SURE 静岡大学学術リポジトリ
Shizuoka University REpository

Characterization of Paecilomyces cinnamomeus from the camellia whitefly, Aleurocanthus camelliae (Hemiptera: Aleyrodidae), infesting tea in Japan

メタデータ	言語: eng 出版者: 公開日: 2012-07-05 キーワード (Ja): キーワード (En): 作成者: Saito, Tsutomu, Takatsuka, Jun, Shimazu, Mitsuaki メールアドレス: 所属:
URL	http://hdl.handle.net/10297/6735

Characterization of *Paecilomyces cinnamomeus* from the camellia whitefly, *Aleurocanthus camelliae* (Hemiptera: Aleyrodidae), infesting tea in Japan

Tsutomu Saito [a], Jun Takatsuka [b], Mitsuaki Shimazu [b]

[a] *Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga, Shizuoka 422-8529, Japan*

[b] *Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan*

ABSTRACT

The whitefly, *Aleurocanthus camelliae* Kanmiya & Kasai (Hemiptera: Aleyrodidae), is an invasive species in Japan that was first discovered in 2004 on tea in Kyoto. Soon after its arrival epizootics of an entomopathogenic fungus were observed in populations of the whitefly in many tea-growing regions. Here we identify this fungus as *Paecilomyces cinnamomeus* (Petch) Samson & W. Gams (Hypocreales: Clavicipitaceae) based on morphological characteristics and molecular analyses. This is the first record of *P. cinnamomeus* in Japan and also the first time it has been recorded from the genus *Aleurocanthus*. A isolate of *P. cinnamomeus* caused greater than 50% and 90% infection in whitefly nymphs at 1×10^6 and 1×10^7 conidia/ml respectively, while the commercial mycoinsecticides Preferd® (*Isaria fumosorosea*) and Mycotal® (*Lecanicillium muscarium*) caused < 10% infection at their recommended field rates (5×10^6 and 9×10^6 conidia/ml, respectively), suggesting that *P. cinnamomeus* may be more useful as a control agent than the currently available mycoinsecticides. Optimum and upper limit temperatures for *in vitro* growth of *P. cinnamomeus* isolates were 22.5-25°C and 32.5°C, respectively. At field rates, the fungicide thiophanate-methyl caused some inhibition of *in vitro* growth of *P. cinnamomeus* isolates, and the bactericide copper oxychloride and the insecticides tolfenpyrad and methidathion were strongly inhibitory. The findings obtained in this study will be useful in the development of microbial control programs using *P. cinnamomeus* against *A. camelliae*.

Keywords:
Tea
Aleurocanthus camelliae
Paecilomyces cinnamomeus
Conoideocrella luteorostrata
Molecular analyses
Pesticides

* Corresponding author. Fax: +81 54 238 4790.
E-mail address: atsaito@ipc.shizuoka.ac.jp (T. Saito).

1. Introduction

In 2004, heavy whitefly infestations were first recorded on tea in Kyoto Prefecture, central Japan, and quickly spread to other Prefectures (Yamashita and Hayashida, 2006; Kasai et al., 2010). Infested tea plants were weakened due to sap loss and to the growth of sooty mold on honeydew-covered leaf surfaces during heavy infestations. Recently this whitefly has been described as a new species, *Aleurocanthus camelliae* Kanmiya & Kasai (Hemiptera: Aleyrodidae), Originally it had been thought to be *A. spiniferus* (Quaintance), a species infesting tea in China (Kanmiya et al., 2011). It is believed that *A. camelliae* was introduced to Japan from China (Kasai et al., 2010).

Simultaneously with outbreaks of the whitefly, fungal epizootics were also observed in *A. camelliae* nymphs in many tea fields. Infected whitefly nymphs all had the common symptom of being covered with a cinnamon-colored mat of mycelium. In this study, the fungus was identified based on morphological characteristics and phylogeny of the ITS rDNA, the β-tubulin gene and the translation elongation factor 1α gene. In addition, the virulences of the fungal isolates were determined and compared with the mycoinsecticides Preferd® (*Isaria fumosorosea*) and Mycotal® (*Lecanicillium muscarium*), which are used commercially to control vegetable whiteflies such as *Bemisia tabaci* (Gennadius) B biotype (formerly *B. argentifolii*) and *Trialeurodes vaporariorum* (Westwood). Moreover, the effects of temperature and pesticides (a bactericide, fungicides and insecticides) on fungal growth were also evaluated. Together, these data inform our understanding of how this fungus might be encouraged under the environmental conditions found in Japanese tea-growing regions and integrated with existing whitefly control strategies.

2. Materials and Methods

2.1. Morphological observation and isolation of the fungus

Infected nymphs were collected from commercial tea plants growing in Kyoto, Shiga and Mie Prefectures, Japan, between August 2009 and February 2010 when heavy infections were observed. Twelve isolates were established as pure cultures on Sabouraud dextrose agar (SDA) (Difco Laboratories Inc., MI, USA) from conidia on cadavers collected in different fields in each Prefecture (Table 1), and deposited in the culture collection of FFPRI (Forestry and Forest Products Research Institute), Tsukuba, Ibaraki, Japan. Infected cadavers were observed and photographed using a stereo microscope (MZ-16, Leica Microsystems GmbH, Wetzlar, Germany) and a scanning electron microscope (SEM; NeoScope JCM-5000, Jeol, Tokyo, Japan). The sizes of conidiogenous cells and conidia of all the isolates were measured using a light microscope (S-Ke, Nikon, Tokyo, Japan).

2.2. Molecular analyses

The twelve isolates were cultured on SDA for 5 days at 25°C. Mycelia were scraped from the plates using a 200-µl pipette tip and suspended in TE buffer (10mM Tris, 1mM EDTA, pH 8.0). Each suspension was homogenized with a hand homogenizer for 30 s and the DNA extracted using a PUREGENE Tissue DNA Purification kit (Qiagen, Tokyo, Japan). Morphological studies of the isolates suggested that they were likely to belong to the genus *Paecilomyces.* To confirm this attribution, or otherwise, DNA sequences and phylogenetic analyses were made on the same three sequence regions that had been used previously to analyze relationships among fungal species placed in *Paecilomyces* and the neighboring *Conoideocrella* and *Isaria* genera (Luangsa-ard et al., 2005; Johnson et al., 2009).

Polymerase chain reactions (PCRs) were performed to amplify the ITS rDNA with primers ITS4 and ITS5 (White et al., 1990), the partial β-tubulin gene (TUB) sequences with primers bt2a and bt2b (Glass and Donaldson, 1995) and the partial translation elongation factor 1α gene (TEF) sequences with primers 983F and 2218R (Rehner and Buckley, 2005). Amplification reactions were made in volumes of 50 µl containing 200 µM dNTPs, 1.25 units of Ex Taq DNA polymerase (Takara Bio Inc., Shiga, Japan), 0.4 µM primer, 1× Ex Taq buffer (Takara Bio Inc. Shiga, Japan) and 10 ng of template DNA. The ITS rDNA was amplified using the same PCR conditions, except for the annealing temperature which was 55°C. PCR conditions for the TUB were 1 cycle at 94°C for 4 min, followed by 35 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 2 min, and then 1 cycle at 72°C for 7 min. Amplification of the TEF was made under the following conditions: 1 cycle at 94°C for 4 min followed by 37 cycles at 94°C for 30 s, 60°C for 1 min, 72°C for 1 min, and then 1 cycle at 72°C for 7 min. Three µl of each PCR suspension was electrophoresed through a 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light to check for amplification of bands of an expected size. Singly amplified products of 630 bp for ITS rDNA were purified using the QIAquick PCR Purification kit (Qiagen, Tokyo, Japan). The amplified products of 380 and 1030 bp for the TUB and TEF, respectively, were excised from the gel and purified using QIAquick Gel Extraction Kit (Qiagen, Tokyo, Japan).

Purified PCR products were sub-cloned into the p3T cloning vector (MoBiTec GmbH, Göttingen, Germany). DNA sequence analysis of both strands was done with a capillary sequencer (ABI 3130XL, Applied Biosystems, CA, USA). Three clones were sequenced for each strand. DNA sequences were subjected to a Basic Local Alignment Search Tool (BLAST) search to identify sequences deposited in GenBank that had a significant homology. The sequences were aligned with the corresponding sequences of the reference isolates and species shown in Table 2 using ClustalW incorporated in MEGA 4 (Tamura et al., 2007). In the ITS rDNA and TUB phylogenies, the fungal isolates that were used previously to clarify relationships amongst fungal species placed in *Isaria* and *Paecilomyces* sect. *Paecilomyces* (Luangsa-ard et al., 2005) were included. A *Paecilomyces* sp. isolate from *A. spiniferus* on tea in China was also included in the ITS phylogeny to understand the relationship between this isolate and our isolates. In the TEF phylogeny, some fungal isolates belonging to Clavicipitaceae were included. These isolates were used in the Johnson et al. (2009) study on systematics of fungi including *Conoideocrella* spp. that had been referred to as species of

the genus *Torrubiella*. Alignment gaps were treated as missing data in the analyses. Phylogenetic trees were constructed by the neighbor-joining method of MEGA 4 (Tamura et al., 2007). Bootstrap analyses of 1000 replications were made to determine the supporting values of branches. *Isaria farinosa* and *I. tenuipes* were the designated outgroups.

