Management of Elongate Hemlock Scale with Entomopathogenic Fungi

Bruce L. Parker[1], Margaret Skinner[1], Vladimir Gouli[1], Svetlana Gouli[1], José Marcelino[1], Jerry Carlson[2], and Lora Schwartzberg[2]

[1] Entomology Research Laboratory, University of Vermont
661 Spear St., Burlington, VT 05405-0105

[2] New York State Department of Environmental Conservation
625 Broadway, Albany, NY 12233

ABSTRACT

The artificial dissemination of entomopathogenic fungi, under suitable environmental conditions, may be an important tool for management of an insect pest and, if established, a seasonal control method to maintain the pest population under an economic threshold level. Recently two entomopathogenic fungi were discovered parasitizing the elongate hemlock scale (EHS), *Fiorinia externa* Ferris (Homoptera: Diaspididae). One was found to cause an epizootic within the population of the scale. Exploratory activities in New York showed the expanding range of the epizootic. To assess the potential and viability of this fungus, a set of biological parameters were measures from 26 of the 66 fungal isolates obtained from EHS. Unique growth and sporulation characteristics were found. Both were higher at 25°C (range of growth conditions between 15, 20, and 25°C [± 1°C] spanning 20 days). Conidial germination occurred after 8 hours at 10°C, but it was significantly higher after 6 hours at 25 and 30°C (range of growth conditions between 10, 15, 20, 25 and 30°C [± 1°C] spanning 24 hours). High viability for mass production was observed.

KEYWORDS

Hemlock, *Fiorinia externa*, entomopathogenic fungi, productivity, fungal growth rate.

INTRODUCTION

The eastern or Canada hemlock, *Tsuga canadensis* (L.) Carrière, plays a key role in forest ecosystems by maintaining their stability. It is particularly important along streams and creeks where their shade provides shelter and sustains aquatic ecosystems and a unique microclimate for forest and wildlife (Howe and Mossman 1995, Wydeven and Hay 1995, Crow 1995, Howard et al. 2000). Hemlock is second only to sugar maple in terms of abundance in the northern forest (Curtis 1959). The present decline of the hemlock forest is due to biotic and abiotic factors often acting together. The primary threat consistently reported is the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), found infesting half of the range of hemlock along the eastern seaboard (Knauer et al. 2002). The elongate

hemlock scale (EHS), *Fiorinia externa* Ferris (Homoptera: Diaspididae), is becoming important not only as a secondary pest enhanced by an initial stress from HWA but as a parallel stress factor in hemlock decline. A rapid spread of EHS within the area of HWA has been observed. Their highest abundance is within a 300-km radius of New York City (Danoff-Burg and Bird 2002). EHS is present in Pennsylvania, Virginia, Massachusetts, Connecticut, Maryland, New Jersey, Rhode Island, Washington D.C., southern New England and western Ohio (Garrett and Langford 1969, Johnson and Lyon 1988, Hoover 2003, USDA 2004). Strong correlations have been found between HWA and scale infestation levels (Johnson and Lyon 1988, Danoff-Burg and Bird 2002).

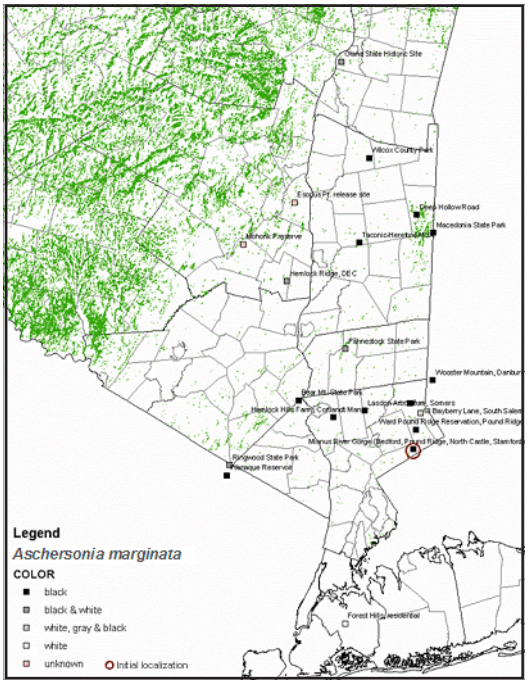
The EHS maybe is a greater problem than HWA, despite its secondary role. Its unique shield-like cover or scale provides protection for the eggs and the adult from contact insecticides, natural enemies and adverse conditions. Losses are soon replaced even with mortality rates of 95% (Baranyovits 1953, Johnson and Lyon 1988). The EHS has probably caused more decline of hemlock in terms of rapid tree mortality than HWA (Greg Hoover, pers. comm.).

