Possibilities for biocontrol of the onion thrips *Thrips tabaci* Lindeman (Thys., Thripidae) using different entomopathogenic fungi from Thailand

Malee Thungrabeab, Peter Blaeser & Cetin Sengonca

Institute of Phytopathology, University of Bonn, Germany

Zusammenfassung: Möglichkeiten zur biologischen Bekämpfung des Zwiebelthrips *Thrips tabaci* LINDEMAN (Thys., Thripidae) durch verschiedene, entomopathogene Pilze aus Thailand

Thrips tabaci LINDEMAN (Thys., Thripidae) ist ein weltweit verbreiteter Schädling mit einem breiten Wirtspflanzenspektrum an verschiedenen Freiland und Gewächshauskulturen, dessen Bekämpfung durch die geringe Größe und ihre versteckte Lebensweise erschwert wird. Entomopathogene Pilze sind wichtige Krankheitserreger zahlreicher Insektenarten und infizieren Individuen verschiedenen Alters und verschiedener Entwicklungsstufen. In der vorliegenden Studie wurden 41 Isolate von 25 entomopathogenen Pilzarten aus 11 Gattungen (*Akanthomyces*, *Aschersonia*, *Beauveria*, *Cordyceps*, *Hirsutella*, *Hymenostilbe*, *Hypocrella*, *Metarhizium*, *Paecilomyces*, *Torrubiella* und *Verticillium*) auf ihre Pathogenität gegenüber *T. tabaci*-Larven untersucht. Bis auf *Hymenostilbe* sp., *Torrubiella* spp., *Hypocrella discoidea* und *Cordyceps pseudomilitaris* stellten sich alle Pilze als Pathogen für *T. tabaci* heraus. Zwischen den Isolaten wurden hoch signifikante Unterschiede festgestellt. Die Mortalität variierte je nach Pilzart, Pilzgattung und Isolat. Fünfzehn Isolate, 7 von *Beauveria*, 4 von *Metarhizium*, 3 von *Paecilomyces* und 1 von *Cordyceps*, wiesen die höchste Pathogenität gegenüber *T. tabaci*-Larven auf.

Key words: Entomopathogenic fungi, *Thrips tabaci*, onion thrips, pathogenicity, *Beauveria*, *Metarhizium*, *Paecilomyces*, *Cordyceps*

M.Sc. Agr. M. Thungrabeab, Dr. P. Blaeser & Prof. Dr. Dr. h.c. C. Sengonca, Dept. of Entomology and Plant Protection, Institute of Phytopathology, University of Bonn, Nussallee 9, D-53115 Bonn, Germany, E-Mail: C.Sengonca@uni-bonn.de.

The onion thrips, *Thrips tabaci* LINDEMAN (Thys., Thripidae) is an important pest of field and greenhouse crops around the world. It causes damage directly through feeding and indirectly through the transmission of lethal plant viruses. It is difficult to control this pest with insecticides because of its small size and cryptic habits (LEWIS 1997).

Entomopathogenic fungi are currently being investigated for the control of many important insect pests on various crops around the world, and some are commercially available. There are many studies on the efficacy of several entomopathogenic fungi on thrips. CARL (1975) reported that *Neozygites parvispora* (MACLEOD, TYRRELL & CARL) REMAUDIERE and KELLER has been found frequently on *T. tabaci* but under field conditions the fungus was less successful than in the greenhouse. In addition, *Entomophthora thripidum* SAMSON, RAMAKERS & OSWALD was found infecting *T. tabaci* in Netherlands, but in field trials the fungus failed to suppress thrips populations below the economic acceptable level (SAMSON et al. 1979). In laboratory studies, *T. tabaci* was susceptible to *Verticillium lecanii* (ZIMMERMANN) VIÉGAS, *Beauveria bassiana* (BALSAMO) VUILLEMIN, *Metarhizium anisopliae* (METSCH.) SOROKIN and *Paecilomyces fumosoroseus* (WIZE) BROWN & SMITH (GILLESPIE 1986, FRANSEN 1990). VESTERGAARD et al. (1995) and BROWNBRIDGE (1995) showed that *B. bassiana*, *M. anisopliae* and *V. lecanii* were more active against the western flower thrips, *Frankliniella occidentalis* (PERGANDE) than *P. fumosoroseus* or *Paecilomyces farinosus* (HOLM ex S.F. GRAY) BROWN & SMITH. HALL et al. (1994) and SAITO (1991) suggested that *Hirsutella* sp., *P. fumosoroseus* and *B. bassiana* may be useful in the management of the melon thrips, *Thrips palmi* KARNY. EKESI et al. (1998) stated that *B. bassiana* and *M. anisopliae* are highly pathogenic to the legume flower thrips, *Megalurothrips sjostedti* (TRYBORN). In the glasshouse, *V. lecanii* has been used successfully to control *T. tabaci* on cucumber (GILLESPIE 1986).VACANTE et al. (1994) noted that *N. parvispora* caused up to 60 per cent mortality in motile developmental stages of *F. occidentalis* and reduced the insect population

