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# EFFECT OF HEAT-STRESS PREDISPOSITION ON THE DEVELOPMENT OF SOOTY CANKER CAUSED BY *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers

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Abstract

Sooty canker, caused by *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, Synon. = *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko, on the inoculated thin bark saplings (12-24 months old) of *Eucalyptus camaldulensis*, *Olea europaea*, and *Populus nigra* was monitored under greenhouse conditions every 2 days until the 8th day, and it was repeated 18, 28, 58 days after inoculation. Predisposition to stem cankers depended on the duration of warm temperature and abundance of fungal inoculum. The infected bark was discolored and revealed a black mass of fungal arthroconidia, particularly on the most susceptible plants of eucalyptus and poplar. The cankers extended to 18.53 mm and 16.11 mm on eucalyptus and poplar, respectively, after 58 days compared to 10 mm for non-inoculated saplings (wounding sites) of control treatment.

The effect of temperature conditions before and after inoculation with *N. dimidiatum* on canker development on the same plants was studied in a growth chamber with two temperature regimes, very hot 40°C and hot 32°C. Among pre-inoculation regimes, very hot and hot temperatures were the most conductive to infection of eucalyptus saplings compared to other hosts, which showed a non-significant dependence between pre- and post-inoculation. Thus, heat stress of 32 and 40°C on the most susceptible host, eucalyptus, sustained the progress of cankers to 17.20-17.56 mm after 3 days and 18.08-18.06 mm after 5 days of inoculation.

**Key words:** sooty canker, heat stress, *Neoscytalidium dimidiatum*

## INTRODUCTION

In the hottest months (June, July, and August) during 2001-2010, sudden limbs wilt or sooty canker invaded thousands of thin or smooth bark trees and ornamentals such as mulberry, ash, walnut, fig, sycamore, apple, apricot, and poplar in Iraq (Hassan et al. 2009).

The fungus was identified as *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko. Furthermore, and the mycelial synanamorph was described as *Scytalidium dimidiatum* (Penz.) B. Sutton & Dyko (Moore, 1988). Crouse et al. (2006) employed DNA sequence data of the 28S rDNA to resolve apparent lineages within the Botryosphaeriaceae, they recognized 10 lineages within the Botryosphaeriaceae, and the new genus is proposed to accommodate this fungus called *Neoscytalidium* (Penz.) Crous & Slippers, and the species called *N. dimidiatum* (Penz.) Crous & Slippers. The colonies grew rapidly, filling the Petri plate within 4-7 days, becoming olivaceous grey to black and overlaid with aerial strands of grayish black mycelia, hyphae fragmented to form cylindrical or barrel-shaped bark brown non-septate or one septate arthroconidia, pycnidial conidia developed one or two septa and a brown median band, with the upper and lower cells being subhyline with tapered ends (Sigler et al. 1997)

The wilt of the middle and upper canopy of grapefruit limbs caused by *Scytalidium lignicola* Pesante was accompanied by gum oozing from the affected branches. The bark of these branches attained a dark colour and the epidermis sloughed off easily revealing a mass of black powder, resulting from copious sporulation of dark conidia (Sadowsky et al. 2007). The fungal hyphae invaded the bark cambium and wood, mostly intracellular, and the cells in the phloem and xylem became dark in colour (Ciesla and Donaubauer, 1994)

The disease was always observed on grapefruit about 3 to 4 weeks after extreme hot and dry weather conditions when temperatures above 45°C and relative humidity 10 to 15% had prevailed for 10-15 consecutive days. Therefore, it was postulated that the affected tress were predisposed by heat stress which triggered

their infection. Sometimes comprehensive pruning preceding the hot spell further enhanced this predisposition effect (Oren et al. 2001).

Recently, it was reported from Oman that in the summer 1998, after a period of heat (up to 45°C) and shortage in water, the dieback of *Albizia lebbeck* and other trees was associated with *N. dimidiatum* (El-shafie and Ba-Omar, 2001).

The aim of the present study was to examine the hypothesis that predisposition to extremely high temperature is a prerequisite for infection of susceptible hosts by *N. dimidiatum*.

## MATERIAL AND METHODS

### Inoculation procedure (pathogenicity) and disease assessment:

Cuttings of poplar, eucalyptus and olive, grown in plastic bags containing sandy loam soil-peat moss, were 12-24 months old at the time of inoculation under greenhouse conditions (25 ± 2°C). Mycelial discs 6mm in diameter were cut out of the margins of 10 day-old culture of *N. dimidiatum* grown on PDA. A similar disc was removed from the bark of the stem using a sharp knife, and an inoculum disc was inserted there and tightly fastened with a flexible polyethylene grafting ribbon (10 mm) which was removed 3 days after inoculation. In control treatment, the stems were injured to a depth of 6 mm with no inoculation. Canker length was measured every 2 day until the 8th day, and then the measurement was repeated 18, 28, and 58 days after inoculation.