A chemical approach to the management of EHS or HWA is not environmentally viable in a forest situation, so alternative measures have been developed: mainly the use of introduced predators. One major factor when considering the use of predators in an open environment is the necessity for a rapid predator population build up before the trees become irreversibly damaged by the pest. In addition, sometimes it is difficult to maintain the population of the predator in levels capable of controlling the pest. The elapse time between the release and achieved biological control is large, sometimes spanning many years.

Scale insects are particularly susceptible to attack by fungi because only one stage is mobile. Quantitative evidence of the importance of entomopathogenic fungi on arthropod populations has been extensively reviewed (Samson et al. 1988), and their impact on armored scales (Homoptera: Diaspididae) has been reported (Helle 1990a and 1990b). An epizootic caused by a “black” fungus within the EHS population in the Mianus River Gorge Preserve in Bedford, New York, was first reported by McClure (2002). Another parasitic ‘white fungus’ has been recently isolated from EHS at the Entomology Research Laboratory (ERL), University of Vermont.

Because the white fungus was found only occasionally and it was believed that the cause of the epizootic was the black fungus, major emphasis was placed on the latter. This fungus was found in a wide range of EHS sites and was easily cultured, producing high concentrations of spores and different pigmentation when cultured, ranging from whitish-pink to dark black (Gouli et al. 2004). It was first identified by Drs. Svetlana and Vladimir Gouli (ERL) as *Hypocrella* sp., and the species was verified as the anamorph *Aschersonia marginata* Ellis and Ever (Ascomycota: Hypocreales: Clavicipitaceae) by Dr. Zengzhi Li, Head of the Entomogenous Fungal Branch of the Mycological Society of China. Doubts concerning the identification still remain because *Aschersonia* spp. mainly grows in tropical or sub-tropical climates, which are different from that in the northeastern US. It has subsequently been identified as *Fusarium merismoides* by Drs. Humber and Rossman, Cornell Univ. and *Myriangium* sp. by Dr. Nigel Hywel-Jones, National Center for Genetic Engineering and Biotechnology, Thailand. DNA analysis is being done currently to clarify identification of this fungus.

Subsequent expansion from the initial focal point of epizootic observation towards other populations of EHS in New York hemlock stands (Map 1) suggests that an alternative low cost control method may be available. For effectiveness in the management of the scale through the use of entomopathogenic fungi, a thoroughly understanding of the biology of the fungi and their interaction with the host must be determined since they directly affect dissemination, establishment, and their self perpetuation of the pathogen. The research reported herein was designed to assess some of these biological parameters: specifically growth, spore productivity, and germination.



Map 1. Distribution of *Aschersonia marginata* within different counties of New York (2004). Source: New York State Department of Environmental Conservation.

METHODS AND MATERIALS

RATE OF GROWTH AND CONIDIAL PRODUCTION

Assays on the rate of growth and conidial production were done on 26 isolates of the fungus selected randomly from a collection of 66 cultures obtained from different EHS fungal infestation sites. These isolates had been held in long-term storage at -80°C. Ten µl of a $1x10^6$ conidial suspension were pipetted onto a 0.64 cm-diameter sterile disc of filter paper (Scleicher and Schuell, Keene, New Hampshire, Grade 740E) in Petri dishes containing ~20 ml Potato Dextrose Agar (PDA) (Difco®). This medium was used because the fungus starts to produce conidial masses after ~72 hours on a high carbohydrate medium. The Petri dishes were held in the dark in growing chambers and maintained at 15, 20, and 25°C (± 1°C). The trial was repeated twice with four replications per trial. The growth of each isolate was monitored at 5, 10, 15, and 20 days and the outer edges of fungal growth marked at each time period. At the end of 20 days, growth at each time period was measured from the center point to the appropriate mark. Different stages of development of this fungus were present at the same time so the Petri dishes containing the full grown cultures were placed individually in a mixer with 100 ml tap water and blended for 1.5 minutes to obtain a suspension representative of the

