Phytopathol. Mediterr. (2009) 48, 454–460

Further investigations on the biology of *Phomopsis cinerascens*, the cause of fig canker in Iran

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Summary. Fig branch canker is a major disease in most parts of Iran, especially in Estahban (Fars province), which has the largest area of dry fig plantations in that country. In 1999–2000 a general survey was conducted in rainfed fig plantations throughout Fars province. In this survey *Phomopsis cinerascens* was consistently isolated from the cankers. The fungus produced pycnidia containing α-conidia on active cankers from fall to mid spring. No β-conidia were found under natural conditions, but many isolates produced β-conidia intermixed with α-conidia in culture. Only α-conidia germinated on agar medium. The optimum temperature for growth, pycnidial formation and pycnidiospore germination was 25°C. Pathogenicity tests revealed that the fungus infected inoculated branches at 15–25°C but no infection occurred at 5°C or at 30°C or higher. Under field conditions, the pathogen infected branches from fall to mid spring, but little infection occurred in summer. The pruning wounds remained receptive to the pathogen from fall to mid spring. Pycnidiospores that over-summered on trees or on branches lying on orchard floors were not viable. Infected branches under moist conditions produced new pycnidia containing viable conidia. Mycelia are considered important for over-summering the pathogen in Fars province.

Key words: *Ficus carica*, Phomopsis canker, rainfed fig, survival.

Introduction

The largest areas of rainfed caprifig (*Ficus carica* L.) used for production of dry figs in Iran are located in Estahban in Fars province. More than 20,000 ha of dry figs are grown in this province, with annual production of over 20,000 tonnes (Javadi, 2001).

In 1978 fig branch canker was detected in Estahban. The causal agent was identified as *Phomopsis* sp. (Zia Banihashemi, unpublished data) and control measures were initiated (Fatemi and Mobayyan, 1983). Further surveys indicated that the pathogen occurred in most rainfed fig plantations in Fars Province (Javadi, 2001). The disease was originally reported from Italy in 1878 by Saccardo and the causal organism was identified as *Phomopsis cinerascens* (teleomorph: *Diaporthe cinerascens*) by Grove in 1935 (cited by Ogawa and English, 1990). It caused a major epidemic on the cultivar Kadota in California, due to heavy pruning (Ferguson *et al.*, 1990). The pathogen affects all commercial figs in California (Ogawa and English, 1991). It survives from one year to the next in cankers on the trees or on infected branches in orchards (Hansen, 1949). After infection, conidia are produced in the cankers during the wet period in winter (English, 1951). Rain splash and pruning tools are the main means whereby the pathogen is dispersed (Hansen, 1949). In California susceptible fig cultivars such as Kadota are prone to infection from November through February, but become resistant thereafter (English, 1951; 1952b). The optimum temperature for growth of the pathogen in culture is 25°C, and isolates do not grow at 4 or 30°C or higher (Ogawa and English, 1991).

The objective of the present study was to investigate the biology of the pathogen concentrating on its mode

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of survival and on the receptiveness of pruning wounds grown in rainfed dry fig plantations in Estahban. Part of this research has been reported earlier (Javadi and Banihashemi, 2005).

Materials and methods

Sample collection and isolation

During 1999–2000 a number of fig plantations in Estahban and other locations in Fars province were visited and symptomatic branches showing wilting or canker were collected and taken to the laboratory. The causal agent was isolated from samples collected from November to May. Diseased tissue on each branch was washed with tap water and the outer bark was carefully removed with a scalpel to expose the layer underlying the discolored tissue. From the margin of healthy and discolored tissues, a few wood fragments (0.5–1×0.2 cm) were excised, surface-sterilized in 0.5% sodium hypochlorite for 1–3 minutes, rinsed with sterile distilled water (SDW), blotted dry with a sterile paper towel, placed on Petri dishes containing potato dextrose agar (PDA) and incubated at 25°C. After 1 week, fungal colonies appeared around each fragment. However, with samples collected after May and throughout the summer, the above isolation method was not suitable. Cankers with pycnidia not older than 3 months were surface-sterilized with 95% ethanol and the pycnidia were removed with a sterile scalpel, transferred to a test tube containing SDW and vortexed. The suspension containing α-conidia was streaked on 2% water agar (WA) in Petri plates and incubated at 25°C. After 24 h, germinated conidia were transferred to PDA plates. If pycnidia were not present upon sampling, the infected branches were surface-sterilized in 0.5% sodium hypochlorite for 5 min., placed in sterilized glass jars contained moist sponge, and incubated at 25°C. After 12–15 days the pycnidia were formed and exuded pycnidiospores as cirri, which were transferred to test tubes containing SDW, vortexed and streaked on 2% WA plates as described above. All cultures for further study were from single conidia, and these were stored at 4°C.