2.3. Bioassays

The three isolates Kyoto-32, Shiga-22 and Mie-1 were used in bioassays because they all sporulated well on medium. Conidia of each isolate were harvested from 20 plate cultures that had been grown for 3 weeks on SDA at 25 ± 1°C in darkness by adding 10 ml of sterile 0.1% Tween 80 to each plate, agitating with a sterile spreader and pipetting the conidia suspension into a sterile flask. Conidial suspensions were filtered through sterile gauze, and conidial concentrations estimated using a Thoma haemocytometer and adjusted to provide suspensions containing 1×10^6, 1×10^7 and 1×10^8 conidia/ml. The mycoinsecticides Preferd® (Tokai Bussan, Nagoya, Japan; active ingredient *I. fumosorosea*) and Mycotal® (Arista LifeSience, Tokyo, Japan; active ingredient *L. muscarium*) were also used at their recommended field application rates (1g/L). The actual conidial concentrations of these field rates were determined as 5×10^6 and 9×10^6 conidia/ml, respectively, using a Thoma haemocytometer.

Field-collected nymphs of *A. camelliae* on tea shoots were used. Tea shoots were collected from an experimental field at the Kyoto Prefectural Tea Experiment Station in Uji, Kyoto, on March 11, 2010. No pesticides had been applied for more than one year, and entomopathogenic fungi infecting *A. camelliae* had not been observed. Each tea shoot collected was approximately 15 cm in length with four or five leaves and approximately 150 (ranging from 135 to 171) overwintering mature nymphs (3^{rd} and 4^{th} instars) of *A. camelliae*. Before inoculation, the viability of each isolate was checked; germination rate of conidia was > 90% in all cases. Three replicate shoots were used for each treatment. The three shoots were inoculated by gentle agitation in 200 ml of the same conidial suspension for 10 sec. Control shoots were treated in the same way but with sterile 0.1% Tween 80 only. The base of each shoot was then inserted into a polyethylene tube containing water, enclosed in a polyethylene bag to maintain a high humidity and incubated under controlled conditions of 16L: 8D and 20 ± 1°C. After inoculation the shoots were checked daily using a stereo microscope to record the first appearance of infection. One, 2 and 3 weeks after inoculation the proportion of nymphs that had become infected by the fungus was calculated from the number of dead nymphs exhibiting mycosis and the number of living nymphs remaining that did not exhibit mycosis, as observed under a stereo microscope.

2.4. Effect of temperature

The three isolates, Kyoto-32, Shiga-22 and Mie-1 used in the bioassays, were cultured on SDA at 20 ± 1°C in darkness for 2 weeks. Plugs were taken from the growing margin of each colony using a 3 mm diameter sterile cork borer and placed individually into the center of sterile 9 cm triple vented

Petri dishes containing 30 ml of SDA. Replicate plates for each isolate were placed into incubators at 10, 15, 20, 22.5, 25, 27.5, 30, 32.5 and 35°C and incubated in darkness for 3 weeks. Colony diameter was then measured in two perpendicular directions, and the mean determined after subtraction of 3 mm to account for the original inoculation plug. Five replicate plates for each isolate were used at each temperature.

2.5. Effect of pesticides

A total of 30 commercial pesticides including 12 fungicides, one bactericide and 17 insecticides were evaluated. These pesticides are in general use to control insect pests and diseases on tea in Japan. Each pesticide was added to sterilized SDA medium at the recommended application rate once it had cooled to below 50°C and mixed using a stirrer before pouring the agar and allowing it to set. Their effects on mycelial growth of Kyoto-32, Shiga-22 and Mie-1 used in the previous experiments were determined using the same procedures as described above for the temperature experiment except that they were incubated at 25 ± 1°C.

2.6. Statistical analyses

The proportion of nymphs infected in bioassays were arcsin square-root transformed and analyzed using ANOVA, followed by Tukey HSD tests to compare infection rates amongst treatments. Data from the pesticide experiment were analyzed using ANOVA, followed by a Dunnett test to detect differences between each pesticide and the control. All analyses were conducted using the software package SPSS (SPSS, 2009).

3. Results

3.1. Description of the fungus

The entomopathogenic fungus was observed infecting nymphs but not eggs or adults in the field. Infected nymphs were covered with a thick cinnamon-colored mat of mycelium that appeared brush-like in form (Fig. 1A). The stromata were raised, pulvinate, tomentose and 1–2 mm in diameter with a hypothallus of thin and smooth-walled hyphae expanding radially to, commonly, 2–3 mm from the host on the leaf surface (Fig. 1B). Large hypothalli expanded to 15–20 mm in total diameter. Conidiogenous cells were phialidic, flask-shaped, and erected loosely on the hypothallus and rarely on the stromata (Fig. 1C). Conidia were fusiform and in basipetal chains (Fig. 1C and D). Perithecia were lacking on the stromata.

All isolates developed flattened, cinnamon-colored colonies *in vitro* and released a deep purple-red diffusible pigment into the medium, with the exception of Mie-1 that produced white colonies and no diffusible pigmentation. Massed conidia were green and produced sparsely on the colony. The mean sizes of conidiogenous cells (n = 30 per isolate) and conidia (n = 50 per isolate) of

198 the 12 isolates ranged between 2.4–2.8 × 9.8–12.5 μm and 2.3–2.6 × 5.2–7.6 μm, respectively (Table
199 1). Perithecia were also lacking *in vitro*.
200 Based on the taxonomic keys of Samson (1974), the fungus was morphologically identical to
201 *Paecilomyces cinnamomeus* (Petch) Samson & W. Gams.
202
203 *3.2. Molecular analyses*
204
205 All isolates used in the present study had an identical sequence for 586 bp of ITS rDNA. A
206 BLAST search indicated that the ITS rDNA sequence of our isolates had 99% alignment with a
207 *Paecilomyces* sp. isolate from *A. spiniferus* on tea in China. The second most significant alignment
208 was 98% with the sequences of *P. cinnamomeus* and *Conoideocrella luteorostrata* (Zimm.) D.
209 Johnson, G. H. Sung, Hywel-Jones & Spatafora (formerly *Torrubiella luteorostrata*) (Johnson et al.,
210 2009). In the ITS rDNA phylogram, all of our isolates were grouped with *Paecilomyces* sp., *C.*
211 *luteorostrata* and *P. cinnamomeus* and were well supported by a bootstrap value of 99% (Fig. 2).
212 The partial sequences of TUB for our isolates had significant alignments of 89–99% with *C.*
213 *luteorostrata* and *P. cinnamomeus.* A TUB phylogram positioned our isolates in a clade that included
214 *C. luteorostrata* and *P. cinnamomeus* and was supported by a bootstrap value of 100% (Fig. 3).
215 The partial sequences of TEF for our isolates had a 99% alignment with *C. luteorostrata* and the
216 second most significant alignment was with the sequences of *Conoideocrella tenuis* (Petch) D.
217 Johnson, G. H. Sung, Hywel-Jones & Spatafora (formerly *Torrubiella tenuis*) in the family
218 Clavicipitaceae (Johnson et al., 2009). A phylogram of TEF indicated that our isolates were
219 positioned with *C. luteorostrata* in a clade that was supported by a bootstrap value of 99% (Fig. 4).
220 The sequences were deposited in GenBank with accession numbers listed in Table 1.
221
222 *3.3 Bioassays*
223
224 Within 5 days of inoculation infected nymphs died and appeared white or cinnamon-colored with
225 hyphae growing from the edges of their bodies. Subsequently the host body became covered with
226 thick-walled cinnamon-colored hyphae. The appearance of the artificially induced infection was
227 similar to that of natural infections in the field.
228 Bioassays were ended 3 weeks after inoculation when whitefly adults began to emerge (Fig. 5).
229 No mycosis was recorded from nymphs in the control treatment. At week 3 there were significant
230 differences among the isolates in the proportion of nymphs that became infected in the low (F =
231 5.51, p = 0.024), medium (F = 71.13, $p < 0.001$) and high conidial concentration treatments (F =
232 83.80, $p < 0.001$). At the low concentration (1×10^6 conidia/ml), Kyoto-32 caused the greatest
233 percent infection (52.3%) which was significantly higher than that caused by Shiga-22 but not Mie-1
234 (Tukey HSD, $p < 0.05$). At the medium concentration (1×10^7 conidia/ml), Kyoto-32 and Shiga-22
235 infected 91.7% and 81.7% of nymphs respectively and these percentages were significantly greater
236 than that for Mie-1 (Tukey HSD, $p < 0.05$). At the high concentration (1×10^8 conidia/ml), Kyoto-32,
237 Shiga-22 and Mie-1 all infected more than 85% of nymphs and there were no significant differences

amongst the isolates (Tukey HSD, $p > 0.05$). Both mycoinsecticides (Preferd® at 5×10^6 conidia/ml and Mycotal® at 9×10^6 conidia/ml) infected < 10% of nymphs which was not significantly different to the control where there was 0% infection (Tukey HSD, $p > 0.05$).

