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The fungus *Nectria aurantiicola* Berk. et Br. (*Fusarium larvarum* Fuckel) a biocontrol agent of the armored scale *Suturaspis archangelskyae* (Lindinger) in Apulia (Southern Italy)*

ABSTRACT

Suturaspis archangelskyae [(Lindinger) 1929] (Homoptera, Diaspididae), an armored scale insect collected in Apulia (Italy) on *Prunus dulcis* (Miller) D. A. Webb (=almond) and *Prunus webbii* (Spach) Vierh., is reported to be infected by *Fusarium larvarum* Fuckel.

The cycle of the fungus, as studied on female lineage of *S. archangelskyae*, shows mycelium in August and September, orange sporodochia from November to March, while mature red perithecia of the teleomorph *Nectria aurantiicola* Berk. et Br. are observed since middle January up to the first half of February. The fungus survives during the hot dry season as mycelium in the dead body of the host.

The fungus is able to kill about 50% of the scales and spreads among demes by conidia carried on crawlers. Both varieties, the creamy var. *larvarum* Fuckel and the carmine-red var. *rubrum* Gerlach, are found on natural infected scales. The male progeny of *S. archangelskyae* seems not to be affected by the fungus.

Ascospores are observed as well, but their role in pathogenesis remains unknown.

Fusarium larvarum seems to be the main "natural" biocontrol agent (sensu Rosen et DeBach, 1990) of *S. archangelskyae* in Apulia.

Key words: semi-arid (mediterranean) habitat, entomopathogens, pathogenic symbiosis, armored scale, coccids, Cheyletidae (Acari preying crawlers), biology, Puglia.

INTRODUCTION

Symbiosis between Diaspididae and fungi may be either mutualistic, as it occurs between armoured scale and *Septobasidium* species (COUCH, 1938; EVANS, 1988), or pathogenic e.g. with many species of fungi (EVANS & PRIOR, 1990). Both occur mainly in tropical and subtropical moist ecosystems because of the environmental needs of the fungi.

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* S. Frisullo identified, isolated and cultured the fungus. All other work was performed by F. Porcelli. A MURST grant is aknowledged.

Several samples of *Suturaspis archangelskyae* [*] (Lindinger) 1929 to study its bionomics were collected on *Prunus webbii* (Spach) Vierh. and *P. dulcis* (Miller) D. A. Webb (=almond). Most of the samples were found plenty of adult females killed by a fungus that was isolated and identified as *Fusarium larvarum* Fuckel (anamorph of *Nectria aurantiicola* Berk. et Br.) (PORCELLI *et al.*, 1995).

Suturaspis archangelskyae is a palaearctic species (KOZÁR et WALTER, 1984) widespread in: Armenia, Turkmenistan, Uzbekistan, Tagikistan, Iran, Iraq (BORCHSENIUS, 1966); in Italy: Sicily sub *Salicicola* 1985 (INSERRA et CALABRETTA, 1985) and Apulia (PORCELLI, 1990). According to BARBAGALLO (*et al.*, 1994) it is an indigenous species.

Several species of *Fusarium* are known as natural enemies of Diaspididae (LOMBARDI, 1937/38; PEREZ, 1972; AOKI, 1974; SALE et FERGUSON, 1975; AGUDELO et FALCON, 1977; GERSON, 1977; GAO et OUYANG, 1981; BARUA, 1983; BLACKBURN et MILLER,1984a; 1984b; DEVNATH, 1987; KOSZTARAB et KOZAR, 1988; WILLIAMS et WATSON, 1988; EVANS et PRIOR, 1990; BAI et CHEN, 1991; FERGUSON et FLETCHER, 1991; DANZIG, 1993; EVANS et HYWEL-JONES, 1997).

However, infections by *F. larvarum* on insects are rarely reported. This species was found by YEN et TSAI (1969, as *Sphaerostilbe aurantiicola*), HSIAO (1978, as *S. aurantiicola*), HORNOK et KOZÁR (1984), HSIAO et HSU, (1985), MORAES et SILVA (1987, as *S. aurantiicola*) and it has been regarded as a natural biocontrol agent.