conidial production per individual colony. The suspension was sub-sampled (0.5 ml) and placed in a test tube with 4.5 ml of a Lactophenol-Cotton Blue Stain (VWR Scientific Products®) to dye spores and stop germination. Subsequent estimation of conidial production per unit volume (1 ml) was assessed with an Improved Neubauer haemacytometer (Propper®).The suspension in the test tube was vortexed for approximately 15 seconds and a drop deposited on each side of the haemacytometer. Under 40x magnification, we randomly selected five squares within each of the two haemacytometer grids and counted the number of spores. The mean number of spores (A) was calculated by multiplying A by the test tube volume dilution factor (10), then by the conversion factor 5,000 (Goettel and Inglis 1997), and finally, by 100 ml (original colony suspension). The data were converted to number of conidia per unit of surface area (cm^2) by dividing the conidial production per colony by the area of growth (Πr^2).

GERMINATION

The quantification of the germination rate under different abiotic conditions (temperatures) was determined by following the conidial development of the 26 colonies (four repetitions) under a compound microscope (40x) after being incubated for 6, 8, 10, 12, 16, 20, and 24 hours on PDA (Difco®) at 10, 15, 20, 25, and 30°C (± 1°C). Germination was present if germ tubes were formed by individual conidia.

STATISTICAL ANALYSES

Data on fungal growth and sporulation were analyzed with an ANOVA (± = 0.05) in SPSS® for Windows® 12.0.2 statistical software to determine differences among treatments per observation period. A Scheffé test (± = 0.05) was employed to identify significant differences among treatments.

RESULTS

The conidial production (Figure 1) did not differ significantly among the 26 isolates (F = 0.87; df = 25,130; P = 0.6). The differences were also not significant for the two trials (F = 3.58; df = 1, 154; P = 0.06) but significant for the three temperatures tested (F = 29.95; df = 2, 153; P < 0.001). Differences among all temperatures except for 15 and 20°C were statistically significant (Figure 2).

The average growth/day (Figure 3) did not differ significantly among the isolates (F = 0.9; df = 25; 130; P = 0.56) but did between trials (F = 4.3; df = 1, 154; P = 0.04) and among temperatures (F = 170; df = 2; 153, P < 0.001). The Scheffé test (± = 0.05) showed differences among all temperatures except for 20 and 25°C (Figure 4).

The conidial germination rate varied according to temperature, following a pattern of increase over time. The maximum rate of conidial germination was achieved at the highest temperatures (25 and 30°C) where 100% germination was reached after 8–10 hours (Figure 5). All isolates had reached 100% germination after 24 hours.

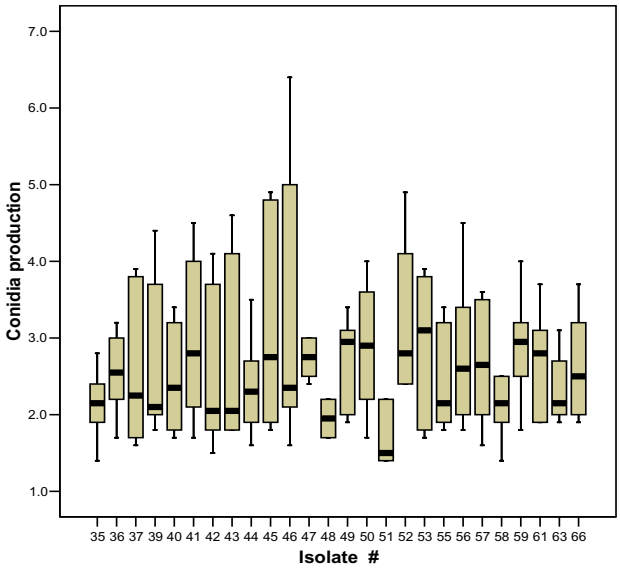


Figure 1. Average conidial productivity per cm^2 ($x10^7$) for the different isolates.

