

Rapid Test of the Suitability of Host-trees and the Effects of Larval History on *Anoplophora glabripennis* (Coleoptera: Cerambycidae)

JAY S. BANCROFT,¹ MICHAEL T. SMITH,² EMMA K. CHAPUT,¹ AND JOSEPH TROPP²

ABSTRACT The invasive cerambycid, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is native to Asia, and threatens numerous species of host trees in Europe and North America. The eradication of breeding populations depends on surveys of host trees, but the suitability of potential hosts has not been measured. Our experiment demonstrates a rapid method to assess the suitability of potential host trees. We compared weight gain over one month by 80 larvae reared in freshly cut logs of eight common hardwood species found in the areas of infestation. From the largest percent weight gain to the smallest, the resulting ranking was Chinese elm, Norway maple, American elm, honeylocust, sugar maple, red oak, white ash, green ash. Although comparisons suggested similar growth by larvae taken from diet to those reared on twigs, larvae from China grew at a greater rate than larvae originating from the Chicago infestation. This technique can be used to rapidly quantify host suitability and identify trap trees to be used for replanting after removal of infested trees.

KEY WORDS host suitability, risk assessment, invasive species

Introduction

The invasive Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), represents a serious threat to deciduous forests of North America and Europe (Haack *et al.*, 1997; Pasek, 2000; FBVA, 2002). Since 1996, more than 7900 infested trees have been removed from areas around New York City and Chicago (Haugen, 2002). An infestation recently discovered in Braunau, Austria, has provoked an eradication program that has removed at least 47 trees since 2000 (FBVA, 2002). Because areas with reproducing beetles are small, the eradication effort represents an important test case for the ability to protect ecosystems from invasive species. Around current infestations, the quarantine of all wood may prevent human-induced spread, and the removal of host-trees within the beetle's dispersal range may prevent natural spread. The need to prevent spread was shown in a risk assessment by Nowak *et al.* (2001), who estimated monetary losses of \$669 billion (all costs in U.S. dollars), assuming *A. glabripennis* spreads to all urban areas in the U.S. The current efforts to eradicate this beetle are expected to cost \$365 million (Stefan and Markham, 2000). The work described here (1) demonstrates a technique for rapid assessment of host suitability by invasive wood boring pests and (2) quantifies suitability of eight tree species for larval growth. For the *A. glabripennis* eradication effort, this timely information helps officials concentrate survey efforts on susceptible trees and replant nonhost trees.

A tremendous number of trees are at risk due to the broad host range of *A. glabripennis*. Of the trees removed from New York, species of maple dominate (95%), followed by elm (2%), ash (<1%), oak (<1%) and others. These values hint at suitability but are a result of the tree's size and prevalence as well as the oviposition preference of adults. Ash trees have been found with beetle oviposition sites, but they are unlikely to be as suitable as maples (Pasek, 2000). Preferred hosts make up approximately 30% or 1.2 billion of the urban trees in the U.S. (Nowak *et al.*, 2001). The wider ecological damage *A. glabripennis*

¹ State of Delaware, Delaware Department of Fish and Wildlife, 225 Bayard Avenue, Dover, Delaware 19901.

² USDA, Agricultural Research Service, 501 S. Chapel Ave., Newark, Delaware 19701

³ Current address, USDA-ARS 17053 N. Shafter Ave., Shafter, California 93263. Email: JSB95003@yahoo.com

nis may cause is reflected in the importance of the maple trees in the eastern U.S. The Forest Service calculated the importance of tree species based on their ecological dominance and spatial distribution, and red maple (1st) and sugar maple (4th) were ranked extremely high (Iverson *et al.*, 1998).

