Biological Control **22,** 51–59 (2001)
doi:10.1006/bcon.2001.0950, available online at http://www.idealibrary.com on IDEAL®

Interactions among the Entomopathogenic Fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes), the Parasitoid, *Aphelinus asychis* (Hymenoptera: Aphelinidae), and Their Aphid Host

Antonio L. M. Mesquita*,† and Lawrence A. Lacey*,‡,[1]

**European Biological Control Laboratory, USDA/ARS, Campus International de Baillarguet, CS 90013, Montferrier sur Lez, 34980 St. Gely du Fesc, Cedex, France; †EMBRAPA/CNPAT, Rua Dra. Sara Mesquita, N 2270, Bairro Pici, Cx. Postal 3761, 60511-110 Fortaleza, Ceará, Brazil; and ‡USDA-ARS-Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, Washington 98951*

Received August 16, 2000; accepted April 2, 2001

The interactions among the hyphomycete, *Paecilomyces fumosoroseus,* the Russian wheat aphid, *Diuraphis noxia,* and its common parasitoid, *Aphelinus asychis,* were investigated under laboratory conditions to determine if fungal infection of the aphid host had an effect on oviposition and feeding behavior of the female parasitoid and on development of parasitoid progeny. Aphids were first treated with 2 times the LD_{95} of *P. fumosoroseus* for *D. noxia* third instars (5.2×10^4 conidia/cm^2). At various intervals afterward they were exposed to 4- to 5-day-old mated parasitoid females for 1 h. Various combinations of treatments were examined: exposure to *P. fumosoroseus* and parasitization simultaneously; exposure to parasitoids 24–96 h after treatment with fungus; exposure to the parasitoid alone; and fungal treatment alone. The average number of aphids probed by the parasitoids was not significantly influenced by host infection with *P. fumosoroseus,* but duration of ovipositor insertion was influenced by the length of the time interval between exposure to *P. fumosoroseus* and subsequent exposure to the parasitoid. Parasitoid females spent considerably less time with their ovipositor inserted in dead aphids and in aphids that had been exposed to *P. fumosoroseus* 72 h prior to contact with the parasitoids. The number of dead aphids fed upon by parasitoids was significantly less than in the other treatment groups. The percentage of successfully parasitized *D. noxia* was significantly reduced as a function of the time between treatment with *P. fumosoroseus* and parasitoid oviposition. The effect of previous parasitization on the ability of the fungus to infect aphids and interfere with parasitoid development within host aphids was also investigated. The number of mummies produced by two female *A. asychis* during 24 h of exposure varied from 20.3 to 23.0 and was not significantly different when the aphids were first exposed to the parasitoids and then treated with *P. fumosoroseus* 24, 48, 72, and 96 h after exposure. No difference in time between oviposition and emergence of the F1 generation of the parasitoid for groups treated with the fungus and the untreated groups was noted, but the percentage of emergence of the F1 generation of *A. asychis* was significantly lower among the aphids exposed to the parasitoid and treated with the fungus 24 h afterward than for the untreated aphids. Various findings of this study demonstrate the potential of synergistic interaction of *P. fumosoroseus* and *A. asychis* for the biological control of *D. noxia.*

***Key Words: Paecilomyces fumosoroseus; Diuraphis noxia; Aphelinus asychis;* Russian wheat aphid; microbial control; interaction.**

Competition between microorganisms and multicellular animals for nutrition and other requisites is pervasive throughout nature (Hochberg and Lawton, 1990). Despite the competition, there is evidence for mechanisms that reduce antagonistic interaction between natural enemies vying for common insect hosts (Hochberg and Lawton, 1990; Brooks, 1993). Several studies on fungal pathogens and parasitoids of homopterous insects have revealed the avoidance of fungal-infected hosts by ovipositing wasps and a degree of host immunity to fungal infection induced by parasitoid larvae within the host (Milner *et al.*, 1984; Powell *et al.*, 1986; Brobyn *et al.*, 1988; Poprawski *et al.*, 1992; Fransen and van Lenteren, 1994).

Aphelinus asychis Walker (Hymenoptera: Aphelinidae) is a common parasitoid of the Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae) and other aphids. In addition to ovipositing into aphids, *A. asychis* females immobilize aphids by stinging and subsequently feed upon their hemolymph and

[1] To whom correspondence should be addressed at USDA-ARS-YARL, 5230 Konnowac Pass Road, Wapato, WA 98951. Fax: (509) 454-5646. E-mail: llacey@yarl.ars.usda.gov.

1049-9644/01

other tissues for longevity and production of eggs (Bai and Mackauer, 1990b). The proportion of aphids killed by host feeding at times exceeds that due to parasitization (Mesquita *et al.*, 1997). Poprawski *et al.* (1992) reported protection of *D. noxia* from infection by the entomophthoralean fungus *Zoophthora radicans* (Brefeld) Batko after parasitization by *A. asychis*. The fungus, *Paecilomyces fumosoroseus* Brown and Smith (Deuteromycotina: Hyphomycetes), has been reported from whiteflies and over 40 other insects, but is not commonly reported from aphids (Samson, 1974; Smith, 1993; Lacey *et al.*, 1996). Laboratory and field studies revealed its potential for control of *D. noxia* in combination with *A. asychis* (Mesquita *et al.*, 1996, 1997). However, under conditions of high humidity, *P. fumosoroseus* can have direct negative impact on *A. asychis* (Lacey *et al.*, 1997).

The objective of this study was to determine if the same antiantagonistic mechanisms reported for hymenopterous parasitoids and fungal pathogens of a common host would also occur with a fungal pathogen and parasitoid that are not commonly found in nature associated with the same host. Specifically, we present information on the influence that the stage of infection of *D. noxia* by *P. fumosoroseus* can have on oviposition, feeding behavior, development time, percentage of emergence, sex ratio, longevity, and size of female *A. asychis*. We also investigated the effect of prior parasitization by the wasp on the ability of the fungus to produce subsequent infection and interfere with parasitoid development within host aphids.