Wood colonization tests in the laboratory

Healthy fig branches 1–2 cm in diameter were collected from the fig plantation and taken immediately to the laboratory. They were cut into 15–20 cm portions and dipped into 0.5% sodium hypochlorite for 5 min., blotted dry with a paper towel and the cut ends were dipped in melted paraffin wax (70°C) to reduce water loss. The middle portion of each branch was surface-sterilized with 95% ethyl alcohol. Three cuts were made in the bark on each side of a square. A block of agar 8 mm in diameter and 2 mm thick colonized by the pathogen was inserted under the bark, covered with the bark and wrapped with Parafilm to reduce desiccation. Experimental controls were inoculated with uncolonized PDA in a similar manner. Inoculated branches were stored in sterilized glass jars containing a moist sponge and incubated at 20–25°C for 10–15 days until the disease symptoms appeared.

Pathogenicity test in the orchard

A few fig trees from one local cultivar and of the same age were selected. From each tree a few branches, 2–3 cm in diameter were inoculated *in situ* as described above. After 12–20 days, the branches were cut from the trees and transferred to the laboratory to check for disease development and to re-isolate the pathogen.

Cardinal temperature for growth and germination

Several isolates of *P. cinerascens* from different locations were transferred to Petri dishes containing PDA and incubated at 15, 20, 25, 30 or 35°C. Five dishes were used for each combination of isolate and temperature. Colony diameters were measured after 12 days. Pycnidiospore suspensions (10^3 conidia mL^{-1}) obtained from PDA cultures were streaked on 2% WA and incubated at different temperatures between 5 and 35°C (5°C increments). Five replications were used for each temperature. Conidial germination and germ tube length were measured after 24 h.

Effect of temperature on disease development

Detached fig branches were inoculated as described above and incubated at 15, 20, 25, 30 or 35°C in jars containing moist sponges. Ten branches were used at each temperature. After 15 days, infection was assessed by re-isolation of the pathogen from beyond each inoculated point.

Effect of temperature on pycnidium formation

Several fig branches were inoculated as described above and kept at room temperature for 10 days before they were incubated at different temperatures between 5 and 35°C (5°C increments). Branches were left until the pycnidia appeared. Ten branches were used at each temperature.

Duration of fig susceptibility to infection

To investigate infection and canker development at different times of the year, ten similar aged fig trees of one local cultivar were selected at the Estahban Fig Research

Station. At the beginning of each month, four branches (2.5–3 cm diameter) from each tree were inoculated as described for detached branches (two branches with the pathogen and two with PDA). At the end of each month, the branches were cut and transferred to the laboratory where the length of the canker on each branch was measured and the pathogen was reisolated. The experiment was repeated each month throughout 1 year.

Receptiveness of pruning wounds to infection

Ten fig trees cv. Sabz of the same age and size were selected. In early December, about 140 branches (diameter of 1.5 cm) were pruned. Every 30 days the pruning wounds of ten branches were inoculated with the pathogen and two branches with sterile PDA as experimental controls. The wounds were then sealed with Parafilm. After 30 days, the inoculated branches were removed and transferred to the laboratory for the re-isolation of the pathogen from beyond the point of inoculation.

Survival of the pathogen

At different seasons, fig plantations throughout the province were inspected several times and pruned branches left on the floor of each plantation were collected, washed and wrapped in wet cloth, placed in a nylon bag and left in the orchard. After 2–3 weeks, a few branches with pycnidia were brought to the laboratory and the germinability of the pycnidiospores was determined.

Host range study

Detached and intact branches of the woody plants grapevine (*Vitis vinifera* L.), apple (*Malus communis* Desf.), pear (*Pyrus communis* L.), pistachio, (*Pistacia vera* L.), apricot (*Prunus armeniaca* L.), peach (*Prunus persica* Stokes), sweet orange (*Citrus sinensis* Otbeck), walnut (*Juglans regia* L.), mulberry (*Morus alba* L.) and plane tree (*Platanus orientalis* L.) were inoculated with *P. cinerascens* using the method described above. Results were recorded after 15 days in the laboratory and 25 days in the field.