This paper reports on the association between *S. archangelskyae* and *F. larvarum* because of: the distribution and the regional economic importance (MILLER et DAVIDSON, 1990; DANZIG, l.c.) of the scale and of almond in Southern Italy (Apulia); the preference of the fungus for the Diaspididae; the chance to observe the fungus in a semi-arid (Mediterranean) habitat. The fact that no other adverse organism, but a Cheyletidae (Acari) preying on crawlers, was found in association with *S. archangelskyae* also influenced our choice.

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[*] The specific name *archangelskayae* was preferred in a previous paper (PORCELLI *et al.*, 1995) because the species was named in honour of ARXANGELSKAJ whose traslitteration is Archangelskaya leading to the latinization *archangelskaiae*. The latter name was also used by BALACHOWSKY (1951, sub *Salicicola*) but not followed by BORCHSENIUS (1966) in his catalog. KOZÁR (1990) used *archangelskayae* (sub *Suturaspis*). However, the latest paper concerning systematic and taxonomic questions is that by Borchsenius so according to him and in order to avoid confusion, I suggest to consider the name *archangelskayae,* I used previously, as a misspelling.
Moreover DANZIG (1993) refers this species as *Salicicola archangelskyae* (Archangelskaya, 1930).

MATERIALS AND METHODS

Bark samples of *P. webbii* and *P. dulcis* trees infested by diaspididae were collected in the "Castel del Monte" area near Andria (Bari, Apulia, Southern Italy) twice a week or weekly for three years (1990-1992). The most frequent sampling was done from late summer to late spring. Furthermore, scattered collections were carried out in all Apulia to get data about the distribution of infected scales.

The percentage of infection was estimated by removing the white wax secretion and looking at the second instar females, whereas the adult females were recovered from the hard second stage exuvia.

From five to forty-three females of *S. archangelskyae* per 10 cm^2 of bark were examined using stereomicroscope, either directly or after mounting in distilled water or Essig's (1948) fluid.

Infected second instars and adult females of the armored scale were screened from the healty ones on the basis of their appearance. Noninfected, living scales are fusiform, pale violet and sligthtly dorsoventrally flattened; the uninfected dead scales of the previous broods appear as semitransparent thin cuticula usually located inside the exuvia near the cephalic end. Infected scales of the current brood show white and waxy thick bodies, shaped like healthy females. Once dead, the infected individuals of the previous broods appear yellowish brittle mummies.

To identify the scale insect, specimens were mounted according to Wilkey (1990); the conidia-bearing crawlers were mounted in lactophenol plus cotton blue or in Essig's fluid plus double stain (Wilkey, l.c.).

Photomicrographies was obtained by means of a Zeiss Phomi III, equipped with bright field and phase contrast.

To study the spread of the fungus, crawlers and Cheyletidae (Acari), predators on *S. archangelskyae,* were collected and plated in Petri dishes on sterile Potato Sucrose Agar (PSA). The plates were incubated at 22°C in the dark.

The fungus was isolated from: living and dead infected adult females, previously washed in sodium hypocloride (4%) in water; sporodochia collected from naturally infected scales in the field and from living first instar crawlers.

Single ascospores were obtained from five perithecia. They were cultured either on APS plates at 22°C in the dark or on water agar+carnation leaves, at 15°C in the dark.

All observations refer to the female progeny of *S. archangelskyae* because no males were infected by *F. larvarum*.

RESULTS

Suturaspis archangelskyae was found in almost all bark collections of *P. webbii* and *P. dulcis* in Apulia and very often in association with *F. larvarum* (fig. 1; 2: a).

Crawlers (L1) were born mostly in May and were found in crevices under partially split bark. They were neither infected nor killed by the fungus. However about 15% of crawlers showed conidia *F. larvarum* laying on their cuticle (fig. 2: b, c). About 40% of plated crawlers originated colonies of *F. larvarum*. Plated Cheyletids did not show any fungus.

