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論文 (Original Article)

Fungal Pathogens of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and Their Virulences[*1]

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Abstract

Entomopathogenic fungi were isolated from infected insects obtained in the field and by the bate method from soil samples in the Ningxia Hui Autonomous Region, China, to develop microbial control of *Anoplophora* (Coleoptera: Cerambycidae)that is attacking poplars in Ningxia. *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces* spp. were isolated from the specimens collected in Ningxia. All isolates from Ningxia were inoculated to *A. glabripennis* larvae for the primary screening of pathogenic isolates with conidial suspensions of 1×10^7 /ml, and several isolates of *B. bassiana* and an isolate of *M. anisopliae* among them had strong pathogenicities. Virulences of 2 isolates from Japanese Cerambycid were calculated by inoculation of various concentration of conidial suspensions with adults and larvae of *Anoplophora glabripennis*. *Beauveria brongniartii* F-1101 from *Anoplophora malasiaca* in Japan showed a very strong virulence against the adults, but weak virulence against the larvae. Virulence of *B. bassiana* F-263 from *Monochamus alternatus* in Japan against the adults was moderate and the same as that against larvae. Using F-1101 against the adults is thought to be promising for control of *A. glabripennis*.

Key words : *Anoplophora glabripennis*, *Anoplophora nobilis*, *Beauveria bassiana*, *Beauveria brongniartii*, microbial control

Introduction

In Ningxia Hui Autonomous Region, China, damage caused by insects, especially cerambycids, has been a serious problem for *Populus* planted for afforestation of deserts. Two species of *Anoplophora* (Coleoptera: Carambycidae), *A. glabripennis*, and *A. nobilis* have caused particularly heavy damage on *Populus* and many trees have been killed (Ningxia Forest editorial committee, 1990). Microbial control may be one way of controlling these insects. In several provinces of China, such as Anhui, Hubei, Hunan, Shanxi, and Gansu, experimental control of poplar-damaging cerambycids have been made using entomogenous fungi, but, microbial control has not been established as a practical control method (Li and Wu, 1993). On the other hand, in Ningxia, most insect pathogens have not ever been identified, and no effective controlling agents against poplar pests have been discovered yet. We therefore collected diseased insects in Ningxia to obtain fungi pathogenic to *Anoplophora*, the most serious pest insect. Entomopathogenic fungi in soil were isolated by burying *Anoplophora* larvae in soil samples. Also, virulences of the isolates, including Japanese ones, were evaluated in the laboratory to use as a microbial control agent of *Anoplophora*.

Materials and Methods

Isolation from diseased insects

Cadavers of *A. glabripennis* and *A. nobilis* those seemed to be fungal diseases collected in the field, were kept in a refrigerator before isolation. Cadavers obtained during rearing were kept at room temperature to allow outer mycerial growth and sporulation.

To extract entomopathogenic fungi existing in soil, the bate method was employed (Shimazu, 1993). The bates for

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Table 1. Infections obtained by bate method.

Collection sites of soil samples	No. of insects		Pathogen[a]	Days to death
	Buried	Infected		
Erlonghe, Liupanshan, Jingyuanxian	4	0	—	—
Shizixiang, Jingyuanxian	4	3	Bb × 3	15 — 17
Kongdongshan, Gansusheng	4	0	—	—
Sanying, Guyuanxian A	4	0	—	—
Sanying, Guyuanxian B	4	2	Ma, bacteria	34

a: Bb=*Beauveria bassiana*, Ma=*Metarhizium anisopliae.*

this method were larvae of *A. glabripennis* and *A. nobilis* obtained by dissecting poplar trunks in Yinchuan City, Ningxia. Soil samples were collected at the various sites in Ningxia shown inTable 1. They were brought back to the laboratory, and kept in a refrigerator before use. Each soil sample was placed in 4 plastic petri dishes of 35 mm in diameter and a small amount of sterilized water was added to the dish. One bate larva was placed in each dish and the dishes were kept at room temperature. Each bate larva was taken out of the dish after 24 h of burying, transferred to a test tube of 18mm × 180 mm with cheese cloth covering, and fed pieces of twig of *Populus opera*. These larvae were checked daily for mortality, and dead ones were placed in 35 mm petri dishes with moistened filter paper after 2-3 days of drying in the tube.

