Evaluation of Biological and Chemical Control Methods for Black Vine Weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), in Container Grown Perennials[1]

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─────────────── **Abstract** ───────────────

Three trials involving the nematode *Heterorhabditis bacteriophora* (Poiner) provided convincing evidence that this entomopathogenic nematode can provide highly effective control of black vine weevil larvae under conventional methods of container plant production. In all trials, the application of nematodes significantly reduced populations of weevil larvae relative to levels found in the untreated containers. The first and second trials, using *Bergenia and Heuchera*, confirmed that *H. bacteriophora* applied at two rates caused substantial mortality to black vine weevil larvae. The third trial revealed that *H. bacteriophora* applied to *Epimedium* provided levels of control comparable to those of imidacloprid and acephate. In all trials, populations of weevil larvae were reduced by 90–100% following the application of *H. bacteriophora*.

Index words: biological control, black vine weevil, herbaceous perennials, nurseries, greenhouses.

Species used in this study: black vine weevil, *Othiorhynchus sulcatus* (Fabricius); *Heterorhabditis bacteriophora* (Poiner); barrenwort, *Epimedium* x *rubrum* C. Morr.; Bergenia *cordifolia* 'Rotblum' (Haw.) Sternb.; alumroot, *Heuchera micrantha* 'Chocolate' Doug. ex Lindl.

Insecticides used in this study: Marathon (imidacloprid), 1-(6-chloro-3-pydrin-3-methyl)-N-nitroimidazolidin-2-ylidenamine; Orthene (acephate), O,S-dimethyl acetylphosphoramidithioate.

Significance to the Nursery Industry

Black vine weevil is a major pest of herbaceous perennial and woody plants grown in landscapes and nurseries. Recent legislative restrictions to the use of insecticides and cancellation of registration of insecticides to control black vine weevil necessitate the identification and evaluation of alternative approaches to managing this pest. Entomopathogenic nematodes have no reentry intervals (REIs) and pose minimal threats to growers, workers, consumers, and beneficial organisms. Entomopathogenic nematodes could be valuable tools to the nursery managers whose access to production areas is restricted following pesticide applications. Evaluation of nematodes as biological control agents provides growers with information useful in deciding the relative value of this tactic for control of black vine weevil.

Introduction

The production of herbaceous perennial crops grew rapidly in the last decade. The combined value of wholesale, retail, and landscape sales of perennials by members of the Perennial Plant Association in the United States and Canada during 1993 was $338.5 million or 25% of an estimated $1.37 billion in gross plant sales (23). Furthermore, recent economic analyses indicate that perennial sales continue to grow in the nursery and greenhouse industries (5).

Growers of herbaceous perennials produce a variety of native and exotic plants having a wide range of insect and disease problems. The black vine weevil, *Otiorhynchus sulcatus* (Fabricius), feeds on many herbaceous perennials and woody plants. Black vine weevil is a pest of aster, astilbe, begonia, bergenia, calceolaria, cyclamen, epimedium, ferns, heuchera, hosta, lily of the valley peony, phlox, polyanthus, primula, sedum, toad lily, and several other herbaceous perennial crops (1, 12, 18, 20). In addition, black vine weevil injures several species of woody landscape plants including balsam, camellia, cotoneaster, elaeagnus, heather, photinia, rhododendron, and taxus (12, 15, 18, 25).

The black vine weevil is a native of Europe and was first noted in the United States in Massachusetts in 1835 (1, 3, 25). The first report of the weevil as a pest was in 1871 from Missouri (3, 25). It is presently reported throughout the continental United States, Europe, and Asia (1, 3). Feeding adults notch foliage and the larvae feed on roots. Infested plants may suddenly wilt and die because of severe injury to the roots or crown area (7, 12).

The black vine weevil reproduces by parthenogenesis. Males are unknown. Adults cannot fly because the elytra are fused. Larvae develop through six instars and are legless, white and wrinkled. Pupation occurs in the soil and lasts three weeks to several months depending on soil temperature. Outdoors, the black vine weevil has one generation per year (12).