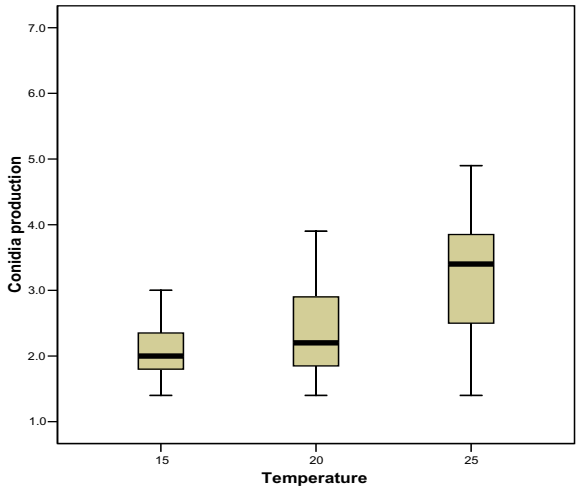


Figure 2. Average conidial productivity per cm^2 ($x10^7$) at different temperatures.

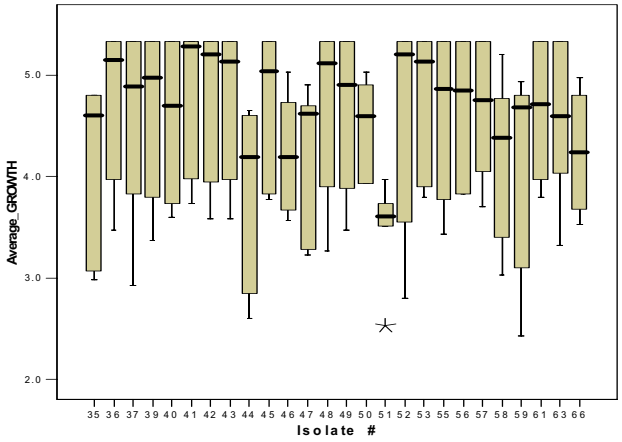


Figure 3. Average growth (mm/day) for the different isolates.

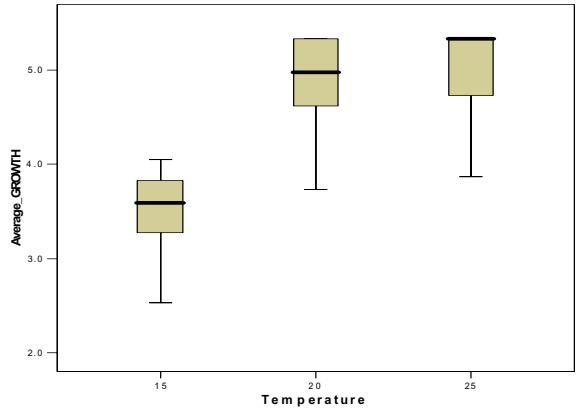


Figure 4. Average growth (mm/day) at different temperatures.

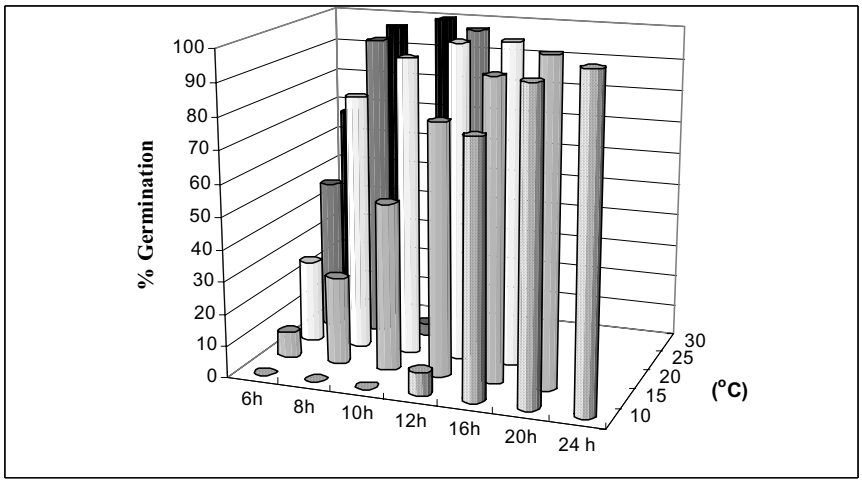


Figure 5. Conidial germination as a function of temperature and time.

Because all isolates did not differ significantly for both growth and conidial productivity within the different temperatures, a ranking system measuring individual performances according to the tested parameters was developed to assess which isolates showed the most promise as a biological control agent. Increasing levels of performance are equivalent to increasing number in the ranking. The values were evaluated for the individual parameters and for an overall measure across parameters (Table 1).

