Journal of Chemical Ecology, Vol. 31, No. 10, October 2005 (©2005)
DOI: 10.1007/s10886-005-7114-z

EFFECTS OF GELDANAMYCIN ON HATCHING AND JUVENILE MOTILITY IN *Caenorhabditis elegans* AND *Heterodera glycines*

ANDREA M. SKANTAR,[1,*] KELI AGAMA,[3] SUSAN L. F. MEYER,[1] LYNN K. CARTA,[1] and BRYAN T. VINYARD[2]

[1]*Nematology Laboratory, USDA-ARS, Beltsville, MD 20705, USA*
[2]*Biometrical Consulting Service, Beltsville, MD 20705, USA*

(Received November 22, 2004; revised May 11, 2005; accepted May 28, 2005)

Abstract—Several *Streptomyces* species are known to produce metabolites that inhibit plant pathogens. One such compound is geldanamycin (GA), a benzoquinone ansamycin originally isolated from *Streptomyces hygroscopicus*. We examined the effect of geldanamycin on egg hatch and juvenile motility in *Caenorhabditis elegans* and in two populations of the plant-parasitic nematode *Heterodera glycines*. When *C. elegans* eggs were exposed to geldanamycin, both hatch and motility were reduced by GA doses between 2 and 50 μg/ml. The *H. glycines* inbred populations TN17 and TN18 exhibited low dose stimulation of hatch and motility, whereas levels occurring at higher GA doses were at or below control levels. These experiments represent the first demonstration of geldanamycin effects in *C. elegans* and *H. glycines* and suggest that the heat shock chaperone Hsp90, the known molecular target of geldanamycin, may be involved in nematode egg hatch and motility. This study also indicates that geldanamycin-producing strains of *Streptomyces* may be useful as biocontrol agents for nematodes.

Key Words—Soybean cyst nematode, *Heterodera glycines*, *Caenorhabditis elegans*, hatching, motility, behavior, eggs, geldanamycin, *Streptomyces*, Hsp90, biocontrol.

* To whom correspondence should be addressed. E-mail: skantara@ba.ars.usda.gov
[3] Current address: Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

INTRODUCTION

Several species of *Streptomyces* have proven effective at reducing plant-parasitic nematode damage (reviewed in Siddiqui and Mahmood, 1999). In addition, metabolites produced by *Streptomyces* spp. were demonstrated to be nematicidal (Nair et al., 1995) or to inhibit nematode hatching (Lee et al., 1996) or motility (Mura et al., 1999). The ability to control bacterial or fungal plant pathogens has been demonstrated for the strains *Streptomyces violaceusniger* YCED-9 (Trejo-Estrada et al., 1998a,b) and *Streptomyces hygroscopicus* spp. *geldanus* EF-76 (Beauséjour et al., 2003), but neither has been tested on nematodes. Both of these strains produce the antibiotic geldanamycin (GA), a specific inhibitor of the heat shock protein chaperone Hsp90 (Whitesell et al., 1994). The impact of pure GA on nematodes was explored in a recent report by David et al. (2003). In that study, plate-grown *Caenorhabditis elegans* were exposed to a single, very high dose of GA, but no discernable effects on the nematodes were detected. We theorized that absorption or ingestion of GA by nematodes might be higher in a liquid culture system than on agar plates, and that the response to GA in plant-parasitic nematodes might differ from *C. elegans*. Thus, the objective of the current work was to determine the effect of GA exposure on *C. elegans* and the soybean cyst nematode *Heterodera glycines* in liquid culture bioassays.

METHODS AND MATERIALS

Test Organisms. *C. elegans* wild-type strain (var. Bristol-N2) and *Escherichia coli* strain OP50 were obtained from the *Caenorhabditis* Genetics Center (St. Paul, MN, USA). *H. glycines* strains TN17 and TN18 are inbred lines that were selected on the basis of relative hatching phenotypes. TN17 was selected for "fast hatching" and TN18 for "slow hatching" as defined by the relative number of eggs hatching early and late during a 2-wk period (Terry Niblack, personal communication). Despite nomenclature preferences that differ in the nematology subdisciplines, for simplicity we refer to immature forms of both *C. elegans* and *H. glycines* as "juveniles" (J2).