Several chemicals and biological control agents have been investigated to control black vine weevil larvae (4, 9, 11, 13,

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17, 18, 21, 24, 25, 27). Blackshaw (4) evaluated seven controlled release insecticides, all of which reduced the number of larvae present, but only chlorpyrifos, fonofos, and aldrin gave 100% control. Halfill (13) obtained the best control of black vine weevil with oxamyl, bendiocarb, carbofuran and chlordane. Nielson and Boggs (21) evaluated efficacy of several pesticides and obtained the highest level of control of black vine weevil with carbofuran. Several of these compounds have been removed from the marketplace and are no longer available for control of black vine weevil. Others have long REIs limiting their utility in nurseries and greenhouses.

Biological control of black vine weevil has included the use of entomopathogenic nematodes in the families Sterinerematidae and Heterorhabditidae. Georgis and Poinar (11) found that *Heterorhabditis heliothidis (= bacteriophora)* gave better control than *Neoaplectana* (= Steinernema) *carpocapsae* (Weiser), for early instars of black vine weevil. Schirocki and Hague (24) confirmed that heterorhabditid species are more effective than steinernematids in controlling black vine weevil larvae. Swier et al. (27) found that multiple applications of *H. bacteriophora* provided levels of control comparable to several conventional insecticides to black vine weevil larvae infesting container grown bridget bloom.

Two conventional insecticides were evaluated in this trial, acephate and imidacloprid. Acephate, an organophosphate, is one of the most widely used systemic insecticides in the ornamental industry (22). Acephate is formulated as Pinpoint® (granule), which is applied to the soil or as Orthene® (75 WP), which is labeled as a foliar spray or drench application. Acephate is an organophosphate insecticide.

Imidacloprid is labeled as a soil drench or foliage spray in nurseries and greenhouses as Marathon® granule (1%) or wettable powder (60%), respectively. This systemic chloronictinyl insecticide controls a number of nursery pests including mealybugs, whiteflies, aphids, and leafminers (6, 19). Imidacloprid binds to the nicotinergic acetylcholine receptor in the postsynaptic nerve. Because it has a unique mode of action, it is effective against pest populations that are resistant to other materials (22, 26).

The plants used in these trials were selected on the basis of problems with black vine weevil reported by growers in Maryland. Alumroot, *Heuchera spp*. (family Saxifragaceae) are low growing perennials native to mountains and deciduous woodlands of North America. Barrenwort, *Epimedium* (family Berberidaceae) includes over 30 species mainly from Asia and Europe. Barrenwort is a low growing, spreading perennial. (2, 28). *Bergenia* (family Saxifragaceae) are Asian species with shiny green foliage. *Bergenia spp*. are used as groundcovers in sun or partial shade landscapes (2, 28).

We examined the efficacy of one species of nematode, *H. bacteriophora*, for control of black vine weevil larvae infesting herbaceous perennial plants growing under standard nursery conditions in containers. Our objective was to evaluate whether single applications of heterorhabditid nematodes and conventional chemical pesticides provided effective control of black vine weevil larvae in the soil of container grown, herbaceous perennial plants.

Materials and Methods

Biological control of black vine weevil in container grown Bergenia. This study was designed to test the efficacy of a single application of a high rate of the nematode, *H.*

bacteriophora, in reducing populations of black vine weevil larvae. Plants and weevil larvae used in this trial were obtained from a commercial grower in central Maryland. The study plants, *Bergenia cordifolia* 'Rotblum', were grown in 1-liter pots planted in the spring of 1998. The growing substrate was a mixture of peat moss, composted leaves, sand, and pine bark (2:3:1:4 by vol). The weevil larvae used in the study were obtained from infested plants in the nursery.

The experiment was initiated on October 19, 1998. Plants selected for the study were visually inspected for signs of infestation by black vine weevil. This included a visual inspection of the foliage for signs of adult feeding damage and removal of the plants from the containers to inspect the root system for larvae. Ten plants showing no signs of infestation were selected for the study. Weevil larvae were obtained from infested *B. cordifolia* 'Rotblum' at the nursery. Sixty second and third instar black vine weevil larvae were removed from infested *B. cordifolia* 'Rotblum' plants. Each study plant received 6 black vine weevil larvae that were distributed over the surface of the soil inside the pot. A thin layer of potting medium was added to each pot to just cover the larvae. All plants were moved from the nursery to the greenhouse at the University of Maryland, College Park, for the remainder of the study.

