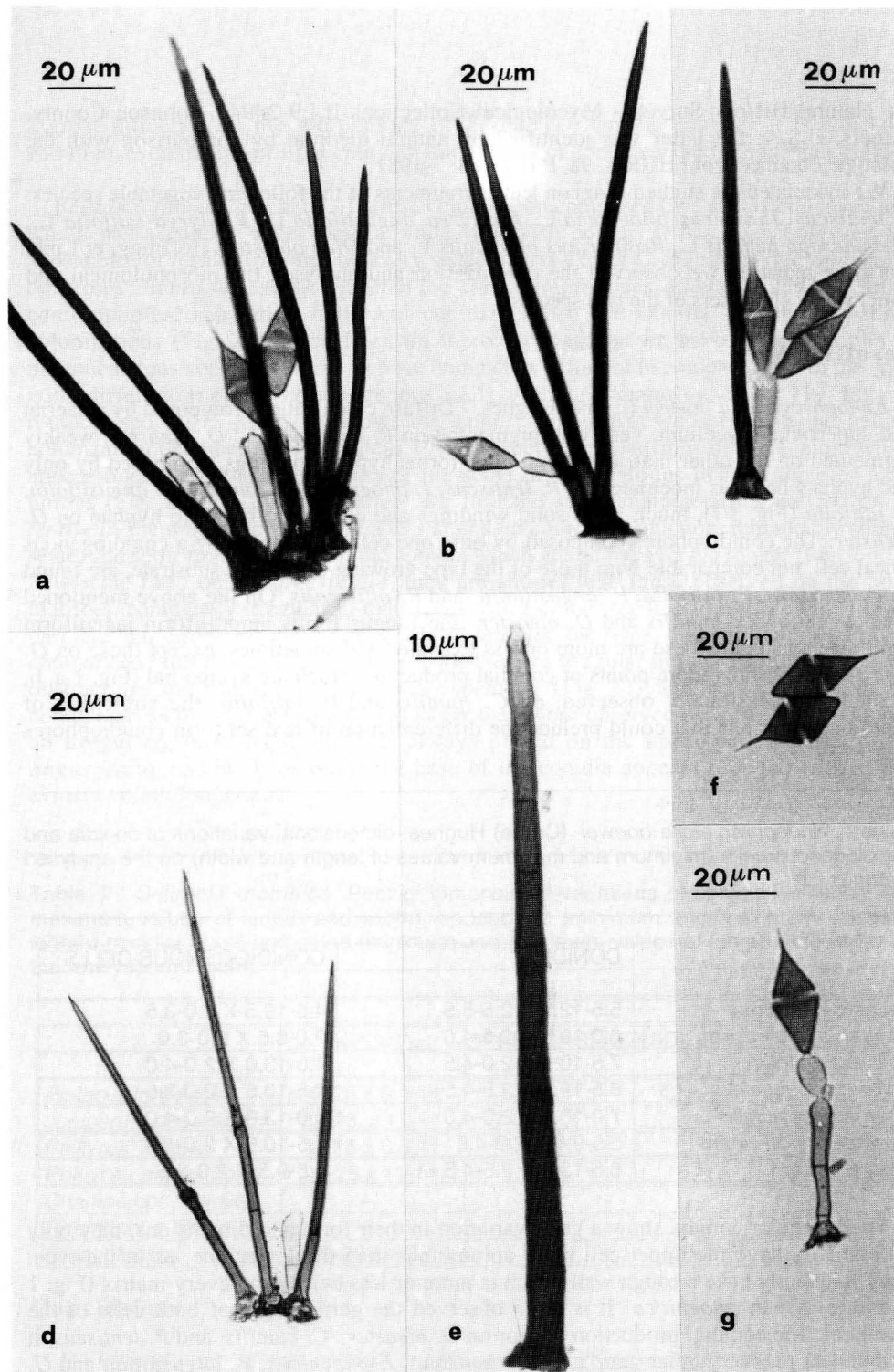


Fig. 2. *Beltrania rhombica* Penzig— a, b, c: Setae, conidiogenous cells and conidia on *P. lentiscus*; d: Conidiogenous cells and conidia on *O. oleaster*; e: Seta on *P. latifolia*; f: Conidia on *J. phoenicea*; g: Conidiophore, separating cells and conidium on *O. oleaster*.

the Natural History Survey - Mycological Collections ILL9 34948, Johnson County, Illinois, U.S.A; the latter was identified on natural medium by comparison with the holotype obtained from HBR n. 9a, P II 23 68; 3-1981.