Results

Isolation and identification

Forty isolates of the pathogen were recovered from cankers collected from different parts of the province. All isolates produced pycnidia on PDA. On fig branches the pycnidia were aggregated, immersed in the bark, globose-depressed, 180–450 µm in diameter, bi- or uni-loculate with the ostiole emerging through the surface of the canker (Figure 1).

Most pycnidia contained α-conidia, which were hyaline, elliptic-fusoid, 7–10×2.5–4 µm, and often biguttulate. No β-conidia were detected in pycnidia collected from the branches. On PDA, however, several pathogen isolates collected from different parts of the province produced pycnidia (Figure 2) containing β-conidia intermixed with α-conidia (Figure 3). They were filiform, mostly hooked, 1×14–25 µm. Only α-conidia germinated on the agar medium, while β-conidia never germinated. No sexual stage of the fungus was found under natural or laboratory conditions. The optimum temperature for growth and

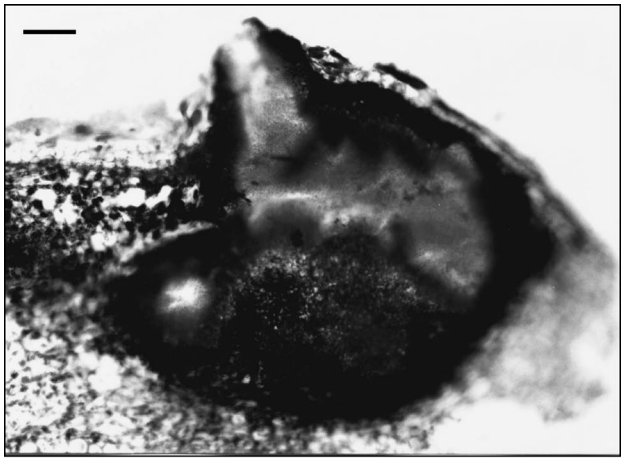

Figure 1. Cross-section through a pycnidium of *Phomopsis cinerascens* on a fig branch (scale bar = 45 µm).

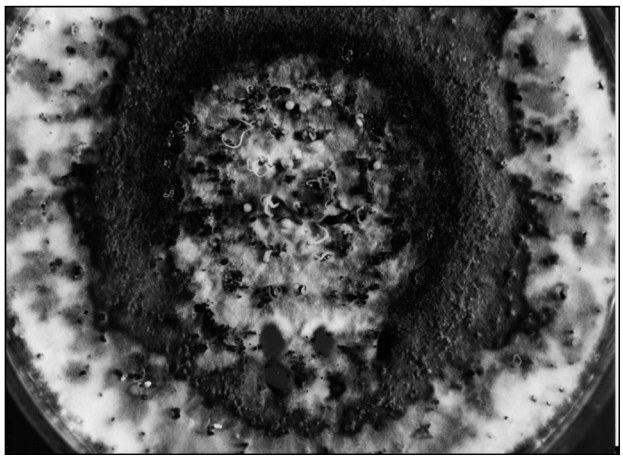

Figure 2. Formation of pycnidium of *Phomopsis cinerascens* on PDA after 4 weeks incubation at 25°C and production of cirri with α and β-conidia.

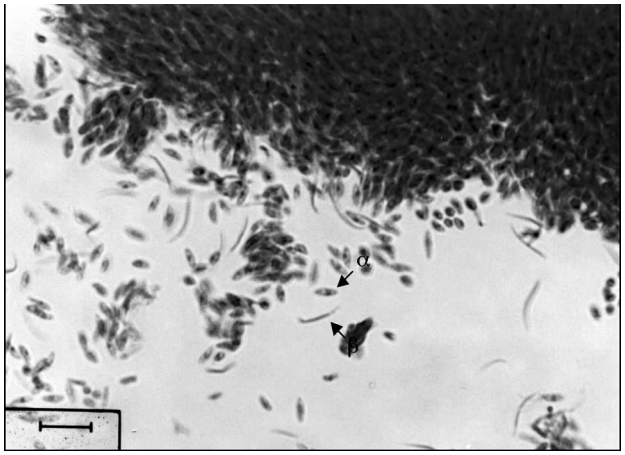

Figure 3. Mixture of α and β-conidia of *Phomopsis cinerascens* (scale bar = 16 μm).

germination was 25°C. No growth or germination occurred at or below 5°C. None of the woody plant species (other than fig) that were inoculated with the fungus became infected by the pathogen. Based on morphological and cultural features and on host specificity the fungus was identified as *Phomopsis cinerascens.*

Temperature effects

The optimum temperature for growth was 25°C (range 15–30°C) with no growth occurring at 35°C (Figure 4). The optimum temperature for conidial germination and germ tube elongation was 25°C (Figures 5 and 6). Although conidial germination was high between 20 and 30°C, germ tube elongation was reduced below 20 and above 30°C.

