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*Laboratory Rearing and Handling of Cerambycids**

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7.1 Introduction

Lack of suitable rearing and handling techniques has hampered research on the biology and control of many species of cerambycids that feed on host species of economic importance. Furthermore, because cerambycids spend most or all of their pre-adult life cycle inside the host plant, the biology of many is not well-known and would be difficult to study in nature. This is especially true for species with extended life cycles where adults only appear briefly in the field either annually, biannually, or less often. The development of laboratory rearing methods, either partially or completely independent of host material, can and has been used to rapidly advance our knowledge of cerambycids.

Several diets and rearing protocols have been published for cerambycid species. These larval diets include artificial diets with no host material incorporated into them, artificial diets with pulverized host material incorporated, and natural diets of cut host material. Diets and handling methods have been formulated for species with adverse economic impacts to help understand their biology and to develop management options. There is one case where methods have been developed as a way to preserve a threatened cerambycid species (Dojnov et al. 2012). Methods have also been developed to produce individuals for biological control of weeds and to allow larvae found in host material to complete development so an

* The mention of trade names or commercial products is solely for the purpose of providing specific information about what was used and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

adult specimen can be procured for identification purposes. Species that can easily be reared on cut host material or on an artificial diet developed for another species have also been reared in a laboratory setting. This chapter will document and summarize the diets and rearing protocols developed for Cerambycidae and provide guidance on how these can be used as models for developing new methods for related species.

7.2 Larval Rearing and Handling Methods

Methods for rearing 140 species of cerambycid larvae, for all or part of their developmental period, have been published. These include species from six of the eight subfamilies of Cerambycidae; 67 lamiines, 41 cerambycines, 20 lepturines, 8 spondylidines, 4 prionines, and 1 parandrine. Table 7.1 provides information on the species reared, their feeding preferences, generation time, geographic distribution, and diets used. A total of 30 species have been reared on artificial diets that contain no host material; another 57 species have been reared on artificial diets that contain dried-pulverized host material; and 8 species have been reared on both types of artificial diet. An additional 33 species have been reared on cut host material, and 12 species have been reared on both cut host material and one or more artificial diets.

In nature, larvae of species reared in the laboratory inhabit stems, roots, inner bark, or wood of living, dying, or dead hosts. The majority of these species feed on multiple hosts (95%), 64 species feed exclusively on deciduous trees, 35 on coniferous trees, 16 on herbaceous hosts, and 21 species feed on plants from two of these groups. Most of the documented species that have been reared are from the Northern Hemisphere, but this could be biased by the accessible literature or resources for conducting this type of work. The species that have been reared inhabit all four host conditions presented in Hanks (1999): healthy host (HH), stressed host (SH), weakened host (WH), and dead host (DH).

7.2.1 Larval Rearing Using Artificial Diets

7.2.1.1 Diets Containing No Host Material

Rearing larvae on an artificial diet that does not contain host material can have its advantages: eliminating labor-intensive and time-consuming efforts needed to harvest and prepare host material for use, producing large numbers of even aged/sized larvae for bioassays, potentially reducing diet contamination and changes needed, rearing year round, and providing a substrate for testing host-derived compounds that may confer resistance. However, a successful artificial diet can take a long time to develop. Therefore, it is important to utilize information, such as is summarized here, from previous artificial diets created for closely related species or species inhabiting the same host (in the same host condition).

Harley and Wilson (1968) were the first to develop an artificial diet for rearing cerambycid larvae that contained no host material. Twelve additional diets have been developed, often based on previously published diets. Six of these diets (Table 7.2: 3, 4, 7, 9, 10, and 13) have been used to rear only one species, and only one (6) has been evaluated for rearing larvae from five different cerambycid subfamilies. These artificial diets have been used to rear larvae that have multiple generations per year, a single generation per year, and those that take multiple years to complete their development. The larvae successfully reared on these diets come from all host types and conditions.