Larvae were allowed to establish on study plants for one week prior to the application of treatments. On October 26, 1998, each study plant was assigned to one of two treatments. Five plants received 50 ml of water and served as a control and five received an application of *H. bacteriophora*. *Heterorhabditis bacteriophora* (Heteromask®) nematodes were mixed in 10 liters (2.6 gal) of water and applied in 50 ml (1.7 oz) to 5 pots at a rate of 66,000 nematodes per pot. Nematodes were mixed onsite and agitated thoroughly by stirring. After agitation, applications were made immediately to the pots using an 11.3-liter (3 gal) compression sprayer. A sample was removed from the solution and examined under a dissecting microscope for viability of the nematodes. Over 95% of the nematodes were determined to be viable (movement evident). The soil temperature at the time of nematode application was measured with a glass laboratory thermometer and determined to be 23C (73.4F). Soil temperatures were not measured over the course of the experiment. However, in the small containers used in this study soil temperatures likely approximated ambient temperatures in the greenhouse that ranged from 15–28C (59–82.4F). Plants were hand watered throughout the course of the study to prevent soils from becoming dry.

Post treatment evaluation was made on November 5, 1998. Plants were removed from the pots and the root system was dissected. Black vine weevil larvae and pupae from all of the treatments were examined under a dissecting microscope to determine if they were infested with entomopathogenic nematodes. No adult weevils were found.

Biological control black vine weevil in container grown Heuchera. This trial evaluated the use of a single, lower rate of *H. bacteriophora* to control black vine weevil larvae than the study described previously. The plants used in this trial, *Heuchera micrantha* 'Chocolate', were grown in 1 liter containers and planted in the spring of 1998 by a commercial grower in Maryland. The growing substrate was a mixture of peat moss, composted leaves, sand, and pine bark (2:3:1:4 by vol). Before the nematodes were applied, 6 plants were

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dissected and thoroughly examined for presence of black vine weevil larvae. The number of larvae per pot was 4.13 ± 0.78 prior to the administration of treatments. The trial consisted of two treatments, the application of nematodes and a water control, and involved 36 plants, 18 randomly assigned to each treatment. Pots were arranged in 6 blocks with 3 replicates in each block.

Treatments were made on October 2, 1998. *Heterorhabditis bacteriophora* (Cruiser®) nematodes were mixed in 10 liters (2.64 gal) of water and applied to 18 plants. The remaining 18 plants received water only (500 ml (17 oz)) and served as a control. Nematodes were mixed onsite and agitated thoroughly by stirring. For each plant in the 6 blocks, 140 ml (4.8 oz) of mixture was applied. This delivered 5,000 nematodes per pot. Applications were made immediately after mixing and agitation using a 1.2 liter (0.32 gal) calibrated container. Samples were removed from the solution and examined under a dissecting microscope for viability of the nematodes. Over 95% of the nematodes were determined to be viable by movement. Following the application of nematodes, the irrigation system was turned on and allowed to run for two hours. A total of 500 ml (17 oz) of water was applied to each pot. The ambient air temperature at time of treatment was 18–20C (64.4–68F) and soil temperature measured with a soil probe thermometer was 17C (62.6F).

Post treatment evaluation was conducted on October 29, 1998. Plants were removed from pots and the root systems were dissected. Black vine weevil larvae, pupae, and adults from both treatments were examined under a dissecting microscope to determine whether they were infested with entomopathogenic nematodes.

Chemical and biological control of black vine weevil in container grown Epimedium. This study compared the efficacy of *H. bacteriophora* and two insecticides in reducing populations of black vine weevil larvae in pots of *Epimedium* x *rubrum* at a commercial nursery in central Maryland. Plants were grown in 1-liter containers for nine months before the initiation of the trial. The growing substrate was a mixture of sand, pine bark, and composted leaves (1:4:5 by vol). A randomized, complete block design was used with 5 treatments in 8 blocks and 12 replicates in each block for each treatment. A buffer of 0.5 m (1.6 ft) was maintained between each treatment within a block to prevent cross contamination of

insecticides among treatments. Ten days before treatments were applied, on April 19, 1998, 8 plants, one per block, were randomly selected and sampled to confirm the presence of black vine weevil larvae. The number of live larvae was 1.36 ± 0.89 (s.e.) per pot.