Table 1. Performance of individual isolates for the different parameters tested.

Isolate #	Conidia cm^2 ($x10^7$)	Growth (mm/day)	Higher germination rate (hour = %)				
			10° C	15° C	20° C	25° C	30° C
35	2.1 (± 0.2) [2]	4.1 (± 0.4) [3]	16h=83% [a, 13]	12h=54% [c, 24]	8h=39% [c, 14]	8h=71% [d, 10]	8h=57% [d, 1]
36	2.5 (± 0.2) [5]	4.7 (± 0.3) [8]	16h=95% [a, 17]	12h=52% [c, 23]	8h=66% [c, 16]	8h=63% [d, 6]	6h=51% [d, 4]
37	2.6 (± 0.4) [6]	4.5 (± 0.4) [6]	16h=44% [a, 1]	8h=41% [b, 6]	8h=40% [b, 1]	6h=49% [d, 14]	6h=74% [d, 13]
39	2.7 (± 0.4) [7]	4.6 (± 0.3) [7]	16h=68% [a, 7]	8h=40% [b, 5]	8h=40% [b, 1]	6h=55% [d, 17]	6h=74% [d, 13]
40	2.5 (± 0.3) [5]	4.5 (± 0.3) [6]	16h=54% [a, 3]	8h=42% [b, 7]	6h=41% [c, 20]	6h=51% [d, 15]	6h=75% [d, 14]
41	3.0 (± 0.4) [10]	4.8 (± 0.3) [9]	16h=81% [a, 12]	10h=50% [b, 14]	6h=45% [c, 21]	6h=57% [d, 18]	6h=68% [d, 10]
42	2.5 (± 0.4) [5]	4.7 (± 0.3) [8]	16h=86% [a, 14]	8h=43% [b, 8]	6h=34% [b, 11]	6h=46% [d, 13]	6h=57% [d, 5]
43	2.7 (± 0.5) [7]	4.7 (± 0.3) [8]	16h=57% [a, 4]	10h=36% [b, 12]	6h=37% [b, 12]	6h=44% [d, 12]	6h=59% \[d, 6]
44	2.4 (± 0.3) [4]	3.8 (± 0.4) [2]	16h=81% [a, 12]	10h=52% [b, 15]	8h=78% [b, 9]	8h=64% [d, 7]	6h=66% [d, 8]
45	3.2 (± 0.6) [11]	4.7 (± 0.3) [8]	16h=78% [a, 10]	6h=33% [b, 22]	6h=43% [b, 13]	6h=57% [d, 18]	6h=77% [d, 15]
46	3.3 (± 0.8) [12]	4.2 (± 0.2) [4]	16h=58% [a, 5]	12h=35% [b, 18]	8h=63% [b, 7]	8h=60% [d, 5]	6h=50% [d, 3]
47	2.9 (± 0.2) [9]	4.2 (± 0.3) [4]	16h=78% [a, 10]	12h=32% [b, 17]	8h=56% [b, 6]	8h=48% [d, 1]	6h=78% [d, 16]
48	2.0 (± 0.1) [1]	4.6 (± 0.3) [7]	16h=63% [a, 6]	8h=50% [b, 9]	8h=50% [c, 15]	6h=54% [d, 16]	6h=72% [d, 12]
49	3.7 (± 0.3) [13]	4.6 (± 0.3) [7]	16h=79% [a, 11]	12h=45% [b, 20]	8h=49% [b, 4]	6h=55% [d, 17]	6h=68% [d, 10]
50	2.9 (± 0.3) [9]	4.4 (± 0.2) [5]	16h=43% [a, 1]	8h=34% [b, 4]	8h=48% [b, 3]	6h=68% [d, 20]	6h=81% [d, 17]
51	2.0 (± 0.4) [1]	3.3 (± 0.3) [1]	16h=86% [a, 14]	12h=52% [b, 21]	8h=45% [b, 2]	8h=68% [d, 9]	6h=44% [d, 2]
52	3.2 (± 0.4) [11]	4.5 (± 0.4) [6]	16h=94% [a, 16]	8h=32% [b, 3]	8h=77% [b, 8]	8h=65% [d, 8]	6h=85% [d, 19]
53	2.9 (± 0.4) [9]	4.7 (± 0.3) [8]	16h=44% [a, 1]	10h=46% [b, 13]	8h=78% [b, 9]	8h=53% [d, 3]	6h=67% [d, 9]
55	2.4 (± 0.4) [4]	4.6 (± 0.3) [7]	16h=72% [a, 8]	8h=28% [b, 2]	8h=76% [c, 17]	6h=57% [d, 18]	6h=66% [d, 8]
56	2.8 (± 0.4) [8]	4.6 (± 0.3) [7]	16h=72% [a, 8]	10h=29% [b, 11]	8h=54% [b, 5]	6h=60% [d, 19]	6h=86% [d, 20]
57	2.7 (± 0.3) [7]	4.6 (± 0.3) [7]	16h=77% [a, 9]	12h=24% [b, 16]	8h=56% [b, 6]	6h=55% [d, 17]	6h=69% [d, 11]
58	2.1 (± 0.2) [2]	4.2 (± 0.3) [4]	16h=77% [a, 9]	6h=66% [c, 25]	6h=74% [c, 22]	6h=89% [d, 21]	6h=95% [d, 21]
59	2.9 (± 0.3) [9]	4.1 (± 0.4) [3]	16h=77% [a, 9]	10h=28% [b, 10]	8h=84% [b, 10]	8h=81% [d, 11]	8h=66% [d, 8]
61	2.7 (± 0.3) [7]	4.6 (± 0.3) [7]	16h=52% [a, 4]	8h=32% [b, 3]	8h=66% [c, 16]	8h=50% [d, 2]	6h=69% [d, 11]
63	2.3 (± 0.2) [3]	4.5 (± 0.3) [6]	16h=79% [a, 11]	12h=36% [b, 19]	8h=78% [c, 18]	8h=57% [d, 4]	6h=63% [d, 7]
66	2.6 (± 0.3) [6]	4.2 (± 0.3) [4]	16h=88% [a, 15]	16h=32% [b, 1]	8h=84% [c, 19]	8h=63% [d, 6]	6h=82% [d, 18]