We inoculated the studied fungi on leaves fragments of the following vegetable species: *P. lentiscus*, *Juniperus phoenicea* L., *Phillyrea angustifolia* L., *Phillyrea latifolia* L., *Chamaerops humilis* L., *Rosmarinus officinalis* L. and *Olea oleaster* Hoffm. et Link. On these matrices we observed the colonization and analysed the morphological and dimensional characters of the two species.

## Results

*Endophragmiella boewei* (Crane) Hughes. - Diffuse colonization, composed by an aerial and superficial mycelium, very well pigmented on *P. lentiscus* and *O. oleaster*, weakly pigmented on the other matrices; the fungus forms hyphal windings, composed by only one hypha when it is inoculated on *P. lentiscus*, *J. phoenicea*, *C. humilis*, *P. angustifolia*, *P. latifolia* (Fig. 1 f), much more solid windings and composed by more hyphae on *O. oleaster*. The conidiophores composed by only one cell surmounted by a conidiogenous apical cell, not comparable with those of the type growing on natural substrate, are found on *P. lentiscus*, *P. latifolia*, *P. angustifolia* and *R. officinalis*. On the above mentioned species and on *C. humilis* and *O. oleaster* the fungus forms ampulliform lageniform conidiogenous cells, these are more or less extended and sometimes, except those on *O. oleaster*, they show more points of conidial production which are sympodial (Fig. 1 a, b, d, c). It is occasionally observed, on *C. humilis* and *P. latifolia*, the stretching of conidiogenous cells that could prelude the differentiation of real setiform conidiophores (Fig. 1 c).

Table 1. *Endophragmiella boewei* (Crane) Hughes: dimensional variations of conidia and conidiogenous cells (minimum and maximum values of length and width) on the analysed matrices.

	CONIDIA	CONIDIOGENOUS CELLS
<i>Pistacia lentiscus</i>	5.5-12.5 X 2.5-5.5	4.5-10.5 X 2.0-3.5
<i>Juniperus phoenicea</i>	6.0-10.5 X 2.5-4.0	2.0-8.5 X 2.0-3.0
<i>Phillyrea latifolia</i>	7.5-10.5 X 2.0-4.5	4.0-13.0 X 2.0-4.0
<i>Phillyrea angustifolia</i>	6.5-12.0 X 2.5-4.5	4.5-10.5 X 2.0-3.5
<i>Chamaerops humilis</i>	7.0-10.5 X 2.0-4.0	4.0-13.0 X 2.0-4.0
<i>Rosmarinus officinalis</i>	5.5-9.5 X 2.0-4.0	4.5-10.5 X 2.0-4.0
<i>Olea oleaster</i>	8.5-13.0 X 2.5-4.5	5.5-9.5 X 2.0-2.5

The bicellular conidia show a great variation in their form and dimensions, they only occasionally have the upper cell more voluminous than the lower one, as in the type. They frequently have a rough wall which is more or less evident on every matrix (Fig. 1 a, b, d, c). On *J. phoenicea* it is often observed the germination of both cells of the conidium. The conidial production is poor on *O. oleaster*, *C. humilis* and *P. lentiscus*, it is abundant on every other matrix. On *P. lentiscus*, *J. phoenicea*, *P. angustifolia* and *O. oleaster* it is observed the presence of globose, pigmented and pluricellular chlamydospores. From what has been said above, it is evident that the substratum has not

a great influence on the morphology of *E. boewei*. On the contrary it is noticed a variation, even though of light entity, in dimensions of conidia and conidiogenous cells.