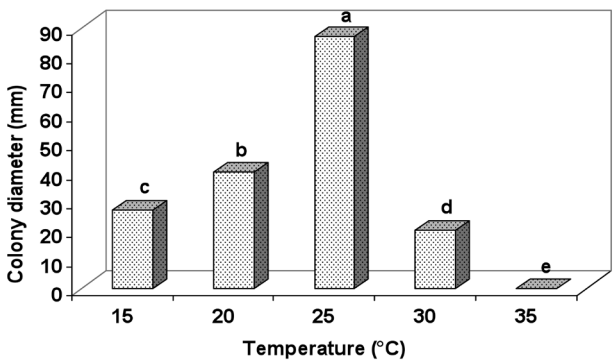

Figure 4. Effect of temperature on colony diameter of *Phomopsis cinerascens* on PDA after 14 days incubation in the dark. Columns followed by the same letter are not significantly different at $P<0.01$ according to Duncan's multiple range test.

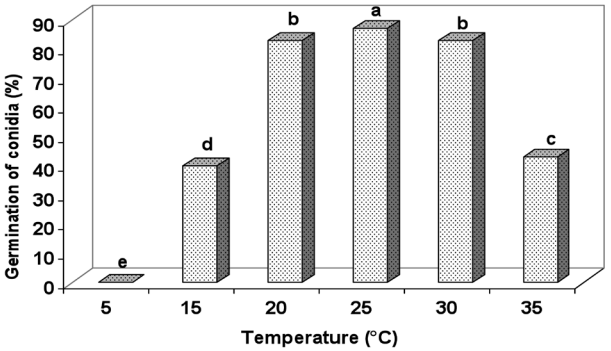

Figure 5. Percentage germination of α conidia of *Phomopsis cinerascens* after 24 hours on water agar at different temperatures. Columns followed by the same letter are not significantly different at $P<0.01$ according to Duncan's multiple range test.

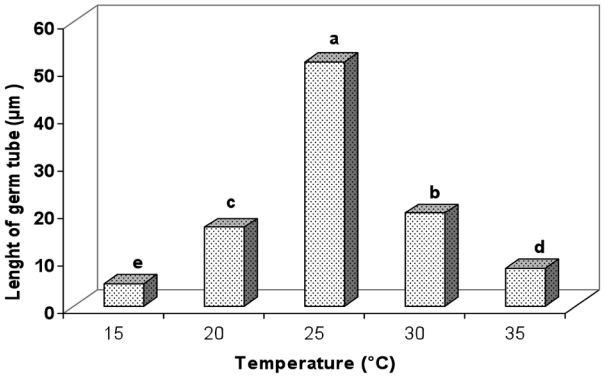

Figure 6. Germ tube length of α conidia of *Phomopsis cinerascens* after 24 hours of incubation at different temperatures. Columns followed by the same letter are not significantly different at $P<0.01$ according to Duncan's multiple range test.

The pathogen infected detached fig branches at 15–25°C but no infection occurred at 5 or 30°C or above.

Pycnidia formed on infected branches between 5 and 25°C, but the time of incubation for pycnidium formation was longer (17–22 days) at 5°C than at 20 or 25°C (4–7 days).

Duration of fig tree susceptibility to the infection

There were statistically significant differences ($P\leq0.01$) in canker development between dates of inoculation. The infection rate was low in July and August. From fall to early spring the trees were susceptible to infection, after which time susceptibility declined (Figure 7). The weather data indicated that temperature was more critical than humidity as a factor affecting infection and disease development.