a - 100 % germination reached after 24 hours c - 100 % germination reached after 16 hours
b - 100 % germination reached after 20 hours d - 100 % germination reached after 10 hours
(± standard error) ranking value

CONCLUSIONS

The 26 isolates tested grew and produced a high number of spores at temperatures ranging from 15 to 25°C. Also, they achieved 100% germination in a short period of time for temperatures ranging from 10 to 30°C. Growth was more affected by temperature than spore production. The rate of growth at 15°C was half that at 20 and 25°C. Although germination of the isolates started at different times 90% of the isolates started to germinate after six hours when the temperature was ≥15°C, and at temperatures 20 and 25° C up to 40-55% germination was achieved for 90% of the isolates after this short period of time. At 30°C, this germination rate was maintained with the exception of some outlier isolates, which reached 80-85% of germination.

Different conclusions on the most promising isolates can be reached depending on the parameters used (Table 1). Isolates 41, 45, and 58 had the highest rankings when considering all the tested parameters together (growth, productivity, and germination). If trying to assess the best isolates to grow under northeastern U.S. environmental conditions, isolates 35, 36, and 45 provided better germination at lower temperatures with good productivity and rate of growth.

Based on these positive results concerning major features required for the potential use of entomopathogenic fungi for insect pest management (high spore productivity, growth, and germination), mass production is critical. Subsequent research will focus in this area and in assessing the virulence of these different strains against the EHS and other pests such as the HWA.

ACKNOWLEDGMENTS

Dr. Scott D. Costa provided valuable advice on preparation of some parts of this manuscript. This research was supported in part by the USDA Forest Service (Project 04-CA-11244225-286).

REFERENCES

Baranyovits, F. 1953. Some aspects of the biology of armoured scales. *Endeavour*, Vol. 12: 202-209.

Crow, T.R. 1995. The social, economic, and ecological significance of hemlock in the Lake States, pp. 11-17. *In* Hemlock Ecology and Management. Proceedings of a Regional Conference on Ecology and Management of Eastern Hemlock, Sept. 27-28, 1995, Iron Mountain, Michigan.

Curtis, J.T. 1959. *The Vegetation of Wisconsin*. The Univ.of Wisconsin Press. 645 p.