Cerambycid natural history poses difficult challenges for eradication and research. Like other cerambycids, *A. glabripennis* causes damage to woody species by enabling secondary infection, weakening physical strength, and girdling the cambium (Hanks *et al.*, 1993). Many forest pests attack saplings and stressed trees, but *A. glabripennis* also attacks apparently healthy full-grown trees (Haack *et al.*, 1997; Cavey *et al.*, 1998). Several years of attack by *A. glabripennis* may be required before large trees exhibit "dieback" or show obvious signs of damage. By this time, larvae in concealed galleries inside the tree may have caused extensive damage and hundreds of beetles may have emerged and dispersed to nearby trees. Survey officials detect infested trees by observing emergence holes and oviposition pits on bark. This is difficult and expensive because beetles first colonize high in the canopy (Bancroft, unpubl. data). *A. glabripennis* has relatively low reproductive (Smith *et al.*, 2002) and dispersal rates (Smith *et al.*, 2001) as compared to other invasive pests (Shelly *et al.*, 1994; Shigesada and Kawasaki, 1997). These are limitations that allow surveyors to concentrate within the limited area of infestation (AHPIS, 2000). Currently, experimental research is challenging because *A. glabripennis* are quarantined, thereby restricting research in the U.S. to quarantine laboratories. To obtain beetles for experiments, larvae must be reared in containers on artificial diet or on twigs. Less labor intensive techniques are being developed but rearing each *A. glabripennis* now costs approximately \$100.

This study is part of an ongoing project to predict population spread by combining dispersal studies with the distribution of suitable host-trees in the landscape (Parker *et al.*, 1999; Neubert and Caswell, 2001). With predictions of *A. glabripennis* density in a given landscape, we may target management efforts and mitigate the beetle's impact on local ecosystems and economies. A first step in this process is to make quantitative measurements of the suitability of host-trees that *A. glabripennis* may encounter in the areas of infestation. The suitability of food has been estimated using weight gain as an index in many animals, including cerambycid beetles (Ferguson *et al.*, 1991; McColl and Noble, 1992; Fox *et al.*, 1994; Futai *et al.*, 1994; Joseph and Kelsey, 1994; Mesfin *et al.*, 1995; Langvatn *et al.*, 1996). This experiment measured larval weight gain when reared on eight common tree species found in the infested areas. The quarantine laboratory experiment demonstrates how freshly cut logs, with growing shoots, may be used to rapidly measure host suitability for invasive tree pests.

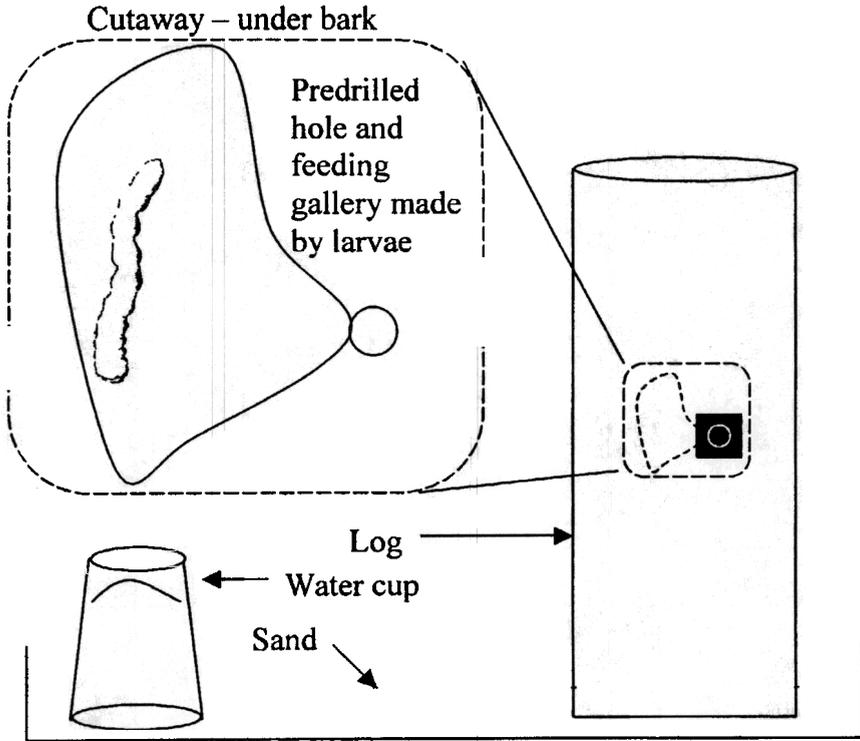


Fig 1 The experimental set-up for logs. Representation of the typical gallery made by a larvac (frass not shown)

et al., 2002; Bancroft, unpubl. data). We chose pairs of tree species in the same genus to test for suitability differences within these genera. Honeylocust is listed as a nonhost for replanting upon removal of infested trees (Bohne, 2002). Finally, honeylocust and red oak have not been removed from the known U.S. infestations, and they are believed to be unsuitable hosts for *A. glabripennis* (*op. cit.*).