Nematode Culturing. *C. elegans* was routinely plate-propagated as previously described (Lewis and Fleming, 1995). *H. glycines* strains TN17 and TN18 were maintained on soybean (*Glycine max* (L.) Merr. cv. Essex) as previously described (Nitao et al., 1999).

Nematode Bioassays. Microwell assay procedures were similar to those described previously (Nitao et al., 1999). Assays were conducted in sterile 96-well tissue culture plates (polystyrene, flat bottom wells; Corning, New York, NY, USA). Each trial consisted of 10 replicate wells per treatment, with ~100

eggs/well (exact counts of eggs/well were determined), and the experiment was repeated for each treatment. Geldanamycin (Sigma-Aldrich, St. Louis, MO, USA) was prepared in dimethyl sulfoxide (DMSO) at 18 mM and subsequently diluted in deionized water as required. Controls and all treatments contained a final concentration of no more than 0.1% DMSO.

Caenorhabditis elegans. N2 embryos were harvested from gravid adults as described (Lewis and Fleming, 1995) and resuspended in M9 buffer. Aliquots (50 µl) of egg suspension were placed in each well of 96-well microtiter dishes, and the exact number of eggs per well was determined. GA dissolved in DMSO, or DMSO alone as control, was added to all eggs, and the plates were incubated in the dark at 20°C. GA concentrations tested were 0, 1, 2, 5, 10, 25, 50, and 100 µg/ml. Because GA is known to be light-sensitive (Queitsch et al., 2002) and unstable in aqueous solution (Wiesgigl and Clos, 2001), the compound was handled with care to minimize the possibility of degradation. Numbers of hatched eggs and of motile, hatched juveniles were counted after 21 hr. Motility of hatched juveniles was defined as any unprovoked movement within a 5-sec interval during which nematodes were observed under an inverted microscope.

Heterodera glycines. Eggs were collected from greenhouse-grown soybean [*Glycine max* (L.) Merr. cv. Essex] and surface-disinfested with 0.5% sodium hypochlorite (Nitao et al., 1999). Eggs were placed in 96-well microtiter plates as described above, treated with GA and/or DMSO, and incubated in the dark at 30°C, a temperature within the optimum growth range for *H. glycines* (Alston and Schmitt, 1988). Egg hatch and motility of second-stage juveniles were counted after incubation for 4 d.

Statistical Analysis. Data were analyzed using SAS® v9.1.3 for Windows (SAS Institute Inc., Cary, NC, UAS). Estimates of the percent hatched and percent motile for each nematode type at each concentration were obtained for each of two trials by summing recorded counts over the 10 wells observed per trial: % Hatched Eggs = 100% (# active + # inactive − # larvae)/# eggs; % Motile J2 = 100% (# active)/(# active + # inactive). For each of the three nematode populations, observed counts associated with each duplicate trial at each of the seven observed concentrations (i.e., $N = 14$ data points) were used to conduct all statistical analyses. For each nematode population, a nonlinear dose-response regression was fit to percent hatched and percent motile vs. GA concentration. A log-normal equation was fitted to the *H. glycines* TN17 and TN18 data, whereas a more general exponential equation was fitted to *C. elegans* data (Ratkowsky, 1990). R^2 is the ratio of the regression sum of squares to the total sum of squares (RegSS/TotSS), which reflects the proportion of the total data variability the regression equation has explained. Significant differences in hatching and motility observed at various GA concentrations vs. no GA were determined by conducting pairwise comparisons within an ANOVA

framework; specifically, a logistic regression using SAS® v9.1.3 Proc GLIMMIX with a logit link function and a binomial distribution were used to model percent hatched eggs and percent motile J2 for each nematode population. All statistical comparisons were conducted at the 95% level of significance.

