Close relationship was found between amoeboid protists and injuries of floral organs of some fruit trees and injuries of holm oak

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Sumário
O vingamento do fruto no damasqueiro (Prunus armeniaca) e na oliveira (Olea europaea) é fortemente reduzido quando existem lesões nos gomos florais ao nível do receptáculo ou istilo, tendo-se verificado uma relação de causa –efeito entre a presença de organismos plasmodiais e lesões florais. Similarmente, em raízes de azinheira tem sido observado um processo anormal de degradação associado à presença de formas plasmodiais. Neste trabalho comparam-se algumas fases de desenvolvimento de plasmódios a invadir raízes de azinheira com formas muito semelhantes às que têm vindo a ser observadas em gomos florais de damasqueiro e oliveira. Foi estudado o aspecto morfológico destes biontes dentro dos tecidos e em meio de cultura para obter um melhor conhecimento do processo degradativo que provocam nas plantas. As observações foram realizadas em microscopia óptica e electrónica de transmissão e de varrimento. As fases iniciais e a progressão das lesões nos vários tecidos foram semelhanas na oliveira e no damasqueiro. A partir da morfologia dos organismos observados nestas duas espécies, assim como das formas isoladas em meio de cultura e esporuladas, no caso da oliveira, podemos concluir que se trata de organismos que pertencem ao grupo dos protistas amibóides. O processo de degradação das raízes de azinheira que se verificou associado à invasão por protistas amibóides segue um padrão muito idêntico, iniciando-se na epiderme até ao cilindro vascular. Foram igualmente observadas formas de resistência como quistos em áreas lisadas do córtex e cilindro vascular. Neste trabalho é discutido o papel dos protistas amibóides no decréscimo do vingamento do damasqueiro e oliveira e na doença do declínio da azinheira.

Abstract
Extensive lesions in apricot (Prunus armeniaca) and olive (Olea europaea) flower bud organs can severely reduce fruit set, when the receptacle or pistil are damaged. A close relationship was found between the presence of plasmodia-like organisms and this disorder. In holm oak roots we also have observed an unusual degradation process, consistently associated with plasmodia-like bionts. In this work we compare some developmental phases of bionts invading the roots of holm oak with similar aspects of bionts in floral bud lesions of apricot and olive. The morphological aspects of bionts on and in plant tissues and in culture media were studied, in order to get a better knowledge of these organisms in the degradation processes of plants. The observations were made by light, transmission and scanning electron microscopy. The initial stages of the bud lesions and the progression of the
injury into the tissues were similar in olive and apricot. We established that the plasmodia-like organisms isolated in culture media are similar to the forms observed on and in floral tissues. The plasmodial aspect of the bionts observed in these crops and the sporulation forms obtained from olive buds suggest its inclusion in the amoeboïd protist group. The degradation of holm oak roots follows a similar pattern of damage from the epidermis towards the vascular cylinder, associated with invasive plasmodia-like bionts. Plasmodial resting cysts were also observed in the lysed areas of the cortex and vascular cylinder. The role of amoeboïd protists in the decrease of fruit set of apricot, olive and holm oak decline is discussed.

INTRODUCTION

Flower bud lesions can severely reduce fruit set, mainly when the receptacle or pistil are damaged. In previous works a close association between the epidermal cell injury and the penetration of plasmodia-like organisms were referred, (Medeira et al., 1992; Maia et al., 2001; Medeira et al., 2002; 2006).

Serious declines of oaks have been reported in Europe and a wide range of associated factors has been identified. Microscopic observations have revealed that the roots of holm oak were frequently injured by plasmodia-like organisms in association with other well-known pathogens as for example the oomycetes, following a similar pattern previously observed in apricot and olive floral buds.

In this work we compare some developmental phases of bionts invading the roots of holm oak with similar aspects of bionts observed in the floral bud lesions of apricot and olive, in order to get a better knowledge of these degradation processes.