The ingredients and amounts required to make 1-L batches of each of the 13 artificial diets that contain no host material are given in Table 7.2. All but one of these diets is made by first bringing an agar and water solution to a boil (diet 12 is baked), mixing in the remaining ingredients either while boiling or at different points in the cooling process, pouring or spooning the diet into containers, and then letting the diet cool and dry for varying amounts of time before use. Several of the diets can then be held at 5–10°C for several weeks until needed without affecting larval performance on the diet.

All of these diets contain the following components: a nitrogen source, lipids (except 6 and 8), carbohydrates, vitamins and minerals, protective agents, and a bulking agent. All but two of these diets include cellulose as a bulking agent. Most of the diets contain large amounts of crude ground or powdered plant products (from corn, wheat, or soybeans), which provide both bulk and other essential components. These plant-based products provide protein, lipids, carbohydrates, and vitamins and minerals, but they

TABLE 7.1

Species of Cerambycid Larvae Reared in the Laboratory with Notes on Hosts Utilized, Generation Time, Occurrence, and Artificial Diets Used*

Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Cerambycinae	*Anelaphus villosus* (Fabricius, 1792)	Deciduous	HH	1–2 years	Eastern North America	11	Keena, unpublished data
Cerambycinae	*Aromia moschata* (Linnaeus, 1758)	Deciduous	WH	N/A	Europe, North Africa, Asia	C	Georgiev et al. 2004
Cerambycinae	*Callidium frigidum* Casey, 1912	Coniferous	DH	1 year	North America	1H	Gardiner 1970
Cerambycinae	*Cerambyx cerdo* Linnaeus, 1758	Deciduous	SH	2–5 years	Europe except north, Turkey	12	Pavlovic et al. 2012, Dojnov et al. 2010
Cerambycinae	*Cerambyx welensii* (Küster, 1846)	Deciduous	WH	N/A	Europe	6	De Viedma et al. 1985
Cerambycinae	*Ceresium guttaticolle* (Fairmaire 1850)	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Cerambycinae	*Ceresium scutellaris* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Cerambycinae	*Ceresium vacillans* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Cerambycinae	*Clytus ruricola* (Oliver, 1795)	Deciduous	DH	N/A	North America	1H	Gardiner 1970
Cerambycinae	*Enaphalodes rufulus* (Haldeman, 1847)	Deciduous	WH	2 years	North America	2, 7	Galford 1985; Galford 1969
Cerambycinae	*Eurphagus lundii* (Fabricius, 1793)	Deciduous	N/A	1 year	Indonesia, Malaysia, Thailand, Puerto Rico, Borneo, Myanmar, Laos, Sumatra, Burma, India, China, Vietnam, Cambodia	10H	Higashiyama et al. 1984
Cerambycinae	*Hylotrupes bajulus* (Linnaeus, 1758)	Coniferous	DH	3–6 years, 1–32 range	Originating in Europe, world distribution	6, 8, 8H	Cannon and Robinson 1982; Notario et al. 1993; Iglesias et al. 1989
Cerambycinae	*Megacyllene caryae* (Gahan, 1908)	Coniferous	SH	1 year	North America	2	Galford 1969
Cerambycinae	*Megacyllene mellyi* (Chevrolat, 1862)	Herbaceous	HH	2–3 per year	South America, imported to Australia	11H	Boldt 1987
Cerambycinae	*Megacyllene robiniae* (Forster, 1771)	Deciduous	WH	1 year	North America	2, 2H	Galford 1969; Wollerman et al. 1969