3.4. Effect of temperature

The three isolates, Kyoto-32, Shiga-22 and Mie-1, grew most rapidly between 22.5°C and 25°C and ceased growth at 32.5°C (Fig. 6).

3.5. Effect of pesticides

There were significant differences in growth rates among treatments for Kyoto-32 ($F = 94.49$, $p < 0.001$), Shiga-22 ($F = 209.03$, $p < 0.001$) and Mie-1 ($F = 225.75$, $p < 0.001$). The bactericide copper oxychloride completely inhibited growth of all three isolates (Fig. 7). Most fungicides were harmful to the isolates, especially difenoconazole and tebuconazole, that completely inhibited growth of Shiga-22, while thiophanate-methyl had no effect on growth of Shiga-22 ($p = 0.78$) and showed only slight inhibition of growth of Kyoto-32 ($p = 0.03$) and Mie-1 ($p = 0.06$). Some insecticides, including tolfenpyrad and methidathion, were extremely inhibitory to all isolates, while imidacloprid, thiacloprid and pyridaben had no effect on growth of any isolate.

4. Discussion

Morphological characteristics of the fungal isolates from *A. camelliae* on tea agreed with those for *P. cinnamomeus* (Samson, 1974) that was reported to be the anamorph (without perithecia) of *C. luterostrata* (the telemorph with perithecia) by Hywel-Jones (1993). The relationship between both states was confirmed by phylogenetic analyses using the 28S rDNA (Artjariyasripong et al., 2001), the 18S rDNA (Luangsa-Ard et al., 2004) and the ITS rDNA and TUB (Luangsa-Ard et al., 2005). These findings are supported by the present study which demonstrated that our isolates fell into a clade with *P. cinnamomeus* and *C. luteorostrata* in the phylogenetic analyses of the ITS rDNA and TUB. Though recent phylogenetic work indicates that *C. luteorostrata* and *C. tenuis* are closely related species forming a sister group within the family Clavicipitaceae (Johnson et al., 2009), phylogenetic analysis using TEF also positioned our isolates with *C. luteorostrata* in a 99% supported clade. In addition, no perithecia could be found on cadavers from the field or from bioassays or *in vitro* cultures. These findings suggest that the fungus should be placed as the anamorph *P. cinnamomeus* of *C. luteorostrata.* Some *Paecilomyces* species (including *P. cinnamomeus*) still have taxonomic issues (Luangsa-Ard et al., 2005, 2011), and these issues remain unresolved. Therefore, here we refer to our isolates as *P. cinnamomeus*.

Paecilomyces cinnamomeus can be distinguished from the other *Paecilomyces* spp. by their

277 fusiform conidia, pigmented hyphae and deep purple-red diffusible pigment in the medium (Samson,
278 1974; Hywel-Jones, 1993). One of our isolates, Mie-1, produced a white colony and no pigmentation
279 *in vitro*, although the isolate did produce cinnamon-colored stromata *in vivo* on hosts as did the other
280 isolates. This suggests that these isolates have morphological and physiological variation in response
281 to media.
282 Since the relationship between *P. cinnamomeus* and *C. luteorostrata* has only recently been
283 discovered, the two species have generally been studied separately. *Paecilomyces cinnamomeus* has
284 been recorded in Thailand, North and Central America, Cuba, Mexico and Ghana, and is known as a
285 pathogen on scale insects and the citrus whitefly, *Dialeurodes citri* (Ashmead) (Samson, 1974;
286 Hywel-Jones, 1993). In contrast, *C. luteorostrata* has been recorded from scale insects and whiteflies
287 in Thailand, Java, Seychelles, Ceylon, Samoa, New Zealand and the Far East of Russia (Petch, 1924;
288 Dingley, 1953; Kobayasi, 1982; Kobayasi and Shimizu, 1982; Hywel-Jones, 1993). Our record of *P.*
289 *cinnamomeus* is the first record of this species in Japan and the first time that the host was from the
290 genus *Aleurocanthus*. However, the fungus possibly occurs on *A. spiniferus* infesting tea in China,
291 because phylogeny of the ITS rDNA of our isolates fell into the clade of a *Paecilomyces* sp. that was
292 isolated from *A. spiniferus* on tea in China (Huang et al., 2002). Moreover, a fungal species reported
293 as *P. aleurocanthus*, which was isolated from *A. spiniferus* on tea in China (Tang et al., 2003),
294 appears to be *P. cinnamomeus* according to attributes such as pigmented hyphae, deep purple-red
295 pigment and green colored conidia. There are also other Chinese records of *Paecilomyces* sp. from *A.*
296 *spiniferus* on tea (e.g. Han and Li, 2001; Tang, 2001; Guo et al., 2006).
297 In Thailand, the *P. cinnamomeus* and *C. luteorostrata* states occur throughout the year apart from
298 the hot dry season, and both were discovered simultaneously on stroma emerging from infected
299 insects (Hywel-Jones, 1993). Moreover, a second strain from a multiple-conidia isolation of the *P.*
300 *cinnamomeus* state produced perithecia *in vitro* after 6 months (Hywel-Jones, 1993). In Japan,
301 however, infected whiteflies attached to tea foliage were found throughout the year including the hot
302 and wet season (summer) and cold dry season (winter), and perithecia could not be found on hosts in
303 the field or in our bioassays. Furthermore, no perithecia formation was observed on 6 month old *in*
304 *vitro* cultures (data not shown). These findings suggest that maybe only the *P. cinnamomeus* state
305 occurs in Japan. Further research directed toward factors inducing both *P. cinnamomeus* and *C.*
306 *luteorostrata* states is necessary.
307 When the relationship between temperature and growth of *P. cinnamomeus* was unknown,
308 Hywel-Jones (1993) suggested that *P. cinnamomeus* should be considered as an entomopathogen
309 only in tropical and subtropical regions. However, in this study, *P. cinnamomeus* grew most rapidly
310 between 22.5°C and 25°C and ceased growth at 32.5°C, suggesting that the fungus prefers moderate
311 temperatures. In Kyoto, Shiga and Mie Prefectures where our isolates were collected, the lowest air
312 temperature is below 0°C in the winter and the highest air temperature is above 35°C in the summer.
313 However, the microclimate of commercial tea bushes, where many plants are grown closely together,
314 may provide not only the moderate temperatures but also the high humidity necessary to promote
315 infection by *P. cinnamomeus* and the development of epizootics in whitefly populations. In China,
316 entomopathogenic fungi are important natural mortality factors of whiteflies in tea-growing areas

(Han and Lin, 2003).

The citrus whitefly, *D. citri*, has been recorded as a host of *P. cinnamomeus* (Samson, 1974). This whitefly is a common pest on Satsuma mandarin, *Citrus unshiu* Marc., which is widely grown in Japan. However, *P. cinnamomeus* has not been found on *D. citri* in Japan. Moreover, *P. cinnamomeus* was highly virulent to the tobacco whitefly, *B. tabaci* B biotype, in the laboratory (Saito, unpublished data), which is also widespread in Japan, but again, infection by *P. cinnamomeus* in the field has never been recorded. It is possible either that *P. cinnamomeus* had been restricted to forests before invasion of *A. camelliae* since the primary habitat of the fungus is forests (Hywel-Jones, 1993), or that it was introduced simultaneously into Japan together with *A. camelliae*.

Preferd® and Mycotal®, mycoinsecticides currently used to control whiteflies, caused very little infection (< 10%) in *A. camelliae* at recommended field application rates (5×10^6 and 9×10^6 conidia/ml, respectively), suggesting that neither are useful as control agents of this whitefly. In contrast, when isolate Kyoto-32 was used at the lower concentration of 1×10^6 conidia/ml, the proportion of nymphs becoming infected (> 50%) was greater than that achieved by the mycoinsecticides. At a concentration of 1×10^7 conidia/ml, the proportion of nymphs infected by isolate Kyoto-32 reached > 90%. These findings suggest that *P. cinnamomeus* may be more useful as a control agent for augmentation into tea fields than the currently available commercial mycoinsecticides.