To isolate the fungus, SDY medium (Sabouraud's dextrose medium supplemented with 1% of east extract) with 10,000 U / 100 ml of penicillin and 10 mg / 100 ml of streptomycin was prepared in 35 mm petri dishes. Conidia of the pathogenic fungi formed on the cadavers were taken by a mycological loop and streaked on SDY plates. After incubation at room temperature around 25℃ for a week, the obtained colonies were transferred to slant medium of SDY for preservation. The isolates were identified by microscopically inspecting the conidia-forming mycelia for conidiogenous structure and conidial morphology according to Samson et al. (1988) and Aoki (1989).

Pathogenicities of the isolates to *Anoplophora glabripennis*

Mortalities of larvae inoculated with the isolates from Ningxia in a single conidial concentration were compared. The isolates shown in Table 2 were cultured on SDY medium for 14 days at 25℃, and conidia were suspended at a concentration of 1×10^7 /ml in sterilized water. Larvae of *A. glabripennis* grown enough for the experiment were collected by dissecting field-infested trees or reared on an artificial diet (Ogura, 2000). The larvae were dipped into the conidial suspension for approximately 30 seconds, drained of excess suspension by filter paper, and reared with a method described later to investigate mortality. Ten to 12 larvae were used for each isolate.

Two Japanese isolates from cerambycid insects were inoculated in various conidial concentrations to calculate LC_{50} for larvae and adults of *A. glabripennis*. *Beauveria bassiana* F-263 (from larva of *Monochamus alternatus* from Kumamoto Prefecture, Japan) and *B. brongniartii* F-1101 (from adult *Anoplophora malasiaca* from Ibaraki Prefecture, Japan)were used. These fungi were cultured on SDY medium for more than 3 weeks at room temperature to produce conidia. Larvae and adults of *A. glabripennis* were collected at Yinchuan, Ningxia. Conidia of fungi were suspended in a 200ppm aqueous solution of Tween 80, and diluted to make concentrations of 10^3, 10^4, 10^5, 10^6, and 10^7 conidia/ml. The control consisted of same solution without

Table 2. Isolates from Ningxia used for inoculation.

F-0001	*Beauveria bassiana*	*A. glabripennis* (L)	Shizuishan, Ningxia
F-0002	*Beauveria bassiana*	*A. nobilis* (A)	Guyuan, Ningxia
F-0003	*Beauveria bassiana*	soil → *A. glabripennis* (L)	Jingyuan, Ningxia
F-0004	*Beauveria bassiana*	soil → *A. glabripennis* (L)	Jingyuan, Ningxia
F-0005	*Beauveria bassiana*	soil → *A. glabripennis* (L)	Jingyuan, Ningxia
F-0006	*Metarhizium anisopliae*	soil → *A. glabripennis* (L)	Guyuan, Ningxia
F-0007	*Beauveria bassiana*	unknown insect	Yinchan, Ningxia
F-0008	*Paecilomyces* sp.	unknown insect	Yinchan, Ningxia
F-0009	*Paecilomyces* sp.	unknown insect	Yinchuan, Ningxia
F-0011	*Beauveria bassiana*	*A. glabripennis* (A)	Huinong, Ningxia
F-0012	*Paecilomyces* sp.	*A. glabripennis* (A)	Yinchan, Ningxia
F-0013	*Paecilomyces* sp.	*A. nobilis* (L)	Pingluo, Ningxia

a : L=Larva, A=Adult, soil→ =burying a larva in soil sample.

the fungus. The insects were dipped in these suspensions for approximately 30 seconds and drained of excess suspension by filter paper. Inoculated larvae were placed into individual test tubes with pieces of twigs of *Populus opera*, and the adults were put into individual plastic cups with leaves and twigs of *P. opera*. Those insects were kept at room temperature which was around 25℃, and checked daily for mortality. For one conidial concentration of each fungus, 25 larvae and 30 adults were used.

When the experimental insects died, the twigs and leaves were removed, the bodies were air dried for 3 days, and then moistened by placing wet filter paper or wet absorbent cotton to promote mycelial growth and conidial production. Fungus on each cadaver was checked with a microscope to identify whether it was the inoculated fungus or not.

