Subtropical Plant Science, 49: 50-52.1997

Antibiotic Treatment of a *Nosema* sp. (Protozoa: Microsporida) Infecting the Ovaries of a Parasitic *Encarsia* Wasp (Hymenoptera: Aphelinidae)

Russell Sheetz[1], John Goolsby[1] and Tadeusz Poprawski[2]

[1]Mission Plant Protection Center, USDA-APHIS-PPQ, P.O. Box 2140, Mission, TX 78573
[2]Beneficial Insects Research Unit, USDA -ARS-SARC, and Texas A&M, Texas Agricultural Experiment Station, 2413 East Highway 83, Weslaco, TX 78596 (corresponding author)

ABSTRACT

A colony of exotic *Encarsia* nr. *pergandiella* parasitic wasps that was being mass-reared for biological control of *Bemisia argentifolii* was found infected with a microsporidium of the genus *Nosema*. One-time *per os* administration of the antibiotic rifampicin to infected parental wasps resulted in effective treatment of microsporidiosis in both the parental wasps and their progeny.

RESUMEN

Una colonia exótica de la avispa parasítica *Encarsia* nr. *pergandiella* que estaba siendo liberada en masa para el control biológico de *Bemisia argentifolii* fue encontrada infectada con un microsporidio del género *Nosema*. Una sola administración oral del antibiótico rifampicina a avispas progenitoras infectadas resultó ser un tratamiento efectivo para la microsporidiosis tanto en las avispas progenitoras como en su progenie.

A species of *Encarsia* nr. *pergandiella* from Sete Lagoas, Brazil (identification number M94055) (Hymenoptera: Aphelinidae) is a uniparental, parasitic wasp of *Bemisia argentifolii* Bellows and Perring [= *Bemisia tababaci* (Gennadius) biotype "B" (Homoptera: Aleyrodidae)] (Goolsby et al., 1996). This wasp was mass-reared at the USDA-APHIS-PPQ Mission Plant Protection Center (MPPC) at Mission, TX, until a steady decline in fecundity severely limited its production. Upon examination of body tissues (see Poinar and Thomas, 1984; Undeen and Vávra, 1997), a microsporidian in the genus *Nosema* (Protozoa, Microspora: Microsporida) (see Poinar and Thomas, 1984; Larsson, 1988) was found infecting the ovaries of the wasp. Microsporidians are parasites of vertebrates and invertebrates and probably occur in all insect orders. Insects are the most important hosts to microsporidia, and approximately half the number of species are parasites of insects (Larsson, 1988); examination of insects in laboratory rearings frequently yields a microsporidium (Shapiro, 1984; Undeen and Vávra, 1997). All microsporidians are obligate pathogens and multiply only in living cells. Many cause severe acute infections in insects, but some produce only inapparent or chronic infections, which however may play an important role in regulating insect populations (Tanada and Kaya, 1993).

Wolbachia species (Rickettsiales: Rickettsiaceae) (Zchori-Fein et al., 1995; Werren, 1997) and other microorganisms (Stouthammer et al., 1990) may induce reproductive cytoplasmic incompatibility in some parasitic wasps. *Wolbachia*-associated asexuality in *Apoanagyrus diversicornis* (Hymenoptera: Encyrtidae) was cured (from thelytoky to arrhenotoky) through feeding on the antibiotics rifampicin, sulphadiazine and tetracycline (Pijis et al., 1996). *Per os* administration of antibiotics including 10% rifampicin to four species of *Trichogramma* (Hymenoptera: Trichogrammatidae) (Stouthamer et al., 1990); 0.05% rifampicin to *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Kajita, 1993); or 5% tetracycline to *E. formosa* (Zchori-Fein et al., 1991) reversed microorganism-induced reproductive incompatibility in these parasitic wasp species. Rifampicin (3-[4-Methylpiperazinyliminomethyl]-rifamycin SV; Rifampin) is a potent inhibitor of DNA-dependent RNA polymerase of bacterial and chloroplast origin; it inhibits initiation of RNA synthesis by binding to ß-subunits of RNA polymerase. It has also been reported to inhibit the assembly of DNA and protein into mature virus particles Yoshikawa and Norman, 1984). The objective of our study was to evaluate the effect of the antibiotic rifampicin on the microsporidian infecting the ovaries of *E.* nr. *pergandiella.*