*Beltrania rhombica* Penzig. - Diffuse colonization composed by a superficial mycelium which has a cottony aspect and a white-yellowish colour. It has been observed the appearance of fertile structures on every tested matrix except *C. humilis* and *R. officinalis*; only on *P. lentiscus* and *O. oleaster* the fungus reproduces all the structures, setae, conidiophores, separating cells and conidia, which are usually present in natural colonizations (Fig. 2 a, b, c, d, g); on the other matrices we have the production of micronematous conidiophores which are completely different in comparison with the type, with differentiation of conidiogenous cells without separating cells. The fungus, reinoculated on *P. lentiscus* shows the same characteristics observed in the natural colonization (Rambelli & Pasqualetti 1990) except the dimensions of the conidia which appear bigger and the aggregation of the setae in small groups of two, three or more unities (Fig. 2 a, b). In *O. oleaster* we find the same characteristics noticed on *P. lentiscus*, the only differences are a less abundant conidia production, remarkably shorter setae, and setiform appendage of the conidia which are on the average longer (Fig. 2 d, g). Few setae form on *P. angustifolia*, they are shorter than those observed on the other matrices, completely irregular in their form with a colour tending to hyaline in the lower part and light-brown on the upper one. In *P. latifolia* the setae, dimensionally superior in comparison with those observed on *P. lentiscus*, are irregular in their form with frequent obstructings and humps (Fig. 2 a). In *J. phoenicea* (Fig. 2 f) and in *P. angustifolia*, the conidia are on the average shorter and larger than those observed on *P. lentiscus* and with an irregularly thick band which is always placed on the upper part; besides, in *P. angustifolia* and in *O. oleaster*, the base of the conidia appears rounded with a little evident point of junction.

Table 2. *Beltrania rhombica* Penzig: dimensional variations of conidia (minimum and maximum values of length and width), appendage (minimum and maximum values of length) conidiophores and setae (minimum and maximum values of length and width) on the analysed matrices.

	CONIDIA	APPENDAGE	CONIDIOPHORES	SETAE
<i>Pistacia lentiscus</i>	22-28 x 8.4-14	5.6-8.4	42 x 5.0	182 x 5.6
<i>Juniperus phoenicea</i>	22-25 x 11-14	7.0-11.2	—	151 x 5.6
<i>Phillyrea latifolia</i>	20-25 x 8.5-11	5.5-8.5	—	327 x 5.5
<i>Phillyrea angustifolia</i>	20-25 x 11-14	5.5-8.5	—	70 x 5.0
<i>Chamaerops humilis</i>	—	—	—	—
<i>Rosmarinus officinalis</i>	—	—	—	—
<i>Olea oleaster</i>	22.5-28 x 8.5-14	5.5-12	42 x 5.5	137 x 4.5

## Conclusions

*E. boewei*, out of the natural conditions, doesn't reproduce on any matrix the morphological structures which, on the contrary, are found on the litter of *P. lentiscus*. This phenomenon is common in other Dematiaceous species (Cole & Samson 1979). The fungus, although invades violently the inoculated matrices, reproduces through holoblastic conidia with deeply different shapes and dimensions in comparison with the original ones

and, above all, it doesn't differentiate percurrent setiform conidiophores typical of the species. The ampulliform conidiogenous cells, monoblastic and polyblastic, with reduced dimensions, mostly originate directly from micronematous conidiophores. It is interesting to note that the behaviour of the fungus on the different matrices doesn't change substantially. In nature the species, found also in very different environments, doesn't show any kind of variation; all this leads us to consider it morphologically steady.

On the contrary the behaviour of *B. rhombica* is deeply different because, reinoculated on dead leaves of *P. lentiscus*, it produce reproductive structures like the original ones, whereas it shows a certain morphological and dimensional variation when it is inoculated on the other matrices. Yet, *B. rhombica*, also in natural conditions, shows a wide variation in dependence on both the environment and the nature of the substratum which it colonizes (Rambelli & Pasqualetti 1990; Di Pietro & Rambelli 1992); this confirms the great ability of adaptation which the fungus shows (Fig. 2).

From the analysis of natural colonizations on the litter of different vegetable species which form the mediterranean vegetation, it seems that we have to consider *E. boewei* a species strictly specialized to *P. lentiscus*, according to the concept expressed by Rambelli (1991), whereas *B. rhombica* a species which is able to colonize more easily other matrices.

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