density. Under greenhouse conditions, *M. anisopliae* was found to be effective in reducing the population growth of *F. occidentalis* on cucumber (AZAIZEH et al. 2002). Whereas, MANIANIA et al. (2001) observed that *M. anisopliae* had the potential to control *F. occidentalis* on chrysanthemum. The studies of MANIANIA et al. (2003) indicated that *M. anisopliae* had a potential to control *T. tabaci* in the field. *Akanthomyces*, *Aschersonia*, *Cordyceps*, *Hypocrella*, *Hynennostilbe* and *Torrubiella* were never observed to control thrips.

Therefore, this study was conducted to investigate the pathogenicity of different entomopathogenic fungi from Thailand against *T. tabaci* larvae under controlled climatic conditions in the laboratory.

Tab. 1: List of entomopathogenic fungi tested to assess pathogenicity.

Species: Isolate number	Original host	Location
Akanthomyces sp.: AK.3497	Hemiptera - Pentotomidae	Ranong
Akanthomyces sp.: AK.3582	Hemiptera - Pentotomidae	Ranong
Aschersonia badia: Ab.917	Homoptera - Coccidae	Nakhon Ratchasima
Aschersonia samoensis: As.4335	Homoptera - Coccidae	Kanchanaburi
Aschersonia samoensis: As.4593	Homoptera - Coccidae	Chanthaburi
Aschersonia tamurai: At.5673	Homoptera - Coccidae	Phetchabun
Aschersonia tamurai: At 6373	Homoptera - Coccidae	Chanthaburi
Beauveria bassiana: Bb.4591	Coleoptera - Curculionidae	Chanthaburi
Beauveria bassiana: Bb.5082	Hymenoptera - bee	Phetchabun
Beauveria bassiana: Bb.5335	Hymenoptera - ant	Phetchaburi
Beauveria bassiana: Bb.6243	Homoptera - cicada	Nakhon Ratchasima
Beauveria bassiana: Bb.7772	Host unknown	Chiangmai
Beauveria sp.:B.6739	Underside of dicotyledonous leaf	Suratthani
Beauveria sp.:B.6988	Host unknown	Suratthani
Beauveria sp.:B.7683	Host unknown	Tak
Cordyceps pseudomilitaris: Cp.951	Lepidoptera/larva	Saraburi
Cordyceps sp.: CO.5598	Lepidoptera - Limacodae-pupa	Chiangmai
Hirsutella citriformis: Hic.7679	Host unknown	Tak
Hirsutella formicarum: Hif.7731	Host unknown	Narathiwat
Hypocrella discoidea: Hd.4385	Homoptera - scale insect	Kanchanaburi
Hymenostilbe sp.: HY.1294	Isoptera-termite	Nakhon Ratchasima
Metarhizium anisopliae: Ma.5035	Homoptera - Cicadellidae	Phetchabun
Metarhizium anisopliae: Ma.6098	Homoptera	Ranong
Metarhizium flavoviride: Ma.6171	Leaf litter	Nakhon Ratchasima
Metarhizium flavoviride: Mf.5744	Hemiptera	Kamphaengphet
Metarhizium flavoviride: Mf.1164	Soil	Lampang
Metarhizium sp.: M.6079	Homoptera	Ranong
Metarhizium sp.: M.7527	Host unknown	Prajeanburi
Metarhizium sp.: M.7965	Host unknown	Nakhon Ratchasima
Paecilomyces farinosus: Pfa.3517	Araneae - spider	Ranong
Paecilomyces fumosoroseus: Pfu.5338	Bupressidae/leaf litter	Phetchaburi
Paecilomyces fumosoroseus: Pfu.2507	Soil	Lampang
Paecilomyces javanicus: Pj.5870	Araneae - spider	Trat
Paecilomyces lilacinus: Pl.5066	Hemiptera - Cydnidae/leaf litter	Phetchabun
Paecilomyces tenuipes: Pt.6718	Lepidoptera - larva	Nakhon Ratchasima
Paecilomyces tenuipes: Pt.7646	Host unknown	Suratthani
Torrubiella petchii: Tp.6200	Homoptera - scale insect	Phetchaburi
Torrubiella tenuis: Tt.345	Homoptera - scale insect	Nakhon Ratchasima
Verticillium hemipterigenum: Vh.6076	Homoptera	Ranong
Verticillium lecanii: Vl.3087	Homoptera - scale insect	Nakhon Ratchasima
Verticillium lecanii: Vl.2321	Host unknown	Lampang