MATERIALS AND METHODS

Fungus. The strain of *P. fumosoroseus* (ARSEF 3877) used for bioassays was isolated from *Bemisia tabaci* (Gennadius) in Multan, Pakistan (Lacey *et al.*, 1993). After passage of the fungus through *D. noxia*, it was grown on Sabouraud maltose-dextrose agar with yeast extract (SMDAY) and subsequently prepared for bioassay using the procedures described by Mesquita *et al.* (1996). The average viability of conidia used in the various tests was 94.7% as determined on SMDAY using standard techniques (Goettel and Inglis, 1997). This involved inoculating a 9-cm petri plate containing SMDAY medium with 100 μl of a suspension containing 10^6 conidia/ml and counting the number of germinated conidia/100 conidia in four separate areas on the plate after incubation at 24°C for 18–20 h. This process was performed for each separate batch of *P. fumosoroseus*. Applications of *P. fumosoroseus* were made with 2 ml aqueous suspensions of 12- to 14-day-old conidia (1.4×10^8 conidia/ml) as described by Mesquita *et al.* (1996) using a Potter spray tower (Potter, 1952). The concentration of conidial suspensions corresponds to an application of 5.2×10^4 conidia/cm^2 and is equal to two times the LD_{95} of *P. fumosoroseus* for third instar *D. noxia*.

Insects. Colonies of *A. asychis* and *D. noxia* derived from collections in the vicinity of Montpellier, France, were used for all tests. Detailed procedures for their production in the laboratory were as described by Mesquita *et al.* (1996, 1997). Briefly, this involved rearing *D. noxia* on barley plants (*Hordeum vulgare* L.) in ventilated plastic cages in incubators at 22–24°C, 55–65% relative humidity (RH), and photoperiod of 16:8 (L:D) until they matured to third instars and then exposing them to mated *A. asychis* females. Four- to 5-day-old *A. asychis* females were used for all experiments except those to determine longevity.

P. fumosoroseus on development, longevity, and fecundity of A. asychis when the host was first exposed to the parasitoid and then treated with the fungus. Four replicate tests were conducted on separate dates, each using five groups of 30 third instar aphids. Each group of aphids was placed on three barley leaves (5 cm long) the bases of which were inserted in pieces of moist cotton and exposed to two 4- to 5-day-old female *A. asychis* for 24 h. The exposure took place in a rectangular plastic box ($9 \times 6 \times 2$ cm) with a 2.5-cm-diameter hole in the lid covered with screen (0.1 mm mesh) for ventilation. After 24 h, the parasitoids were removed and the leaves bearing live aphids were transferred to petri dishes that had each been lined with a 9-cm-diameter filter paper. Parafilm (5×2.5 cm) prevented contact between the moist cotton and the filter paper. The petri dish covers were also ventilated with a screened hole. For each replicate test, one group of parasitized aphids (23–24 individuals) was sprayed with $2 \times LD_{95}$ *P. fumosoroseus* either 24, 48, 72, or 96 h after exposure to the parasitoids. The fifth group of parasitized aphids was treated with distilled water only. Additionally, four replicate dishes with 30 aphids each that were not exposed to parasitoids were sprayed with fungus only, and an equal number of dishes were treated with distilled water only. In the two latter cases, the insects from each repetition were sprayed over 4 consecutive days, coinciding with the treatment of aphids that had been parasitized and subsequently treated with the fungus. During the first 24 h following fungal treatment, the petri dishes were hermetically sealed with parafilm to maintain saturated humidity and enable germination. The covers were then replaced by others with mesh-covered apertures as described above to allow aeration and prevent condensation. The barley leaves were replaced with fresh ones every 3 days. Dead aphids were recorded daily for 1 week and were placed on water agar (3 g of agar/liter of water) to confirm infection by *P. fumosoroseus*. Mummies were placed individually into gelatin capsules and observed for 12 days or until the emergence of adult parasitoids.

To determine the effect of the fungus on longevity

and fecundity of the F1 generation of *A. asychis,* 20 females that emerged from aphids treated with the fungus 24 h after exposure to *A. asychis* were raised individually on barley plants (10–12 leaves, 10–15 cm long) infested with aphids. A cylindrical plastic cage (25 cm long × 3.8 cm in diam), with five screened ventilation windows (1.5 cm diam) were used to isolate the plants and insects. To study longevity, the females were counted daily and every 3 to 4 days the survivors were transferred to different cages onto aphid-infested plants. Mummies were counted 15 days after transfer of the females. The trial was conducted at a temperature of 22–24°C, 70 to 80% RH and 16-h photoperiod.

P. fumosoroseus on oviposition, feeding, and the development of A. asychis when the host was first treated with the fungus and then exposed to the parasitoid. Fifteen replicate tests were conducted, each using 240 third instar *D. noxia,* distributed equally into eight petri dishes on three barley leaves/dish. Six dishes were treated with 2 × LD_{95} of *P. fumosoroseus.* The aphids in each of four dishes were then exposed to a 4- to 5-day-old female *A. asychis* at different time intervals following application of the fungus. The following combinations of treatments were tested: exposure to the parasitoid 0, 24, 48, or 72 h after application of the fungus, exposure of parasitoids to aphids killed by fungal infection (maximum 24 h after death), and exposure to aphids sprayed with water only. Also, for each repetition of treatment with the fungus and parasitoid, 30 aphids were treated with the fungus only and an equal number were treated with water only.

As described in the previous experiment, during the first 24 h following treatments, the petri dishes were hermetically sealed. Then the cover was replaced with one having a screened opening. The petri dishes were placed into a plastic container (35 × 25 × 13 cm) at 82–87% RH, 22–24°C, and 16-h photoperiod. This humidity was obtained using a solution of 160 g potassium chloride per 400 ml of distilled water. The petri dishes were set on a plastic support 3.5 cm high which kept them above the solution.

One hour before exposure to aphids, female *A. asychis* were individually isolated into gelatin capsules, without food, to stimulate predation and oviposition when subsequently exposed to the aphids. The exposure time of the aphids to the parasitoid was 1 h, beginning with the first probing of aphids. The aphids were exposed on a barley leaf (5 cm long) placed at the center of a rectangular plastic box (9 × 6 × 2 cm). Twelve aphids were initially exposed to a female for each treatment. The number of aphids probed, the duration of probing, the type of probing (oviposition or feeding), and the time spent feeding were recorded for each female for each treatment. Parasitized aphids or aphids killed by predation were removed after the female parasitoid moved on to another aphid. The parasitized aphids were placed on barley leaves and then set into petri dishes as previously described. When five aphids remained in the exposure chamber, seven new aphids from the same treated lot were added. The exposures of the aphids to the parasitoids occurred between 0800 and 1700 h at 25°C under ambient room light.