MATERIAL AND METHODS

Light (LM) and transmission electron microscopy (TEM)

Fragments of primary and lateral fine roots of seedlings of holm oak and floral buds of olive and apricot was fixed in 3% glutaraldehyde in 0,1M sodium cacodylate buffer, pH 7.4, overnight at 4ºC. The specimens were washed three times for 30 min in the same buffer, post fixed in 1% osmium tetroxide in 0,1M sodium cacodylate buffer, dehydrated in an ethanol gradient to absolute ethanol and embedded in Spurr’ resin. Sections of 1-2 µm and 60-80 nm were cut in a Leica Ultracut R using diamond knives. Thick sections were observed by light microscopy (LM), stained in 0,05% toluidine blue in 1% sodium carbonate. Thin sections were stained in an aqueous saturated solution of uranyl acetate for 30 min and post-stained in a 3% aqueous solution of lead citrate at room temperature for 15 min. The observations were made with a FEI Morgagni Transmission Electron microscope at 80 Kv.

Scanning electron microscopy (SEM)

Fragments of floral organs were fixed, post fixed and dehydrated by the same procedure used in the preparation of the material for TEM. Specimens were critical point dried and mounted on the metal stubs using a neutral varnish. Each sample was coated with a thick layer of gold using the ‘Sputter-coating’ technique and scanned with an ISI Scanning Electron Microscope, model DS- 130, at 10 kV accelerating voltage.

Isolation in culture medium

Small pieces of floral buds without fungicide or bactericide disinfections were placed on Petri dishes with poor medium of agar in water (3 % w/v) and hay-agar culture
medium (0.5% (w/v) agar in “hay juice”) in order to isolate the plasmodia-like organisms. The Petri dishes were incubated at 21-22°C with a photoperiod of 12 hours (fluorescent light 200 μmoles⁻¹ s⁻¹).

RESULTS

The initial stages of bud lesions and the progression of the injury into the tissues were similar in apricot and olive. The lesions seemed to be originated by the penetration of amoeboid plasmodia-like organisms, mainly in the receptacle coming from bracts into the floral primordia. The epidermal cell injury was always restricted to minute areas where plasmodia-like organisms were adherent or penetrated. These organisms were seen on epidermis and inside the tissues of several organs, digesting areas of the parenchyma and progressing into the buds (Fig.1). Pleomorphic plasmodia-like organisms were seen on several organs, often emerging from resting cysts (Fig.2). The external aspect of a plasmodial resting cyst observed by scanning electron microscopy (SEM) on a section of the receptacle is shown on Fig.2 (S.E.M. detail) Dense and filamentous structures were observed in the areas of the plasmodia-like organism penetration. The breakdown of cuticle and cell walls occurred in a narrow zone (Fig.2).

In the sites where the plasmodia-like organisms were adherent (Figs. 3 and 4) we could see the epidermal cell damaged close to epidermal and sub-epidermal cells preserving their integrity (Fig.4).

The organisms isolated in culture medium from apricot floral organs (Fig.5) were similar to the plasmodia-like masses that appeared inside the pistils, anthers and other floral pieces of apricot (Fig.6).

In olive floral buds extensive lesions in ovary were often visible (Fig.7). The signs of disruption could be seen associated with plasmodia-like organisms (Figs7- 9). A detail of a thin section of a plasmodia-like biont on a pistil is visible on Fig.8. A detail of injury into a pistil can be seen on Fig.9. The tubular circunvoluted form of the biont inside the tissues corresponds to the similar tubular organization on epidermal cells (Fig.8). Inside the ovary wall the tissues appeared lysed around the plasmodia - like organism.

Plasmodia-like bionts growing in culture medium isolated from olive floral buds are shown on Fig.10. Similar aspect revealed a squashed plasmodial biont with numerous nuclei collected from a pistil strongly injured (Fig.11).

In holm oak roots an extensive injury process was frequently observed. It starts by minute disruptions of epidermal cells associated with the germination of plasmodia-like encysted forms (Fig.12). The injury progresses towards vascular cylinder (Fig.13) and results in the break of the cell walls of the cortical and vascular parenchyma and lyses of the cell contents (Figs.13 and 14). The observation of damaged areas by TEM revealed the presence of protoplasmatic masses rich in ribosomes and vesicles suggesting a plasmodia-like biont (Fig.15).

The injury process leads to the lysis of the vascular cylinder, break-down of xylem vessels, remaining the encysted forms among the vessel debris (Fig.16).