MATERIALS AND METHODS

Fifty mg crystalline rifampicin (Sigma, St, Louis, MO) were mixed with 0.18 ml glycerin, 0.5 ml distilled water, and 1 ml honey, to give a final concentration of 30,000 ppm (3%) antibiotic. The glycerin was added first to wet the rifampicin crystals; the water was added next and the mixture was stirred for 5 min; the honey was then added which made the mixture a homogenous, red, viscous solution. The test *Encarsia* wasps originated from a colony that had been maintained at MPPC for more than five generations. Fifty female wasps were placed in a culture tube that was streaked on the inside with the

Table 1. Intensity of *Nosema* infection and percent infection in rifampicin-treated and untreated parental *Encarsia* wasps and in their respective progeny.

Sample	Sample size	Intensity of infection[a] and percent infected			
		Heavy	Light	Very Light	No infection
Parental (P_1) control	6	33.3	0	0	66.7
Parental (P_1) treated	10	11.1	11.1	0	77.8
F_1 form P_1 control	9	0	100	0	0
F_1 from P_1 treated	10	0	0	30.0	70.0

[a]See text for details.

antibiotic-treated honey and 50 additional females were placed in a separate tube which contained untreated honey (control). Both tubes were observed for 1 h for foraging and feeding by the wasps, and then placed in an environmental growth chamber in which they were maintained for 2 d at 24 to 29°C and 50-70% RH under a photophase of 14:10 (L:D) h. The treated and untreated wasps were then transferred into two separate rearing cages which each contained a hibiscus plant infested with 2nd- and 3rd-instar *B. argentifolii*. These P_1 (parental) wasps were allowed to oviposit, and after 3 d the live P_1 wasps were collected at random from each cage and immediately frozen at 0°C. Hosts were reared until parasitoid emergence (F_1). Live wasps of the emerged F1 progeny were also randomly collected from each cage and frozen at 0°C.

Microscopic examination on both samples was performed 2 wk after emergence of the F_1 generation. The wasps were thawed at room temperature, dissected using minute insect pins, and the ovaries transferred onto microscope slides. Squash preparations of the ovaries were made as described in Larsson (1988). The preparations were stained using the Giemsa stain for microsporidian spores (Poinar and Thomas, 1984), before examination under oil immersion (phase contrast). The intensity of infection in each sample was scored as heavy, light, very light, and no infection, corresponding to an average of over 6, 3 to 5, 1 to 2, and no microsporidian spores present, respectively, for each field of view scanned (3 field of views were scanned for each sample and the counts averaged).

RESULTS AND DISCUSSION

The ovaries of 35 wasps were examined (Table 1). The ovaries of 66.7% of the control group were not infected by *Nosema* and 33.3% were heavily infected. One hundred percent of the F_1 progeny from the control group were lightly infected. This indicates that without antibiotic treatment, the microsporidian infection is easily transmitted vertically (from one generation to the next), either on the egg surface (transovum) or in the egg (transovarial). In the treated wasps, a drop of 7.8% occurred in uninfected wasps from the P_1 to the F_1. However, the overall intensity of infection decreased compared to the controls, as evidenced by the loss of heavily and lightly infected females from the P_1 to the F_1. Further treatment over several generations or prolonged P_1 treatment with rifampicin could result in a complete cure of the infection.

Protozoa, mainly microsporidia, can represent serious problems in the rearing of many insects. Microsporidiosis (infection cause by a microsporidian) may affect egg production which may result in eventual decline of the colony. The disease can also cause high mortality in the infected hosts. For example, in one biological control program, larvae of the cinnabar moth, *Tyria jacobaeae* (L.), which is used for control of the tansy ragwort, *Senecio jacobaea* L., were heavily infected by a microsporidian. This led Bucher and Harris (1961) to conclude that the microsporidian may play an important role in the failure of mass-reared lepidopterans to control weeds. Microsporidia and other protozoan pathogens are usually enzootic in field populations of insects (Goodwin, 1984). Some microsporidians are known from parasitic Hymenoptera (Brooks, 1974). Parasitoids infected in the field or in the insectary may survive to reproduce and spread the infection throughout the environment. This implies that the elimination of microsporidians from introduced parasitic wasp species could be a practical means of improving classical biological control through improving parasite activity in new habitats. Rifampicin may be a useful curative and preventative treatment of *Nosema* infection in mass production of *Encarsia* and other aphelinid parasitoids. Additional studies to determine the long term efficacy of the treatment are warranted.