In December 2000, we acquired host material from two locations in the Newark, Delaware area that had not received any pesticides in the preceding year. All trees were leafless so no phloem was moving, and nutrient levels would be relatively low in the cambium. Healthy branches were selected from 1 or 2 trees of the eight species, and logs were cut (30 × 8 cm) with a volume of approximately 1500 cm³. The distal end of each log was waxed and their bases were placed in moist sterile sand (Fig. 1). The quarantine room for the experiment was held at 22 ± 2°C, 50 ± 5% R.H., and 16:8 L:D cycle. Logs taken into quarantine grew shoots when placed on moist sand. Within 24 hours, a 3 × 3 cm bark flap was cut out with a chisel and a 1 × 4 cm hole was drilled into each log. The bark flap kept the beetles in the log and the hole allowed larvae in living wood to choose the tissue for feeding. Larvae were weighed (accuracy ± 10⁻⁴ g), placed in drilled holes, and bark flaps were replaced and held in place with masking tape.

We used four categories of larvae in the experiment. Eggs were obtained from trees in Hohhat, Inner Mongolia, China and transported as larvae to the above quarantine room where they

Table 1. Summary data: Number of replicates (one beetle per log), mean initial and final weights (g), and note on survival

Tree species		China twigs	China diet	Lab twigs	Lab diet	Notes
American elm	Number of beetles	3	3	2	2†	1 missing
	Initial weight	0.11	0.15	0.66	0.40	
	Final weight	0.33	0.31	0.82	0.88	
Chinese elm	Number of beetles	3†	3	1	3	1 died
	Initial weight	0.09	0.11	0.76	0.08	
	Final weight	0.34	0.46	1.02	0.32	
Green ash	Number of beetles	2	4†	3†	1	2 died
	Initial weight	0.28	0.13	0.68	0.26	
	Final weight	0.35	0.21	0.55	0.12	
Honeylocust*	Number of beetles	3†	3	3	2	1 died
	Initial weight	0.35	0.08	0.64	0.38	
	Final weight	0.34	0.34	0.56	0.32	
Norway maple	Number of beetles				10†	1 died
	Initial weight				0.30	
	Final weight				0.64	
Red oak	Number of beetles	3	3	3	1	
	Initial weight	0.26	0.10	0.75	0.05	
	Final weight	0.39	0.19	0.72	0.08	
Sugar maple	Number of beetles	3†	3	2	1	1 killed
	Initial weight	0.32	0.17	0.68	0.15	
	Final weight	0.72	0.31	0.82	0.11	
White ash	Number of beetles	3†	4†	1	2	2 died
	Initial weight	0.11	0.07	0.72	0.04	
	Final weight	0.13	0.09	0.44	0.05	

*Honeylocust had 11 replicates

†Indicates group that note refers to

were reared in cups of an artificial *Anoplophora* diet (Dubois *et al.*, 2002) for early instars. Third or later instars fed on freshly split willow twigs within large test tubes. This regimen for rearing small and large larvae was developed to reduce desiccation in early instars and mortality due to unsuccessful molting in older instars. We also used larvae reared from eggs laid in the quarantine laboratory. We removed infested timber from Chicago and collected emerging adults. The females were mated and laid eggs on red and sugar maple logs, which were subsequently transferred onto diet. The number of larvae in each category was 23 small larvae from China, 20 large larvae from China, 22 small larvae from U.S., and 15 large larvae from U.S. One larvae was placed in each log, and larvae from the four categories were placed on tree species in approximately equal numbers, except for Norway maple. After we had allocated the other larvae to host-logs, we gained access to Norway maple. This is the most frequently attacked species, and was added to test larval growth on a highly preferred host. We used 10 larval replicates, one per log, for each host species (Table 1, first column).