Results

Isolation and identification of fungi

After rearing *A. glabripennis* larvae in soil samples from various regions, infections with entomopathogenic fungi were obtained from 2 of the 5 soil samples (Table 1). They were identified as *Beauveria bassiana* and *Metarhizium anisopliae* by their morphological features. Both are commonly known entomopathogenic fungi, and have been detected from soil specimens (Beilharz, et al.,1982; Doberski and Tribe, 1980; Liu et al., 1993; Mietkiewski et al., 1991; Mietkiewski, et al., 1992; Sato et al.,1994). An especially high 75% of the larvae reared in the samples from Shizixiang, Jingyuanxian were infected with *B. bassiana*, and this indicated that a considerably high density of *B. bassiana* was contained in this soil.

Fungi shown in Table 3 were isolated from the infected insects. This includes infected larvae obtained by burying in soil samples. *B. bassiana* isolates from soil samples produced a red pigment that stained the SDY medium into a wine red. Isolates of *Beauveria* from soil or soilborne insects often produce such red pigments (Shimazu et al., 1984).

Pathogenicities of isolates to *Anoplophora*

Mortalities of larvae inoculated with the isolates from Ningxia are shown in Table 4. Among the tested isolates, *B. bassiana* F0001, F0003, F0004, and F0005, and *M. anisopliae*, F0006 produced relatively high mortality. Species of *Paecilomyces* showed only slight mortality, even those inoculated with a high concentration of 10^7

Table 3. Entomopathogenic fungi isolated from infected insects.

Species	*Anoplophora glabripennis*		*Anoplophora nobilis*	
	Adult	Larva	Adult	Larva
Beauveria bassiana	+	+		
Metarhizium anisopliae		+		
Paecilomyces sp.1	+			
Paecilomyces sp.2				+

Table 4. Mortalities of *Anoplophora glabripennis* larvae by isolates from Ningxia.

Isolate	Insects used		Days after inoculation					LT_{50}[a]
	number	source	10	20	30	40	50	
F0001	11	field-collected	9.1	63.6	63.6	63.6	63.6	17.7
F0002	11	field-collected	0.0	9.1	36.4	45.5	54.5	42.5
F0006	10	field-collected	10.0	70.0	70.0	70.0	80.0	14.0
Control	10	field-collected	0.0	0.0	0.0	0.0	0.0	-
F0003	12	artificial diet	16.7	75.0	100.0	100.0	100.0	16.0
F0004	12	artificial diet	8.3	50.0	66.7	83.3	83.3	17.0
F0005	12	artificial diet	0.0	25.0	41.7	58.3	58.3	32.0
F0007	12	artificial diet	0.0	0.0	0.0	0.0	0.0	-
F0008	12	artificial diet	0.0	0.0	0.0	0.0	0.0	-
F0009	12	artificial diet	0.0	0.0	0.0	0.0	0.0	-
F0011	12	artificial diet	0.0	0.0	0.0	0.0	0.0	-
F0012	12	artificial diet	0.0	0.0	8.3	8.3	8.3	>60
F0013	12	artificial diet	0.0	0.0	0.0	0.0	0.0	-
Control	12	artificial diet	0.0	0.0	0.0	0.0	0.0	-
F-263	25	field-collected	16.0	60.0	72.0	72.0	72.0	17.5

a : Median lethal time in days.

Table 5. Median lethal time in days for gross and net mortalities of *Anoplophora glabripennis* by Japanese isolates of *Beauveria* spp.

Isolate	Conidial concentration (/ml)	Larvae		Adults	
		Gross	Net	Gross	Net
control	0	>29	—	10.7	—
F-263	10^3	>29	>29	9.0	>19
	10^4	>29	>29	10.0	>19
	10^5	>29	>29	8.8	>19
	10^6	>29	>29	10.0	>19
	10^7	14.8	17.5	8.1	10.0
F-1101	10^3	>29	>29	10.5	>19
	10^4	>29	>29	9.5	>19
	10^5	>29	>29	8.3	11.0
	10^6	>29	>29	6.8	8.9
	10^7	19.5	>29	7.1	8.1