The aphids in which apparent egg deposition took place were observed daily until death or mummification. The surviving aphids were transferred onto new leaves every 72 h whereas the dead ones were placed onto water agar to confirm infection. The total number of mummies produced per female was recorded for each treatment for a total of 15 females/treatment. The mummies were individually placed into gelatin capsules and observed daily for 12 days or until emergence of adults. The time from oviposition to mummification, from mummification to emergence, percentage emergence, sex ratio, and longevity of the emerged females were recorded. Morphometric measurements were also made on the lengths of the tibia of the metathoracic leg and the anterior costal wing vein of emerging wasps to determine if stress due to fungal invasion of the host during development of parasitoid larvae resulted in stunting in size. For comparative purposes, measurements were also made on wasps that developed in hosts that were not treated with fungus.

To determine longevity of emerging *A. asychis,* the females were fed only honey (60%) and kept in 3.5-cm petri dishes (22–24°C, 82–87% RH, 16-h photoperiod), the covers having a 1-cm-diameter screened ventilation opening through which the honey was administered. To measure the tibia and costal vein, the females were placed into a solution of 10% potassium hydroxide for 24 h and then mounted on a slide in Hoyer's medium. The measurements were taken using an ocular micrometer mounted on an Olympus microscope at 200× magnification.

Statistical analyses of the data. Analyses of variance (ANOVA) were used to test the effect of treatments on the number of mummies produced, the number of days from oviposition to emergence of the parasitoid, and percentage emergence of the F1 generation of *A. asychis* when the aphids were first exposed to the parasitoid then treated with the fungus. Percentage data were arcsine square-root transformed. The averages of the three variables were compared using Tukey's test at the 0.05 level. The comparison of longevity and the number of mummies produced per female/day from individuals that had emerged from fungus-treated aphids 24 h after exposure to the parasitoid and individuals that had emerged from untreated aphids was performed with the *t* test.

ANOVA were also used to test the effect of time

TABLE 1

The Effect of Parasitism of *Diuraphis noxia* by *Aphelinus asychis* (*Aa*) Followed by Treatment with *Paecilomyces fumosoroseus* (*Pfr*) (5.2×10^4 Conidia/cm^2) on the Number of Mummies Produced, Duration of Parasitoid Development, and Percentage of Parasitoid Emergence

Treatments	No. aphids exposed to two *A. asychis* females/24 h (mean of four replicate tests)	No. aphids treated with fungus after exposure to *A. asychis* (mean ± SE)[a]	No. mummies produced by two *A. asychis* females in 24 h (mean ± SE)[a]	Days from oviposition until parasitoid emergence (mean ± SE)[a]	Emergence of F1 generation of *A. asychis* (%) (mean ± SE)[a]
Aa alone	30	—	23.0 ± 1.5a	11.5 ± 0.1a	92.6 ± 0.1a
Aa + *PFr* (24 h)	30	23.5 ± 1.3	20.3 ± 1.9a	11.8 ± 0.1a	71.1 ± 0.1b
Aa + *Pfr* (48 h)	30	24.0 ± 0.4	22.3 ± 0.5a	11.6 ± 0.1a	90.9 ± 0.1a,b
Aa + *Pfr* (72 h)	30	23.5 ± 0.6	23.0 ± 0.7a	11.7 ± 0.1a	89.2 ± 0.1a,b
Aa + *Pfr* (96 h)	30	22.5 ± 0.6	21.5 ± 1.0a	11.3 ± 0.1a	81.2 ± 0.1a,b

[a] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Tukey's test for mean separation.

interval between application of *P. fumosoroseus* and parasitism by *A. asychis* on the number of aphids receiving ovipositional probing, the number of mummies produced, the number of aphids consumed per female during the course of 1-h exposure, when the aphids were first treated with the fungus and then exposed to the parasitoid. The three variables were transformed by $\sqrt{x + 0.5}$. The duration of ovipositor insertion for egg deposition and feeding, the duration of the preimaginal stages, the percentage emergence of the parasitoid, the proportion of females emerged, the longevity, and the size of the *A. asychis* females emerged were all analyzed using ANOVA. The proportions were arcsine square-root transformed. A multivariate analysis of variance (MANOVA) was performed to test the effect of the stage of infection by *P. fumosoroseus* on the frequency of aphids being probed as a function of the duration of ovipositor insertion by *A. asychis*, after arcsine square-root transformation of the data. The averages of all these variables were compared using Tukey's test at the 0.05 level. All statistical analyses were performed with the logical Statistical Analysis System (SAS, 1989).

RESULTS

Cumulative mortality of third-instar *D. noxia* treated with $2 \times LD_{95}$ (5.2×10^4conidia/cm^2) of *P. fumosoroseus* and not exposed to the parasitoid increased progressively from the second day after treatment and averaged 96.5% six days after treatment over the course of our studies. Control insects that had been treated with water and not exposed to the parasitoid averaged 11.0% mortality. After incubation on water agar, 96.7% of dead fungus-treated aphids showed signs of infection with *P. fumosoroseus*.

P. fumosoroseus on the Development, Longevity, and Fecundity of A. asychis When the Host Was First Exposed to the Parasitoid and Then Treated with the Fungus

The number of mummies produced by two female *A. asychis* during 24 h of exposure varied from 20.3 ± 1.9 to 23.0 ± 0.7 and was not significantly different when the aphids were first exposed to the parasitoids and then treated with water or with *P. fumosoroseus* 24, 48, 72, or 96 h after exposure (Table 1). No difference in time between oviposition and emergence of the F1 generation of the parasitoid for groups treated with the fungus and the untreated groups was noted. However, the percentage of emergence of the F1 generation of *A. asychis* was significantly lower among the aphids exposed to the parasitoid and treated with the fungus 24 h afterward than for the untreated aphids ($F = 3.97$; $df = 4, 15$; $P = 0.028$). The female *A. asychis* emerging from aphids treated 24 h after exposure to the parasitoid had a longevity of 14.6 ± 1.8 days and a reproductive capacity of 8.6 ± 0.5 mummies/day and did not differ from the females that emerged from nontreated aphids (Table 2).

