BioOne COMPLETE

Susceptibility of biological stages of the horn fly, Haematobia irritans, to entomopathogenic fungi (Hyphomycetes)

Authors: Angel-Sahagún, C. A., Lezama-Gutiérrez, R., Molina-Ochoa, J., Galindo-Velasco, E., López-Edwards, M., et al.

Source: Journal of Insect Science, 5(50) : 1-8

Published By: Entomological Society of America

URL: https://doi.org/10.1673/1536-2442(2005)5[1:SOBSOT]2.0.CO;2

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Susceptibility of biological stages of the horn fly, *Haematobia irritans*, to entomopathogenic fungi (Hyphomycetes)

C. A. Angel-Sahagún[1], R. Lezama-Gutiérrez[2], J. Molina-Ochoa[2], E. Galindo-Velasco[1], M. López-Edwards[2], O. Rebolledo-Domínguez[2], C. Cruz-Vázquez[3], W. P. Reyes-Velázquez[4], S. R. Skoda[5], and J. E. Foster[6]

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad de Colima, Km 40 Autopista Colima-Manzanillo, Tecomán, Colima, CP. 28100.
[2]Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, Km 40 Autopista Colima-Manzanillo, Tecomán, Colima, CP. 28100
[3]Instituto Tecnológico Agropecuario de Aguascalientes. A.P. 74-2, Admón. Postal No. 2, C.P. 20041, Aguascalientes, Aguascalientes, Mexico
[4]Centro Universitario de Ciencias Biológicas y Agropecuarias. Universidad de Guadalajara. Km. 15.5 Carretera Guadalajara-Nogales "Las agujas"
Zapopan, Jalisco, Mexico
[5]American Embassy, Unit 0945, APO, AA 34002 (located in Panama City, Panama)
[6]University of Nebraska Lincoln, Insect Genetics Laboratory, Department of Entomology, 312 F Plant Industry Building, Lincoln, Nebraska 68583-0938

Abstract

The susceptibility of the egg, pupa, and adult of *Haematobia irritans* (L.) (Diptera: Muscidae) to isolates of the fungi *Metarhizium anisopliae* (Metsch.) Sor., *Beauveria bassiana* (Bals.) Vuill., and *Paecilomyces fumosoroseus* (Wize) Brown and Smith, was evaluated under laboratory conditions. Groups of 20 eggs than 4 h old, pupae less than 48h old and adults were sprayed with a conidial suspension of each isolate. Eggs, pupae and adults of horn fly were susceptible to these entomopathogenic fungi. For treated eggs, the isolates Ma3, Ma 15, Ma25, Pfr1, and Pfr8 reduced adult emergence to 3.8% to 6.3% in comparison with the control (72%). The mortality of pupae infected by the isolates Ma2, Ma25, and Pfr10 ranged between 50% and 71.3%. Mortality of adults after treatment with the isolates Ma6, Ma 10, Ma 14, Ma 15, Pfr 1, Pfr 9, Pfr 10, Pfr 11, and Pfr12 were higher than 90%. The isolate Ma6 produced the lowest LC_{50} against adult horn flies (8.08 x10^2conidia/ml). These findings supported the hypotheses that isolates of *M. anisopliae*, and *P. fumosoroseus* are pathogenic against the different biological stages of horn flies by reducing adult emergence when applied on groups of eggs and pupae, and producing mortality when applied to adults.

Resumen

La susceptibilidad de los estados biológicos de larva, pupa y adulto de *Haematobia irritans* (L.) (Diptera: Muscidae) a aislados de los hongos *Metarhizium anisopliae* (Metsch.) Sor., *Beauveria bassiana* (Bals.) Vuill., y *Paecilomyces fumosoroseus* (Wize) Brown y Smith, fue evaluada bajo condiciones de laboratorio. Grupos de 20 huevos de 4 h de edad fueron colocados sobre 50 g de excremento de vaca fresco en una proporción de 10:1 de excremento de vaca: harina de pescado deshidratada y ellos se asperjaron con una suspensión a una concentración de 1 X 10^6 conidias/ml. Otros grupos de 20 pupas de menos de 48 h de