RESULTS

To establish whether GA would interfere with nematode hatching or motility, we exposed *C. elegans* eggs to GA over a 100-fold range of concentrations, including 1, 2, 5, 10, 25, 50, and 100 μg/ml. Pairwise comparisons of percent hatched eggs or percent motile J2 among GA doses and the control are shown in Table 1. The relationships between percent egg hatch and percent juvenile motility vs. GA concentration were plotted (Figure 1). Statistical tests for linearity of the data failed; subsequently, nonlinear equations of exponential form exhibited the best fit to the data among all possibilities examined.

$$\%\,\text{Hatched Eggs} = 100\%[0.77 - 0.038 \cdot \%C_{GA} \cdot \exp(-0.038 \cdot \%C_{GA})]\; r^2 = 32.1\%$$

$$\%\,\text{Motile J2} = 100\%[0.498 - 0.038 \cdot \%C_{GA} \cdot \exp(-0.038 \cdot \%C_{GA})]\; r^2 = 37.5\%$$

Eggs that were exposed to GA concentrations of between 2 and 100 μg/ml exhibited hatch rates that were significantly lower than the control, but each was

TABLE 1. EFFECT OF GELDANAMYCIN ON EGG HATCH AND MOTILITY OF *Caenorhabditis Elegans*

GA conc. (μg/ml)	% Hatched eggs	% Motile J2
0	79.6 ± 7.1a	59.5 ± 7.1 a
2	60.9 ± 10.3 bc	28.1 ± 6.0 c
5	58.6 ± 10.5 cd	38.4 ± 7.0 b
10	54.8 ± 10.7 de	17.1 ± 4.3 d
25	47.6 ± 10.8 f	25.2 ± 5.6 c
50	51.1 ± 10.8 ef	19.3 ± 4.7 d
100	63.9 ± 10.0 b	34.8 ± 6.7 b
Total no. of eggs or J2	13,875	9,160

Means followed by different letters are different ($\alpha = 0.05$) by pairwise comparisons conducted on logit-transformed binomial response data in a logistic regression ($N = 14$; duplicate trials at each concentration).
Reported means ± standard errors were back-transformed to percentages from logit-scale estimates.

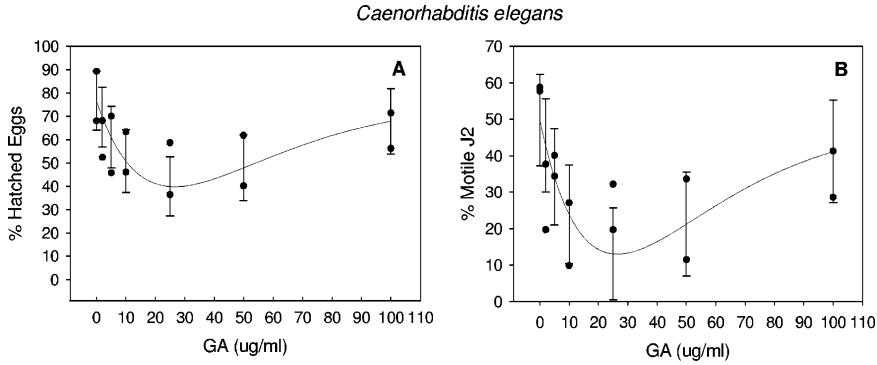

FIG. 1. Effect of geldanamycin (GA) on hatching and motility in *Caenorhabditis elegans*. Duplicate trials were used to fit a nonlinear dose-response regression to % hatched eggs and % motile juvenile values vs. GA concentration. Closed circles at each dose represent data means for the separate trials. Error bars were based upon the variation between duplicate trials pooled across the observed GA concentrations ($N = 14$) and indicate 95% confidence intervals for individual estimates of % hatched or % motile. (A) Percentage of *C. elegans* eggs that hatched after incubation for 20 hr in various concentrations of GA or DMSO control. (B) Percentage of *C. elegans* hatched juveniles that were motile after 20 hr incubation with GA or DMSO control.

similar to the hatch rate of at least one other GA concentration (Table 1). The dose showing the greatest decrease in egg hatch (47.6%) compared to control (79.6%) was 25 μg/ml GA. However, the suppressive effect on hatch rate reversed between 25 and 100 μg/ml GA, with the highest concentration (63.9% hatch) showing a similar effect to 2 μg/ml (60.9%) (Figure 1A). Geldanamycin also reduced the motility of *C. elegans* juveniles that hatched from treated eggs (Table 1, Figure 1B). Motility responses paralleled the hatch responses, with all GA levels showing significantly lower motility than the controls (Table 1). The greatest reductions in motile J2 were observed at 10 and 50 μg/ml GA (17.1% and 19.3% vs. 59.5% control). According to the nonlinear regression model, 25 μg/ml GA were shown to cause the greatest reduction in J2 motility. Curves constructed with nonlinear regression equations were U-shaped for both hatching and motility.