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TABLE 7.1 ***(Continued)***

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Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Cerambycinae	*Meriellum proteus* (Kirby in Richardson, 1837)	Coniferous	DH	2 year	North America	1H	Gardiner 1970
Cerambycinae	*Neoclytus acuminatus* (Fabricius, 1775)	Deciduous	WH	1–3 per year	North America, introduced to Europe	2	Galford 1969
Cerambycinae	*Neoclytus caprea* (Say, 1824)	Deciduous	WH	1 year	North America	2	Galford 1969
Cerambycinae	*Neoclytus leucozonus* (Laporte and Gory, 1835)	Coniferous	DH	N/A	North America	1H	Gardiner 1970
Cerambycinae	*Oemona hirta* (Fabricius, 1775)	Deciduous	WH	2 years	New Zealand	21H	Wang et al. 2002
Cerambycinae	*Phoracantha recurva* Newman, 1840	Deciduous	SH	2–3 per year	Australia, introduced pest in many countries	C	Bybee et al. 2004
Cerambycinae	*Phoracantha semipunctata* (Fabricius, 1775)	Deciduous	SH	2–3 per year	Australia, introduced pest in many countries	6, C	De Viedma et al. 1985; Hanks et al. 1993; Bybee et al. 2004
Cerambycinae	*Phymatodes testaceus* (Linnaeus, 1758)	Deciduous and coniferous	DH	1–2 years	Europe, Japan, North Africa, North America	6	De Viedma et al. 1985
Cerambycinae	*Plagionotus arcuatus* (Linnaeus, 1758)	Deciduous	DH	2 years	Europe, North Africa, Caucasus, Iran	6	De Viedma et al. 1985
Cerambycinae	*Plagionotus detritus* (Linnaeus, 1758)	Deciduous	DH	1–2 years	Europe, Russia, Caucasus, North Kazakhstan, Transcaucasia, Iran, Near East	6	De Viedma et al. 1985
Cerambycinae	*Plagithmysus bilineatus* Sharp, 1896	Herbaceous	HH	1 year	Oceania (Hawaii)	9H	Stein and Haraguchi 1984
Cerambycinae	*Plagithmysus funebris* Sharp, 1896	Deciduous	HH	1 year	Oceania (Hawaii)	9H	Stein and Haraguchi 1984
Cerambycinae	*Plagithmysus varians* Sharp, 1896	Deciduous	HH	1 year	Oceania (Hawaii)	9H	Stein and Haraguchi 1984
Cerambycinae	*Purpuricenus ferrugineus* (Fairmaire, 1851)	Herbaceous	N/A	N/A	Southern Europe	6	De Viedma et al. 1985
Cerambycinae	*Sarosesthes fulminans* (Fabricius, 1775)	Deciduous	SH	N/A	North America	1H	Gardiner 1970
Cerambycinae	Scituloglaucytes muriri (Gressitt, 1940)	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014

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Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Cerambycinae	*Semanotus japonicus* Lacordaire, 1869	Coniferous	WH	1 year	East Asia, Japan, Taiwan, China, introduced into Canada	20H, C	Kitajima 1999; Shibata 1995
Cerambycinae	*Semanotus ligneus* (Fabricius, 1787)	Coniferous	WH	1 year	North America	1H	Gardiner 1970
Cerambycinae	*Semanotus litigiosus* (Casey, 1891)	Coniferous	SH	N/A	North America	1H	Gardiner 1970
Cerambycinae	*Trichoferus holosericeus* (Rossi, 1790)	Deciduous	DH	2–3 years	Mediterranean region	21H	Palanti et al. 2010
Cerambycinae	*Xylotrechus arvicola* (Olivier, 1795)	Deciduous and herbaceous	WH	2 years	Europe, Russia, Caucasus, North Kazakhstan, Transcaucasia, Turkey, Iran, Near East, North Africa	12H,6	García-Ruiz et al. 2012; De Viedma et al. 1985
Cerambycinae	*Xylotrechus colonus* (Fabricius, 1775)	Deciduous and coniferous	WH	1 year	Eastern North America, west to central Texas	1H, 3, C	Galford 1969; Haack and Petrice 2009; Gardner 1970
Cerambycinae	*Xylotrechus pyrrhoderus* Bates, 1873	Herbaceous	HH	1 year	Korea, China, Japan	C	Ashihara 1982
Cerambycinae	*Xylotrechus sagittatus* (Germar, 1821)	Coniferous	WH	1 year	North America	1H	Gardiner 1970
Cerambycinae	*Xylotrechus undulatus* (Say, 1824)	Coniferous	WH	1 year	Canada, plus Alaska and northern United States	1H	Gardiner 1970
Lamiinae	*Acalolepta australis* (Boisduval, 1835)	Deciduous and coniferous	N/A	1 year	Australia, Papua New Guinea, Moluccas, Ireland, New Britain	10H	Higashiyama et al. 1984
Lamiinae	*Acalolepta holotephra* (Boisduval, 1835)	Deciduous	N/A	1 year	Papua New Guinea, Samoa, Vanuatu, Solomon Islands	10H	Higashiyama et al. 1984
Lamiinae	*Acalolepta luxuriosa* (Bates, 1873)	Deciduous	N/A	1 year	Russia, Korea, China, Taiwan, Japan	5H	Akutsu et al. 1980
Lamiinae	*Acalolepta tincturata* (Pascoe, 1866)	Deciduous	N/A	1 year	Papua New Guinea, Indonesia	10H	Higashiyama et al. 1984
Lamiinae	*Acalolepta vastator* (Newman, 1847)	Deciduous	WH	1 year	Australia	1	Goodwin and Pettit 1994