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

edad fueron asperjados con una suspensión conteniendo 1 X 10^8 conidias/ml, y cuatro grupos de 20 adultos fueron asperjadas con una suspensión de cada aislado. Los huevos, pupas y adultos de la mosca de los cuernos fueron susceptibles a los hongos entomopatógenos. Los aislados Ma2, Ma3, Ma 15, Ma25, Pfr1, y Pfr8 causaron una reducción en la emergencia de los adultos desde 3.8 a 6.3% en comparación con el testigo (72%). Las pupas fueron micosados por los aislados Ma2, Ma25 y Pfr10 en un rango entre 50 y 71.3%; los porcentajes de mortalidad de adultos mayor que 90% con los aislados Ma6, Ma 10, Ma 14, Pfr 9, Pfr 10, Pfr 11 y Pfr12. La CL_{50} más baja (8.08 x10^2conidias/ml) en contra de adultos de moscas de los cuernos, la produjo el aislado Ma6. Los resultados apoyan la hipótesis de que los aislados de *M. anisopliae* y *P. fumosoroseus* son patógenos de diferentes estados biológico de las moscas del cuerno y que estos hongos reducen la emergencia de adultos cuando son aplicados a grupos de huevos y pupas y producen mortalidad cuando son aplicados a adultos de esta plaga.

Cite this paper as:
Angel-Sahagún CA, Lezama-Gutiérrez R, Molina-Ochoa J, Galindo-Velasco E, López-Edwards M, Rebolledo-Domínguez O, Cruz-Vázquez C, Reyes-Velázquez WP, Skoda SR, Foster JE. Susceptibility of biological stages of the horn fly, *Haematobia irritans*, to entomopathogenic fungi (Hyphomycetes). 8pp. *Journal of Insect Science* 5:50, available online: insectscience.org/5.50

Keywords: *Metarhizium anisopliae, Beauveria bassiana, Paecilomyces fumosoroseus,* horn fly, biological control
Descriptores: *Metarhizium anisopliae, Beauveria bassiana, Paecilomyces fumosoroseus,* moscas del cuerno, control biológico
Correspondence: jmolina@ucol.mx, jmolina18@hotmail.com, rlezama@ucol.mx
Received 15 February 2005 | Accepted 8 October 2005 | Published 31 December 2005

Introduction

The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae) lay eggs on fresh cow manure. Maggots hatch in a few days, finish development in 8-10 days and leave the manure to pupate. Adults feed on cattle blood. *H. irritans* is widely distributed in North and Latin America and is considered to be a major bloodsucking pest of pastured cattle due to significant losses in cattle production (Guglielmone et al., 1997). This pest has also been recently reported attacking confined dairy cattle in Central Mexico (Cruz-Vázquez et al., 1999). The economic impact on livestock production by *H. irritans* has been estimated to approach $1 billion annually in the North America (Cupp et al., 1998). A horn fly population of about 700-1000 adults per animal caused weight losses between 40-90 g per day in beef cattle during a 4-month period (Kunz et al., 1984). In 1992, the horn fly was the most important and widespread cattle pest in the US, with an annual economic impact on cattle production estimated at $730.3 million (Byford et al., 1992), but the stable fly has taken its place in most of the country. Occasionally, *H. irritans* feeds on other species such as goats, horses, mules, dogs, but only rarely attacks humans (Greer and Butler, 1973; Torres and Prieto, 1993).

Chemical insecticides have bee used to control this pest, but widespread resistance has occurred throughout the United States (Sheppard and Joyce, 1992). Capsules of insecticides fed orally to cattle are used to control dung-inhabiting flies but also adversely affect the abundance of biological control agents in dung (Wall and Strong, 1987).

Horn fly control is becoming increasingly difficult due to development of resistance to chlorinated hydrocarbons, carbamates, pyrethroids, and organophosphates from Canada (Mwangala and Galloway, 1993) to Argentina (Guglielmone et al., 2001). Therefore, alternative means of control that are not potentially harmful to humans, other animals and the environment have been sought. A number of resistance management strategies for the horn fly have been proposed (Sparks et al., 1985) to delay or prevent insecticide resistance (Byford et al., 1987).

Biological control is an alternative being investigated for control of livestock ectoparasites (Hogsette, 1999). The horn fly is susceptible to entomopathogenic fungi (Steenberg et al., 2001a). *Beauveria bassiana* (Bals.) Vuill, *Lecanicillium (=Verticillium) lecanii* (Zimmermann) Viegas, *V. fusisporum* (W. Gams) and *Furia americana* (Thaxter) Humber have been isolated from this insect pest (Steenberg et al., 2001a). The species, *B. bassiana* and *P. farinosus* killed 100%, and 90% of adults 4 days postreatment, respectively, at a concentration of 1.7x10^9 spores under laboratory conditions (Steenberg et al., 2001b).

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Watson et al. (1995) evaluated the larvicidal activity of *B. bassiana* against the house fly, *Musca domestica* with the aim of controlling larvae in the bedding of confined cattle.