Treatments were applied on April 27, 1998. The treatments consisted of *H. bacteriophora*, acephate, and imidacloprid. The water used to dilute the nematodes was 19C (66.2F) with a pH of 6.1. *Heterorhabditis bacteriophora* (Cruiser®) nematodes were mixed in 10 liters (2.6 gal) of water and applied to 72 pots (140 ml of mixture applied to each pot) for each treatment in the 8 blocks at a rate of 5,000 nematodes per pot. Nematodes were mixed onsite and agitated thoroughly by stirring. Samples were removed from the solution and examined under a dissecting microscope for viability of the nematodes. Over 92% of the nematodes were determined to be viable by movement. After agitation, applications were made immediately to the pots applying 140 ml (4.8 oz) for each of the 72 pots. A total of 500 ml (17 oz) of water was applied to each pot through irrigation.

Two acephate 15% tablets were split in half and placed on the surface of the soil in each acephate treated pot. Two rates, 1.25 g and 2.50 g, of imidacloprid 1% granule were also evaluated. Imidacloprid was applied directly onto the soil surface for each of the treated pots. The irrigation system was turned on following all treatments and allowed to run for two hours.

Soil temperature probes (Reotemp®, model 'A', bimetal temperature probe) were placed in 5, randomly selected pots to record soil temperatures on the day of the treatments and at the final evaluation. The soil temperature was 15C (59F) at the beginning of the day of nematode and insecticide application and rose to 16C (60.8F) by the end of the day. The ambient temperature was 21C (69.8F) and it was sunny with a light breeze.

Plants were evaluated on May 27, 1998, 30 days after treatment. At the time of evaluation the soil temperature was 25C (77F). Five plants were selected randomly from each treatment in each of the eight treatment blocks for the post treatment evaluation. Plants were removed from the pots and the root system was dissected. The number of living black vine weevil larvae, pupae, and adults was recorded. Larvae and pupae treated with nematodes were examined under a dissecting scope to determine whether they were infested with

Table 1. Effects of *Heterorhabditis bacteriophora* and conventional insecticides on abundance of black vine weevil immatures infesting perennial plants grown in 1 liter containers.

Host plant	Treatment	Rate	Weevils mean (s.e.)
Bergenia cordifolia 'Rotblum'	Control	0/pot	5.4 (0.4)a[z]
	H. bacteriophora	66,000/ pot	0.0 (0.0)b
Heuchera micrantha 'Chocolate'	Control	0/pot	5.00 (0.58)a
	H. bacteriophora	5,000/pot	0.17 (0.17)b
Epimedium x *Rubrum*	Control	0/pot	1.40 (1.16)a
	H. bacteriophora	5,000/pot	0.13 (0.11)b
	Acephate	2 tablets/pot	0.23 (0.07)b
	Imidacloprid	1.25 g/pot	0.08 (0.71)b
		2.50 g/pot	0.45 (0.14)b

[z]For trials with *Bergenia* and *Heuchera*, means that share the same letter do not differ by the results of a Kruskal-Wallace test (P = 0.05). For the trial with *Epimedium*, means that share the same letter do not differ by a Nemenyi test (P = 0.05).

entomopathogenic nematodes. Weevil larvae in the chemical treatments were also examined to confirm the presence or absence of nematodes.

Statistical analysis. Variances among treatments were large and heteroscedastic in all three studies. Homogeneity of variance could not be achieved through transformation of the data. Therefore, in the first two studies nematode treatments were compared to controls with a Kruskal-Wallis nonparametric analysis of variance (29). In the third study differences among nematodes and insecticide treatments were resolved following the Kruskal-Wallis analysis with a Nemenyi test (29).