DISCUSSION

Floral bud injury in olive was detected during intensive growth period of organogenesis marked by high mitotic activity. Differently in apricot the destructive process starts earlier in healthy buds, during the rest period. The epidermal and sub-epidermal cell injury always occurs at sites of attachment of the biont to the floral organs in olive, and in apricot. The damage process in olive is similar to the previously described
in apricot associated with plasmodia-like organisms (Medeira et al., 1992; 1995; 1997). The disorganisation of epidermal and sub-epidermal cells after biont penetration in healthy tissues suggests the importance of these organisms in the appearance of bud lesions once extensive damaged areas of pistils or receptacle leads to floral bud failure.

The first stages of plasmodia, grown in culture medium, isolated from olive floral buds resemble those isolated in culture medium from apricot buds. They are naked, pleomorphic and polynucleotide masses, common to the amoeboid protists (Sleigh, 1989). They were morph and cytologically similar to the plasmodia developed on and in the lesioned pistils, anthers, receptacles, or other floral organs of both fruit species.

The plasmodia grown in culture medium from olive floral pieces suggest being a Myxogastria (Medeira et al., 2006) and the sporulation of the plasmodia suggests that these bionts belongs to the genus Didymium (Cohen, 1960; Olive, 1975; Medeira et al., 2006).

In apricot, the plasmodia-like forms grown in culture medium encysted not allowing their morphological identification.

In holm oak plasmodia-like masses germinated from cysts penetrated the roots, lysing and invading extensive areas of the cortex and of the vascular cylinder.

A similar process of damage, disruption of cell walls, membranar degradation and cytoplasmic lysis was observed in floral organs of olive and apricot, as well as in the roots of holm oak. The presence of plasmodia-like bionts associated with the injury process is the common feature leading to the death of several organs in these species.

Floral bud drop in apricot and in olive occurs often due to several abiotic and biotic factors. Among these, the plasmodia-like (amoeboid protists) may have an important role in the decrease of fruit set.

The root death in holm oak by lysis of the cortical and vascular tissues, disruption of the cell walls, including those of the xylem vessels, associated to plasmodia-like bionts leads to establish these bionts as potent destroyers of root holm oak stands.

References
Figs. 1, 2. Apricot. (L.M.). Fig. 1. Pleomorphic plasmodia-like organism (P) on and inside the receptacle. Fig. 2. Plasmodia-like organism emerging from a resting cyst (R) on a pistil. Dense and filamentous structures in the penetration area, breakdown of cuticle and cell walls (arrow) are visible. P- plasmodia-like organisms, R- resisting cyst. Insert: (S.E.M.) - external aspect.

Figs. 3 and 4. Apricot (T.E.M.). Details of the previous organism adherent to the epidermis. The damaged epidermal cell (Dc) (Fig. 4) is close to cells that maintained their integrity (*) in areas where the plasmodia-like organisms (P) are adherent or start to penetrate. D- dictiosome, Dc- damaged epidermal cell, P- plasmodia-like organism, Pc- pistil cell wall, Pl- plasmalemma of plasmodial-like organism, V- vesicle, Mos- granular osmiophylic material. Fig. 5. A plasmodia-like organism growing in culture medium after squash. N- nuclei. Fig. 6. A similar plasmodia-like organism inside an anther. N- nuclei.
Figs. 7 to 11. Olive. Fig.7 (L.M.) Extensive lesion (**) in an ovary. Fig. 8 (T.E.M.). A plasmodia-like organism (P) on a pistil. E-epidermal cell.
Figs. 9 to 11 (L.M.). Fig.9. A plasmodia-like biont (P) presenting a tubular and convoluted feature in a lysed area of the receptacle. Figs. 10 and 11. Plasmodia-like organism. Fig.10. In culture medium. Fig.11. In an injured pistil after squash.

Figs. 12 to 16. Holm oak roots. Fig.12 (L.M.). Resting cyst on the epidermis. Fig.13. (L.M.). Injuries (*) in the cortex and in vascular cylinder. Fig. 14 (L.M.) Injured (*) vascular cylinder. Fig.15 (T.E.M.). A plasmodia-like mass inside the vascular cylinder. Fig.16. (L.M.). Lysis of the vascular cylinder remaining the resting cysts (C) among the vessel debris.