ACKNOWLEDGMENTS

This research was conducted in partial fulfillment (RS) of the requirements of a Biological Problems course at the University of Texas Pan-American, Edinburg, TX. We thank M. Hunter (Univ. of Arizona) for advice on the use of rifampicin. The manuscript was improved through reviews kindly provided by M. Ciomperlik (USDA-APHIS-PPQ, Mission, TX) and S. Greenberg (USDA-ARS, Weslaco, TX). Mention of a proprietary product does not constitute an endorsement by the USDA.

REFERENCES

Brooks, W. M. 1974. Protozoan infections. p.237-300. In G. E. Cantwell (ed.). Insect Diseases, vol.1. Marcel Dekker, New York.

Bucher, G. E. and P. Harris. 196l. Food-plant spectrum and elimination of disease of cinnabar moth larvae, *Hypocrita jacobaeae* (L.) (Lepidoptera: Arctiidae). Can. Entomol. 93:931-936.

Goodwin, R.H. 1984. Recognition and diagnosis of diseases in

insectaries and the effects of disease agents on insect biology. p.96-129. In E.G. King and N. C. Leppla (eds.). Advances and Challenges in Insect Rearing. US Department of Agriculture, Agricultural Research Service, New Orleans, LA.

Goolsby, J.A., J.C. Legaspi and B.C. Legaspi. 1996. Quarantine evaluation of exotic parasitoids of the sweet potato whitefly, *Bemisia tabaci* (Gennadius). Southwest. Entomol. 21:13-21.

Kajita, H. 1993. Induction of males in the thelytokous wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae). Appl. Entomol. Zool. 28:115-117.

Larsson, J.I.R. 1988. Identification of microsporidian genera (Protozoa, Microspora) - a guide with comments on the taxonomy. Arch. Protistenkd. 136:1-37.

Pijls, J.W.A.M., H.J. van Steenbergen, and J.J.M. van Alphen. 1996. Asexuality cured: the relations and differences between sexual and asexual *Apoanagyrus diversicornis*. Heredity 75:506-513.

Poinar, G.O., Jr. and G.M. Thomas. 1984. Laboratory Guide to Insect Pathogens and Parasites. Plenum Press, New York and London. 392 pp.

Shapiro, M. 1984. Micro-organisms as contaminants and pathogens in insect rearing. p. 130-142. In E. G. King and N. C. Leppla (eds.). Advances and Challenges in Insect Rearings. US Department of Agriculture, Agricultural Research Service, New Orleans, LA.

Stouthamer, R., R.F. Luck and W.D. Hamilton. 1990. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera: Trichogrammatidae) to revert to sex. Evolution 87: 2424-2427.

Tanada, Y. and H.K. Kaya. 1993. Insect Pathology. Academic Press, San Diego. 666 pp.

Undeen, A.H. and J. Vávra. 1997. Research methods for entomopathogenic Protozoa. p.117-151. In L. Lacey (ed.). Manual of Techniques in Insect Pathology. Academic Press, San Diego.

Werren, J.H. 1997. Biology of *Wolbachia*. Annu. Rev. Entomol. 42:587-609.

Yoshikawa, T.T. and D.C. Norman. 1984. Rifampicin. p.335-337. In A. M. Ristuccia and B.A. Cunha (eds.). Antimicrobial Therapy. Raven, New York.

Zchori-Fein, E., R.T. Roush and M.S. Hunter. 1991. Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species. Experientia 48:102-105.

Zchori-Fein, E., O. Faktor, M. Gottlieb, H. Czosnek, and D. Rosen. 1995. Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). Insect Mol. Biol. 4:173-178.