After 31 days, the logs were dissected and live larvae were reweighed. Recovered larvae were kept for reproductive stock, and host material was autoclaved before removal from quarantine. The suitability of host trees was represented by the larval weight gain (= end wt. - start wt.). We used a general linear model with the categorical variables: species, origin, and diet (Statsoft, 1999). To control for variation in initial weight, the original weight of the larvae was used as a covariate. Because larvae originating from U.S. or China were divided into groups based on previous diet type (artificial or twigs), the diet variable was nested within the larval origin. The final categorical variable was the species

of host tree. The design was unbalanced in each cell because Norway maple logs were only infested with larvae originating from the lab and reared on diet. Therefore, the data were analyzed as an incomplete design, and interaction between larval regimen and tree species was not tested. We tested for mean separation among levels of each variable using Scheffe's test at $\alpha = 0.05$, which is much more conservative than least significant difference or other tests with multiple comparison (Winer, 1962). In summary, the experiment tested for differences in weight gain due to host species and larval origin while accounting for initial weight.

Results

During dissection of the logs, we found that larvae typically flattened themselves and fed on the cambium tissue in a gallery just under the bark (Fig. 1). Some utilized the predrilled hole while others chewed into the heartwood from a feeding gallery under the bark. Larger individuals (>0.5 g) were always found in the xylem tissue although many had made galleries in the cambium.

The analysis with a general linear model showed significant differences in weight gain among tree species (d.f. = 7, 59; $F = 7.23$; $P < 0.001$). The two types of larval food, which were nested within the two sources of larvae, did not show any effect (d.f. = 2, 59; $F = 0.148$; $P = 0.863$), but an effect on weight gain was suggested due to larval origin (d.f. = 1, 59, $F = 6.2$; $P = 0.016$). The origin of the larvae suggested that wild-caught larvae grew faster than those reared from eggs in the lab (Fig. 2a).

The larval weight gain among host-trees showed considerable variation (Fig. 2). The ranking generally corroborates observations made in tree removal data and Chinese management efforts (Qin *et al.*, 1985; Pasek, 2000; APHIS, 2002). Maple and elm are desirable shade trees, and both have been frequently attacked and removed in New York and Chicago. Our results confirm that larvae readily gained weight on maple and elm, with the greatest weight gain observed in Old World species. Our study suggests that honeylocust, a less common ornamental than the other species, and red oak should be surveyed to make sure no emergence holes are found. We believe further study of the specific characteristics of host material and larval behavior will improve our ability to predict larval suitability and the adaptation of *A. glabripennis* to U.S. trees.

Discussion

Perhaps the main result of this study is that *A. glabripennis* grew more on honeylocust and red oak than either ash species, which seems to contradict tree removal data suggesting ashes were of intermediate suitability. The number of removed trees is likely to reflect the frequency distribution of tree species in infested areas, as well as their suitability. Ashes have been found with oviposition sites, but few emergence holes, which suggests they are not high quality hosts. The red oak and honeylocust may occur at low frequency in infested areas and have small amounts of infestation, but these trees should not be neglected by eradication surveyors. Oak timber is highly valued and the trees provide many important ecosystem functions, so their protection from more pests is important (Jones *et al.*, 1998). There are many trees *A. glabripennis* cannot use (Pasek, 2000), and we prescribe that honeylocust not be replanted in infested areas until it has been verified as a nonhost (Haugen, 2002). There may be benefit to eradication efforts if *A. glabripennis* females are "trapped" by indiscriminate attack on ashes. Because so ash may cause high mortality of larvae. As stated earlier, these results need to be compared with long-term studies on egg to adult development, now underway.