Results and Discussion

Results of the three trials involving nematodes provide convincing evidence that applications of entomopathogenic nematodes provide highly effective control of black vine weevil larvae under conventional methods of container plant production. In all trials, the application of nematodes and insecticides significantly reduced populations of weevil larvae relative to levels found in the untreated containers (Table 1). The first and second trials using the plants *Bergenia* and *Heuchera* confirmed that entomopathogenic nematodes at high and low rates provide significant reductions of black vine weevil populations compared to untreated controls (*Bergenia*, $\chi^2 = 7.97$, P < 0.0047; *Heuchera*, $\chi^2 = 8.97$, P < 0.0028). The last trial demonstrated that all materials provided significant reductions of black vine weevil larvae in containers relative to untreated controls ($\chi^2 = 72.07$, P < 0.0001). *Heterorhabditis bacteriophora* applied to *Epimedium* provided levels of control comparable to those obtained by using imidacloprid and acephate (Table 1). Populations of weevil larvae were reduced by 90–100% following the application of *H. bacteriophora* in all trials (Table 1). Immature stages of black vine weevil recovered from treatments where nematodes were applied were all infested with nematodes. No immatures recovered from controls or insecticide treatments were found to contain nematodes.

Black vine weevils in soilless substrate distribute themselves through the root zone but are found in largest numbers in the upper strata of the containers near the base of the plant during the active growing season (Gill, personal observation). We believe that nematodes had no problem contacting weevil larvae found in our containers that were maintained under conditions of high soil moisture due to regular irrigation. Hanula (14) found that in native soil columns, black vine weevils were found as deep as 30 cm (12 in) but over 90% were found in the top 15 cm (6 in). Distribution of larvae and pupae were similar. *Heterorhabditis bacteriophora* did not infect larvae below 12.5 cm (5 in) (14). Hanula (14) found that *H. bacteriophora* was ten times more virulent (LC95 = 77 nematodes per cm³) than *S. carpocapsae* (LC95 = 794 nematodes per cm³). Both species were equally effective in a field trial at the highest rate tested (2×10^5 nematodes per plant).

The plant injury sustained by black vine weevils on the herbaceous perennial plants varied with species. Plants such as *Heuchera* with 4 to 5 larvae in a 1-liter pot had the roots completely severed. In other trials we have observed Toad lily, *Tricytis spp.*, with 6 to 8 larvae per 1-liter pot to show no significant injury. Toad lily has an extremely vigorous, fleshy root system that appears to sustain more feeding injury but

regenerate rapidly after injury. The *Heuchera* and *Epimedium* plants that were used in this study but not destructively sampled recovered very rapidly after the application of nematodes and insecticides. Weevil free plants regenerated new root systems in less than thirty days from October through November (Gill, personal observation).

The advantages to growers of using entomopathogenic nematodes are numerous. First, *H. bacteriophora* can be extraordinarily lethal to black vine weevil larvae. Second, entomopathogenic nematodes do not require EPA labeling and there is no restrictive Re-Entry Interval (REI) as with conventional chemical controls. Third, entomopathogenic nematodes are safe for the applicator and have minimal impact on non-target organisms. Fourth, entomopathogenic nematodes are relatively easy to apply and readily available from commercial suppliers.

The disadvantages to the use of nematodes include thermal limitations to survival and infectivity (8, 10). Entompathogenic nematodes must be used shortly after purchase because their storage interval is limited to 6 months or less depending on the type of formulation. Suppliers of entompathogenic nematodes vary widely in the price charged and rate recommended. This makes a cost comparison to conventional pesticides difficult. In general, nematodes will be more expensive to use than conventional pesticides.

Nonetheless, our studies, conducted with two formulations of nematodes on three species of perennials, provide strong evidence that *H. bacteriophora* controls black vine weevil larvae in containers. Nursery managers and greenhouse growers have an efficacious biological control option for dealing with this pest.

Literature Cited

1. Alford, D.V. 1995. Pests of Ornamental Trees, Shrubs and Flowers. Halsted Press: an Imprint of John Wiley and Sons, New York, NY. 448 pp.

2. Armitage, A. 1989. Herbaceous Perennial Plants. Stipes Publishing, Champaign, IL. 1141 pp.

3. Baker, J.R. 1994. Insect and related pests of flowers and foliage plants. N.C. State Agric. Publication, AG–136, 130 pp.

4. Blackshaw, R.P. 1986. An evaluation of seven controlled-release insecticides for control of vine weevil larvae. J. Hort. Sci. 61:109–111.

5. Decker, D. 1997. Floriculture and environmental horticulture: situation and outlook report. USDA-ERS, Washington, DC.104 pp.