Whether *P. cinnamomeus* can be used as a control agent for augmentative control or natural control will be influenced by interactions with pesticides that are currently in use in tea. For example, previous studies have shown that fungicides may disrupt control by entomopathogenic fungi, thereby resulting in extremely high populations of insect pests (Steinkraus, 2007). In this study, all the fungicides evaluated significantly inhibited growth of *P. cinnamomeus* although thiophanate-methyl was relatively safe for the fungus. The high susceptibility of the fungus to the bactericide copper hydroxide is interesting since this bactericide has different effects depending on the fungus species and whether *in vitro* growth or germination are being considered; it strongly affects *Lecanicillium* spp. (Saito, 1988; Saito and Yabuta, 1996) but not *Beauveria bassiana* (Saito, 1984). It is necessary to note that some insecticides e.g., tolfenpyrad and methidathion, were also highly inhibitory to the fungus. Further studies will be needed to determine what effect these pesticides may have on fungal efficacy in the field.

Acknowledgements

We are grateful to Y. Yoshiyasu and A. Kasai (Kyoto Prefectural University) for promoting this program and to K. Yamashita (Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center) for providing the whitefly. We thank co-operators in Kyoto, Shiga and Mie Prefecture for collecting infected whiteflies. We thank Dr. J.K. Pell for critical review and copyediting of the manuscript. We also appreciate critical comments from X-F. Chen and M-J. Tang (Tea Research Institute, Chinese Academy of Agricultural Science). This study was funded in part by a

Grant-in-Aid for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries (21002) supported by MAFF.

References

Artjariyasripong, S., Mitchell, J.I., Hywel-Jones, N.L., Jones, E.B.G., 2001. Relationship of the genus *Cordyceps* and related genera, based on parsimony and spectral analysis of partial 18S and 28S ribosomal gene sequences. Mycoscience 42, 503-517.

Clausen, C.P., 1978. Biological control of citrus insects. In: Reuter, W., Calavan, E.C., Carman, G.E. (Eds.), The Citrus Industry Vol. IV, University of California, Division of Agricultural Sciences, pp. 276-303.

Dingley, J.M., 1953. The Hypocreales of New Zealand. V. The genera *Cordyceps* and *Torrubiella*. Trans. Roy. Soc. N.Z. 81, 329-343.

Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1323-1330.

Guo, L., Qiu, B-L., Ren, S-X., Wu, H-J., 2006. Summary on the classification of natural enemy germplasm resources of *Aleurocanthus spiniferus* (Homoptera: Aleyrodidae). Guangdong Agric. Sci. No. 2, 9-11 (in Chinese with English abstract).

Han, B-Y., Li, Z-Z., 2001. Growing characteristics on Czapek medium of 8 kinds of entomogenous fungi from *Aleurocanthus spiniferus* and their infection ratios. Entomol. J. East China 10, 39-43 (in Chinese with English abstract).

Han, B-Y., Lin, C., 2003. Natural population life table of citrus spiny whitefly (*Aleurocanthus spiniferus*) in tea garden. Acta Ecol. Sin. 23, 1781-1790 (in Chinese with English abstract).

Huang, B., Yu, C-X., Chen, X-L., Fan, M-Z., Li, Z-Z., 2002. Classification and identification of species and strains in *Peacilomyces* by using RAPD. Mycosystema 21, 33-38 (in Chinese with English abstract).

Hywel-Jones, N.L., 1993. *Torrubiella luterostrata*: a pathogen of scale insects and its association with *Paecilomyces cinnamomeus* with a note on *Torrubiella tenuis*. Mycol. Res. 97, 1126-1130.

Johnson, D., Sung, G-H., Hywel-Jones, N.L., Luangsa-ard, J.J., Bischoff, J.F., Kepler, R.M., Spatafora, J.W., 2009. Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). Mycol. Res. 113, 279-289.

Kanmiya, K., Ueda, S., Kasai, A., Yamashita, K., Sato, Y., Yoshiyasu, Y., 2011. Proposal of new specific status for tea-infesting populations of the nominal citrus spiny whitefly *Aleurocanthus spiniferus* (Homoptera: Aleyrodidae). Zootaxa 2797, 25-44.

Kasai, A., Yamashita, K., Yoshiyasu, Y., 2010. Tea-infesting population of the citrus spiny whitefly, *Aleurocanthus spiniferus* (Homoptera: Aleyrodidae), does not accept citrus leaves as host plants. Jpn. J. Appl. Entomol. Zool. 54, 140-143 (in Japanese with English abstract).

Kobayasi, Y., 1982. Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. Trans. Mycol. Soc. Japan 23, 329-364.

396 Kobayasi, Y., Shimizu, D., 1982. Monograph of the genus *Torrubiella*. Bull. Natn. Sci. Mus., Tokyo,
397 Ser. B 8, 43-78.
398 Luangsa-ard, J.J., Hywel-Jones, N.L., Samson, R.A., 2004. The polyphyletic nature of *Paecilomyces*
399 *sensu lato* based on 18S-generated rDNA phylogeny. Mycologia 96, 773-780.
400 Luangsa-ard, J.J., Hywel-Jones, N.L., Manoch, L., Samson, R.A., 2005. On the relationships of
401 *Paecilomyces* sect. *Isarioidea* species. Mycol. Res. 109, 581-589.
402 Luangsa-ard, J.J., Houbraken, J., van Doorn, T., Hong, S., Borman, A.M., Hywel-Jones, N.L.,
403 Samson, R.A., 2011. *Purpureocillium*, a new genus for the medically important *Paecilomyces*
404 *lilacinus*. FEMS Microbiol. Lett. 321, 141-149.
405 Petch, T., 1924. Studies in entomogenous fungi III. *Torubiella*. Trans. Br. Mycol. Soc. 9, 108-129.
406 Peterson, G.D., 1955. Biological control of the orange spiny whitefly in Guam. J. Econ. Entomol. 48,
407 681-683.
408 Rehner, S.A., Buckley, E., 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF-*α*
409 sequences: evidence for cryptic diversification and link to *Cordyceps* teleomorphs. Mycologia
410 97, 84-98.
411 Saito, T., 1984. Effect of pesticides on conidial germination and hyphal growth of the
412 entomopathogenic fungus *Beauveria bassiana*. Jpn. J. Appl. Ent. Zool. 28, 87-89 (in Japanese).
413 Saito, T., 1988. Control of *Aphis gossypii* in greenhouses by a mycoinsecticidal preparation of
414 *Verticillium lecanii* and effect of chemicals on the fungus. Jpn. J. Appl. Ent. Zool. 32, 224-227
415 (in Japanese with English abstract).
416 Saito, T., Yabuta, M., 1996. Laboratory studies on effect of pesticides on entomopathogenic fungus,
417 *Verticillium lecanii*. Jpn. J. Appl. Ent. Zool. 40, 71-76 (in Japanese with English abstract).
418 Samson, R.A., 1974. *Paecilomyces* and some allied Hyphomycetes. Stud. Mycol. 6, 1-119.
419 SPSS, 2009. PASW Statistics 18. SPSS Inc., Chicago.
420 Steinkraus, D.C., 2007. Documentation of naturally occurring pathogens and their impact in
421 agroecosystems. In: Lacey, L.A., Kaya, H.K. (Eds.), Field Manual of Techniques in Invertebrate
422 Pathology. Second ed. Springer, Dordrecht, pp. 267-282.
423 Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. *MEGA4*: Molecular Evolutionary Genetics
424 Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596-1599.
425 Tang, M-J., 2001. Progress of research on the biological control of spiny black whitefly
426 (*Aleurocanthus spiniferus* Quaintance). J. Tea Sci. 21, 4-6 (in Chinese with English abstract).
427 Tang, M-J., Yin, K-S., Chen, X-F., 2003. Studies on the cultural characteristics and host range of the
428 entomogenous fungus *Paecilomyces aleurocanthus*. J. Tea Sci. 23, 46-52 (in Chinese with
429 English abstract).
430 White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal
431 ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J.
432 (Eds.), *PCR Protocols: a Guide to Methods and Aplications*, Academic Press, San Diego, pp.
433 315-322.
434 Yamashita, K., Hayashida, Y., 2006. Occurrence and control of the citrus spiny whitefly,
435 *Aleurocanthus spiniferus* (Quaintance), on tea tree in Kyoto Prefecture. Plant Prot. 60, 378-380

(in Japanese).
Yamashita, K., Saito, T., 2009. Occurrence of entomopathogenic fungus on the whitefly, *Aleurocanthus spiniferus*, on tea and its susceptibility to mycoinsecticides. Tea Res. J. 108, 24-25 (in Japanese).

Captions of Figures

Fig. 1. *Paecilomyces cinnamomeus* as an entomopathogenic fungus on the camellia spiny whitefly, *A. camelliae.* (A) Naturally infected larvae in a tea growing field, (B) cinnamon-colored stroma on the host and conidia on hypothalli expanding on a tea leaf, (C) conidiogenous cells and conidia, and (D) conidia.