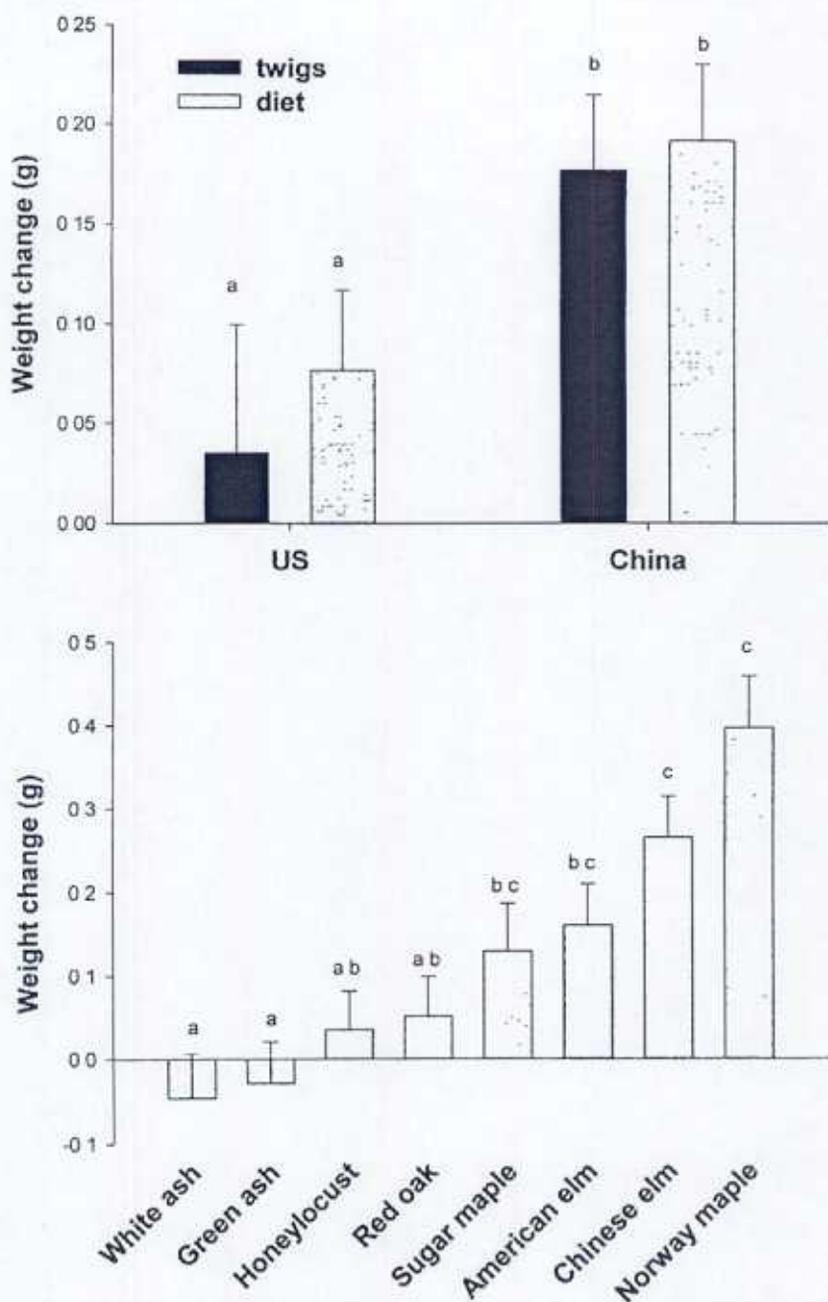


Fig. 2. Weight change of larval *A. glabripennis* represented by least square means with standard error bars. Scheffe's test identified homogeneous groups, which are shown with the same letter ($\alpha = 0.05$). a) Weight gain for each food type and larval origin, while controlling for initial weight and tree species. b) Weight change for each tree species while controlling for initial weight, food type, and larval origin.

The effects of larval history on growth help us identify areas in need of further research. Slower growth was observed for beetles originating from the Chicago population (Fig. 2a). One interpretation is that culturing methods need to be improved so adults may be fed and oviposit healthy eggs in the laboratory. Although we cannot discount maternal effects, we believe the larvae originating from China or the US were exposed to very similar environments. Another explanation is that the beetles in Chicago are a slower growing sub-population than those from Hohhat. In other words, there could be genetic and environmental effects influencing larval development. This may be elucidated by rearing studies based on estimated relationships among *A. glabripennis* lineages. These experiments could involve rearing larvae from different populations on various hosts in controlling conditions. This could be used to identify host specificity and the coevolutionary adaptations among beetle populations. The methods for measuring host suitability that are described here form a basis for such studies. Our results reaffirm that interpretations of *A. glabripennis* biology from laboratory studies must explicitly state the population origin.