There are no reports of the effects of *Metarhizium anisopliae* (Metsh.), *P. fumosoroseus*, and *B. bassiana* against the eggs, and pupae of *H. irritans*. The objective of this study was to evaluate the susceptibility of eggs, pupae, and adults of *H. irritans* to these entomopathogenic fungi.

Materials and Methods

Stock colonies

Horn flies were provided by the Centro Nacional de Parasitología Animal (CENAPA-SAGARPA) (National Center for Animal Parasitology) in Jiutepec, Morelos, Mexico. The colony was maintained under laboratory conditions following the methodology and techniques of Schmidt et al. (1967). Constituents of the larval medium included fresh cow manure, and sardine meal Mazarina (Maz Industrial SA de CV, Estero de Urias, Mazatlán, Sinaloa, México) at a 10:1 ratio. Larvae were maintained at 30° C, and 60-70% RH. Pupae were collected by flotation, air dried, and placed in plastic containers with a capacity of 500 ml. Adults were fed *ad libitum* with bovine defibrined blood on saturated cellucotton pads covered with gauze, and maintained at 30° C, and 70-80% RH, in total darkness (Mendes and Linhares, 1999).

Table 1 shows the origin of the fungal isolates used. For egg inoculation, 9 isolates of *M. anisopliae*, 8 of *P. fumosoroseus*, and 2 of *B. bassiana* were used. For evaluating the susceptibility of pupae, 10 isolates of *M. anisopliae*, 9 of *P. fumosoroseus*, and 2 of *B. bassiana* were used. To evaluate the susceptibility of adults, 11 isolates of *M. anisopliae*, 10 of *P. fumosoroseus*, and 2 of *B. bassiana* were used. All the fungal isolates are maintained in the Entomopathogenic Fungi Collection of the Facultad de Ciencias Biológicas y Agropecuarias, of the Universidad de Colima. The isolates of *P. fumosoroseus* were provided by the Centro Nacional de Referencia de Control Biológico-Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (CNRCB-SAGARPA), located in Tecomán, Colima, México, and the Ma 1 strain of *M. anisopliae* was graciously provided by Dr. A. Mendoça (BIOTEC, Alagaos, Brasil).

Fungi were mass produced on Sabouraud dextrose Agar medium with 1% yeast extract and chloramphenicol at 500 ppm (Moorhouse et al., 1993). They were incubated at 25 ± 1° C, and 70% RH for three weeks. Conidia of each 21-day-old

Table 1. Origin of *M. anisopliae* (Ma), *P. fumosoroseus* (Pfr) and *B. bassiana* (Bb) fungal isolates used against different host life stages of the horn fly, Haematobia irritans.

Isolate Code	Source of isolation	Host life stage used	Country	Date
Ma1	*Aeneolamia postica* Walker (Homoptera: Cercopidae)	E, P, A	Brasil	1990
Ma2	*Diatraea saccharalis* Fabr. (Lepidoptera: Pyralidae)	E, P, A	Mexico	1988
Ma3	*D. saccharalis*	E, P, A	Mexico	1987
Ma6	Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae)	P, A	Mexico	1989
Ma7	*Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae)	E, P, A	Mexico	1987
Ma10	*Macrodactylus murinus* Bates (Coleoptera: Scarabaeidae)	E, P, A	Mexico	1993
Ma 13	Soil	E, P, A	Mexico	1993
Ma 14	Soil	E, P, A	Mexico	1993
Ma15	*Schistocera piceifrons* (Walker) (Orthoptera: Acrididae)	E, P, A	Mexico	1993
Ma25	*A. postica*	E, P, A	Mexico	1993
Ma 38	Soil	A	Mexico	
Pfr1	*Bemisia tabaci* Genn. (Homoptera: Aleyrodidae)	E, P, A	Mexico	1991
Pfr 3	*B. tabaci*	E, P, A	Mexico	1994
Pfr 4	*B. tabaci*	A	Mexico	1994
Pfr 5	*B. tabaci*	E, P, A	Mexico.	1995
Pfr7	*B. tabaci*	E, P, A	Mexico	1994
Pfr8	S. piceifrons	E, P, A	Mexico	1992
Pfr 9	*S. piceifrons*	E, P, A	Mexico	1992
Pfr10	*Toxoptera aurantii* (Fonsc.) (Homoptera: Aphididae)	E, P, A	Mexico	1998
Pfr 11	*B. tabaci*	E, P, A	Mexico	1994
Pfr 12	*B. tabaci*	P, A	Mexico	1992
Bb17	*B. tabaci*	E, P, A	Mexico	1992
Bb21	*Geraeus senilis* Gyll (Coleoptera: Curculionidae)	E, P, A	Mexico	1990

E=eggs, P= pupae, A=adults.