Fig. 2. Neighbor-joining tree based on analysis of the ITS rDNA sequences showing the link between isolates from *A. camelliae* and *Paecilomyces* sp., *C. luteorostrata* and *P. cinnamomeus*. Numbers above or below the nodes indicate bootstrap values (> 50%) generated after 1000 replications. Branch termini are labelled according to species and GenBank accession numbers. Species names were expressed as the anamorph or telemorph according to the morph of the analyzed samples.

Fig. 3. Neighbor-joining tree based on analysis of the partial β-tubulin gene sequences showing the link between isolates from *A. camelliae* and *C. luteorostrata* and *P. cinnamomeus*. Numbers above or below the nodes indicate bootstrap values (> 50%) generated after 1000 replications. Branch termini are labelled according to species and GenBank accession numbers. Species names were expressed as the anamorph or telemorph according to the morph of the analyzed samples.

Fig. 4. Neighbor-joining tree based on analysis of the partial translation elongation factor 1α gene sequences showing the link between isolates from *A. camelliae* and *C. luteorostrata*. Numbers above or below the nodes indicate bootstrap values (> 50%) generated after 1000 replications. Branch termini are labelled according to species and GenBank accession numbers. Species names were expressed as the anamorph or telemorph according to the morph of the analyzed samples.

Fig. 5. Proportion of nymphs infected 1, 2 and 3 weeks after inoculation with isolates Kyoto-32, Shiga-22, Mie-1 and the mycoinsecticides (Preferd® and Mycotal®). The isolates were inoculated at three different concentrations of conidia, the low concentration (1×10^6 conidia/ml), the medium concentration (1×10^7 conidia/ml) and the high concentration (1×10^8 conidia/ml). The mycoinsecticides Preferd® and Mycotal® were inoculated at their recommended field application rates that were measured as conidial concentrations of 5×10^6 and 9×10^6 conidia/ml, respectively. Error bars represent one standard error of 3 replicates. Columns followed by different letters in the same week of each inoculum are significantly different at the 5% level using Tukey HSD test.

476

477 Fig. 6. Effect of temperature on growth of the three isolates, Kyoto-32, Shiga-22, Mie-1. Colony
478 diameter was determined 3 weeks after inoculation. Error bars represent standard error of 5
479 replicates.

480

481 Fig. 7. Effect of pesticides on growth of the three isolates, Kyoto-32, Shiga-22 and Mie-1. Colony
482 diameter was measured 3 weeks after inoculation. Error bars represent one standard error of 3
483 replicates. Asterisks indicate significant differences between each pesticide and the control at the 5%
484 level using the Dunnett test.

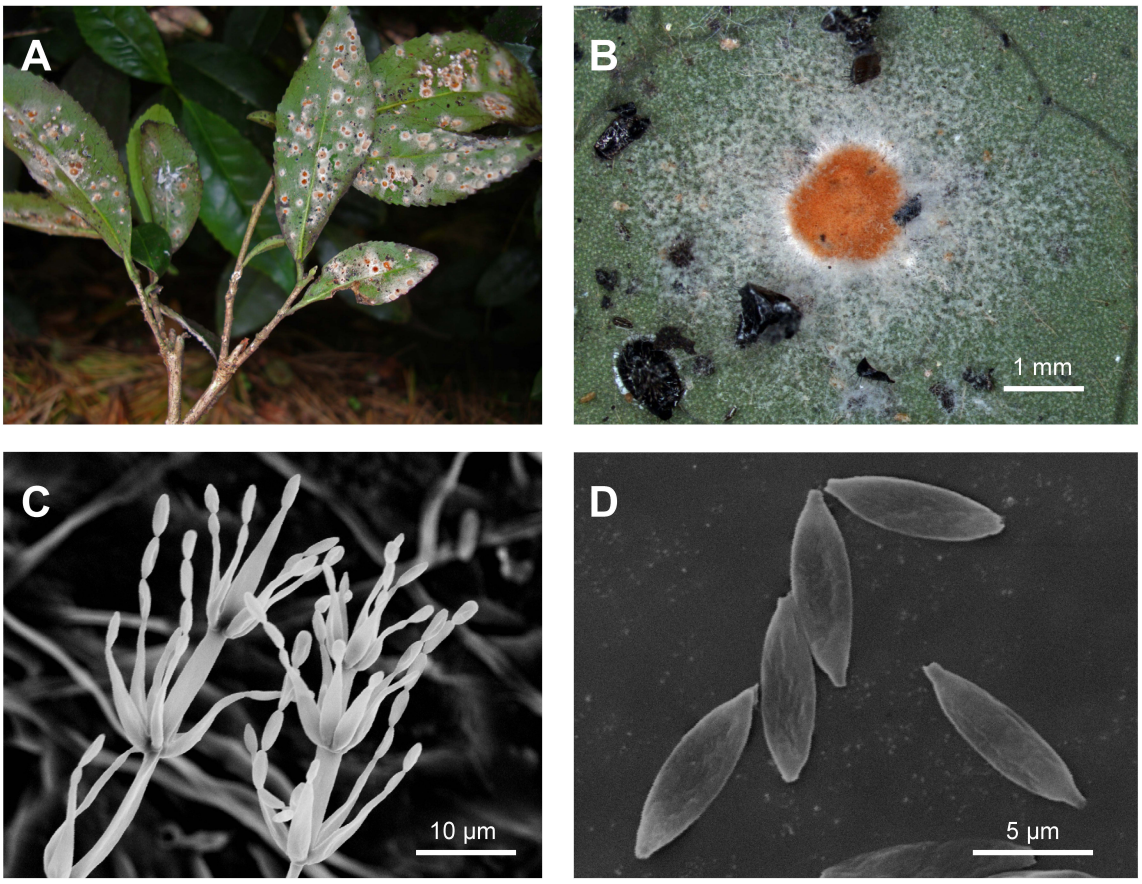
485

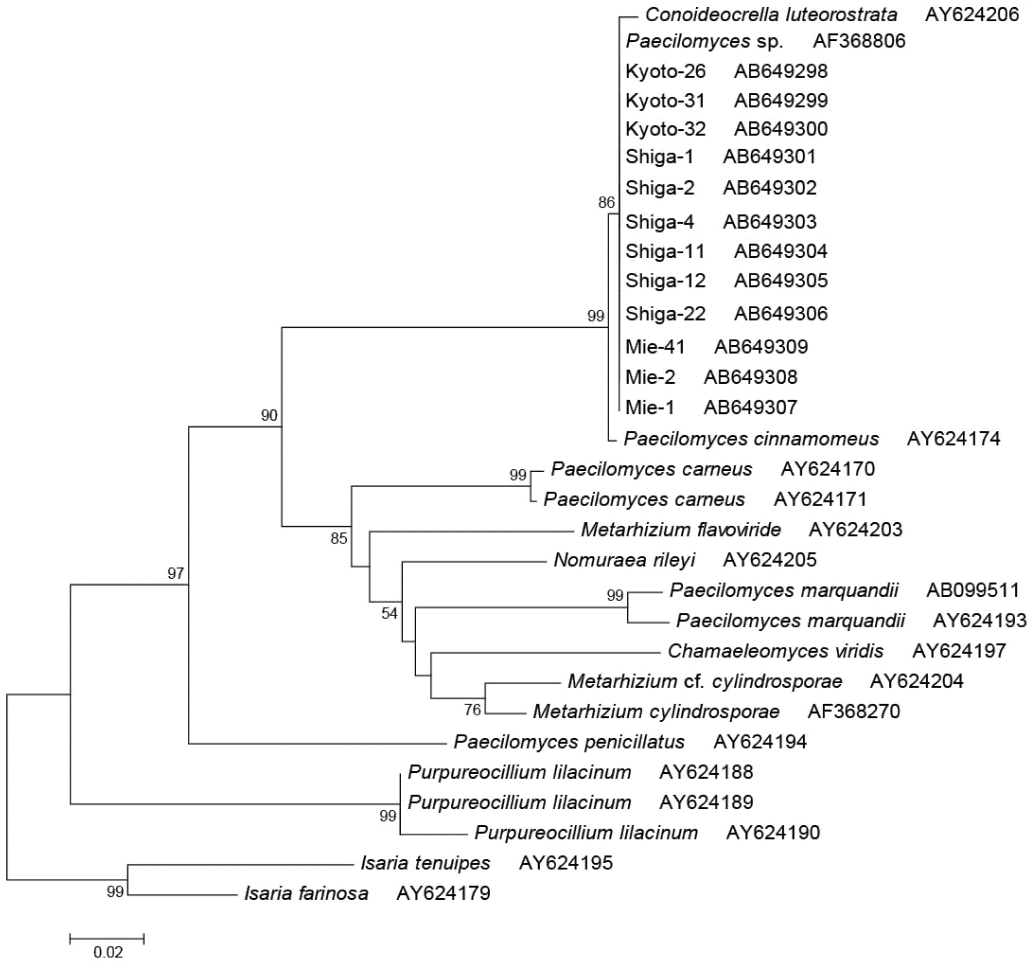
Table 1. List of isolates from *Aleurocanthus camelliae* in this study