Larvae from both sources grew at about the same rate whether they were fed twigs or diet. Even though large larvae were fed twigs, the covariate analysis accounted for the influence of size on rearing protocol. It is not clear if there is a required nutritional shift, but large larvae in China are usually found deep in the heartwood (Bancroft, unpubl. data). We believe deeper boring by *A. glabripennis* larvae may afford protection from parasitoids and woodpeckers. Further study may suggest how *A. glabripennis* discerns differences between live heartwood, twigs, and diet, but the relatively equal growth in spite of feeding history suggests the nutrition of the artificial diet is adequate for larval development. These results also indicate that the twigs did not precondition the larvae to exploit woody host-trees provided in the experiment. Finally, the results of diet comparison suggest that healthy *A. glabripennis* may be reared and manipulated in the laboratory.

The quantitative measure of suitability is important for ongoing modeling efforts. Prediction of population spread depends on the relative suitability among available hosts (Shigesada and Kawasaki, 1997). The reproductive success on a host is a combination of female propensity to oviposit and suitability. Adult females clearly discern host suitability based on previous allocation of eggs on host material (Smith and Bancroft, 2002). This may be combined with dispersal and mortality to predict population spread (Smith *et al.*, 2001). The predictions of spread will provide a foundation for assessing adaptive management strategy for *A. glabripennis* (Sharov and Liebhold, 1998).

This technique provides a fast assessment of larval suitability, which may be used on other wood boring pests. The measurement of developmental success is often impractical on live trees by natural oviposition, which would require yearlong studies, many trees of sufficient diameter, and large quarantine greenhouses. Although the logs were sprouting and beetles gained weight when placed on tissue of the various trees, the accuracy of host-suitability indices from the artificial infestation needs to be verified with longer-duration field studies. When improved artificial rearing produces large numbers of beetles, field experiments will be able to measure mortality within host-trees. United States Department of Agriculture scientists are growing these species of host-trees and others in China for just such experiments. These studies using the same host-tree species will test whether local effects from trees grown in Delaware account for some of the observed variation among host species. Further physiological work is also needed to assess how *A. glabripennis* larvae differentiate various host tissues and how these characteristics interact with different host species. Females readily oviposit on branches with diameters of 2 cm or larger (Bancroft, unpubl. data), but additional studies on the optimal diameter of suitable branches can

help predict whole tree exploitation. Our suitability measures corroborate anecdotal evidence from tree removal data and previously reported hosts (APHIS, 2002). We believe this technique will be verified with long-term studies and may enable suitability estimates from experiments of about 2 weeks duration. The immediate threat to host trees may be quickly screened using this technique.

Acknowledgement

We thank John Fret, University of Delaware, for assisting in the acquisition of host-tree material. We also thank both the Chinese Academy of Forestry and the State Forestry Administration for efforts in facilitating this cooperative research. Finally, the authors wish to thank USDA-ARS for funding.