Materials and Methods

The rearing of *T. tabaci* and the laboratory experiments were carried out under the same climatic conditions of 25±1°C, 60±10% RH and 16:8 h (L:D) photoperiod.

The stock culture of *T. tabaci* was maintained on leek plants in a rearing room. The uniformly aged *T. tabaci* individuals were obtained by using rounded plastic cages, 5.5cm in diameter, and with a meshed hole in the lid to allow air exchange. The rounded plastic cages were filled with a 0.5cm-thick-layer of 0.7% water agar. Freshly excised leek leaf discs 4.5cm in diameter were placed upside down onto the water agar. Ten adult *T. tabaci* females were picked up from the stock culture and placed on the leek leaf discs for 24 hours in each rounded plastic cage for egg laying. The rounded plastic cages were kept in climatically controlled chambers. The females were gently transferred to new similarly prepared cages. The eggs obtained in the old cages were reared further until the thrips reached the age desired for the experiments.

Forty-one isolates of different entomopathogenic fungi from different insect hosts and several locations in Thailand were used (Tab. 1).

The fungi were cultured on malt peptone agar and were incubated at continuous light at 25±1°C. The conidia were harvested from 1 to 3- week-old surface cultures by flooding the plates with sterile 0.1% Tween 80 in water. The concentration of conidia was determined with an improved haemocytometer and adjusted to a concentration of $1x10^8$ conidia/ml with sterile 0.05% Tween 80 in water. Each replication was performed by spraying 1 ml of the conidia suspension onto the first larval instars of thrips using the sprayer (Eco spray; Labo chimic France). Control was sprayed only with 0.05%Tween 80 in water. The round plastic cages were sealed with transparent tape and kept in climatically controlled chambers. The mortality caused by the fungi was confirmed by microscopic examination of hyphae and spores on the surface of the thrips larvae and was recorded daily for 7 days. Each isolate was replicated three times with 30 thrips individuals per replicate, and the assay was normally repeated twice for each isolate.

Percentages of mortality were transformed by arcsine square root to normalize the mean percentages (GOMEZ & GOMEZ 1984) after correcting for natural mortality (ABBOTT 1925) and subjected to analysis of variance appropriate for a completely randomized design. The percentages of mortality were combined with normal distribution curve to classify pathogenicity level.