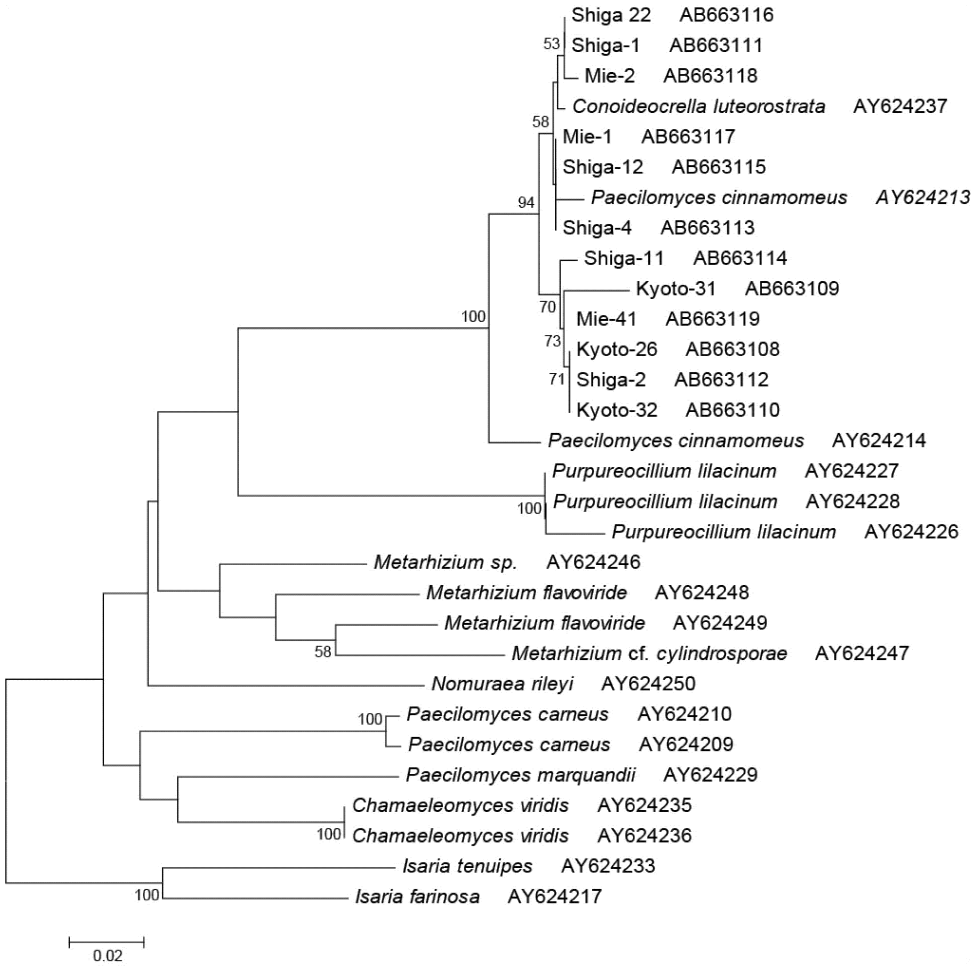
Isolates	Sites and dates of collection	Cultural observation		Size (μm, mean ± SE)		GenBank accession no.		
		Color of colony	Diffusible pigmentation	Conidiogenous cells	Conidia	ITS rDNA	β-tubulin gene	TEF 1α gene
Kyoto-26	Soraku, Kyoto, 2 Nov. 2009	Cinnamon	Yes	2.5 ± 0.12 × 9.8 ± 0.44	2.5 ± 0.04 × 6.7 ± 0.18	AB649298	AB663108	AB663120
Kyoto-31	Soraku, Kyoto, 14 Oct. 2009	Cinnamon	Yes	2.6 ± 0.12 × 11.1 ± 0.75	2.7 ± 0.04 × 6.2 ± 0.16	AB649299	AB663109	AB663121
Kyoto-32	Soraku, Kyoto, 14 Oct. 2009	Cinnamon	Yes	2.5 ± 0.06 × 11.4 ± 0.86	2.3 ± 0.04 × 5.9 ± 0.16	AB649300	AB663110	AB663122
Shiga-1	Koga, Shiga, 17 Sep. 2009	Cinnamon	Yes	2.7 ± 0.14 × 12.5 ± 0.77	2.6 ± 0.04 × 6.1 ± 0.12	AB649301	AB663111	AB663123
Shiga-2	Gamo, Shiga, 17 Sep. 2009	Cinnamon	Yes	2.8 ± 0.09 × 10.5 ± 0.77	2.5 ± 0.05 × 6.4 ± 0.16	AB649302	AB663112	AB663124
Shiga-4	Koga, Shiga, 19 Nov. 2009	Cinnamon	Yes	2.9 ± 0.13 × 10.3 ± 0.70	2.3 ± 0.04 × 5.6 ± 0.11	AB649303	AB663113	AB663125
Shiga-11	Koga, Shiga, 19 Nov. 2009	Cinnamon	Yes	2.6 ± 0.11 × 12.1 ± 0.80	2.5 ± 0.04 × 6.5 ± 0.17	AB649304	AB663114	AB663126
Shiga-12	Koga, Shiga, 19 Nov. 2009	Cinnamon	Yes	2.8 ± 0.08 × 10.8 ± 0.65	2.3 ± 0.04 × 5.2 ± 0.08	AB649305	AB663115	AB663127
Shiga-22	Koga, Shiga, 19 Nov. 2009	Cinnamon	Yes	2.7 ± 0.16 × 12.1 ± 0.58	2.5 ± 0.03 × 6.6 ± 0.15	AB649306	AB663116	AB663128
Mie-1	Taki, Mie, 20 Oct. 2009	White	No	2.8 ± 0.05 × 12.2 ± 0.94	2.6 ± 0.03 × 7.6 ± 0.21	AB649307	AB663117	AB663129
Mie-2	Watarai, Mie, 20 Oct. 2009	Cinnamon	Yes	2.6 ± 0.06 × 12.3 ± 0.57	2.4 ± 0.04 × 6.6 ± 0.16	AB649308	AB663118	AB663130
Mie-41	Kameyama, Mie, 11 Mar. 2010	Cinnamon	Yes	2.4 ± 0.12 × 10.2 ± 0.64	2.5 ± 0.06 × 6.2 ± 0.15	AB649309	AB663119	AB663131

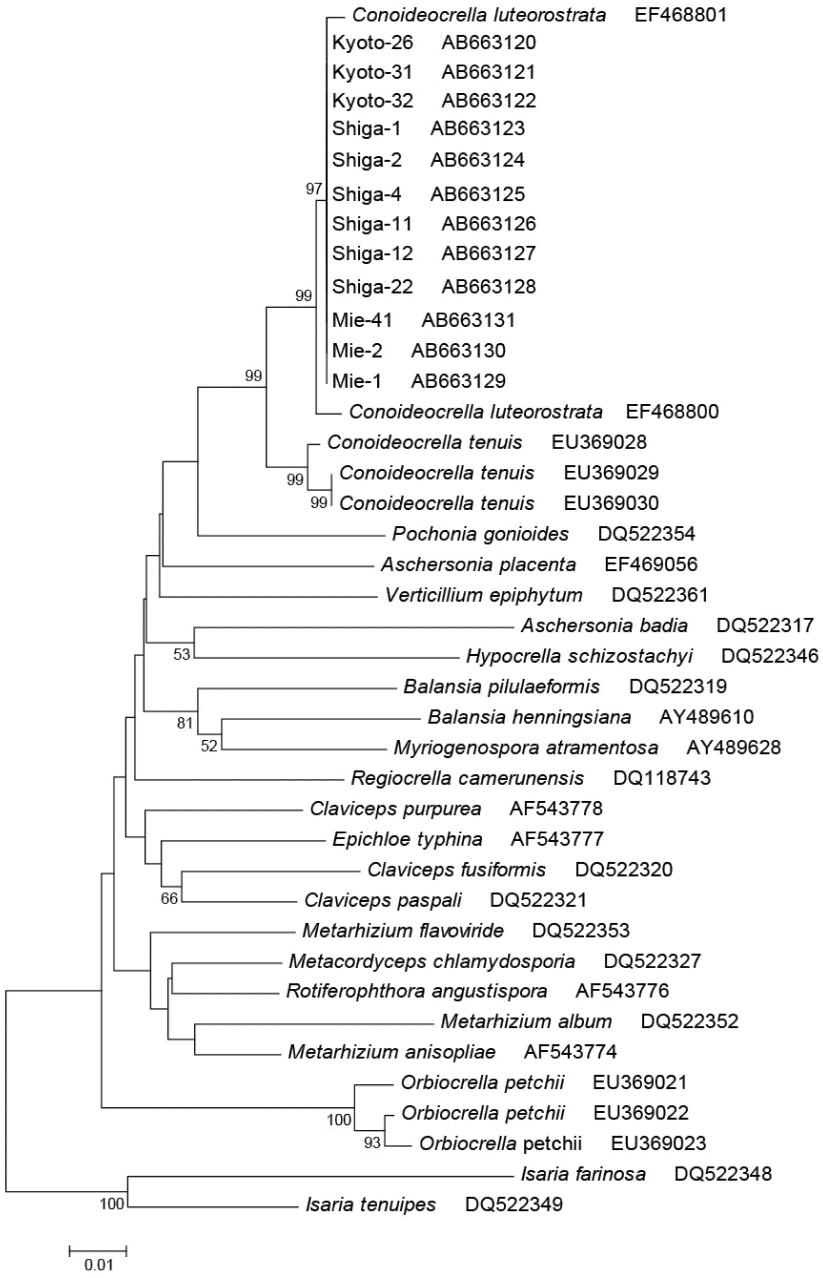
Table 2. List of specimens used in phylogenetic analyses