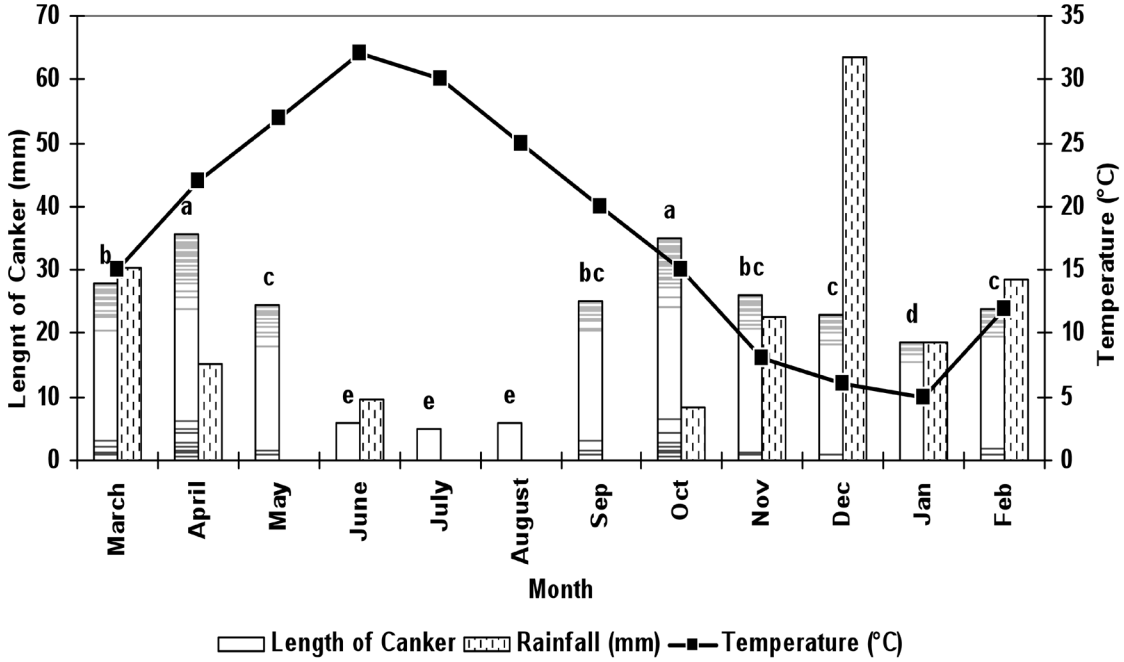

Figure 7. Relationship between the mean temperature and *Phomopsis cinerascens* canker development on fig trees during 2000–2001 under Estahban (Fars, Iran) conditions. Columns followed by the same letters are not significantly different at $P<0.01$ according to Duncan's multiple range test.

Receptiveness of pruning wound to infection

Pruning wounds made in December remained receptive to the pathogen throughout the year but they were most receptive from fall to mid spring (Figure 8).

Survival of the pathogen

Infected branches collected in the fall, winter and early spring produced pycnidia after they were incubated for 2–3 weeks under humid conditions. No pycnidia were seen on the branches collected in summer; these branches were colonized by saprophytic fungi. The old pycnidia left on the branches from before the fall contained non-viable conidia.

Host range study

Inoculated and non-inoculated, attached and detached branches of different plant species were inspected 15 and 25 days after inoculation. Only inoculated fig branches developed cankers and produced pycnidia containing pycnidiospores of *P. cinerascens*, and only from these branches was the pathogen re-isolated. There was limited infection on mulberry but the pathogen did not spread beyond the point of inoculation. No symptoms developed on any of the other inoculated hosts, and the pathogen was not re-isolated from these inoculated plants.

Discussion

This is the first comprehensive study of *P. cinerascens* in rainfed fig plantations in Iran. The pathogen was active during the dormant period of the host, but when growth resumed the host resisted infection. Similar results were reported in California (English, 1951, 1952a, 1952b, 1962). Kadota fig trees in Californian orchards were immune to infection from April and through the growing season, but were highly susceptible from November to February. New pruning cuts were susceptible to infection when moisture and temperature conditions were favourable (English 1952, 1958). The present study found that pruning cuts under Estahban conditions remained receptive to the pathogen in fall, winter and early spring but became resistant thereafter.