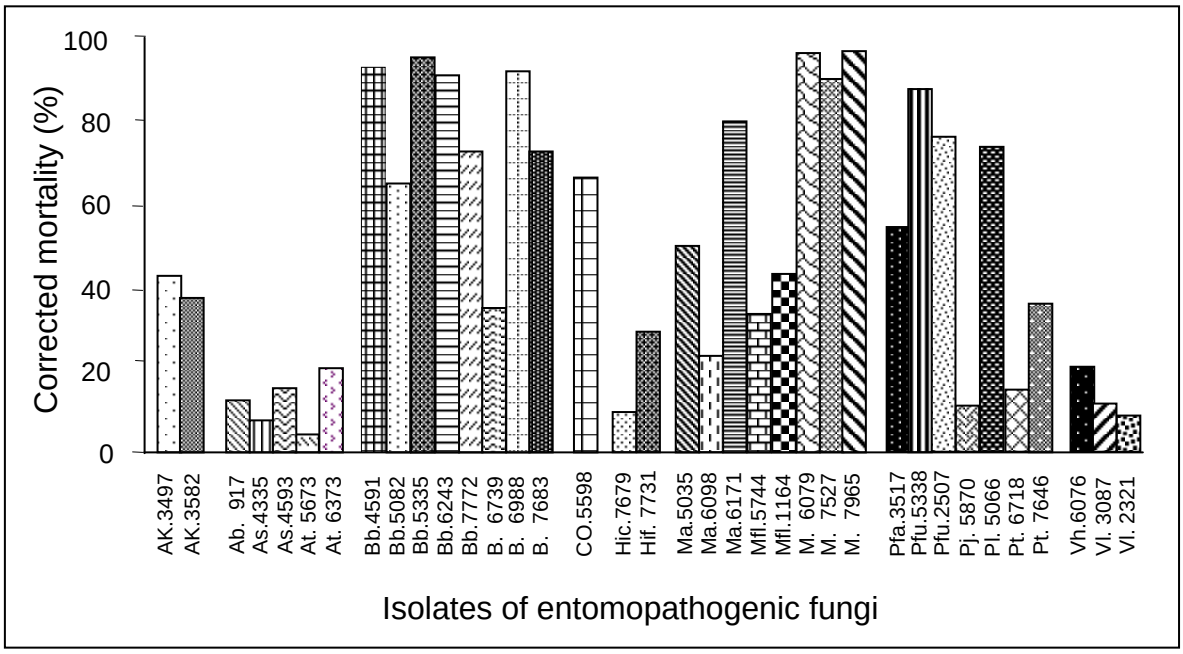

Fig. 1: Percentage corrected mortality of *Thrips tabaci* larvae caused by different entomopathogenic fungi at 1x108 spores/ml after 7 days and 25°C

Results

The results of the bioassay showed that 36 out of 41 isolates were pathogenic to *T. tabaci*. *Hymenostilbe* sp., *Torrubiella* spp., *Hypocrella discoidea* and *Cordyceps pseudomilitaris* were found to be non-pathogenic. There were greatly differences among the isolates. All isolates caused mortalities that differed from those in the control. The control mortality in 7 days was 4.5 %. In addition, different levels of pathogenicity were detected in the bioassay within genera and species as well as isolates (Fig. 1).

For example, mortalities caused by *Metarhizium* spp. ranged from 23.5% to 97.3%, in which *Metarhizium* sp. isolates were more pathogenic than *M. anisopliae*. *M. anisopliae* isolates were also more pathogenic than *Metarhizium flavoviride* GAMS & ROZSYPAL, whereas *Beauveria* spp. caused 35.1% to 95.5%. Among the five isolates tested, *B. bassiana* recorded the highest and the lowest mortality with 95.5% and 65%, respectively. In addition, mortalities caused by *Paecilomyces* varied greatly among fungal species and isolates and ranged from 11.1 to 87.7%.

The entomopathogenic fungi used in this study can be classified into three classes. In class 1 the mortality rate is greater than 64.49%, while in class 2 the mortality is between 64.49 to 30.99% and in class 3 less than 30.99% (Tab. 2). Class 1 consists of 15 highly pathogenicity isolates, which were identified as virulent and will be used in further experiments for management of *T. tabaci*.

Tab. 2: Pathogenicity level of different entomopathogenic fungi against *Thrips tabaci* larvae

Pathogenicity level	Mortality (%)	Isolates
Highly pathogenicity	>64.49	M.7965, M.6079, Bb.5335, Bb.4591, B.6988, Bb.6243, M.7527, Pfu.5338, Ma.6171, Pfu.2507, Pl.5066, B.7683, Bb.7772, CO.5598, Bb. 5082
Moderately pathogenicity	64.49–30.99	Pfa.3517, Ma.5035, Mfl.1164, AK.3497, AK.3582, Pt.7646, B.6739, Mfl.5744
Low pathogenicity	<30.99	Hif.7731, Ma.6098, At.6373, Vh.6076, At.5673, Pt.6718, Ab. 917, Vl.3087, Pj.5870, Hic.7679, Vl.2321, As.4335, As.4593

Discussion

The use of entomopathogenic fungi for biocontrol of thrips has long been recognized. However, the success in the use entomopathogenic fungi largely depends on the selection of highly virulent isolates. The results of the bioassays indicated that all isolates tested were found to be pathogenic to *T. tabaci*, except *Hymenostilbe* sp., *Torrubiella* spp., *Hypocrella discoidea* and *C. pseudomilitaris*. This is the first study, which stated that *Akanthomyces*, *Aschersonia* and *Cordyceps* are pathogenic to thrips.