Danoff-Burg, J.A. and S. Bird. 2002. Hemlock Woolly Adelgid and Elongate Hemlock Scale: Partners in crime?, pp. 254-268. *In* Proceedings of Hemlock Woolly Adelgid in the Eastern United States Symposium, Feb. 5-7, 2002, East Brunswick, New Jersey, ed. B. Onken, R. Reardon, and J. Lashomb. Rutgers University, New Brunswick, New Jersey.

Garrett, W.T. and G.S. Langford. 1969. Control of *Fiorinia externa* on hemlock in Maryland. *J. Econ. Entomol*. 62 (6): 1449-1450.

Goettel, M.S. and G.D. Inglis. 1997. Fungi: Hyphomycetes, pp. 213-249. *In* Manual of Techniques in Insect Pathology. Academic Press.

Gouli, S., B. Parker, and V. Gouli. 2004. Biological properties of a new entomopathogenic fungus *Aschersonia marginata*, p. 85. *In* Society for Invertebrate Pathology. 37th Annual Meeting. 7th Intern. Conf. on *Bacillus thuringiensis*, 1-6 August 2004, Helsinki, Finland. (Book of Abstracts).

Helle, W. 1990a. Armored Scale Insects. Their Biology, Natural Enemies and Control. [Series title: World Crop Pests, Vol. 4A: The Armored Scale Insects], ed. D. Rosen. Amsterdam, The Netherlands: Elsevier. 384 p.

Helle, W. 1990b. Armored Scale Insects: Their Biology, Natural Enemies and Control. [Series title: World Crop Pests, Vol. 4B: The Natural Enemies], ed. D. Rosen. Amsterdam, The Netherlands: Elsevier. 690 pp.

Hoover, G.A. 2003. Elongate Hemlock Scale. *Entomological Notes:* www.ento.psu.edu/extension/factsheets/pdfs/elongateHemlockScale.pdf. The Penn. State Univ.

Howard, T., P. Sendak, and C. Codrescu. 2000. Eastern Hemlock: A Market Perspective, pp. 161-166. *In* Proc. of the Sustainable Management of Hemlock Ecosystems in Eastern North America Symposium. June 22-24, 1999, Durham, New Hampshire.

Howe, R.W. and M. Mossman. 1995. The Significance of Hemlock for Breeding Birds in the Western Great Lakes Region, pp. 125-140. *In* Hemlock Ecology and Management. Proc. of a Regional Conf. on Ecology and Management of Eastern Hemlock, September 27-28, 1995, Iron Mountain, Michigan.

Johnson, W.T. and H.H. Lyon. 1988. Insects That Feed on Trees and Shrubs. 2nd Edition. Cornell Univ. Press. 556 p.

Knauer, K., J. Linnane, K. Shields. and R. Bridges. 2002. An Initiative for Management of Hemlock Woolly Adelgid. *In* Proceedings of Hemlock Woolly Adelgid in the Eastern United States Symposium, Feb. 5-7, 2002, East Brunswick, New Jersey, ed. B. Onken, R. Reardon, and J. Lashomb. Rutgers University, New Brunswick, New Jersey.

McClure, M.S. 2002a. The Elongate Hemlock Scale, *Fiorinia externa* Ferris (Homoptera: Diaspididae): A new Look at an Old Nemesis, pp. 248-253. *In* Proceedings of Hemlock Woolly Adelgid in the Eastern United States Symposium, Feb. 5-7, 2002, East Brunswick, New Jersey, ed. B. Onken, R. Reardon, and J. Lashomb. Rutgers University, New Brunswick, New Jersey.

USDA 2004. Forest Insects and Diseases Conditions in the United States 2003. USDA, Forest Service Pub. 142 p.

Samson, R.A., H.C. Evans, and J.P. Latge. 1988. Atlas of Entomopathogenic Fungi. Berlin; Springer Verlag. 187 p.

Wydeven, A.P. and R.W. Hay. 1995. Mammals, Amphibians and Reptiles of Hemlock Forests in the Lake Superior Region, pp. 115-123. *In* Hemlock Ecology and Management. Proc.of a Regional Conf. on Ecology and Management of Eastern Hemlock, September 27-28, 1995, Iron Mountain, Michigan.