6. Dotson, J.A. 1994. Merit Product Information. M. Bayer Corp. 110 pp.

7. Garth, S. and C.H. Shanks. 1978. Some factors affecting infestation of strawberry fields by black vine weevil in western Washington. J. Econ. Entomol. 71:443–448.

8. Gaugler, R. and H.K. Kaya. 1990. Entomopathogenic nematodes in biological control. CRC Press. Boca Roton, FL. 265 pp.

9. Georgis, R. 1984. Greenhouse control of black vine weevil, *Othiorhynchus sulcatus* (Coleoptera: Curculionidae) by Heterorhabditid and Steinernematid nematodes (*Neoaplectana carpocapsae*, *Neoaplectana glaseri*, *Heterorhabditis heliothidis*). Environ. Entomol. 13:1138–1140.

10. Georgis, R and R. Gaugler. 1991. Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol. 84:713–720.

11. Georgis, R. and G. Poinar. 1984. Greenhouse control of black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) by Heterorhabditid and Steinernematid nematodes. Environ. Entomol. 13:1138–1140.

12. Gill, S.A. and J. Sanderson. 1998. Greenhouse Pests and Beneficials. Ball Publishing, Batavia, IL. 245 pp.

13. Halfhill, J.E. 1985. Larvicides for black vine weevil on woody ornamentals. J. Agric. Entomol. 2:292–296.

14. Hanula, J.L. 1993. Vertical distribution of black vine weevil (Coleoptera: Curculionida) immatures and infection by entomogenous nematodes in soil columns and field soil. J. Econ. Entomol. 86:340–347.

15. Johnson, W.T. and H.H. Lyon. 1988. Insects that Feed on Trees and Shrubs. 2nd ed. Cornell University Press, Ithaca, NY. 556 pp.

16. Kaya, H.K. and R. Gaugler. 1993. Entomopathogenic nematodes. Ann. Rev. Entm. 38:181–206.

17. Kakouli, T., N.G. Hague, and A.A. Otto. 1993. Evaluation of the entomopathogenic nematode, *Steinernema carpocapsae*, against the black vine weevil, *Otiorhynchus sulcatus*. Tests of agrochemicals and cultivars, Supplement to Ann. Appl. Biol. 122:190–191.

18. Moorhouse, E.R., A.K. Charney, and A.T Gillespie. 1992. A review of the biology and control of black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Cucurlionidae). Ann. Appl. Bio. 121:431–454

19. Mullins, J.W. 1993. Imidacloprid: A new nitroguanidine insecticide. Am. Chem. Soc. Symp. Ser. 524:183–198.

20. Neiswander, R.B. 1976. Control of the black vine weevil. J. Econ. Entomol. 46:234–237.

21. Nielson, D.G. and J. Boggs. 1985. Topical toxicity of insecticides to first-instar black vine weevil (Coleoptera: Curculionidae). J. Econ. Entomol. 78:1114–1117.

22. Onofrey, D. 1998. FQPA Special Report. Prevent the squeeze on our food and flowers. Greenhouse Grower 16:3.

23. Rhodus, T. and J. Hoskins. 1995. Results of the 1995 PPA perennial sales survey. Perennial Plants 3:1–10.

24. Schirocki, A. and N. Hague. 1997. Evaluation of UK Heterorhabditis and Steinernematids for the control of the black vine weevil (*Otiorhynchus sulcatus*). Ann. Appl. Biol. 130:46–47.

25. Smith, F.F. 1927. The black vine weevil (*Brachyrhinus sulcatus* Fabr.) as a pest in greenhouses and nurseries. J. Econ. Entomol. 20:127–131.

26. Steward, B.V., G. Braness, and S.A.Gill. 1998. Ornamental pest management using Imidacloprid applied with the Kioritz soil injector. J. Arboriculture 24: 344-346.

27. Swier, S.R., A. Rollins, and R. Lamarche. 1998. Efficacy of talstar formulations and entomopathogenic nematodes toward black vine weevil in potting media: 1997. Arthropod Manag. Tests 23:341.

28. Woods, C. 1992. Encyclopedia of Perennials: A Gardener's Guide. Facts on File, Inc, New York, NY. 350 pp.

29. Zar, J. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ. 633 pp.

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