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

isolate were harvested by scraping, then suspending them in sterile distilled water containing 0.1% Tween 80. The suspension was poured into a sterile glass tube and mixed using a vortex mixer. Conidial concentrations were measured with an improved Neubauer hemocytometer (Reichert Scientific Instruments, Buffalo, New York) and adjusted by dilution with 0.1% Tween 80 for each life stage of horn fly as described below.

Susceptibility of eggs to entomopathogenic fungi

Fresh cow manure (50 g), mixed with fish meal in a ratio 10:1 (wt:wt), was placed in clear plastic containers (6 cm diameter x 6 cm height). Twenty eggs of *H. irritans* less than 4 h old were placed on the cow manure: fish meal mixture in each container using a camel hair brush. Four groups of 20 eggs each were inoculated with 4 ml sprays of a conidial suspension of each isolate of the entomopathogenic fungi at a concentration of 1 x 10^6 conidia/ml ($1.1x10^5$ conidia/cm^2 of manure surface). The conidial suspensions were sprayed using a hand sprayer. Four control groups of eggs were sprayed with sterile distilled water with 0.1% Tween 80. After the inoculation, the eggs were incubated at 30o C, and 58% RH, in total darkness. The emergence of adults was observed and recorded at 48 h-intervals until adult emergence was complete. Dead horn flies were incubated in sterile Petri dishes on filter paper moistened with sterile distilled water at 25° C and ≈100% RH for 4 days to allow growth of surface mycelia of the fungi.

Susceptibility of pupae to entomopathogenic fungi

Groups of 20 pupae less than 48 h old were placed in Petri dishes (60 X 15 mm) lined with two moistened layers of Whatman No. 1 filter paper. Pupae were inoculated with 4 ml of conidial suspension using a hand sprayer. The suspension was adjusted to a concentration of 1 x 10^8conidia/ml to achieve $3.5x10^6$ conidia/cm^2. Untreated controls were sprayed with sterile distilled water containing 0.1% Tween 80 to similar groups of 20 pupae. The Petri dishes were incubated at 25 ± 1° C and 70% RH, in total darkness. Mycosis in the pupae was recorded during 24 h intervals until adult emergence. Pupae showing symptoms or signs of mycosis were placed in Petri dishes (60 x 15 mm) with a moistened double-lined filter paper to allow for the growth of surface mycelia.

Susceptibility of adults to entomopathogenic fungi

A suspension of conidia of each 21 day-old isolate (1 x 10^8conidia/ml) was used to inoculate with hand sprayer adults less than 48 h old to determine their susceptibility to entomopathogenic fungi (Watson et al., 1995). Four groups of 20 adults were counted out (Cantagui et al., 1995) and immobilized by exposure to cold temperature (4 ± 1° C) for 2 minutes (Crosby et al., 1991). Adults were placed on a moistened double-lined filter paper in plastic 500-ml containers (10 cm height x 10 cm diameter) and, spayed with a 1 x 10^8 conidia/ml suspension of each isolate. Untreated controls consisted of four groups of 20 flies, sprayed with sterile distilled water containing Tween 80 (Poprawsky et al., 1985). After inoculation, a 10-cm lid was placed on each container. The interior of the lid was replaced with a 2 mm mesh metallic mosquito screen to allow for air circulation and feeding the flies. Adults were fed *at libitum* with bovine defibrined blood in saturated cellucotton pads covered with gauze (Schmidt et al., 1967). All containers were placed in insulated boxes with a 2-cm layer of water and maintained at 27 ± 1° C.

Twenty four treatments with four replications each were evaluated using a completely randomized design. Emergence data, the percent of pupae, and adults exhibiting mycosis were transformed using arcsine transformation and subjected to ANOVA. Beginning 48 hours after inoculation, dead adults were recorded at 24-h intervals. Dead adults were placed in Petri dishes (60 x 15 mm) with a moistened double-lined filter paper to allow the growth of surface mycelia (Arthurs and Thomas, 2001). Means were separated by Tukey test ($P < 0.05$) (SAS Institute, 1989).

Mean lethal concentration of the most pathogenic fungi against horn fly adults

The most pathogenic isolates of *M. anisopliae*, and *P. fumosoroseus* from the adult susceptibility studies were selected to determine the mean lethal concentrations (LC_{50}). Conidia of 20 d-old incubations of each isolate were used and the concentrations were adjusted to 1 x 10^2, 1 x 10^3, 1 x 10^4, 1 x 10^5, 1 x 10^6, and 1 x 10^7 conidia/ml. Groups of 20 adults 24-48 d-old were counted out (Butt *et al.* 1994) and immobilized as described above. Adults were placed on a moistened double-lined filter paper in plastic 500-ml containers (10 cm height x 10 cm diameter) and, each group was sprayed with the correspondent concentration of

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

the suspension of each isolate. Untreated controls consisted of four groups of 20 flies, sprayed with sterile distilled water with 0.1% Tween 80 (Poprawsky et al., 1985). After inoculation, adults were handled as described above.