The second instar females (L2) were found in June under the bark, in clefts or galleries of woodbores. They were infected by the fungus in a low percentage (8%) before metamorphosis. Few L2 scales survived the Winter and will be found killed by the fungus in the following Spring while others died soon.

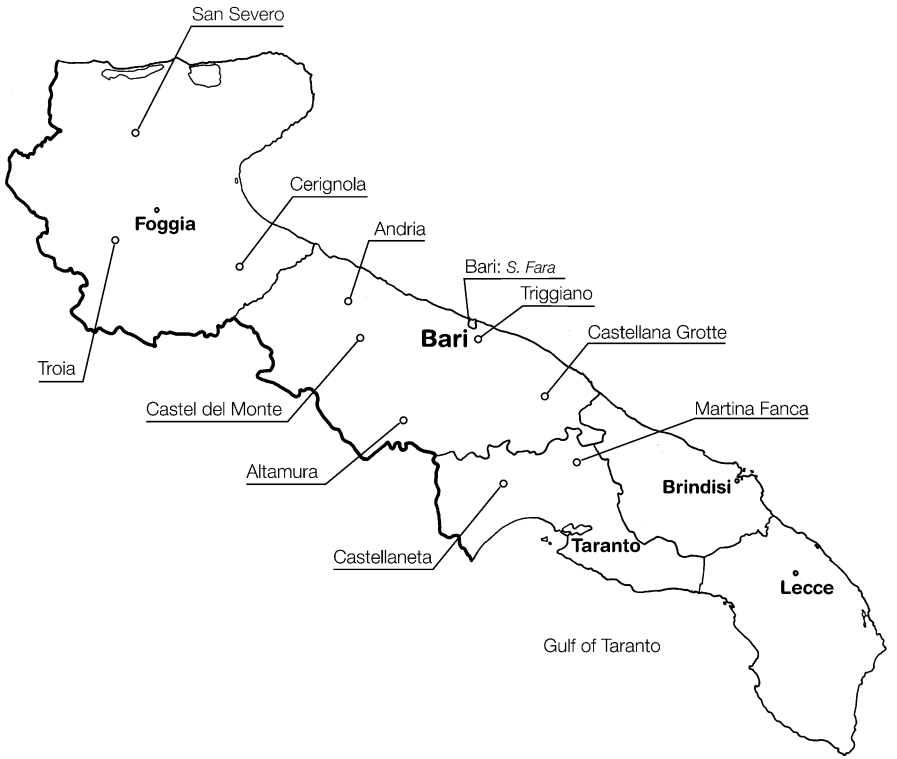

Fig. 1 - Collection localities (white dots) in Apulia of *Suturaspis archangelskyae* and of its pathogen *Fusarium larvarum*.

Usually about 30% of the individuals of a deme were infected. It was possible to recognise, infected L2 and adult females of the previous years, which are very common.

The infection by *F. larvarum* turns the scale body into a waxy mummy completely colonizing the body tissues with hyphae. Neither sporodochia nor perithecia of the fungus were found on L2 females.

Living, apparently uninfected, L2 scales were observed moulting in late June by thickening the body wall into a hard wall. Sometimes it was possible to detect hyphae growing very close to the body of the scales, but the species of the fungus was not identified.

The first adult females were found in July; the fungus enters the body of the females, invading the tissues with its hyphae. The infected female body becomes a waxy mummy that differs from that of L2 because of the general shape and the presence of the hard exuvia.

The percentage of infection changed greatly from one deme to another: some groups of scales were completely killed by the fungus, while others escaped the infection.

Sporodochia were formed on infected dead adult females from September to March.

Sporodochia are bean-shaped, creamy-orange, sticking out of the body of the adult scale at the level of the vulva. Sporodochia are less than a 1/4 millimeter large and are produced by hyphae passing through the pygidial margin split in the exuvia (fig. 2: a).