P. fumosoroseus on Oviposition, Feeding Behavior, and Development of A. asychis When the Host Was First Treated with the Fungus and Then Exposed to the Parasitoid

Number of aphids probed, duration of ovipositor insertion, and number of mummies produced. The number of aphids probed per female over the course of 1 h of exposure was not significantly different when the aphids were first treated with *P. fumosoroseus* and then exposed to the parasitoid 0, 24, 48, or 72 h after application of the fungus, or when the aphids had been killed by fungal infection (Table 3).

The time interval between application of *P. fu-*

TABLE 2

Comparative Longevity and Fecundity of *Aphelinus asychis* Females That Emerged from *Diuraphis noxia* Treated with *Paecilomyces fumosoroseus* (5.2×10^4 Conidia/cm^2)24 h after Exposure to the Parasitoid with Those Emerging from Untreated Aphids

Treatments	Longevity (days) (mean ± SE)[a]	Mummies produced (mean ± SE)[a]
Females emerging from fungus-treated aphids	14.6 ± 1.8a	8.6 ± 0.5a
Females emerging from nontreated aphids	14.0 ± 1.3a	7.4 ± 0.1a

[a] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Student's t test.

mosoroseus to *D. noxia* and exposure to *A. asychis* had a significant effect on the duration of ovipositional probing ($F = 25.71$; $df = 5, 84$; $P = 0.0001$). The duration of the probing of aphids exposed only to the parasitoid (2.2 ± 0.2 min) was not significantly different from that of the aphids exposed 0, 24, or 48 h after treatment with the fungus. However, 72 h after fungal application, the time during which the ovipositor was inserted in the host was significantly shorter. The duration of probing dead aphids was 0.4 ± 0.05 min and was significantly shorter than all other treatments (Table 3).

For the same duration of ovipositional probing, the percentage of aphids probed by *A. asychis* varied significantly depending on the treatment (MANOVA; Wilk's $\lambda = 0.19$; $F = 12, 2$; $P = 0.0001$) (Table 4). Aphids exposed to the parasitoid 72 h after application of the fungus and insects that died from fungal infection received a greater proportion of probings lasting less than 1 min than all the other treatments, 36.6 ± 7.7 and 90.6 ± 3.0%, respectively. On the other hand, the dead insects showed the lower percentage (9.4 ± 3.0%) of aphids probed with durations being between 1 and 3 min.

The number of mummies produced by females during 1 h of exposure varied significantly as a function of the time interval between application of the fungus and exposure to the parasitoid ($F = 31.69$; $df = 5, 84$; $P = 0.0001$) (Table 3). Twenty-four hours after fungal application, the number of aphids successfully parasitized per female (2.5 ± 0.5) was significantly lower than that from aphids exposed only to the parasitoid (5.4 ± 0.5). Forty-eight hours between application of the fungus and exposure to parasitoids, the number of mummies produced decreased and approached zero at 72 h.

Number of aphids consumed by predation, duration of nutritional stings, and feeding time. During 1 h exposure, *A. asychis* females killed an average of 1.1 ± 0.09 aphids by host feeding when the aphids were exposed only to the parasitoid (Table 5). Host feeding did not vary significantly in relation to the aphids exposed to the parasitoid 0, 24, 48, or 72 h after application of the fungus. However, for aphids killed by the fungus, the number of aphids used for feeding was significantly lower (0.3 ± 0.1) compared to other treatments ($F = 11.64$; $df = 5, 84$; $P = 0.0001$). The duration of nutritional stings and the time spent feeding by the parasitoid were not significantly different among the treatments. When the aphids were exposed to the parasitoid only, the duration of nutritional stings and feeding time were 6.7 ± 0.4 and 17.1 ± 1.8 min, respectively.

Preimaginal development, percentage of emergence, sex ratio, longevity, and size of emerging females. The number of days from oviposition to mummification and from mummification to emergence of the F1 generation of the parasitoid did not vary significantly among the treatments and was 6 and 7 days, respectively (Table 6). No significant effect due to the interval of time between application of *P. fumosoroseus* and exposure of the aphids to the parasitoid was noted on the percentage of emergence of the F1 generation nor on the proportion of females emerged (Table 6). Longevity of the females that emerged from aphids parasitized 0, 24, or 48 h after application of the fungus and fed only with honey varied from 8.2 to 10.8 days and was not significantly different from those females that emerged from aphids not exposed to the fungus. The length of the metathoracic tibia and the length of the costal nerve of the anterior wing of these females did not show significant variation among the treatments, indicating that females emerging from fungus-treated aphids were the same size as those from untreated hosts (Table 7).

TABLE 3

The Effect of Treatment of *Diuraphis noxia* with *Paecilomyces fumosoroseus* (*Pfr*) (5.2×10^4 conidia/cm^2) and Exposure to *Aphelinus asychis* (*Aa*) 0–96 h afterward on the Number and Duration of Ovipositional Probings and the Number of Mummies Produced per *A. asychis*

Treatments	No. aphids probed/female/h (mean ± SE)[a]	Duration of probing (min) (mean ± SE)[a]	No. mummies produced/female/h (mean ± SE)[a]
Aa alone	6.7 ± 0.5a	2.2 ± 0.2a	5.4 ± 0.5a
Pfr + *Aa* (0 h)	7.3 ± 0.6a	2.1 ± 0.1a	4.1 ± 0.6ab
Pfr + *Aa* (24 h)	6.9 ± 0.6a	2.2 ± 0.1a	2.5 ± 0.5b
Pfr + *Aa* (48 h)	7.5 ± 0.7a	2.2 ± 0.2a	0.8 ± 0.2c
Pfr + *Aa* (72 h)	7.9 ± 1.3a	1.6 ± 0.2b	0.2 ± 0.1c
Fungus-killed aphid + *Aa*	9.8 ± 1.0a	0.4 ± 0.1c	0.0 ± 0.0c

[a] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Tukey's test for mean separation.