Specimens	Host taxon	GenBank accession no.		
		ITS rDNA	β-tubulin gene	TEF 1α gene
Aschersonia badia	Scale insect (Hemiptera)			DQ522317
A. placenta	Scale insect (Hemiptera)			EF469056
Balansia henningsiana	*Panicum* sp. (Poaceae)			AY489610
B. pilulaeformis	Poaceae			DQ522319
Chamaeleomyces viridis	*Chameleo lateralis* (Chamaeleonidae)	AY624197	AY624235	
	Chameleo lateralis (Chamaeleonidae)		AY624236	
Claviceps fusiformis	Poaceae			DQ522320
C. paspali	Poaceae			DQ522321
C. purpurea	*Dactylis glomerata* (Poaceae)			AF543778
Conoideocrella luteorostrata	Hemipteran insect	AY624206	AY624237	
	Scale insect (Hemiptera)			EF468800
	Scale insect (Hemiptera)			EF468801
C. tenuis	Scale insect (Hemiptera)			EU369028
	Scale insect (Hemiptera)			EU369029
	Scale insect (Hemiptera)			EU369030
Epichloe typhina	*Festuca rubra* (Poaceae)			AF543777
Hypocrella schizostachyi	Scale insect (Hemiptera)			DQ522346
Isaria farinosa	Soil	AY624179	AY624217	
	Lepidopteran pupa			DQ522348
I. tenuipes	Lepidopteran larva	AY624195	AY624233	
	Lepidopteran pupa			DQ522349
Metacordyceps chlamydosporia	Egg of slug (Diplopoda)			DQ522327
Metarhizium album	*Cofana spectra* (Hemiptera)			DQ522352
M. anisopliae	*Oryctes rhinoceros* (Coleoptera)			AF543774
M. cylindrosporae	Hemipteran adult	AY624204	AY624247	
			AF368270	
M. flavoviride	Hemipteran adult	AY624203	AY624248	
	Nilaparvata lugens (Hemiptera)		AY624249	DQ522353
Metarhizium sp.	Coleopteran larva		AY624246	
Myriogenospora atramentosa	*Andropogon virginicus* (poaceae)			AY489628
Nomuraea rileyi	*Trichoplusia ni* (Lepidoptera)	AY624205	AY624250	
Orbiocrella petchii	Scale insect (Hemiptera)			EU369022
	Scale insect (Hemiptera)			EU369023
	Scale insect (Hemiptera)			EU369021
Paecilomyces carneus	Soil	AY624170	AY624210	
	Dune sand	AY624171	AY624209	
P.cinnamomeus	Coccidae insect		AY624214	
	Living leaf of *Syzygium jambos* (Myrtaceae)	AY624174	AY624213	
P. marquandii	Soil	AY624193	AY624229	
		AB099511		
P. penicillatus	Rotting mushroom	AY624194	AY624232	
Paecilomyces sp.	*Aleurocanthus spiniferus* (Hemiptera)	AF368806		
Pochonia gonioides	Nematode			DQ522354
Purpureocillium lilacinum	*Aethus* sp. (Hemiptera)	AY624190		
	Egg mass of Meloidogyne		AY624228	
	Egg mass of Meloidogyne	AY624188		
	Soil	AY624189	AY624227	
	Cydnid bug (Hemiptera)		AY624226	
Regiocrella camerunensis	Scale insect (Hemiptera)			DQ118743
Rotiferophthora angustispora	Rotifer (Rotifera)			AF543776
Verticillium epiphytum	*Hemileia vastatrix* (Uredinales)			DQ522361

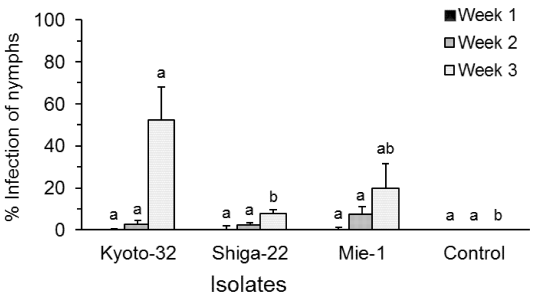

A
B
1 mm
C
10 µm
D
5 µm


Conoideocrella luteorostrata AY624206
Paecilomyces sp. AF368806
Kyoto-26 AB649298
Kyoto-31 AB649299
Kyoto-32 AB649300
Shiga-1 AB649301
Shiga-2 AB649302
Shiga-4 AB649303
Shiga-11 AB649304
Shiga-12 AB649305
Shiga-22 AB649306
Mie-41 AB649309
Mie-2 AB649308
Mie-1 AB649307
Paecilomyces cinnamomeus AY624174
Paecilomyces carneus AY624170
Paecilomyces carneus AY624171
Metarhizium flavoviride AY624203
Nomuraea rileyi AY624205
Paecilomyces marquandii AB099511
Paecilomyces marquandii AY624193
Chamaeleomyces viridis AY624197
Metarhizium cf. cylindrosporae AY624204
Metarhizium cylindrosporae AF368270
Paecilomyces penicillatus AY624194
Purpureocillium lilacinum AY624188
Purpureocillium lilacinum AY624189
Purpureocillium lilacinum AY624190
Isaria tenuipes AY624195
Isaria farinosa AY624179
86
99
90
99
85
97
99
54
76
99
0.02

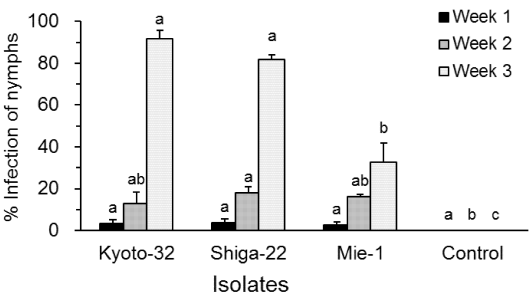

Shiga 22 AB663116
53
Shiga-1 AB663111
Mie-2 AB663118
Conoideocrella luteorostrata AY624237
58
Mie-1 AB663117
Shiga-12 AB663115
Paecilomyces cinnamomeus AY624213
94
Shiga-4 AB663113
Shiga-11 AB663114
Kyoto-31 AB663109
70
100
Mie-41 AB663119
73
Kyoto-26 AB663108
71
Shiga-2 AB663112
Kyoto-32 AB663110
Paecilomyces cinnamomeus AY624214
Purpureocillium lilacinum AY624227
100
Purpureocillium lilacinum AY624228
Purpureocillium lilacinum AY624226
Metarhizium sp. AY624246
Metarhizium flavoviride AY624248
Metarhizium flavoviride AY624249
58
Metarhizium cf. cylindrosporae AY624247
Nomuraea rileyi AY624250
100
Paecilomyces carneus AY624210
Paecilomyces carneus AY624209
Paecilomyces marquandii AY624229
Chamaeleomyces viridis AY624235
100
Chamaeleomyces viridis AY624236
Isaria tenuipes AY624233
100
Isaria farinosa AY624217
0.02