Phomopsis cinerascens is a wound invader: the pathogen invades host branches mainly through pruning cuts, but sunburn and frost probably also enable infection. English (1951) also suggested that wounds and bark killed by frost and sunburn were the chief avenues of infection,

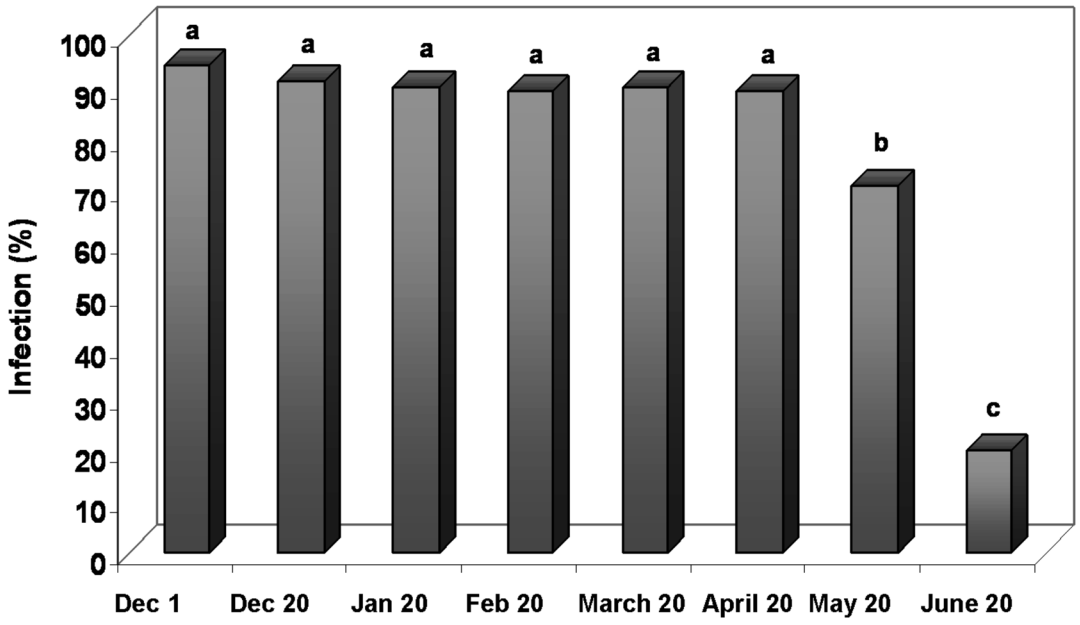

Figure 8. Duration of pruning wound receptiveness to *Phomopsis cinerascens* on fig trees from November to June. Columns followed by the same letter are not significantly different at $P \leq 0.01$ according to Duncan multiple range test.

and that leaf scars were only of minor importance.

Fig branches were susceptible to infection in fall, winter and early spring, but branches were not susceptible in July and August. The lack of rapid canker spread from April to October has been assumed to be due to the active growth of the host (Ogawa and English, 1991).

Under laboratory conditions, the optimum temperature for growth and spore germination of *P. cinerascens* was 25°C and no infection occurred at 5ºC or below or 30°C or above. A suitable temperature seemed to be more critical than moisture. The weather data in Estahban over the last 13 years indicated that temperature plays an important role in canker development.

In the present study it was found that oversummered pycnidiospores of *P. cinerascens* were not viable on cankers recovered from canker-affected field-grown branches, so we assume that mycelium is the main survival agent. With appropriate humidity and temperature, branches with oversummered pycnidia on trees and on the orchard floors produce new pycnidia with viable conidia. These conidia disseminate the pathogen and cause new infections when temperature and moisture conditions are favourable.

Pycnidia collected under natural conditions contained only α-conidia, which are capable of germination. Under laboratory conditions some isolates on PDA produced β-conidia intermixed with α-conidia, but these β-conidia did not germinate on any of the media examined in this study. Uddin *et al*. (1995) reported that *Phomopsis* sp., which causes peach Phomopsis canker, did not produce β-conidia on some media and under certain environmental conditions. The role of β-conidia in the epidemiology of these diseases is therefore unclear.

Since pruning cuts remain receptive to *P. cinerascens* for long periods, the best strategy to manage the disease would be to delay pruning as much as possible. However, late pruning has been reported to adversely affect fruit maturity (English, 1953). Uddin and Stevenson (1998) suggested that in peach late pruning may reduce infection by *Phomopsis* sp. The effect of late pruning of rainfed caprifig in Estahban has not been investigated.

Acknowledgements

The authors are grateful to the Shiraz University Research Council for financial support (project number 79-AG-1344-211).

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Accepted for publication: August 31, 2007