TABLE 4

Mean Percentages of *Diuraphis noxia* Receiving Ovipositional Probings of Various Durations by 15 Female *Aphelinus asychis* (*Aa*) as a Function of the Interval Following Treatment with *Paecilomyces fumosoroseus* (*Pfr*)(5.2×10^4 Conidia/cm^2) and Then Exposure to the Parasitoid

Treatments	Percentage of parasitoids and duration of probing as a function of treatment[a]			Total number of aphids probed/15 females/h
	<1 min (mean % ± SE)[b]	1–3 min (mean % ± SE)[b]	>3 min (mean % ± SE)[b]	
Aa alone	4.0 ± 2.3a	82.2 ± 4.4a	13.8 ± 4.7ab	100
Pfr + *Aa* (0 h)	5.4 ± 1.9a	83.3 ± 3.9a	11.3 ± 3.5ab	109
Pfr + *Aa* (24 h)	6.2 ± 2.3a	78.1 ± 5.4ab	15.7 ± 2.9a	104
Pfr + *Aa* (48 h)	12.5 ± 4.2a	72.1 ± 5.4ab	15.4 ± 5.2a	112
Pfr + *Aa* (72 h)	36.6 ± 7.7b	57.3 ± 7.4b	6.1 ± 3.0ab	119
Fungus-killed aphid + *Aa*	90.6 ± 3.0c	9.4 ± 3.0c	0.0 ± 0.0b	147

[a] Nutritional stings were not counted.
[b] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Tukey's test for mean separation.

DISCUSSION

The high cumulative mortality of *D. noxia* 6 days after application of *P. fumosoroseus* confirms the results previously presented by Mesquita *et al.* (1996) and Vandenberg (1996) on the virulence and pathogenicity of this fungus for *D. noxia*. The absence of a significant difference between the number of untreated *D. noxia* probed by *A. asychis* and aphids that had been pretreated with *P. fumosoroseus* and then exposed to the parasitoid indicates that the parasitoids still require probing with their ovipositors to determine host suitability, in addition to using other host cues (Gerling *et al.*, 1990). The significantly shorter duration of probing of insects that had been exposed to the parasitoid 72 h after application of the fungus and of fungus-killed aphids probably indicates strong internal cues and subsequent rejection of most of these insects for oviposition or feeding. According to Boyle and Barrows (1978) and Bai and Mackauer (1990a), parasitoids probe all potential host aphids using their ovipositor and the final acceptance or rejection of a host is determined by internal stimuli. Prolonged insertions of the ovipositor into the host (>1 min, not counting nutritional stings) are correlated with acceptance of the host for oviposition, and short probings (<1 min) indicate general rejection of the host. We did not dissect probed insects to confirm the presence of eggs. However, the highest percentage of aphids receiving short duration probing (<1 min) and the lower percentage of insects probed for a duration of 1 to 3 min indicate that insects exposed to the parasitoid 72 h or longer after application of the fungus probably received a lower number of eggs than aphids from other treatment groups. These data also correlate well with the lower number of mummies produced. The steady decline in numbers of mummies from the fungus-treated aphids is distinctly related to the time interval between fungal treatment and subsequent exposure to parasitoids. It may also indicate a lack of survival of parasitoid larvae in infected hosts in addition to decreased oviposition in the 72 h and fungus-killed groups.

Parasitoid rejection of diseased hosts has already been demonstrated by other authors. Landa (1984) and Fransen and van Lenteren (1993) demonstrated that the parasitoid *Encarsia formosa* Gahan laid fewer eggs in the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), infected by the fungus *Aschersonia aleyrodis* Webber than in uninfected hosts. Brobyn *et al.* (1988) found that the frequency with which *Aphidius rhopalosiphi* de Stefani Perez deposited eggs in the aphid *Metopolophium dirhodum* (Walker) infected with *Pandora* (*Erynia*) *neoaphidis* (Brefeld) Batko di-

TABLE 5

Mean Number of *Diuraphis noxia* Consumed per Female *Aphelinus asychis* (*Aa*) during 1 h of Exposure, Duration of Nutritional Sting, and Time Spent Feeding on Untreated Aphids and Those That Had Been Treated Immediately after Parasitization or 24–96 h Earlier with *Paecilomyces fumosoroseus* (*Pfr*) (5.2×10^4 Conidia/cm^2)

Treatments	No. aphids consumed/female/h (mean ± SE)[a]	Duration of nutritional sting (min) (mean ± SE)[a]	Time spent feeding (min) (mean ± SE)[a]
Aa alone	1.1 ± 0.1a	6.7 ± 0.4a	17.1 ± 1.8a
Pfr + *Aa* (0 h)	1.1 ± 0.1a	7.6 ± 0.4a	19.4 ± 2.7a
Pfr + *Aa* (24 h)	0.9 ± 0.1a	8.4 ± 0.4a	18.6 ± 2.4a
Pfr + *Aa* (48 h)	1.1 ± 0.1a	8.4 ± 0.4a	15.8 ± 1.9a
Pfr + *Aa* (72 h)	1.1 ± 0.2a	8.3 ± 0.6a	17.4 ± 3.3a
Fungus-killed aphid + *Aa*	0.3 ± 0.1b	7.6 ± 0.8a	19.5 ± 6.1a

[a] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Tukey's test for mean separation.

TABLE 6

Number of Mummies Produced by 15 *Aphelinus asychis* (*Aa*) Females during 1 h of Exposure to Untreated *Diuraphis noxia* and Those That Had Been Treated Simultaneously or Earlier with *Paecilomycesfumosoroseus* (*Pfr*) (5.2×10^4 Conidia/cm^2)

Treatments	No. mummies produced/15 females/h (total)	Days from oviposition until parasitoid emergence (mean)	Emergence of F1 generation (%) (mean ± SE)[a]	Percentage of females in F1 generation (mean ± SE)[a]
Aa alone	81	13.0	91.2 ± 3.4a	83.3 ± 4.1a
Pfr + *Aa* (0 h)	62	13.0	72.0 ± 9.2a	84.4 ± 5.2a
Pfr + *Aa* (24 h)	38	13.0	70.3 ± 8.4a	89.5 ± 5.0a
Pfr + *Aa* (48 h)	12	13.0	69.0 ± 5.6a	91.6 ± 8.3a

[a] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Tukey's test for mean separation.

minished when aphids were infected 3 days before exposure to the parasitoid. Conversely to *A. asychis*, *A. rhopalosiphi* did not attempt to oviposit in fungus-killed aphids. The presence of hyphal bodies or fungal metabolites in the hemolymph of the host, detected by the parasitoid at the time of insertion of the ovipositor, is ostensibly the cause of rejection of these insects for oviposition. The difference between the number of mummies produced per *A. asychis* female in our studies, as a function of the time interval between application of the fungus and exposure to the parasitoid, demonstrates the importance of fungal development between initial treatment and oviposition on the success of parasitism of infected insects.