### Effect of temperature on canker development on thin bark hosts

An experiment was conducted in a growth chamber and two temperature regimes were maintained: very hot (40°C) and hot (32°C), to study the effect of pre- and post-inoculation temperature on the disease development. Twelve months old plants for each host were wounded, as mentioned in exp. 1, and exposed to 32°C and 40°C for 8 days (pre-inoculation temperature). The same plants were maintained under the post-inoculation conditions at both temperatures for 3 and 5 days. In control treatment, the stems were injured to a depth of 6 mm with no inoculation. There were five plants per treatment in each of three replications. Differences between the treatments were determined by ANOVA and Duncan's test at P≤ 0.05 with SAS software (SAS, 1999).

## RESULTS

Stem cankers of the infected poplar, eucalyptus and olive saplings in the greenhouse, which were measured every 2 days until the 8th day and repeated again after 18, 28 and 58 days after inoculation (the measurement results are presented in Fig. 1), revealed that the occurrence of cankers and their subsequent development strongly depended on the longevity of fungal inoculum because the length of these cankers increased after 48 hrs and continued to increase; the cankers reached the highest length (15.58 mm) after 58 days from inoculation.

Conspicuous cankers were characterized by bark discoloration and cracks and they exposed black masses of fungal arthrospores. The inoculated saplings failed to callus around the wounded sites; parts of inner bark were removed, resulting in the collapse of the infected tissues after 2-4 days of inoculation.

Cankers 17.00 and 15.10 mm in length developed after 28 days and they extended to 18.53 and 16.11 mm 58 days after inoculation of both susceptible hosts, eucalyptus and poplar (Table 1). Olive bark did not develop significant cankers after 8 days and no differences were found in the canker area even after 58 days. However, the first evidence of cankers was detected on the bark tissues of eucalyptus and poplar after 48 hrs of inoculation, with canker length of 11.06 mm and 11.05 mm, respectively. Therefore, necrosis caused by Neoscytalidium might be limited within wounded tissues of such tolerant hosts as olive, since cankers developed to a length of only 11.40 mm, compared with 13.60 mm and 14.30 mm on poplar and eucalyptus, respectively (Fig. 2).

Table 1.
Development of cankers on three *Neoscytalidium dimidiatum* hosts in the greenhouse at 25 ± 2 °C

| Host | Average canker length (mm) after | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 days | 4 days | 6 days | 8 days | 18 days | 28 days | 58 days |
| Poplar | 11.05* ij | 11.67 hi | 12.17 gh | 12.83 fg | 13.89 e | 15.06 d | 16.11 c |
| Eucalyptus | 11.06 ij | 12.11 gh | 12.50 gh | 13.36 ef | 15.17 d | 17.00 b | 18.53 a |
| Olive | 10.00 k | 10.17 k | 10.33 jk | 11.83 gh | 12.11 gh | 12.22 gh | 12.94 fg |

* Means followed by different letters are significantly different based on Duncan's multiple range test (P=0.05). Control treatments consist of 6 mm wounding courts for each host.

Table 2.
Development of cankers on three *Neoscytalidiium dimidiatum* hosts maintained at two temperature regimes before and after inoculation

| Hosts | Temperature regime (°C) | Average canker length (mm) | | |
|---|---|---|---|---|
| | | 8 days before inoculation** | 3 days after inoculation | 5 days after inoculation |
| Poplar | 32 | 12.61* d-f | 13.11 de | 13.72 d |
| | 40 | 12.45 d-f | 13 de | 13.89 d |
| Eucalyptus | 32 | 16.06 c | 17.20 a-c | 18.06 ab |
| | 40 | 16.44 bc | 17.56 a-c | 18.58 a |
| Olive | 32 | 10.81 f | 12 d-f | 12.17 d-f |
| | 40 | 11.42 ef | 12.03 d-f | 12.61 d-f |

* Means followed by different letters are significantly different based on Duncan's Multiple Range test (P=0.05)
** Numbers are the results of control (wounded tissues with no inoculation)

The effect of temperature regimes before and after inoculation on the development of cankers (Table 2) shows that the wounded control saplings, to which sterile agar discs were applied, did not develop diagnostic cankers in any of the inoculation regimes. Among the pre-inoculation regimes, hot and very hot temperatures were the most conducive to infection of eucalyptus saplings. Eucalyptus cankers developed under very hot temperature and reached a length of 17.56 mm and 18.58 mm after 3 and 5 days of inoculation, respectively.