We also measured the effect of GA on egg hatch and juvenile motility in TN17 and TN18, two inbred populations of *H. glycines*. These strains were chosen on the basis of their selected hatching phenotypes (TN17, fast hatching; TN18, slow hatching), to determine whether they would respond differently to GA exposure. The hatch rate for each population was determined after 4 d of incubation in various GA doses suspended in DMSO or in the control treatment of DMSO alone. The dose–response relationships for TN17 and TN18 were

nonlinear. Log-normal equations were fit to the data to generate the curves shown in Figure 2.

TN17

$$\%\text{Hatched} = 100\% \cdot \left[0.098 + 0.086 \cdot \exp\left(-0.5 \cdot \left\{\log_e\left[(\%C_{GA} + 1)/5.3\right]/1.1\right\}^2\right)\right]$$

$$r^2 = 55.5\%$$

$$\%\text{Motile} = 100\% \cdot \left[0.20 + 0.38 \cdot \exp\left(-0.5 \cdot \left\{\log_e\left[\%C_{GA} + 1)/4.5\right]/1.7\right\}^2\right)\right]$$

$$r^2 = 65.5\%$$

TN18

$$\%\text{Hatched} = 100\% \cdot \left[-0.15 + 0.32 \cdot \exp\left(-0.5 \cdot \left\{\log_e\left[(\%C_{GA} + 1)/7.7\right]/3.7\right\}^2\right)\right]$$

$$r^2 = 31.7\%$$

$$\%\text{Motile} = 100\% \cdot \left[-0.05 + 0.36 \cdot \exp\left(-0.5 \cdot \left\{\log_e\left[\%C_{GA} + 1)/3.1\right]/2.2\right\}^2\right)\right]$$

$$r^2 = 89.8\%$$

Pairwise comparisons of percent hatched *H. glycines* eggs or percent motile J2 at each GA dose compared to control are shown in Table 2. For TN17, GA stimulated egg hatch between 2 and 25 μg/ml, but at 50 and 100 μg/ml, the percent egg hatch was similar to the control (Figure 2A). For TN18, a similar stimulation of egg hatch was observed, with both curves exhibiting an asymmetric inverse U shape (Figure 2C). None of the GA doses tested reduced the hatch rate to levels lower than the controls.

For TN17, percent motile J2 was higher than control at 2 and 5 μg/ml and declined to near control levels at 10–50 μg/ml GA (Figure 2B). At 100 μg/ml, the percent motile J2 was significantly lower (27.6%) than control (43.1%). For TN18, GA doses of 25 μg/ml and higher significantly reduced the motility of juveniles (Figure 2D).

DISCUSSION

Our experiments demonstrating the sensitivity of *C. elegans* to GA initially appear to contradict recent *in vitro* and *in vivo* evidence for GA resistance of *C. elegans* Hsp90 (David et al., 2003). In that study, nematodes were contin-