The elevated level of mummification observed with aphids initially exposed to the parasitoid and then treated with the fungus shows that *A. asychis* escaped the lethal action of the fungus, especially if the parasitoid had 48 h or more to develop before exposure to the fungus. There is evidence reported by other researchers that the presence of a fungistatic substance secreted by the parasitoid into the hemolymph of the host impedes the development of mycosis and enables normal parasitoid development and emergence (El-Sufty and Führer, 1981b; Willers *et al.*, 1982; El-Sufty and Führer, 1985). However, in some cases, prior oviposition by a parasitoid can predispose the host to fungal infection by reducing the resistance of the cuticle to penetration of the fungus (Führer and El-Sufty, 1979; El-Sufty and Führer, 1981a).

TABLE 7

Longevity and Length of Metathoracic Tibia and Anterior Wing Vein of Female *Aphelinus asychis* (*Aa*) That Emerged from Untreated *Diuraphis noxia* and Those That Had Been Treated Immediately after Parasitization or 24–48 h Earlier with *Paecilomyces fumosoroseus* (*Pfr*)(5.2×10^4 Conidia/cm^2)

Treatments	*N*	Longevity (days) (mean ± SE)[a]	Tibial length (mm) (mean ± SE)[a]	Anterior wing vein length (mm) (mean ± SE)[a]
Aa alone	26	9.7 ± 0.8a	0.359 ± 0.003a	0.448 ± 0.006a
Pfr + *Aa* (0 h)	23	10.8 ± 0.9a	0.369 ± 0.004a	0.445 ± 0.007a
Pfr + *Aa* (24 h)	18	8.5 ± 0.7a	0.363 ± 0.005a	0.468 ± 0.006a
Pfr + *Aa* (48 h)	6	8.2 ± 3.5a	0.371 ± 0.010a	0.465 ± 0.017a

[a] Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined with Tukey's test for mean separation.

The inhibitory effect of parasitoids on the development of mycosis in parasitized hosts is normally linked to the interval of time between parasitism and contamination of the host by the fungus (King and Bell, 1978; Powell *et al.*, 1986). However, there are cases where simultaneous exposure to parasitoids and fungus resulted in emergence of adults of the F1 generation (Milner *et al.*, 1984). In our study, treating aphids with fungus 1 day before exposure to *A. asychis* was sufficient to result in significant decrease in mummy production compared to the aphids that were only exposed to parasitoids. Conversely, a 2-day advance for parasitization by *A. asychis* prior to fungal treatment was sufficient for no harmful effects of the fungus on parasitoid larvae to occur. According to Brooks (1993), the premature death of the host is the more frequent consequence of host-parasitoid-pathogen interaction. Several examples are known where premature death of the infected host has also caused death of the parasitoid (Bilioti, 1955; Laigo and Paschke, 1968; Thomas and Watson, 1986). Histological observations of parasitized aphids that were infected by *P. neoaphidis* showed that invasion by the fungus was limited to host tissues (Powell *et al.*, 1986). The only evidence of invasion of parasitoid larval tissue was observed by Keller (1975) in 2 out of 26 aphids that were analyzed. However, it was not possible to determine if the fungus had killed the parasitoid directly or if it was colonized after death.

Although feeding by *A. asychis* on the host did not vary with the stage of infection of living aphids, the parasitoids fed on a significantly smaller number of dead aphids than living aphids, infected or not. On the other hand, when the parasitoid fed on dead insects, the sting which preceded feeding as well as the duration of feeding time, was similar to that on living aphids. The possibility of

negative effects of feeding on infected hosts for survival of the female parasitoids was not studied. The rejection of dead aphids for feeding purposes did not occur until after insertion of the ovipositor.

The increased frequency of probing of fungal-infected or killed aphids could increase the chances for horizontal dissemination of *P. fumosoroseus* by *A. asychis* when conditions are optimal for the fungus. Such dissemination was observed for another homopteran, *T. vaporariorum*, infected with *A. aleyrodes*. Fransen and van Lenteren (1994) observed transmission of *A. aleyrodes* by *E. formosa* after probing infected whiteflies.

Various aspects of *A. asychis* biology were unaffected in the F1 generation that emerged from *D. noxia* treated with *P. fumosoroseus* regardless of the timing of fungal treatment. These included *A. asychis* preimaginal development time, sex ratio, longevity, fecundity, and size of F1 females. Generally, secondary effects of entomopathogenic fungi on endoparasitic insects are little known (Flexner *et al.*, 1986; Goettel *et al.*, 1990). According to El Maghraby *et al.* (1988) the larval stage of *Microplitis rufiventris* Kok. was prolonged when the parasitoid developed in larvae of *Spodoptera littoralis* (Boisduval) infected by *Beauveria bassiana* (Bals.) Vuill. or treated with *Bacillus thuringiensis* Berliner. Milner *et al.* (1984) showed that adult *Trioxys complanatus* Quilis emerged normally from aphids infected by *Z. radicans* without reduction in size. The effect of fungal infection of the host insect on the fecundity of emerging parasitoids has not been well studied. Females of *E. formosa* that emerged from *T. vaporariorum* infected by *A. aleyrodis* demonstrated parasitoid behavior similar to that shown by females that emerged from noninfected whiteflies (Fransen and van Lenteren, 1994). According to Bethke and Parrella (1989), the presence of *Aphis gossypii* Glover infected by *Verticillium lecanii* (Zimm.) did not affect the period of preoviposition, fecundity, or the longevity of parents or progeny of the parasitoid *Digyphus beginii* Ashmead.