Literature Cited

- APHIS 2002 Host trees <http://www.aphis.usda.gov/oa/alb/hostsp.html>. 22 January 2002
- Banerjee, S N., and D K Nath 1971 Life-history, habits and control of the trunk-borer of orange, *Anoplophora versteegi* (Ritsema) (Cerambycidae Coleoptera) Indian Journal of Agricultural Science 41 765-71
- Bohne, M 2002 Asian longhorned beetle <http://www.uvm.edu/albeetle/index.html> 22 January 2002
- Cavey, J. F., E R Hoebeke, S Passoa, and S W Lingafelter 1998 A new exotic threat to North American hardwood forests: an Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera Cerambycidae) I Larval description and diagnosis Proceeding of the Entomological Society of Washington 100 373-381
- Dubois, T, A E Hajek, and S Smith 2002 Laboratory rearing methods of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera Cerambycidae) Annals of the Entomological Society of America 95 223-230.
- FBVA 2002 Austrian Federal Forest Research Centre <http://fbva.forvic.ac.at/400/1517.html> 22 January 2002
- Ferguson, C, M Lunt, and G Krause 1991 Host-plant preference of the asiatic oak weevil (Coleoptera, Curculionidae) Environmental Entomology 20 1427-1432
- Fox, C W, K J Waddell, and T A Mousseau 1994 Host-associated fitness variation in a seed beetle (Coleoptera, Bruchidae)—evidence for local adaptation to a poor quality host Oecologia 99 329-336.
- Futai, K, S Shirakikawa, and I Nakai 1994 The suitability of Korean pine (*Pinus koraiensis* Sieb Et Zucc) and Japanese red pine (*P. densiflora* Sieb Et Zucc) as a host of the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera, Cerambycidae) Applied Entomology and Zoology 29 167-177
- Haack, R A., K. R Law, V C Mastro, H S Ossenbruggen, and B J Raimo 1997 New York's battle with the Asian long-horned beetle Journal of Forestry 95 11-15
- Hanks, L M., Paine, T D., and J G Millar 1993 Host species preference and larval performance in the wood-boring beetle *Phoracantha semipunctata* F Oecologia 95 22-29
- Haugen, D 2002 Asian longhorn <http://www.na.fs.fed.us/spfo/alb/general/replanting.htm> 22 January 2002
- Iverson, L R., A M Prasad, B J Hale, and E K Sutherland 1998 Atlas of current and potential future distributions of common trees of the eastern United States USDA-FS Technical Report 265 Delaware, Ohio.
- Jones, C G, R S Ostfeld, M P Richard, E M. Schaubert, and J O. Wolff 1998 Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk Science 279:1023-1026
- Joseph, G., and R. G Kelsey 1994 Acceptability and suitability of Douglas-fir as a secondary host for gypsy-moth (Lepidoptera, Lymantridae). Environmental Entomology 23 396-405
- Langvatn, R., S D Albon, T. Burkey, and T H Clutton-Brock 1996 Climate, plant phenology and variation in age of first reproduction in a temperate herbivore Journal of Animal Ecology 65 653-670.
- McColl, A L., and R M Noble 1992 Evaluation of a rapid mass-screening technique for measuring antibiotic to *Helicoverpa* spp in cotton cultivars Australian Journal of Experimental Agriculture 32:1127-1134
- Mesfin, T, J D Hollander, and P G Markham 1995 Feeding activities of *Cicadulina mbila* (Hemiptera, Cicadellidae) on host-plants Bulletin of Entomological Research 85 387-396
- Neubert, M G., and H Caswell 2000 Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations Ecology 81 1613-28
- Nowak, D, J Pasek, R A Sequiera, D Crane, and V Mastro 2001 Potential effect of the Asian longhorned beetle (Coleoptera Cerambycidae) on urban trees in the United States Journal of Economic Entomology 94:116-122

- Parker, I M., D Simberloff, W M Lonsdale, K Goodell, M Wonham, P M Kareiva, M H Williamson, B V Holle, P B Moyle, J E Beyers, and L Goldwasser. 1999 Impact toward a framework for understanding the ecological effects of invaders *Biological Invasions* 1 3-9
- Pasek, J E 2000 Pest Risk Assessment for Importation of Solid Wood Packing Materials into the United States 311 pp. USDA APHIS
- Qin, X., R Gao, J Li, W Hao, and K Liu 1985 A preliminary investigation on the resistance of different clones of poplars to *Anoplophora glabripennis* (Motsch) *Scientia Silvae Sinicae* 21 310-314
- Sharov, A A., and A M Liebhold 1998 Bioeconomics of managing the spread of exotic pest species with barrier zones *Ecological Applications* 8 833-845
- Shelly, T E., T S Whittier, and K Y Kaneshiro. 1994 Sterile insect release and the natural mating system of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera, Tephritidae) *Annals of the Entomological Society of America* 87 470-481
- Shigesada, N., and K Kawasaki 1997 *Biological Invasions Theory and Practice* Oxford Univ Press, Oxford
- Smith, M T., and J Bancroft 2002 Age-specific fecundity of the Asian longhorned beetle on black willow, Norway maple and red maple *Environmental Entomology* 31 76-84.
- Smith, M. T., J Bancroft, G Li, R Gao, and S Teale 2001 Dispersal of the Asian longhorned beetle, *Anoplophora glabripennis* Motsch (Cerambycidae) *Environmental Entomology* 30 1036-1040
- Statsoft 1999 *Statistica v. 5.5* computer program. Tulsa, OK
- Stefan, M., and C Markham 2000 Strategic plan for the eradication of the Asian longhorned beetle for New York and Chicago. Riverdale, MD 25 p. U S D A -A P H. I. S. Plant Protection and Quarantine.
- Winer, B J 1962 *Statistical Principles in Experimental Design* McGraw-Hill, New York