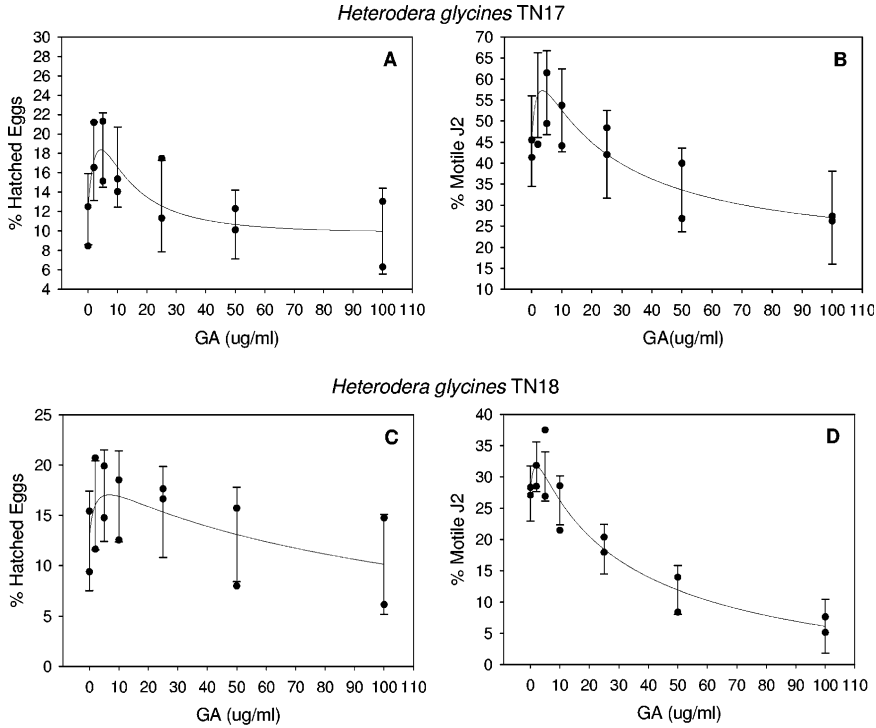

FIG. 2. Effect of geldanamycin (GA) on hatching and motility in *Heterodera glycines* inbred lines. Duplicate trials were used to fit a nonlinear dose-response regression to % hatched eggs and % motile juvenile values vs. GA concentration. Closed circles at each dose represent data means for the separate trials. Error bars were based upon the variation between duplicate trials pooled across the observed GA concentrations ($N = 14$) and indicate 95% confidence intervals for individual estimates of % hatched or % motile. (A) Percentage of *H. glycines* TN17 eggs that hatched after incubation for 4 d in various concentrations of GA or DMSO control. (B) Percentage of *H. glycines* TN17 hatched J2 that were motile after 4 d in GA or DMSO control. (C) TN18, egg hatch vs. GA concentration. (D) TN18, juvenile motility vs. GA concentration.

uously exposed to a single dose of GA, equivalent to our highest level (178 μM = 100 μg/ml GA), but no defects in viability, dauer formation, fertility, or life span were detected over several generations. This study followed the common procedure for testing compounds on nematodes, in which a high concentration is initially used to overcome the relative impermeability of the *C. elegans* cuticle (Rand and Johnson, 1995). In contrast, our multiple dose bioassays allowed us to uncover a complex response pattern that was not detectable with a single GA dose. The reversals of hatch and motility that we observed in *C. elegans* exposed

TABLE 2. EFFECT OF GELDANAMYCIN ON EGG HATCH AND MOTILITY OF THE PLANT PARASITIC NEMATODE *Heterodera glycines*, STRAINS TN17 AND TN18

	TN-17		TN-18	
GA conc. (μg/ml)	% Hatched eggs	% Motile J2	% Hatched eggs	% Motile J2
0	10.5 ± 0.7c	43.1 ± 6.5 cd	12.2 ± 2.7 b	27.5 ± 3.7 a
2	18.8 ± 0.9 a	61.6 ± 6.0 a	16.0 ± 3.4 a	30.0 ± 3.7 a
5	18.1 ± 0.9 a	55.6 ± 6.3 ab	17.0 ± 3.6 a	32.3 ± 3.8 a
10	14.7 ± 0.8 b	48.9 ± 6.5 bc	15.3 ± 3.3 a	25.0 ± 3.4 ab
25	14.3 ± 0.8 b	44.5 ± 6.4 c	16.4 ± 3.5 a	19.2 ± 3.0 b
50	11.3 ± 0.8 c	33.3 ± 6.0 de	11.9 ± 2.7 b	11.3 ± 2.3 c
100	9.7 ± 0.7 c	27.6 ± 5.5 e	10.3 ± 2.4 b	6.4 ± 1.7 c
Total no. eggs or J2	14,926	2330	13,260	2416

Means followed by different letters are different ($\alpha = 0.05$) by pairwise comparisons conducted on logit-transformed binomial response data in a logistic regression ($N = 14$; duplicate trials at each concentration).
Reported means ± standard errors were back-transformed to percentages from logit-scale estimates.

to high GA concentrations are, therefore, congruent with the prior study's inability to detect a phenotype at 100 μg/ml GA. Also, because the prior study administered GA to the surface of agar plates, it is not possible to discern whether uptake of GA by the nematodes was comparable in both studies. Comparison of low dose effects in both plate and liquid assay systems would be necessary to answer this question. These issues underscore the sensitivity of *C. elegans* to experimental conditions when testing responses to pharmacologic agents, and stress the importance of testing a range of doses and assay systems.