A high proportion of male offspring in many parasitic Hymenoptera may indicate virginity or sperm depletion in parental (P1) females or manipulated sex ratio (i.e., deposition of unfertilized eggs to produce male offspring) (Godfray, 1994). Manipulated sex ratio may be in response to host size and quality, parasite to host ratio, or local mate competition (Charnov, 1982; Godfray, 1994). The strong proportion of F1 females in our study indicates that P1 females were sufficiently fertilized and that they did not modify the sex ratio of their descendants as a function of the stage of infection of *D. noxia* by *P. fumosoroseus*.

Various findings of our study demonstrate the potential of synergistic interaction of *P. fumosoroseus* and *A. asychis* for the biological control of *D. noxia*. These include the discrimination by *A. asychis* of *D. noxia* infected by *P. fumosoroseus* reducing the possibility of eggs being laid into infected aphids, the protection of hosts that are parasitized by *A. asychis* from infection by the fungus, and the absence of significant negative effects on the F1 generation of the parasitoid that emerge from fungus-treated aphids. The possibility of transmission of the fungus by *A. asychis* after probing of infected or dead aphids warrants further study.

For the most part, the interaction between fungal pathogens of insects and other natural enemies is positive (Roy and Pell, 2000). Environmental conditions that favor hymenopteran parasitoids or fungi will influence the type of interaction and compatibility or antagonism of these two groups of biological control agents. Elucidation of the degree of competition or compatibility will require study under specific field conditions in order to know what these interactions entail and how to best utilize the two groups of agents for biological control. Coexistence is favored by complementarity between parasitoid and pathogens in terms of extrinsic and intrinsic qualities (Begon *et al.*, 1999). The key component of complementarity under the manipulated conditions of our studies is the ability of *A. asychis* to protect itself from fungal infection within the host and to avoid, to a certain degree, unsuitable hosts.

ACKNOWLEDGMENTS

We thank Guy Mercadier for technical assistance during the course of this study and Remi Debire for assistance with statistical analyses. Reviews by Drs. Tad Poprawski, Jeff Lord, Don Hostetter, and Lisa Neven provided constructive criticism for improving the manuscript.

REFERENCES

Bai, B., and Mackauer, M. 1990a. Host discrimination by the aphid parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae): When superparasitism is not adaptive. *Can. Entomol.* **122,** 363–372.

Bai, B., and Mackauer, M. 1990b. Oviposition and host-feeding patterns in *Aphelinus asychis* (Hymenioptera: Aphelinidae) at different aphid densities. *Ecol. Entomol.* **15,** 9–16.

Begon, M., Sait, S. M., and Thompson, D. J. 1999. Host-pathogen-parasitoid systems. *In* "Theoretical Approaches to Biological Control" (B. A. Hawkins and H. V. Cornell, Eds.), pp. 327–348. Cambridge Univ. Press, Cambridge, UK.

Bethke, J. A., and Parrella, M. P. 1989. Compatibility of the aphids fungus *Cephalosporium lecanii* with the leafminer parasite, *Diglyphus beginii* (Hymenoptera: Eulophidae). *Pan-Pacific Entomol.* **64,** 385–390.

Bilioti, E. 1955. Survie des larves endophages de Tachinaires à une mort prématurée de leur hôte par maladie. *C. R. Acad. Sci.* **240,** 1021–1023.

Boyle, H., and Barrows, E. M. 1978. Oviposition and host feeding behavior of *Aphelinus asychis* (Hymenoptera: Chalcidoidea: Aphelinidae) on *Schizaphis graminum* (Homoptera: Aphididae) and some reactions of aphids to this parasite. *Proc. Entomol. Soc. Wash.* **80,** 441–455.

Brobyn, P. J., Clark, S. J., and Wilding, N. 1988. The effect of fungus infection of *Metopolophium dirhodum* [Hom.: Aphididae] on the

oviposition behaviour on the aphid parasitoid *Aphidius rhopalosiphi* [Hym.: Aphidiidae]. *Entomophaga* **33,** 333–338.

Brooks, W. M. 1993. Host-parasitoid-pathogen interactions. *In* "Parasites and Pathogens of Insects." Vol. 2, "Pathogens" (N. E. Beckage, S. N. Thompson, and B. A. Federici, Eds.), pp. 231–272. Academic Press, San Diego.

Charnov, E. L. 1982. "The Theory of Sex Allocation." Princeton Univ. Press, Princeton, NJ.

El-Maghraby, M. M. A., Hegab, A., and Yousif-Khalil, S. I. 1988. Interactions between *Bacillus thuringiensis* Berl., *Beauveria bassiana* (Bals) Vuill. and the host/parasitoid system *Spodoptera littoralis* (Boisd.)/*Microplitis rufiventris* Kok. *J. Appl. Entomol.* **106,** 417–421.

El-Sufty, R., and Führer, E. 1981a. Interrelationships between *Pieris brassicae* L. (Lep., Pieridae), *Apanteles glomeratus* L. (Hym., Braconidae) and the fungus *Beauveria bassiana* (Bals.) Vuill. Z. *Angew. Entomol.* **92,** 321–329.

El-Sufty, R., and Führer, E. 1981b. The effect of entomophagous endoparasites on the cuticle of the host *Pieris brassicae* and *Cydia pomonella*. *Entomol. Exp. Appl.* **30,** 134–139.

El-Sufty, R., and Führer, E. 1985. Interrelationships between *Cydia pomonella* L. (Lep., Tortricidae), *Ascogaster quadridentatus* Wesm. (Hym., Braconidae) and the fungus *Beauveria bassiana* (Bals.) Vuill. *Z. Angew. Entomol.* **99,** 504–511.

Flexner, J. L., Lighthart, B., and Croft, B. A. 1986. The effects of microbial pesticides on non-target, benefical arthropods. *Agric. Ecosys. Environ.* **16,** 203–254.

Fransen, J. J., and van Lenteren, J. C. 1993. Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum,* in the presence of hosts infected with the fungus *Aschersonia aleyrodis*. *Entomol. Exp. Appl.* **69,** 239–249.