Conidia (macroconidia) of *F. larvarum* var. *larvarum* arise laterally on hyphae of the mycelium as simple phialides (fig. 2: d).The conidia (fig. 2: e) are allantoid, falcate or sublunate, quite uniform in size. Most conidia are 1 to 5 septate, 16 to 30 µm long, 3 to 4 µm thick, depending on the number of septa.

Perithecia (fig. 2: f-white arrows) formed on killed adult females, were observed in January and February: they arise from a small stroma (fig. 2: f-slender arrow) exiting the exuvia (fig. 2: f-bordered arrow) by pygidial margin split. Perithecia are 200 x 350 µm in diameter, globose or pyriform, red-orange, with a smooth outer wall. The cylindrical asci bearing a rounded apex are 70-100 µm long and 6,5-9 µm thick. Ascospores 12-15 x 5.5-6.5 µm (fig. 2: g), are broadly fusoid, hyaline and smooth, with a single transvere septum.

In culture ascosporae (fig. 2: h, i) produced colonies of *F. larvarum* var. *larvarum* only, whereas conidia produced colonies of both *F. larvarum* var. *larvarum* and *F. larvarum* var. *rubrum*.

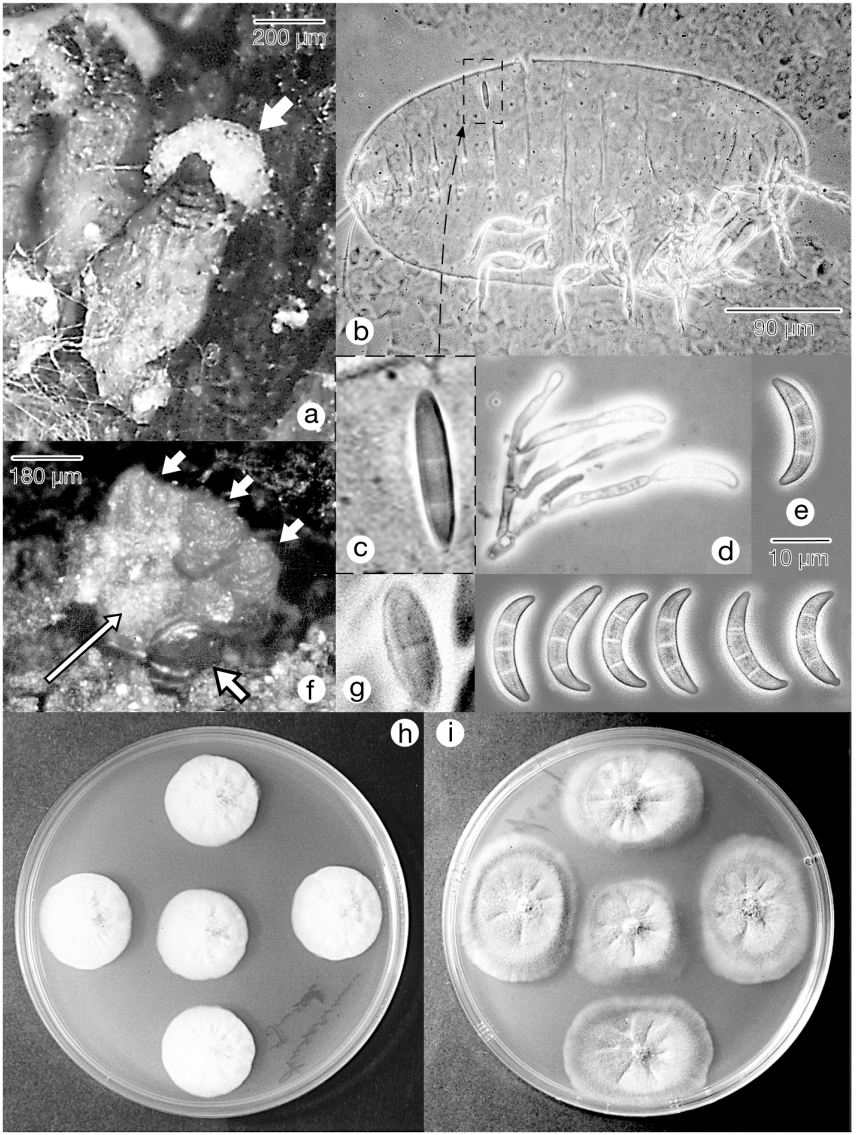

Fig. 2 - Aspects of entomopathogeny on *Suturaspis archangelskyae* and *Fusarium larvarum*: a: adult female of *S. archangelskyae* killed by *F. larvarum*, bearing a sporodochium (arrow); b, c: a crawler of *S. archangelskyae* bearing a conidium of *F. larvarum*; d: conidiophores and (e) conidia of *F. larvarum*; f: perithecia (white arrows) arising from a small stroma (slender arrow) on infected dead adult female of *S. archangelskyae* (bordered arrow); g: ascospores; h: a plate with colonies of *F. larvarum* var. *larvarum*; i: a plate with colonies of *F. larvarum* var. *rubrum*.

DISCUSSIONS

In Southern Italy the armored scale *S. archangelskyae* is well controlled on *P. webbii* and *P. dulcis* by *F. larvarum*. The latter appears to be the main "natural" biological control agent (sensu ROSEN et DEBACH, 1990).