Seven treatments with four replications each for each selected fungal isolate were evaluated using a completely randomized design. Beginning 48 h after inoculation, dead adults were recorded at 24-h intervals. Dead adults were placed in Petri dishes (60 x 15 mm) with a moistened double-lined filter paper to allow for the growth of surface mycelia (Butt *et al.* 1994; Smith *et al.* 2000; Arthurs and Thomas, 2001). Data of mortality percentage were normalized by arcsine transformation prior to the analysis. Mean lethal concentrations and associated statistics were estimated by Probit analysis (SAS Institute, 1989).

Results

Effects of application of entomopathogenic fungi on eggs

Application of the entomopathogenic fungi *M. anisopliae,*, *P. fumosoroseus*, and *B. bassiana*, to eggs of *H. irritans* caused reduced adult emergence. The most effective isolates were, Ma 3, Ma 25, Ma 15, Pfr 1, Pfr 8, and Bb 17 (Table 2). Significant differences between some treatments compared to controls were demonstrated (F = 8.65, P < F=0.0001, df = 19) by the following fungal strains: Ma3, Ma15, Ma25, Pfr1, and Pfr8, all with adult horn fly emergence values lower than 7%.

Effects of application of entomopathogenic fungi on pupae

The pupae of *H. irritans* were also susceptible to isolates of the entomopathogenic fungi. The highest mortality was obtained with the isolates Ma2, Ma25, Pfr9, Pfr10, Pfr11 and Bb 24 (Table 2). The ANOVA showed differences between treatments (F = 11.7, F < P = 0.0001, df = 21). The Tukey test indicated no significant differences between isolates Ma2, Ma14, Ma25, Bb21, Pfr9, Pfr10, and Pfr11 and these treatments produced the highest levels of mortality (Table 2).

Susceptibility of adults to entomopathogenic fungi

Application of entomopathogenic fungal isolates to adults caused a range of mortality between 5, and 100%. The most effective isolates were Ma6, Ma10, Ma14, Ma15, and Ma25, Bb21, Bb17, Pfr3, Pfr7, Pfr8, Pfr9, Pfr10, Pfr11, and Pfr12 (Table 2). The ANOVA showed differences between treatments (F = 28.66; F < P = 0.0001; df = 23). Tukey test showed no significant differences (P<0.05) between the isolates Pfr9, Pfr12, Ma10, Ma14; Ma6, Pfr11, Ma15, Pfr3, Pfr10, Pfr1, Pfr7, Pfr8, Pfr5, Ma25, and Bb17 all of which produced the highest mortality (Table 2).

Table 2. Percentage horn fly adult emergence and mortality of pupae and adults, respectively, as response to *M. anisopliae* (Ma), *P. fumosoroseus* (Pfr), and *B. bassiana* (Bb) at doses of 1 x 10^6 conidia/ml on eggs and 1 x 10^8 conidia/ml on pupae and adults.

Fungi isolates	Effect of application on eggs (% adult emergence)	The effect of application on pupae (% mortality)	The effect of applicaton on adults (% mortality)
Ma 1	63.8 a	16.3 cdefg	6.25 h
Ma 2	15.0 bcdef	71.3 a	53.8 cdef
Ma 3	3.8 f	6.3 fg	7.5 gh
Ma 6	----	8.8 defg	97.5 a
Ma 7	38.8 abcde	17.5 bcdefg	50.0 def
Ma10	50 abc	1.3 fg	100.0 a
Ma 13	26.3 abcdef	6.3 fg	5.0 gh
Ma14	46.3 abc	46.3 abcde	98.8 a
Ma 15	5.0 def	22.5 bcdef	92.5 ab
Ma 25	6.3 ef	68.8 a	85.0 abcd
Ma 38	-------	-----	65.0 bcde
Pfr 1	3.8 ef	13.8 bcdefg	92.5 abc
Pfr 3	27.5 abcdef	23.8 bcdef	87.5 abc
Pfr 4	---	---	17.5 fgh
Pfr 5	51.3 abc	7.5 efg	82.5 abcd
Pfr 7	20.0 bcdef	21.3 bcdefg	85.0 abcd
Pfr 8	3.8 ef	17.5 bcdefg	85.0 abcd
Pfr 9	26.3 abcdef	41.3 abcde	100.0 a
Pfr 10	68.8 a	53.8 ab	90.0 abc
Pfr 11	55.0 ab	50.0 abc	97.5 a
Pfr 12	-----	15.0 bcdefg	100.0 a
Bb17	11.3 cdef	0 (0) f	73.8 abcde
Bb 21	43.8 abcd	44.0 (48.8) abc	40.0 efg
Control	72.0 a	00.0 g	0.0 h

Means between columns followed by the same letter are not significantly different (Tukey test, P < 0.05).