It is well established that Hsp90 is the definitive molecular target of geldanamycin binding (Prodromou et al., 1997; Stebbins et al., 1997). This fact, combined with information about the role of Hsp90 (*daf-21*) in *C. elegans*, provides clues about the possible pathways affected by geldanamycin. The different hatch responses to GA that we observed in *C. elegans* and *H. glycines* reflect differences in the way eggs hatch in each species. In *C. elegans,* all eggs are equally competent to hatch without any external stimulation (Albert and Riddle, 1988). The sensitivity of *C. elegans* egg hatch to GA exposure suggests the involvement of Hsp90 in this process, which is consistent with evidence for the presence of Hsp90/DAF-21 within the germline (Inoue et al., 2003). In contrast to *C. elegans*, the hatching process in cyst nematodes is complex, asynchronous, and influenced by environmental and host-derived factors (reviewed in Perry, 1997). The *H. glycines* TN17 and TN18 hatch responses to GA were similar to each other but very different from *C. elegans*. Because the hatching phenotypes of these inbred lines are temporal, exposure of eggs to one GA dose over time might reveal differences between these strains.

With respect to motility, in *C. elegans* the Hsp90 mutation *daf-21(p673)* is known to cause lethargy and defective chemotaxis to volatile odorants (Vowels and Thomas, 1994; Birnby et al., 2000). Also, the *C. elegans* protein UNC-45 functions as a cochaperone with Hsp90 for the folding and assembly of myosin, an integral component of muscle (Barral et al., 2002). Together, these associations predict a possible role for Hsp90 in movement or muscle development pathways in *C. elegans*, and GA inhibition of *C. elegans* juvenile motility is consistent with this interpretation. Overall, the differences in GA responses observed in *C. elegans* and *H. glycines* reflect the likely influence of genetic background upon the Hsp90-dependent pathways underlying hatching and motility. Because the gene for Hsp90 from *H. glycines* has recently been described (Skantar and Carta, 2004), further details of the Hsp90–GA interaction may be elucidated.

The complex nonlinear hatch and motility profiles in both nematode species display characteristics of hormesis, a model that describes the nonlinear responses of an organism to a wide range of compounds, including peptides, metals, and toxins (Stebbing, 2000; Calabrese and Baldwin, 2003). Whereas the external agent and the measured biological endpoint may vary, hormesis is commonly characterized by U- or inverse U-shaped dose–response curves. Prior studies pointed to the existence of hormetic effects in *C. elegans*. It has been established that different concentrations of pharmacological agents can cause contradictory effects in nematodes, and may indicate that the compound acts upon multiple targets (Rand and Johnson, 1995). Also, a hormetic link between thermotolerance and longevity has been described in *C. elegans* (Butov et al., 2001; Cypser and Johnson, 2002). The fact that *C. elegans daf-21*/Hsp90 is known to be involved in thermotolerance and upregulated by DAF-16 (McElwee et al., 2003) further supports involvement of Hsp90 in a hormetic biological switch involved in stress responses. Hsp90 could provide rheostatic control of client protein activities (e.g., protein kinases or hormone receptors), in response to cellular indicators of nutrient, thermal, or oxidative stress (Morano and Thiele, 1999; Knowlton and Sun, 2001). The reversal of egg hatching and J2 motility effects observed at GA doses may be such a manifestation of Hsp90-mediated hormesis. By profiling the GA doses that show observable effects, these studies should open the door to further investigations of Hsp90 and hormesis in nematodes.