Conoideocrella luteorostrata EF468801
Kyoto-26 AB663120
Kyoto-31 AB663121
Kyoto-32 AB663122
Shiga-1 AB663123
Shiga-2 AB663124
Shiga-4 AB663125
Shiga-11 AB663126
Shiga-12 AB663127
Shiga-22 AB663128
Mie-41 AB663131
Mie-2 AB663130
Mie-1 AB663129
Conoideocrella luteorostrata EF468800
Conoideocrella tenuis EU369028
Conoideocrella tenuis EU369029
Conoideocrella tenuis EU369030
Pochonia gonioides DQ522354
Aschersonia placenta EF469056
Verticillium epiphytum DQ522361
Aschersonia badia DQ522317
Hypocrella schizostachyi DQ522346
Balansia pilulaeformis DQ522319
Balansia henningsiana AY489610
Myriogenospora atramentosa AY489628
Regiocrella camerunensis DQ118743
Claviceps purpurea AF543778
Epichloe typhina AF543777
Claviceps fusiformis DQ522320
Claviceps paspali DQ522321
Metarhizium flavoviride DQ522353
Metacordyceps chlamydosporia DQ522327
Rotiferophthora angustispora AF543776
Metarhizium album DQ522352
Metarhizium anisopliae AF543774
Orbiocrella petchii EU369021
Orbiocrella petchii EU369022
Orbiocrella petchii EU369023
Isaria farinosa DQ522348
Isaria tenuipes DQ522349
97
99
99
99
99
53
81
52
66
100
93
100
0.01

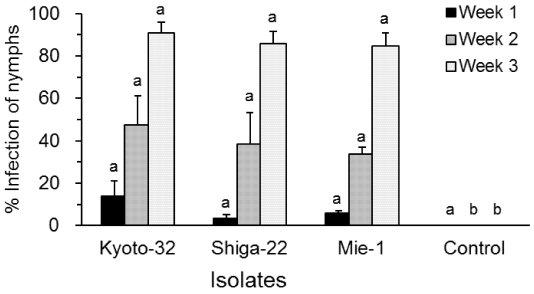
1 × 10^6 conidia/ml



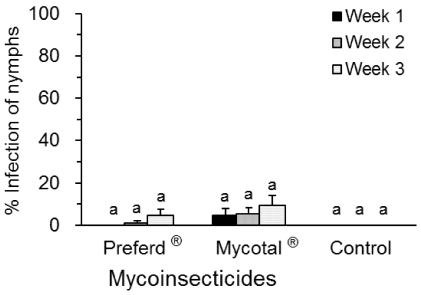
1 × 10^7 conidia/ml

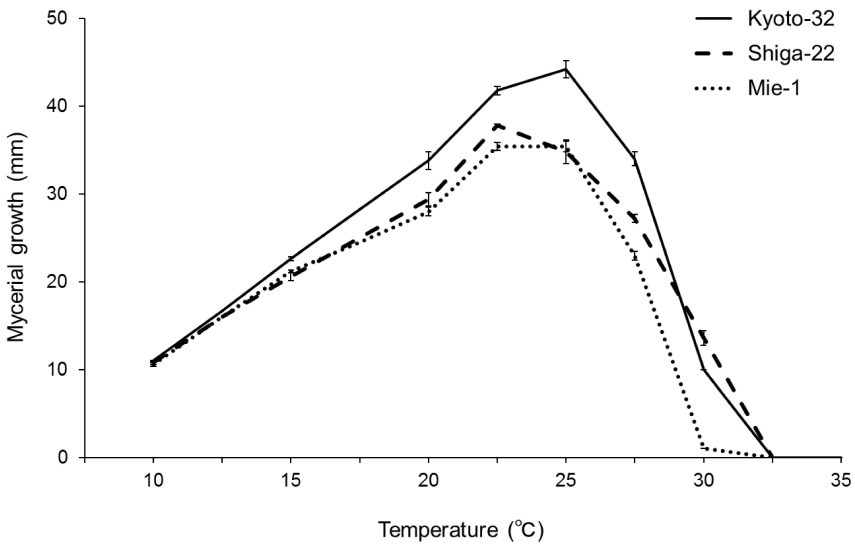


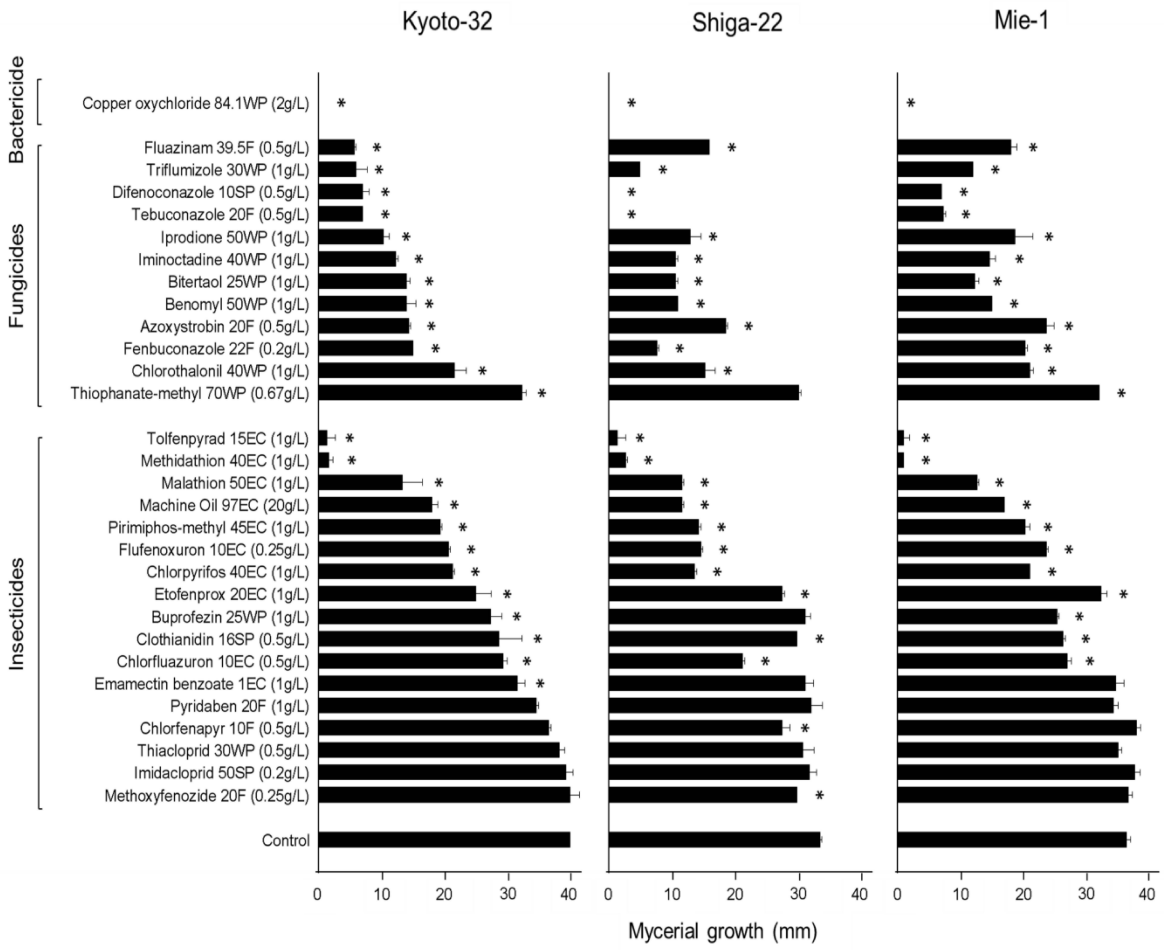
1 × 10^8 conidia/ml



Field application rates




Kyoto-32
Shiga-22
Mie-1
Mycerial growth (mm)
0
10
20
30
40
50
10
15
20
25
30
35
Temperature (℃)


Kyoto-32
Shiga-22
Mie-1
Bactericide
Fungicides
Insecticides
Copper oxychloride 84.1WP (2g/L)
Fluazinam 39.5F (0.5g/L)
Triflumizole 30WP (1g/L)
Difenoconazole 10SP (0.5g/L)
Tebuconazole 20F (0.5g/L)
Iprodione 50WP (1g/L)
Iminoctadine 40WP (1g/L)
Bitertaol 25WP (1g/L)
Benomyl 50WP (1g/L)
Azoxystrobin 20F (0.5g/L)
Fenbuconazole 22F (0.2g/L)
Chlorothalonil 40WP (1g/L)
Thiophanate-methyl 70WP (0.67g/L)
Tolfenpyrad 15EC (1g/L)
Methidathion 40EC (1g/L)
Malathion 50EC (1g/L)
Machine Oil 97EC (20g/L)
Pirimiphos-methyl 45EC (1g/L)
Flufenoxuron 10EC (0.25g/L)
Chlorpyrifos 40EC (1g/L)
Etofenprox 20EC (1g/L)
Buprofezin 25WP (1g/L)
Clothianidin 16SP (0.5g/L)
Chlorfluazuron 10EC (0.5g/L)
Emamectin benzoate 1EC (1g/L)
Pyridaben 20F (1g/L)
Chlorfenapyr 10F (0.5g/L)
Thiacloprid 30WP (0.5g/L)
Imidacloprid 50SP (0.2g/L)
Methoxyfenozide 20F (0.25g/L)
Control
0
10
20
30
40
Mycerial growth (mm)