The heat stress conditions at 32°C and 40°C were favourable and conducive to canker development on eucalyptus compared to poplar and olive (Fig. 3). The duration of wounding and fungal invasion in the inoculated sites sustained canker development particularly, on the most susceptible eucalyptus plants (Fig. 4).

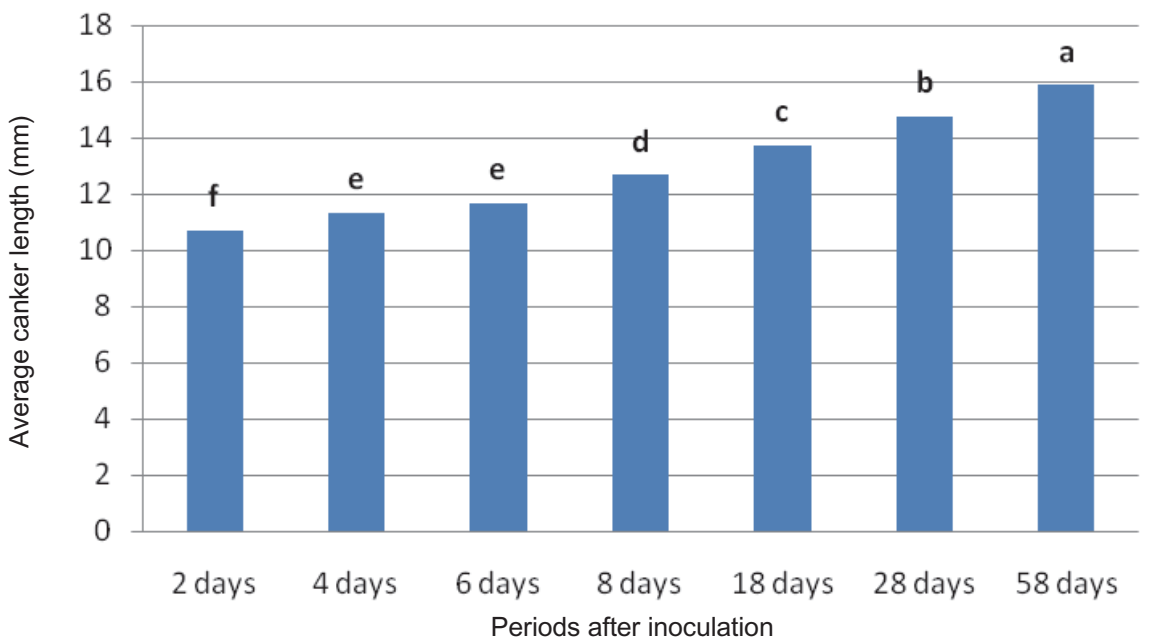


Fig. 1. The effect of inoculation longevity on the length of canker caused by *N. dimidiatum* in the greenhouse at 25 ± 2°C

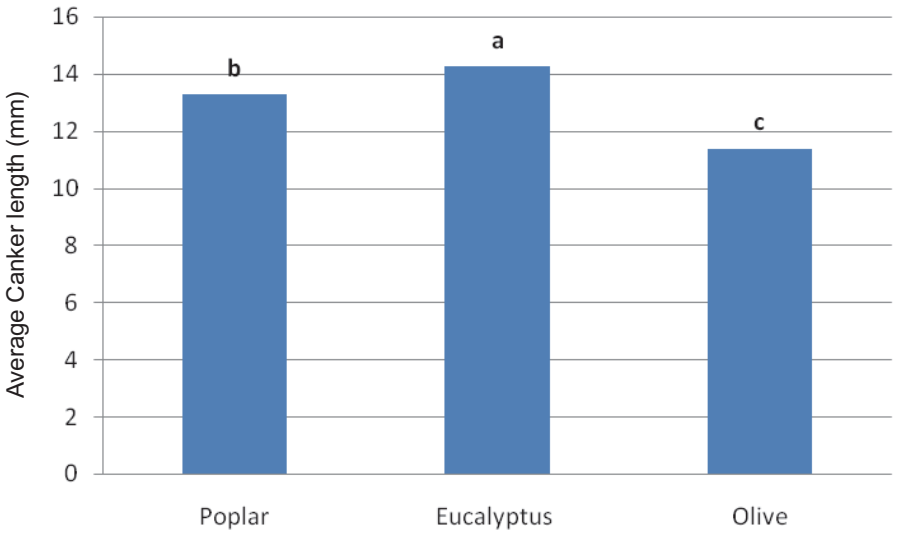


Fig. 2. Canker length on hosts after infection by *N. dimidiatum* in the greenhouse at 25 ± 2 ˚C