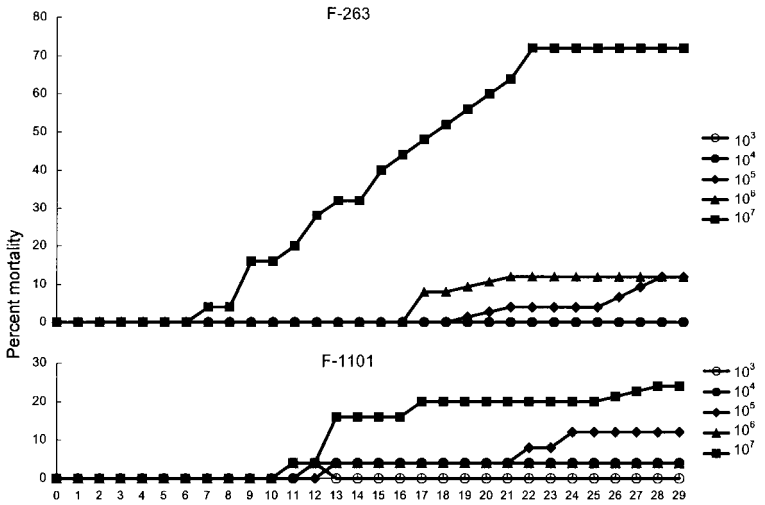

Fig. 1. Accumulated net mortalities of *Anoplophora glabripennis* larvae inoculated with various conidial concentrations of *Beauveria bassiana* F-263 and *Beauveria brongniartii* F-1101.

conidia/ml. Inoculation experiments to larvae were performed in 2 different periods, and the experimental insects included both field-collected and ilaboratory-reared ones. However, no tendency of mortality specific to the larval origins was found.

The Japanese isolates of *B. bassiana* and *B. brongniartii* showed pathogenicity to larvae and adults of *A. glabripennis* (Table 5, Fig. 1, 2). No mycosis was found on insects of the control plot, and there was apparently no fungal contamination among the wild populations used as the experimental insects. Only the larvae inoculated with 10^7 conidia/ml of F-263 were killed at a high rate of mortality, and mortalities of those inoculated with less than 10^6 conidia/ml of F-263 and F-1101 were low (Fig. 1). No larva molted within a few days after inoculation, and the inoculated fungi were thought to have completed penetration into larval hemocoel, although some molted many days after the inoculation.

On the other hand, many adults inoculated with F-1101 were infected, while those inoculated with F-263 were killed in moderate numbers (Fig. 2). The gross mortalities in Table 5 are the mortalities calculated regardless of outer mycelial growth after incubation in a humid chamber, whereas the net mortalities are calculated based on the number of insects confirmed with outer growth of the inoculated fungus after incubation in a humid chamber. More than half of the adults died after 10 days of rearing regardless of the inoculum concentration, and the median lethal period for gross mortality of adults was around 10 days. Since the adults were collected in the field, their age at the moment of collection was unknown and thought to be uneven. Thus, their life spans were thought to be considerably short. However, net mortality of the inoculated adults increased after around day 6.

The median lethal concentrations (LC_{50}) were calculated from net mortalities to evaluate the virulence of the fungi (Table 6). Those values were calculated from net mortalities after the end of the respective experimental periods: 29 days for larvae and 19 days for adults. Since the highest concentration of F-1101 did not produce more

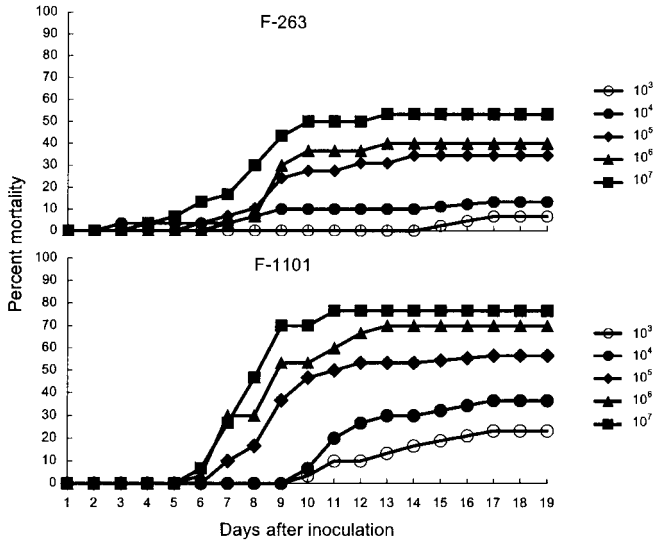

Fig. 2. Accumulated net mortalities of *Anoplophora glabripennis* adults inoculated with various conidial concentrations of *Beauveria bassiana* F-263 and *Beauveria brongniartii* F-1101.