The three years detection of infections on L2 and adult females indicates that the fungus is active in about the same way over the years. The persistence of the fungus in the area here considered, as demostrated by the remainders of the past infections, shows that the pathogen is well estabilished.

Spreading of *F. larvarum* is assured by crawlers, and this way of dissemination shows some degree of specificity; in fact Cheyletidae, collected in almost all samples and close to the armored scale, do not carry the fungus.

Fusarium larvarum seems to skip the first and the bulk of the second stage of the host and to infect and to kill mainly adult females. This was demonstrated by direct observation and may be explained by accepting that the fungus waits until its host is large enough to provide food for the production of conidia. Another hypothesis is that conidia remain embedded in the waxy secretion of the armor, waiting until its host is large enough to start the infection; this is summarized in fig. 3.

Usually entomopathogenic fungi are common in hot, humid areas (EVANS et PRIOR, l.c.; ROSEN, 1990). However *F. larvarum* prefers low temperatures to grow and to produce both sporodochia and perithecia. A moist and sometimes a wet microhabitat is guaranteed to the fungus by the particular location of *S. archangelskyae* in crevices of the bark. Moreover the fungus spends the hot season within the infected scales.

Difficulties experimented in inoculing the same host (KUNO et FERRER, 1973) could be a consequence of neglecting the stage of the scale, the microclimate, or the susceptibility of the scales (FERRARI, 1996)*.

CONCLUSIONS

Suturaspis archangelskyae on *P. webbii* and *P. dulcis* is controlled mainly by *F. larvarum,* which is the only natural enemy of the adult female of the scale observed in Apulia. The fungus is a perennial control factor of the scale insect population.

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* Prova di controllo biologico per incremento contro *Melanaspis inopinata* (Leonardi) con *Fusarium larvarum* (Fuckel). Dipl. Thesis: Università degli studi di Bari, 46 pp.