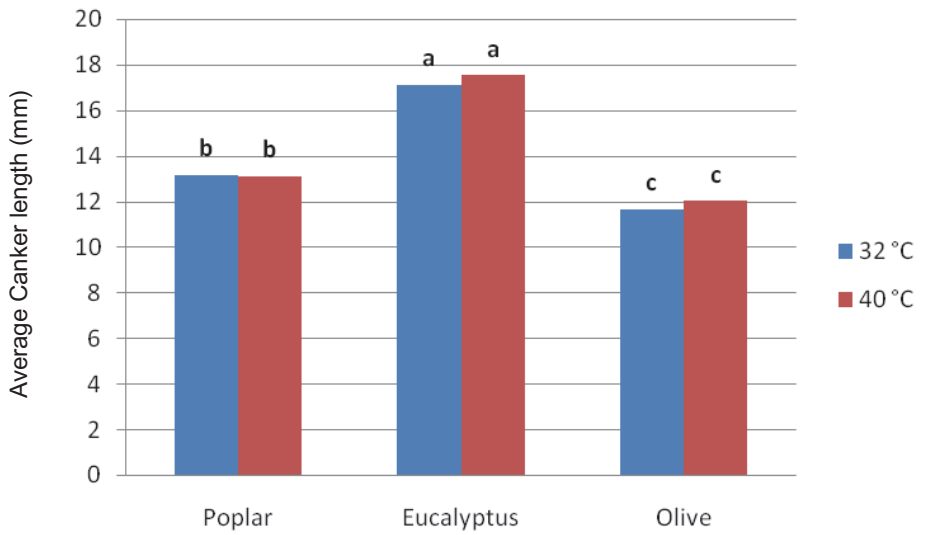


Fig. 3. The effect of temperature on the length of *N. dimidiatum* canker in three different hosts at 32°C and 40°C

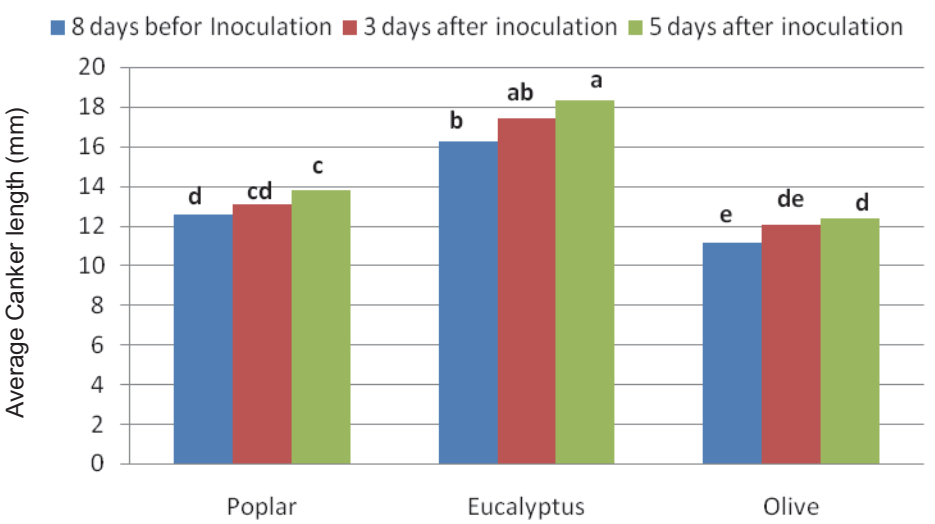


Fig. 4. The development of canker before and after inoculation with *N. dimidiatum* at 32°C and 40°C

## DISCUSSION

It is common that plant diseases are greatly influenced by both biotic factors and environmental conditions. Under the hot and relatively dry climate of Iraq, research on specific effects of elevated temperature on plant diseases has become more interesting. Sadowsky et al. (2007) reviewed the predisposing effect of 25, 32 and 40°C on Neoscytalidium wilt of grapefruit and confirmed a hypothesis that predisposition induced by extremely hot temperatures is pre-requisite for infection of susceptible hosts by *N. lignicola.*

Sooty canker (the most common name of the disease caused by *N. dimidiatum*) increased during June and July on thin or smooth bark trees of poplar and eucalyptus as well as other fruit or ornamental trees when temperature reached a very hot regime (42°C) in Iraq, particularly on non-shaded trunks or limbs that face the sun (Hassan et al. 2009). Bressette (1995) reported that cracking and collapse of inoculation sites become conspicuous before fungal invasion, as well as effects of sunburn that prevented callus growth.