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Mean lethal concentration of the most pathogenic fungi against horn fly adults

The LC_{50}s for the *M. anisopliae* isolates Ma6, Ma10, and Ma14 ranged from 8.08 x 102 to 9.28 x 106conidia/ml (Table 3) with the most virulent isolate being Ma 6. The LC_{50}s for the *P. fumosoroseus* isolates were similar to the less virulent *M. anisopliae* strains (Table 3).

Table 3. LC_{50} for selected isolates of *Metarhizium anisopliae* (Ma) and *Paecilomyces fumosoroseus* (Pfr) against horn fly adults.

Isolate	LC_{50}	95% CI	Equation	X^2
Ma6	8.08×10^2	1.35×10^2-2.74×10^3	Y=0.25 X+3.76	33.1
Ma10	1.18×10^5	8.08×10^4-1.73×10^5	Y=0.99 X-1.99	10.38
Ma14	9.28×10^6	6.06×10^6-1.5×10^7	Y=0.9 X-3.03	47.76
Pfr9	7.56×10^5	4.65×10^5-1.25×10^6	Y=0.59 X+0.32	20.75
Pfr11	4.49×10^6	3.01×10^6-6.91×10^6	Y=0.59 X=0.32	18.31

Discussion

The hypothesis of this research was that egg, pupa and adult of *Haematobia irritans* are susceptible to the entomopathogenic fungi *M. anisopliae, P. fumosoroseus*, and *B. bassiana,* by reducing adult emergence when applied on groups of eggs and pupae and producing mortality when applied to adults. The results showed that these stages are susceptible to the different isolates of the evaluated fungi. However, differences were detected in the susceptibility between and among isolates of these three fungal species.

Treatments with the fungi *M. anisopliae,* and *P. fumosoroseus*, on horn fly eggs reduced adult emergence. When the entomopathogenic fungi attacked the eggs, the eggs did not hatch and we recovered the fungi from cadavers. Healthy eggs hatched in about 36 hours, and pupae produced healthy horn flies. We did not observe saprophytic contamination in controls. The isolates Ma 2, Ma 3, Ma 15, Ma 25, Pfr 1 and Pfr 8 reduced emergence to less than 7%, in comparison with the emergence of about 72.5% in the control treatments. These results corroborate the need to evaluate fungal isolates of different species, origins, and strains for selecting the most effective isolate or species with the object of regulating insect pest populations. These findings support the previous reports of Lezama-Gutiérrez et al. (1996), who reported that the eggs of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) are susceptible to entomopathogenic fungi, resulting in control before the pest can harm the crop.

The use of entomopathogenic fungi as a strategy for larval control of *Musca domestica* has been suggested by Watson et al. (1995), particularly if the larvae remain in confined areas. They reported mortality of about 56% after applying a concentration of 1×10^{10} conidia/cm^3 to manure. In our research, the results suggest that the use of entomopathogenic fungi could reduce the horn fly populations although it would be necessary to evaluate the fungal persistence in the inoculated manure. Because of the effects of manure pH, and microbial flora on the fungal persistence in naturally dried manure remain unknown, as well as the impact of fungi on parasitoids, it will be necessary to evaluate their performance under field conditions (Hu and Frank, 1996).

Horn fly pupae were also susceptible to entomopathogenic fungi. The isolates Ma2, and Ma25 of *M. anisopliae* caused high levels of mycosis in pupae. The remaining isolates caused less than 50% of infection in pupae. Similar results have been reported by Ekesi et al. (2002) using entomopathogenic fungi against *Ceratitis capitata* (Wiedeman) pupae with mortality of 94%.

There was considerable variability in the pathogencity of the fungal isolates. Three isolates of *M. anisopliae* (Ma6, Ma10, and Ma14), and four of *P. fumosoroseus* (Pfr9, Pfr10, Pfr11, and Pfr12) exhibited the highest pathogenicity causing adult mortality rates higher than 90%. The isolates of *B. bassiana* caused adult mortality rates between 40% and 73.8%. Steenberg et al. (Steenberg et al., 2001a) reported that *B. bassiana* caused 100% adult mortality in *H. irritans* seven days post inoculation. Their mortality values are higher than ours, but it could be due differences in the concentrations used. Steenberg et al. (Steenberg et al., 2001a) also reported susceptibility of horn fly adults to *Paecilomyces farinosus* with mortality rates of 90% after 7 days of inoculation. They also mentioned that other fungi such as *M anisopliae* and *P. fumosoroseus* showed high pathogenicity against horn flies.