Beauveria, *Metarhizium* and *Paecilomyces* were more pathogenic than isolates from other genera of entomopathogenic fungi. Similarly, GILLESPIE (1986) and FRANSEN (1990) reported that in laboratory studies, *T. tabaci* was susceptible to *M. anisopliae*, *B. bassiana*, *P. fumosoroseus* and *V. lecanii*. EKESI et al. (1998) screened 22 strains of entomopathogenic fungi against *M. sjostedti* and found two strains of *B. bassiana* and four strains of *M. anisopliae* are highly pathogenic to *M. sjostedti*. VESTERGAARD et al. (1995) identified strains of *M. anisopliae*, which were more pathogenic than any of the *V. lecanii* strains assayed on *F. occidentalis*. Wheras, AZAIZEH et al. (2002) found that *M. anisopliae* was able in reducing the population growth of *F. occidentalis* on cucumber. *Metarhizium*, *Beauveria* and *Peacilomyces* are the best known fungi with respect to their wide geographical distribution, broad host range as well as their great potential as microbial control agents (HALL & PAPIEROK 1982, MOORHOUSE et al. 1993). Furthermore, MANIANIA et al. (2003) observed that *M. anisopliae* had the potential to control *T. tabaci* in the field.

In the present study, the genera and species varied in their pathogenicity. Seven from eight isolates of *Beauveria* were highly pathogenic, while among eight isolates of *Metarhizium*, only four were highly pathogenic. From seven isolates of *Peacilomyces*, only three were highly pathogenic. Within isolates, the

differences in pathogenicity were more pronounced for *Metarhizium* than for *Beauveria*. This contrasts with EKESI et al. (1998), who found that the differences in virulence were more pronounced for *B. bassiana* strains than for *M. anisopliae* ones. Differences in pathogenicity between fungal species and isolates have also been reported for other insects' species (MOORHOUSE et al. 1993, POPRAWSKI et al. 1985).

The entomopathogenic fungi used in this study were isolated from different hosts. Many investigators reported that the pathogenicity is not always related to the original host or geographic origins (PRIOR 1990, MOORHOUSE et al. 1993). VESTERGAARD et al. (1995) found that *V. lecanii* isolated from thrips was weakly pathogenic to *F. occidentalis*, while EKESI et al. (1998) reported that *B. bassiana* isolated from *M. sjostedti* was only moderately pathogenic to its original host. FENG & JOHNSON (1990) noted that the original host has no significant influence on the virulence. Moreover, they found that entomopathogenic fungi isolated from soil showed a high pathogenicity to insects. EKESI et al. (1998) found that one isolate of *B. bassiana* and two isolates of *M. anisopliae* from soil were highly virulent to the legume flower thrips, and MANIANIA (1992) reported that they were highly pathogenic to the stem borers, *Chilo partellus* (Swinhoe) and *Busseola fusca* (Fuller). Furthermore, EKESI (1999) noted that *M. anisopliae* isolated from the soil showed high level of pathogenicity to the pod bug, *Clavigralla tomentosicollis* Stål.

In conclusion, from 41 isolates of entomopathogenic fungi tested, only 36 isolates were found pathogenic against *T. tabaci*, while the pathogenicity varied greatly among fungal species, genera and isolates.

References

ABBOTT, W.S. (1925): A method of computing the effectiveness of an insecticide. – J. Econ. Entomol. 18: 265-267.

AZAIZEH, H., GALINA GINDIN, SAID, O. & BARASH, I. (2002): Biological control of the Western Flower Thrips *Frankliniella occidentalis* in cucumber using the entomopathogenic fungus *Metarhizium anisopliae*. – Phytoparasitica 30(1): 18-24.

BROWNBRIDGE, M. (1995): Prospect for mycopathogens in thrips management. – In: PARKER, M., SKINNER, M. LEWIS, T. (eds.): Thrips Biology and Management. Plenum Press, New York: 281-295.

CARL, K.P. (1975): An *Entomophthora* sp. (Entomophthorales: Entomophthoraceae) pathogenic to *Thrips* spp. (Thysanoptera, Thripidae) and its potential as a biological control agent in glasshouses. – Entomophaga 20: 381-388.

EKESI, S. (1999): Selection of virulent isolates of entomopathogenic hyphomycetes against *Clavigralla tomentosicollis* Aátl and evaluation in cage experiments using three cowpea varieties. – Mycopathologia 148: 131-139.

EKESI, S., MANIANIA, N.K., ONU, I. & LÖHR, B. (1998): Pathogenicity of entomopathogenic fungi (Hyphomycetes) to the legume flower thrips, *Megalurothrips sjostedti* (TRYBOM) (Thys., Thripidae). – J. Appl. Entomol. 122: 629-634.