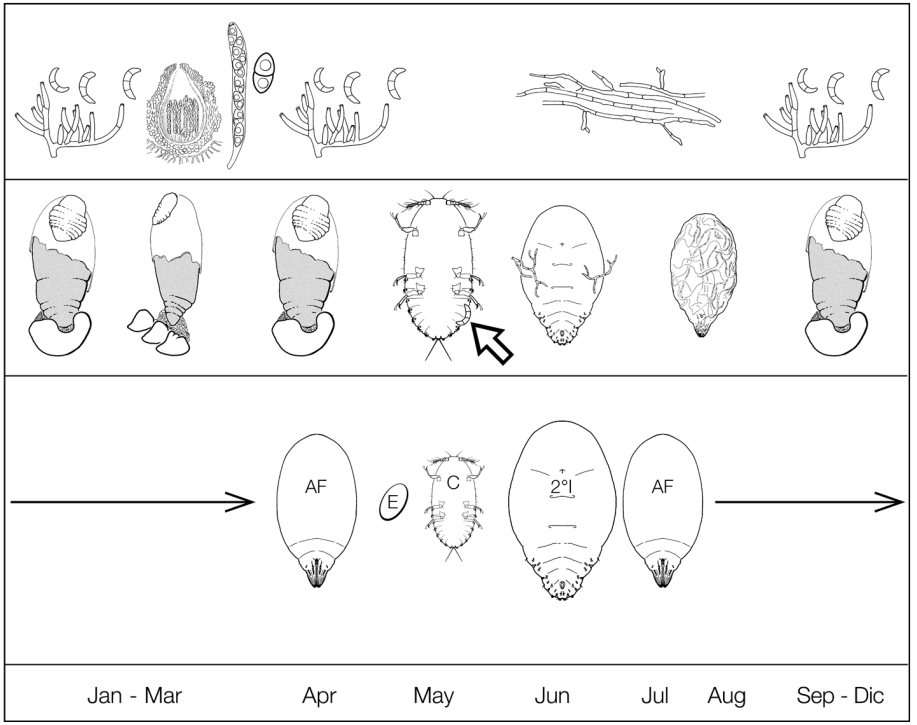

Fig. 3 - Schematic view of: biology of the female lineage of *Suturaspis archangelskyae*, bottom row; pathogenic symbiosis between *S. archangelskyae* and *Fusarium larvarum*, middle row and cycle of *Fusarium larvarum* upper row. Arrow = a conidium on a crawler.

Fusarium larvarum is spread by conidia-carrying crawlers, while the role of ascospores is unknown. Infected crawlers have a chance to moult into second instar larvae up to adult females, but the fungus kills them before they reproduce.

The use of this fungus by inoculation or increment in a biological control program could be possible if key (ecological) factors were studied in depth. *F. larvarum* prefers low temperatures and a moderately moist environment in contrast with the majority of entomopathogenic fungi. This behaviour makes it suitable for use in temperate areas of the world.

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RIASSUNTO

Nectria aurantiicola Berk. et Br. (*Fusarium larvarum* Fuckel) antagonista di *Suturaspis archangelskyae* (Lindinger) in Puglia (Italia meridionale)

Gli autori descrivono la simbiosi fra un fungo entomopatogeno, *Fusarium larvarum* Fuckel (teleomorfo *Nectria aurantiicola* Berk. et Br.), ed il diaspino *Suturaspis archangelskyae* (Lindinger, 1929) su *Prunus dulcis* (Miller) D. A. Webb e *P. webbii* (Spach) Vierh. presso "Castel del Monte" (Andria, BA) ed in numerose località della Puglia.

Sono state osservate le modalità di disseminazione del fungo ed il procedere dell'infezione, che culmina con l'uccisione delle cocciniglie prima che queste possano riprodursi. Durante i mesi invernali il fungo produce sporodochi reniformi di colore arancio, ricchi di conidi falciformi, e periteci globoso-piriformi di colore rosso arancio, contenenti le ascospore.

Il fungo risulta essere il principale fattore biotico limitante del diaspino del quale uccide le femmine adulte. Esso sembra suscettibile di utilizzo come agente di controllo biologico per inoculazione od incremento, sempre che vengano assicurati a fondo i fattori, ecologici che regolano l'infezione.

Aspetto favorevole all'uso di *F. larvarum* nelle regioni temperate è la sua predilezione per le temperature moderate. Non è stato possibile chiarire il ruolo delle ascospore nella diffusione del fungo.

Parole chiave: mandorlo, habitat semi-aridi (mediterranei), coccoidei, Cheyletidae (Acari predatori di neanidi).

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