Differences in virulence (LC_{50}) between selected isolates of *M. anisopliae*, and *P. fomosoroseus* were determined against horn fly adults and showed that Ma6 had the lowest LC_{50} (8.08×10^2conidia/ml). Several studies on other Diptera mentioned that high mortalities were produced with isolates of *M. anisopliae* (Lezama-Gutiérrez et al., 2000), *B. bassiana* (Watson et al., 1995, Meadow et al.,

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

1996), and *P. fumosoroseus* (Steenberg et al., 2001b) but we highlight the low concentration needed to diminish horn fly populations to 50% with the isolates Ma6. Our findings corroborate their results and the hypothesis that selected isolates of *M. anisopliae*, and *P. fumosoroseus* can be considered as microbial control agents for use in an IPM program against the horn flies.

Overall, our results support the selection of the isolates Ma2, Ma 3 and Ma25, as candidates for biological control of different biological stages of *H. irritans*. The isolate Ma25 reduced the adult emergence to 6.3%, caused mycosis on 69% of the horn fly pupae bioassayed, and reduced adult emergence when applied to eggs.

Acknowledgements

The authors acknowledge the support of this work by the grant SAGARPA-2002-CO1-0853/A-10obtained from the Fondo Sectorial de Investigación en Materia Agrícola, Pecuaria, Acuacultura, Agrobiotecnología y Recursos Fitogenéticos of the CONACYT-Mexico, and to CONACYT for granting to the first author with the grant No. 174412. We also thank Dr. John J. Hamm (USDA-ARS Crop Protection and Management Research Unit, Tifton, GA) for review of the manuscript. This paper is a contribution of the Universidad de Colima-Facultad de Ciencias Biológicas y Agropecuarias, Tecomán, Colima, México, and the University of Nebraska Agricultural Research Division, Lincoln, NE 68583. Journal Series No 14797, Department of Entomology, University of Nebraska Lincoln.

References

Arthurs S, Thomas MB. 2001. Effect of dose pre-mortem host incubation temperature and thermal behaviour on host mortality, mycosis and sporulation of *Metarhizium anisopliae* var. *acridum* in *Schistocerca gregaria*. *Biocontrol Science and Technology 11*:411-420.

Byford RL, Lockwood JA, Smith SM, Sparks TC, Luther DG. 1987. Insecticide mixtures as an approach to the management of pyrethroid-resistant horn flies (Diptera: Muscidae). *Journal of Economic Entomology* 80: 111-116.

Byford RL, Craig ME, Crosby BL. 1992. A review of ectoparasites and their effects on cattle production. *Journal of Animal Science* 70: 597-602.

Cantagui MA, Campbell JB, Thomas GD, Boxler DJ. 1995. Average daily gains of Brahman-Crossbred term exposure to stable flies (Diptera: Muscidae). *Journal of Economic Entomology* 88: 1349-1352.

Crosby BL, Byford RL, Kinzer HG. 1991. Insecticide resistance in the horn fly, *Haematobia irritans* (L.), in New Mexico: survey and control. *Southwestern Entomologist* 16: 301-309.

Cruz-Vázquez C, Vitela-Mendoza I, Ramos-Parra M, Quintero-Martínez MT, García-Vázquez, M. 1999. Presencia de *Haematobia irritans* (L.)(Diptera: Muscidae) en ganado lechero estabulado de Aguascalientes, México: Informe preliminar. *Veterinaria Mexico* 30(2): 205-208.

Cupp EW, Cupp MS, Ribeiro JM, Kunz, SE. 1998. Blood-feeding strategy of *Haematobia irritans* (Diptera: Muscidae). *Journal of Medical Entomology* 35(4): 591-595.

Ekesi S, Maniania NK, Lux SA. 2002. Mortality in three African tephritid fruit fly puparia and caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. *Biocontrol Science and Technology* 12: 7-17.

Finney DJ. 1971. Probit analysis. Cambridge University Press, England.

Greer NI, Butler DF. 1973. Comparisons of horn fly development in manure of five animal species. *Florida Entomologist* 56(3): 197-199.

Guglielmone AA, Anziani OS, Mangold AJ, Lorgi RE, Volpogni MM, Flores SG. 1997. Seasonal variation of *Haematobia irritans* (Diptera: Muscidae) in a recently infested region of Central Argentina. *Bulletin of Entomological Research* 87: 55-59.