Table 6. Median lethal concentration [a] of F-263 and F-1101 against *Anoplophora grablipennis*.

Isolate	Larvae[b]	Adults[c]
F-263	4.1×10^6 (1.0×10^6 - 1.7×10^7)	4.1×10^6 (3.4×10^5 - 5.0×10^7)
F-1101	5.0×10^8	6.2×10^4 (1.5×10^4 - 2.5×10^5)

a: Mean conidial concentration /ml with 95% confidence intervals in parentheses.
b: Calculated from net mortalities at the day 29 for larvae.
c: Calculated from net mortalities at the day 19 for adults.

than 50% mortality in larvae, 95% confidence intervals could not be calculated.

Discussions

Li and Wu (1993) reported *Aspergillus* sp., *B. bassiana*, *Cephalospoium* sp., *Crinula* sp., *Chaetomium funicolum*, *Fusarium* spp., *M. anisopliae*, *Penicillium* sp., *Scopulariopsis brevicaulis*, *Syngliocladium* sp., and *Verticillium* sp. as fungal pathogens of *Anoplophora*. Among these, *Cephalosporium* (=*Acremonium*, Gams; 1971), *Aspergillus*, *Beauveria*, *Fusarium*, *Metarhizium*, *Verticillium* include entomopathogenic species (Samson et al., 1988), but other fungi could have grown on insect bodies as saprophytes. In the present study, *Paecilomyces* species were isolated from larvae of *A. nobilis*. There have already been records of isolating *Paecilomyces* fungi from other species of Cerambycidae (Soper and Olson, 1963; Kunimi, 1993), but, there has been no record of the genus *Anoplophora*. Although these *Paecilomyces* have weak virulence, Li and Wu (1993) stated that an isolate of *Paecilomyces farinosus* was infectious to *A. glabripennis* when inoculated. Thus, it will be possible to find isolates or species of *Paecilomyces* that have pathogenicity against this insect.

In the present experiments, there were numerous examples of insects which had been inoculated with a fungus but did not show outer growth of mycelia even after being moistened. It is not rare for an entomopathogenic fungus not to proliferate in insect hemocoel for some reason, but contaminants such as enteric bacteria can spread in the hemolymoph even if the insect has been inoculated with a pathogenic fungus and killed by the fungal pathogenicity (Shimazu, 1994). In such a case, the fungus cannot sporulate on the insect body, so that type of cadaver is not counted as a net mortality. The same phenomenon perhaps occurred in the present study, and the true virulences of fungi probably exceeded the net mortality obtained in the experiment. The ages of adults at the moment of inoculation were unknown and could have been uneven because they were captured in the wild, nevertheless, the lives of wild adults are thought to be considerably short, since more than half died within 10 days of rearing regardless of inoculum size.

The LC_{50} of *B. brongniartii* F-1101 to adults of *A. glabripennis* was around 10^4 conidia/ml which can be said to be very strong, even when calculated from the net mortality. However, LC_{50} of this fungus to the larvae was more than 10^8 conidia/ml, and was only 1/10000 of that to the adults. Generally, larvae are more susceptible to fungi than adults, which have thicker cuticles than the larvae. Our result of higher susceptibility of adults was unique and

interesting. It is not clear that this phenomenon is common with *Anoplophora malasiaca*, which is the origin of isolate F-1101, since the virulence of the cerambycid-parasitic type of *B. brongniartii* on *A. malasiaca* larvae has not been investigated.

In contrast, LC_{50} of *B. bassiana* F-263 did not differ between adults and larvae, with both being around 10^6 to 10^7 conidia/ml. This value was stronger than that of *B. brongniartii* F-1101 to the larvae. However, virulence of F-263 to *Monochamus alternatus*, the original host, was far stronger; even when calculated from the net mortality, it was 2×10^3 conidia/ml (Shimazu, 1994) and 1000 times more infectious than to *A. glabripennis*.