Results of the present work demonstrated that wounded and inoculated saplings of susceptible plant species of eucalyptus and poplar exposed to very hot and hot regimes are invaded by *N. dimidiatum*. This finding supports the hypothesis based on field observations that Neoscaytalidium cankers develop on forest trees as a consequence of a predisposition induced by heat stress. In Iraq events of extreme heat are accompanied by very low humidity, which exposes the trees to extreme transpiration. Therefore, the foliage of these trees usually turns yellow or blighted, particularly on saplings and trees grown in poor soil. We found a similar appearance in saplings after a week's exposure to the very hot regime. Oren et al. (2001) showed that the damage to uninfected saplings of several hosts, such as grapefruit, was reversible; they regained their normal foliage 2-3 months after relief from the heat stress.

On the other hand, fungal spores enter the plant mainly through wounds and disseminate in the field under a temperature of 40-44°C that stimulates germination, growth and reproduction (Mc Gough et al. 1993). Therefore, reports reviewed by Sadowsky et al. (2007) about parasitism of Neoscytalidium isolates suppose that the fungus is an opportunistic pathogen after it had been exposed to extreme heat. Two rare reports of its pathogenicity to other stressed crops, such as citrus, have been published (Oren et al. 2001). Schoeneweiss (1975) suggested that stress exerts the most pronounced effect predisposing plants to facultative parasites, particularly weak or non-aggressive ones. He concluded that in most cases plants tolerate or adapt to stress without permanent injury in the absence of disease-causing organisms.

Olive saplings were found resistant to both heat stress and *N. dimidiatum* cankers compared with poplar and eucalyptus. This resistance might have been due to phenolic compounds, often implied in plant defense to pathogens and associated with plant host resistance. Zine El Aabidine et al. (2010) distinguished 15 major phenolic compounds by high-performance liquid chromatography (HPLC) analysis, according to their chromatographic and spectral characteristics belonging to five phenolic families (hydroxycinnamic derivatives, flavonoids, verbascoside derivatives, tyrosol derivatives, oleuropein derivatives). The tyrosol and its derivatives were associated with constitutive resistance of olive to diseases.

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## Wpływ podatności spowodowanej przez stres cieplny na rozwój *sooty canker* wywoływanej przez *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers

Streszczenie

Choroba *Sooty canker*, wywoływana przez *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers, Synon. = *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko, była obserwowana na inokulowanych sadzonkach drzew o cienkiej korze (w wieku 12-24 miesięcy) *Eucalyptus camaldulensis*, *Olea europaea* oraz *Populus nigra* w warunkach szklarniowych co dwa dni do dnia 8, a następnie obserwacje powtarzano dnia 18, 28 i 58 po inokulacji. Podatność na powstawanie nekroz na pędzie zależała od czasu trwania ciepłej temperatury oraz obfitości inokulum grzybowego. Porażona kora była odbarwiona i odsłaniała czarną masę artrokonidii grzyba, w szczególności na najbardziej podatnych roślinach eukaliptusa i topoli. Nekrozy osiągały długość 18,53 mm i 16,11 mm odpowiednio na eukaliptusie i topoli po 58 dniach w porównaniu z 10 mm na drzewkach nieokulowanych (w miejscach zranienia) stanowiących obiekt kontrolny.

Wpływ warunków temperatury przed i po inokulacji grzybem *N. dimidiatum* na rozwój nekroz na tych samych roślinach badano w fitotronie w dwóch zakresach temperatury, w temperaturze bardzo gorącej 40ºC i gorącej 32ºC. Jeśli chodzi o temperatury zastosowane przed inokulacją, temperatura bardzo gorąca i gorąca najbardziej sprzyjały porażeniu sadzonek eukaliptusa w porównaniu z innymi roślinami żywicielskimi, które nie wykazywały istotnej zależności, jeśli chodzi o oddziaływanie temperatury przed i po inokulacji. Zatem stres cieplny w zakresie temperatur 32 i 40ºC sprzyjał rozwojowi nekroz na najbardziej podatnej roślinie żywicielskiej, eukaliptusie; nekrozy te osiągnęły długość 17,20-17,56 mm po 3 dniach oraz 18,08-18,06 po 5 dniach po inokulacji.