Fransen, J. J., and van Lenteren, J. C. 1994. Survival of the parasitoid *Encarsia formosa* after treatment of parasitized greenhouse whitefly larvae with fungal spores of *Aschersonia aleyrodis*. *Entomol. Exp. Appl.* **71,** 235–243.

Führer, E., and El-Sufty, R. 1979. Produktion fungistischer Metabolite durch Teratocyten von *Apanteles glomeratus* L. (Hym., Braconidae). *Z. Parasitenkd.* **59,** 21–25.

Gerling, D., Roitberg, B. D., and Mackauer, M. 1990. Instar-specific defense of pea aphid, *Acyrthosiphon pisum:* Influence on oviposition success of the parasite *Aphelinus asychis* Hymenoptera: Aphelinidae). *J. Insect Behav.* **3,** 501–514.

Godfray, H. C. J. 1994. "Parasitoids: Behavior and Evolutionary Ecology." Princeton Univ. Press, Princeton, NJ.

Goettel, M. S., and Inglis, G. D. 1997. Fungi: Hyphomycetes. *In* "Manual of Techniques in Insect Pathology" (L. A. Lacey, Ed.), pp. 213–249. Academic Press, London.

Goettel, M. S., Poprawski, T. J., Vandenberg, J. D., Li, Z., and Roberts, D. W. 1990. Safety to nontarget invertebrates of fungal biocontrol agents. *In* "Safety of Microbial Insecticides" (M. Laird, L. A. Lacey, and E. W. Davidson, Eds.) pp. 209–232. CRC Press, Boca Raton, FL.

Hochberg, M. E., and Lawton, J. H. 1990. Competition between kingdoms. *Trends Ecol. Evol.* **5,** 367–371.

Keller, S. 1975. Histological examinations of parasitized, Entomophthora-infected pea aphids, *Acyrthosiphon pisum*. *Bull. Soc. Entomol. Suisse.* **48,** 248–252.

King, E. G., and Bell, J. V. 1978. Interactions between a braconid, *Microplitis croceipes*, and a fungus, *Nomuraea rileyi*, in laboratory-reared bollworm larvae. *J. Invertebr. Pathol.* **31,** 337–340.

Lacey, L. A., Fransen, J. J., and Carruthers, R. 1996. Global distribution of naturally occurring fungi of *Bemisia,* their biologies and use as biological control agents. *In* "Bemisia 1995: Taxonomy, Biology, Damage, and Management" (D. Gerling and R. Mayer, Eds.), pp. 401–433. Intercept, Andover.

Lacey, L. A., Kirk, A. A., and Hennessey, R. D. 1993. Foreign exploration for natural enemies of *Bemisia tabaci* and implementation in integrated control programs in the United States. *Proc. ANPP Int. Conf. Pests Agric.* **1,** 351–360.

Lacey, L. A., Mesquita, A. L. M., Mercadier, M., Debire, R., Kazmer, D. J., and Leclant, F. 1997. Acute and sublethal activity of the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) on adult *Aphelinus asychis* (Hymenoptera: Aphelinidae). *Environ. Entomol.* **26,** 1452–1460.

Laigo, F. M., and Paschke, J. D. 1968. *Pteromalus puparum* L. parasites reared from granulosis and microsporidiosis infected *Pieris rapae* L. chrysalids. *Phillip. Agric.* **52,** 430–439.

Landa, Z. 1984. Control of glasshouse whitefly in the programs of integrated protection of glasshouse cucumbers. *Zahradnictvi.* **11,** 215–228.

Mesquita, A., Lacey, L. A., Mercadier, G., and Leclant, F. 1996. Entomopathogenic activity of a whitefly derived isolate of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against the Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae) with the description of an effective bioassay method. *Eur. J. Entomol.* **93,** 69–75.

Mesquita, A. L. M., Lacey, L. A., and Leclant, F. 1997. The individual and combined effects of the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) and the parasitoid, *Aphelinus asychis* (Hymenoptera: Aphelinidae) on confined populations of the Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae) under field conditions. *J. Appl. Entomol.* **121,** 155–163.

Milner, R. J., Lutton, G. G., and Bourne, J. 1984. A laboratory study of the interaction between aphids, fungal pathogens and parasites. Proc. 4th Aust. Appl. Entomol. Res. Conf., pp. 375–381.

Poprawski, T. J., Mercadier, G., and Wraight, S. P. 1992. Interactions between *Diuraphis noxia*, *Zoophthora radicans* and *Aphelinus asychis:* Preliminary results of laboratory studies. Proc. V Ann. Russian Wheat Aphid Conf., pp. 1–9.

Potter, C. 1952. An improved apparatus for applying direct sprays and surface films with data on the electrostatic charge on atomized spray fluids. *Ann. Appl. Biol.* **39,** 1–28.

Powell, W., Wilding, N., Brobyn, P. J., and Clark, S. J. 1986. Interference between parasitoids [Hym.: Aphidiidae] and fungi [Entomophthorales] attacking cereal aphids. *Entomophaga* **31,** 293–302.

Roy, H. E., and Pell, J. K. 2000. Interactions between entomopathogenic fungi and other natural enemies: Implications for biological control. *Biocontrol Sci. Technol.* **10,** 737–752.

Samson, R. A. 1974. "*Paecilomyces* and some allied Hyphomycetes." Studies in Mycology, No. 6, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

SAS 1989. "SAS/STAT Users Guide," version 6, Vols. 1, 2. SAS Institute Inc. Cary, NC.

Smith, P. 1993. Control of *Bemisia tabaci* and the potential of *Paecilomyces fumosoroseus* as a biopesticide. *Biocontrol News Inform.* **14,** 71N–78N.

Thomas, E. M., and Watson, T. F. 1986. Effect of Dipel (*Bacillus thuringiensis*) on the survival of immature and adult *Hyposoter exigua* (Hymenoptera: Ichneumonidae). *J. Invertebr. Pathol.* **47,** 178–183.

Vandenberg, J. D. 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against the Russian wheat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* **89,** 1418–1423.

Willers, D., Lehmann-Danzinger, H., and Führer, E. 1982. Antibacterial and antimycotic effect of a newly discovered secretion from larvae of an endoparasitic insect, *Pimpla turionellae* L. (Hym.). *Arch. Microbiol.* **133,** 225–229.