On the other hand, isolates from Ningxia varied in their virulences; although their inoculation experiments were conducted at a single conidial concentration of 1 x 10^7 /ml. Among the 12 isolates tested, *B. bassiana* F0003 isolated from soil produced higher mortality of *A. glabripennis* larvae than F-263 did, and was the strongest. This isolate may have had higher virulence to *A. glabripennis* larvae, so further evaluation of virulence deriving LC_{50} will be necessary. Most of the other *B. bassiana* isolates caused little or no mortality in *A. glabripennis* larvae. *M. anisopliae* F0006 produced nearly the same mortality that F-263 did. *Paecilomyces* sp., F0012 killed only one larva and F0013 did not cause any mortality, even when dipped in a high conidial concentration of 10^7 /ml, and their pathogenicity to *A. glabripennis* was thought to be weak.

According to De Hoog (1972), which is the most popular key to identify the genus *Beauveria*, all species of *Beauveria* having oval conidia are *B. brongniartii*. However, detailed observation revealed that *Beauveria* species identified as *B. brongniartii* can be divided into 2 types which Shimazu (1994) called the cerambycid-parasitic type and the scarabaeid-parasitic type. The cerambycid-parasitic type of *B. brongniartii* has very weak pathogenicity to the silkworm, *Bombyx mori*, while it has a strong pathogenicity to the adults of *Psacothea hilaris* (Kawakami, 1978) and *Anoplophora malasiaca* (Kashio and Ujiie, 1988). This fungus therefore has been studied for utilization as a microbial control agent of these cerambycid beetles (Hashimoto,1989; Kashio and Tsutsumi, 1990; Tsutsumi et al., 1990), and the fungus has been commercially sold as a mycoinsecticide in Japan.

In the present study, the cerambycid-parasitic type of *B. brongniartii* F-1101 isolated from *A. malasiaca* in Japan was found to have a strong pathogenicity against the Chinese species of this genus, *A. glabripennis*. Although this fungus showed a low pathogenicity to the larvae of this insect, there is little need to consider them as the target stage, because they live deep in the wood and cannot easily make contact with the fungus. However, at its larval stage this insect injures the wood, and when the target stage of the control is adults, the control effect can be induced by the fertility of the adults. Therefore, it is necessary for the fungus to kill the adults before they complete oviposition; consequently, the oviposition curve of this insect must be clarified for the correct evaluation of this fungus. A few isolates of *B. bassiana* had relatively strong pathogenicity to the larvae of *A. glabripennis*, so they should be considered when the larvae must be controlled.

These experiments were carried out in the laboratory; and field experiments examining the pathogenicity of the isolates in a desert environment should be done in Ningxia.

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ツヤハダゴマダラカミキリの病原糸状菌とその病原力

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要　旨

中国，寧夏回族自治区でポプラを加害するゴマダラカミキリ類の微生物的防除法を開発するため，野外採集および土壌からの釣り餌法で得た感染虫から，昆虫病原糸状菌を分離した。寧夏産の標本から*Beauveria bassiana*，*Metarhizium anisopliae*，*Paecilomyces* spp.などを分離した。寧夏のすべての分離菌について，ツヤハダゴマダラカミキリに対する病原性を調べるため，1×10^7 /mlの濃度で一次スクリーニングしたところ，*B. bassiana*の数株と*Metarhizium anisopliae*の１株が強い病原力を有していた。カミキリムシに強い病原力を持つことがわかっている２株の日本産の菌の，ツヤハダゴマダラカミキリに対する病原力を明らかにするため，数種濃度段階の懸濁液を，幼虫と成虫に接種して半数致死濃度を求めた。日本のゴマダラカミキリ由来の*Beauveria brongniartii* F-1101は，成虫に対し非常に強い病原力があったが，幼虫に対する病原力は弱かった。日本のマツノマダラカミキリ由来の*B. bassiana* F-263は幼虫にも成虫にも中程度の病原力を有していた。ツヤハダゴマダラカミキリ成虫の微生物的防除にはF-1101の利用が有望であると考えられた。

キーワード：ツヤハダゴマダラカミキリ，キボシゴマダラカミキリ，昆虫病原糸状菌，微生物的防除

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