FENG, M.G. & JOHNSON, J.B. (1990): Relative virulence of six isolates of *Beauveria bassiana* on *Diuraphis noxia* (Homop: Aphididae). – Environ. Entomol. 19: 785-790.

FRANSEN, J.J. (1990): Fungi on aphids, thrips and whitefly in the greenhouse environment. – In: Proceeding, 5th International Colloquium on Invertebrate Pathology and Microbial Control. Society for Invertebrate Pathology, Adelaide, Australia: 376-380.

GILLESPIE, A.T. (1986): The potential of entomogenous fungi as control agents for onion thrips, *Thrips tabaci*. – In: Proceeding of Brighton Crop Protection Conference: Biotechnology and Crop Improvement and Protection, Brighton, UK: 237-242.

GOMEZ, K.A. & GOMEZ, A.A. (1984): Statistical procedures for agricultural research. 2nd ed. – John Wiley and Sons, Inc., New York, USA: 680 pp.

HALL, R.A. & PAPIEROK, B. (1982): Fungi as biological control agent of arthropods of agriculture and medical importance. – Parasitology 84: 205-240.

HALL, R.A., PETERKIN, D. & ALI, B. (1994): Fungal control of whitefly, *Thrips palmi* and sugarcane froghopper in Trinidad and Tobago – In: Proceeding, 6th International Colloquium on Invertebrate Pathology and Microbial Control. Society for Invertebrate Pathology, Montpellier, France: 277-282.

LEWIS, T. (1997): Pest thrips in perspective. – In: LEWIS, T. (ed.): Thrips as Crop Pests. CAB International, Wallingford, UK: 1-14.

MANIANIA, N.K. (1992): Pathogenicity of entomogenous fungi (Hyphomycetes) to larvae of stem borers, *Chilo partellus* (SWINHOE) and *Busseola fusca* FULLER. – Insect Sci. Applic. 13: 691-696.

MANIANIA, N.K., EKESI, S., LOEHR, B. & MWANGI, F. (2001): Prospects for biological control of the western flower thrips, *Frankliniella occidentalis*, with the entomopathogenic fungus, *Metarhizium anisopliae*, on chrysanthemum. – Mycopathologia 155: 229-235.

MANIANIA, N.K., SITHANANTHAM, S., EKESI, S., AMPONG-NYARKO, K., BAUMGÄRTNER, J., LÖHR, B. & MATOKA, C.M. (2003): A field trial of the entomopathogenous fungus *Metarhizium anisopliae* for control of onion thrips, *Thrips tabaci*. – Crop Protection 22: 553-559.

MOORHOUSE, E.R., GILLESPIE, A.T. & CHARNLEY, A.K. (1993): Laboratory selection of *Metarhizium* spp. isolates for control of vine weevil larvae (*Otiorhynchus sulcatus*). – J. Invertebr. Pathol. 62: 15-21.

POPRAWSKI, T.J., MARCHAL, M. & ROBERT, P.H. (1985): Comparative susceptibility of *Otiorhynchus sulcatus* and *Sitona lineatus* (Coleoptera:Curculionidae) early stages to five entomopathogenic hyphomycetes. – Environ. Entomol. 14: 247-253.

PRIOR, C. (1990): The biological basis for regulating the release of microorganisms with particular reference to the use fungi for the pest control. – Aspects of Applied Biology 24: 231-238.

SAITO, T. (1991): A field trial of an entomopathogenic fungus, *Beauveria bassiana* (BALSAMO) VUILLEMIN., for the control of *Thrips palmi* KARNY (Thysanoptera, Thripidae) – Jap. J. Appl. Entomol. Zool. 35: 80-81.

SAMSON, R.A., RAMAKERS, P.M.J. & OSWAL, T. (1979): *Entomophthora thripidum*, a new fungal pathogen of *Thrips tabaci*. – Can. J. Bot. 57: 1317-1323.

VACANTE, V., CACCIOLA, S.O. & PENNISI, A.M. (1994): Epizootiological study of *Neozygites parvispora* (Zygomycota: Entomophthoraceae) in a population of *Frankliniella occidentalis* (Thysanoptera, Thripidae) on pepper in Sicily. – Entomophaga 39: 123-130.

VESTERGAARD, S., GILLIESPIE, A.T., BUTT, T.M., SCHREITER, G. & EILENBERG, J. (1995): Pathogenicity of the hyphomycetes fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis*. – Biocontrol Sci. Tech. 5: 185-192.