Recent studies have shown that GA-producing strains of *Streptomyces* are effective against a variety of plant pathogens. The GA-producing strain *S. violaceusniger* YCED-9 was shown to antagonize the growth of seven fungal pathogens of turfgrass *in vitro* (Trejo-Estrada et al., 1998b). In greenhouse studies, this strain partially controlled grass seedling disease caused by *Rhizoctonia solani* and crown-foliar disease caused by *Sclerotinia homeocarpa* (Trejo-Estrada et al., 1998a). *S. hygroscopicus* spp. *geldanus* strain EF-76, was

shown to inhibit the causal agent of potato common scab, *Streptomyces scabies*, under field conditions, but did not affect potato yield (Beauséjour et al., 2003). In light of our GA results, these strains may have promise as biocontrol agents for nematodes as well.

Acknowledgments—The authors thank Maria Hult, Paula Crowley, Sharon Ochs, and Steven Rogers for technical assistance, and Dr. Terry Niblack for strains TN17 and TN18. This study was supported in part by grant #1241 from the United Soybean Board. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

AGBESSI, S., BEAUSÉJOUR, J., DERY, C., and BEAULIEU, C. 2003. Antagonistic properties of two recombinant strains of *Streptomyces melanosporofaciens* obtained by intraspecific protoplast fusion. *Appl. Microbiol. Biotechnol.* 62:233–238.

ALBERT, P. S. and RIDDLE, D. L. 1988. Mutants of *Caenorhabditis elegans* that form dauer-like larvae. *Dev. Biol.* 126:270–293.

ALSTON, D. G. and SCHMITT, D. L. 1988. Development of *heterodera glycines* life stages as influenced by temperature. *J. Nematol.* 20:366–372.

BARRAL, J. M., HUTAGALUNG, A. H., BRINKER, A., HARTL, F. U., and EPSTEIN, H. F. 2002. Role of the myosin assembly protein *unc-45* as a molecular chaperone for myosin. *Science* 295:669–671.

BEAUSEJOUR, J., CLERMONT, N., and BEAULIEU, C. 2003. Effect of *Streptomyces melanosporofaciens* strain EF-76 and of chitosan on common scab of potato. *Plant Soil* 256:463–468.

BIRNBY, D. A., LINK, E. M., VOWELS, J. J., TIAN, H., COLACURCIO, P. L., and THOMAS, J. H. 2000. A transmembrane guanylyl cyclase (*daf-11*) and Hsp90 (*daf-21*) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. *Genetics* 155:85–104.

BUTOV, A., JOHNSON, T., CYPSER, J., SANNIKOV, I., VOLKOV, M., SEHL, M., and YASHIN, A. 2001. Hormesis and debilitation effects in stress experiments using the nematode worm *Caenorhabditis elegans*: the model of balance between cell damage and hsp levels. *Exp. Gerontol.* 37:57–66.

CALABRESE, E. J. and BALDWIN, L. A. 2003. Toxicology rethinks its central belief. *Nature* 421:691–692.

CYPSER, J. R. and JOHNSON, T. E. 2002. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *J. Gerontol., Biol. Sci. Med. Sci.* 57:B109–B114.

DAVID, C. L., SMITH, H. E., RAYNES, D. A., PULCINI, E. J., and WHITESELL, L. 2003. Expression of a unique drug-resistant hsp90 ortholog by the nematode *C. elegans*. *J. Cell Stress, and Chaperones* 8:93–104.

INOUE, T., TAKAMURA, K., YAMAE, H., ISE, N., KAWAKAMI, M., TABUSE, Y., MIWA, J., and YAMAGUCHI, Y. 2003. *Caenorhabditis elegans daf-21* (Hsp90) is characteristically and predominantly expressed in germline cells: spatial and temporal analysis. *Dev. Growth Differ.* 45:369–376.

KNOWLTON, A. A. and SUN, L. 2001. Heat-shock factor-1, steroid hormones, and regulation of heat-shock protein expression in the heart. *Am. J. Physiol. Heart Circ. Physiol.* 280:H455–H464.

LEE, M. D., TSAY, T. T., and LIN, Y. Y. 1996. Physiological characteristics and effect of *Streptomyces saraceticus* on hatching ratio of southern root-knot nematode. *Plant Protec. Bull. (Taipei)* 38:235–245.