Guglielmone AA, Castelli ME, Volpogni MM, Medus PD, Martins RJ, Duarez VH, Anziani OS, Mangold AJ. 2001. Toxicity of cypermethrin and diazinon to *Haematobia irritans* (Diptera: Muscidae) in its American Southern range. *Veterinary Parasitology* 101: 67-73.

Hogsette JA. 1999. Management of ectoparasites with biological control organisms. *International Journal of Parasitology* 29: 147-151.

Hu GY, Frank JH. 1996. Effect of the red imported fire ant (Hymenoptera: Formicidae) on dung-inhabiting arthropods in Florida. *Environmental Entomology* 25: 1290-1296.

Kunz SE, Miller AJ, Simms PL. 1984. Economics of controlling horn flies (Diptera: Muscidae) in range cattle management. *Journal of Economic Entomology* 77: 657-660.

Lezama-Gutiérrez R,Alatorre-Rosas R, Bojalil-Jaber LF, Molina-Ochoa J, Arenas-Vargas M, González-Ramírez M, Rebolledo-Domínguez O. 1996. Virulence of five entomopathogenic fungi (Hyphomycetes) against, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs and neonate larvae. *Vedalia* 3: 35-39.

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Lezama-Gutiérrez R, Trujillo de la Luz A, Molina-Ochoa J, Rebolledo-Domínguez O, Pescador-Rubio A, López-Edwards M, Aluja M. 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboaratory and field trials. *Journal of Economic Entomology* 93(4): 1080-1084.

Meadow R, Vandenberg JD, Shelton AM, Watson DW. 1996. Microbial control of cabbage maggot-Preliminary screenings of fungal isolates. *Arthropod Management Tests* 21: 443.

Mendes J, Linhares AX. 1999. Diapause, pupation, sites and parasitism of the hornfly, *Haematobia irritans*, in South-eastern Brazil. *Medical and Veterinary Entomology* 13: 1-6.

Moorhouse ER, Gillespie AT, Charnley AK. 1993. Selection of virulent and persistent *Metarhizium anisopliae* isolates to control black vine weevil (*Otiorhynchus sulcatus*) larvae on glasshouse begonia. *Journal of Invertebrate Pathology* 62: 67-52.

Mwangala FS, Galloway TD. 1993. Susceptibility of horn flies, *Haematobia irritans* L. (Diptera: Muscidae), to pyrethroids in Manitoba. *Canadian Entomologist* 125: 47-53.

Poprawsky TJ, Robert RH, Majchrowicz L, Boivin G. 1985. Susceptibility of *Delia antiqua* (Diptera. Anthomyiidae) to eleven isolates of entomopathogenic hyphomycetes. *Environmental Entomology*. 14: 557-561.

SAS Institute. 1989. SAS/STAT users guide, version 6, 4th ed. SAS Institute, Cary, NC.

Schmidt CD, Harris RL, Hoffman RA. 1967. Mass rearing of the horn fly, *Haematobia irritans* (Diptera: Muscidae), in the laboratory. *Annals of the Entomological Society of America* 60: 508-510.

Sheppard DC. Joyce JA. 1992. High levels of pyrethroid resistance in horn flies (Diptera: Muscidae) selected with cyalothrin. *Journal of Economic Entomology*. 85: 1587-1593.

Sparks TC, Quisenberry SS, Lockwood JA, Byford RL, Roush RT. 1985. Insecticide resistance in the horn fly, *Haematobia irritans*. *Journal of Agricultural Entomology* 2: 217-233.

Steenberg T, Jensen KMV, Jespersen JB. 2001a. Microbial control of flies on pastured cattle. *DJF-rapport NR*. 49: 87-90.

Steenberg T, Jespersen JB, Jensen KMV, Nielsen BO, Humbert RA. 2001b. Entomopathogenic fungi in flies associated with pasture cattle in Denmark. *Journal of Invertebrate Pathology* 77: 186-197.

Torres P, Prieto O. 1993. La mosca de los cuernos *Haematobia irritans*, pp. 357-457. *In* Enfermedades parasitarias de importancia económica en bovinos, bases epidemiológicas para su prevención y control. Nari, A. y Fiel, C. (Eds). Editorial Hemisferio Sur. Montevideo, Uruguay.

Wall R, Strong L. 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. *Nature* 327: 418-421.

Watson DW, Geden CJ, Rutz DA. 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). *Biological Control* 5: 405-411.

Downloaded From: https://bioone.org/journals/Journal-of-Insect-Science on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use