LEWIS, J. A. and FLEMING, J. T. 1995. Basic culture methods, pp. 4–27, *in* H. F. Epstein and D. C. Shakes (eds.). *Caenorhabditis elegans*: Modern Biological Analysis of an Organism. Academic Press, San Diego, CA.

MCELWEE, J., BUBB, K., and THOMAS, J. H. 2003. Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. *Aging Cell* 2:111–121.

MORANO, K. A. and THIELE, D. J. 1999. Heat shock factor function and regulation in response to cellular stress, growth, and differentiation signals. *Gene Express.* 7:271–282.

MURA, S., ENOMOTO, Y., SHINOSE, M., TAKAHASHI, Y., IWAI, Y., and SHIOMI, K. 1999. Isolation and structure of a new antibiotic viridomycin F produced by sp. K96-0188. *J. Antibiot.* 52:61–64.

NAIR, M. G., CHANDRA, A., THOROGOD, D. L., and DAVIS, R. M. G. 1995. Nematicidal and mosquitocidal aromatic nitro compounds produced by *Streptomyces* spp. *Pest. Sci.* 43:361–365.

NITAO, J. K., MEYER, S. L. F., and CHITWOOD, D. J. 1999. *In-vitro* assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. *J. Nematol.* 31:172–183.

PERRY, R. N. 1997. Plant signals in nematode hatching and attraction, pp. 38–50, *in* C. Fenoll. (ed.). Cellular and Molecular Aspects of Plant–Nematode Interactions. Kluwer Academic Publishers, The Netherlands.

PRODROMOU, C., ROE, S. M., O'BRIEN, R., LADBURY, J. E., PIPER, P. W., and PEARL, L. H. 1997. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 90:65–75.

QUEITSCH, C., SANGSTER, T. A., and LINDQUIST, S. 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417:618–624.

RAND, J. B. and JOHNSON, C. D. 1995. Genetic pharmacology: interactions between drugs and gene products in *Caenorhabditis elegans*, pp. 187–204, *in* H. F. Epstein and D. C. Shakes (eds.). *Caenorhabditis elegans*: Modern Biological Analysis of an Organism. Academic Press, San Diego, CA.

RATKOWSKY, D. A. 1990. Handbook of Nonlinear Regression Models. Marcel Dekker Inc., New York.

SIDDIQUI, Z. A. and MAHMOOD, I. 1999. Role of bacteria in the management of plant parasitic nematodes: a review. *Bioresour. Technol.* 69:167–179.

SKANTAR, A. M. and CARTA, L. K. 2004. Molecular characterization and phylogenetic evaluation of the Hsp90 gene from selected nematodes. *J. Nematol.* 36:466–480.

STEBBING, A. R. 2000. Hormesis: interpreting the beta-curve using control theory. *J. Appl. Toxicol.* 20:93–101.

STEBBINS, C. E., RUSSO, A. A., SCHNEIDER, C., ROSEN, N., HARTL, F. U., and PAVLETICH, N. P. 1997. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* 89:239–250.

TREJO-ESTRADA, S. R., PASZCZYNSKI, A., and CRAWFORD, D. L. 1998a. Antibiotics and enzymes produced by the biocontrol agent *Streptomyces violaceusniger* YCED-9. *J. Indus. Microbiol. Biotech.* 21:81–90.

TREJO-ESTRADA, S. R., SEPULVEDA, I. R., and CRAWFORD, D. L. 1998b. *In vitro* and *in vivo* antagonism of *Streptomyces violaceusniger* YCED9 against fungal pathogens of turfgrass. *World J. Microbiol. Biotechnol.* 14:865–872.

VOWELS, J. J. and THOMAS, J. H. 1994. Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. *Genetics* 138:303–316.

WHITESELL, L., MIMNAUGH, E. G., DE COSTA, B., MYERS, C. E., and NECKERS, L. M. 1994. Inhibition of heat shock protein HSP90-pp60(v-src) heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. *Proc Natl. Acad. Sci. U.S.A.* 91(18):8324–8328.

WIESGIGL, M. and CLOS, J. 2001. Heat shock protein 90 homeostasis controls stage differentiation in *Leishmania donovani*. *Molec. Biol. Cell* 12:3307–3316.