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Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lamiinae	*Acanthocinus aedilis* (Linnaeus, 1758)	Coniferous	WH	1–2 years	Europe, Russia, Caucasus, Kazakhstan, Korea, Mongolia, northern China	6	Notario et al. 1993
Lamiinae	*Acanthocinus obsoletus* (Olivier, 1795)	Coniferous	N/A	1 year	North America, Caribbean	1H, C	Haack and Petrice 2009; Gardiner 1970
Lamiinae	*Acanthocinus pusillus* Kirby, 1837	Coniferous	N/A	1 year	North America	1H	Gardiner 1970
Lamiinae	*Aegomorphus modestus* (Gyllenhal in Schoenherr, 1817)	Deciduous	N/A	N/A	North America	11, 1H	M. A. Keena, unpublished data; Gardiner 1970
Lamiinae	*Aerenicopsis championi* Bates, 1885	Herbaceous	HH	1 year	Middle America, Oceania, introduced to Hawaii and Australia	C	Palmer et al. 2000
Lamiinae	*Agapanthia asphodeli* (Latreille, 1804)	Herbaceous	HH	2–3 per year	Europe, Algeria, Tunisia, Turkey	6	De Viedma et al. 1985; Baragaño Galan et al. 1981; Notario and Baragaño 1987
Lamiinae	*Agapanthia cardui* (Linnaeus, 1767)	Herbaceous	HH	1 year	Europe and near East	6	De Viedma et al. 1985
Lamiinae	*Agapanthia irrorata* (Fabricius, 1787)	Herbaceous	HH	1 year	Europe, North Africa	6	De Viedma et al. 1985
Lamiinae	*Agapanthia villosoviridescens* (De Geer, 1775)	Herbaceous	HH	1 year	Europe, Russia, Caucasus, Kazakhstan, Turkey, Near East	6	De Viedma et al. 1985
Lamiinae	*Anoplophora glabripennis* (Motschulsky, 1854)	Deciduous	WH	1–2 years	China and Korea	15H, 19H, 11, 10, C	Zhang and Hu 1993; Zhao et al. 1999; Keena 2005; Dubois et al. 2002; Ludwig et al. 2002; Morewood et al. 2005
Lamiinae	*Anoplophora malasiaca* (Thomson, 1865)	Deciduous and coniferous	HH	1–2 years	Japan, Korea, Taiwan, China, Hong Kong, Malaysia, Myanmar, Viet Nam	7H, 8H, 18H	Murakoshi and Aono 1981; Lee and Lo 1998; Adachi 1994

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Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lamiinae	*Apriona germari* (Hope 1831)	Deciduous	HH	1–3 years	China, Korea, Japan, India, Laos, Malaysia, Myanmar, Nepal, Pakistan, Taiwan, Thailand, Vietnam, Far east Russia	17H	Yoon et al. 1997
Lamiinae	*Batocera laena* Thomson 1858	Deciduous	N/A	N/A	Australia, Papua New Guinea, Moluccas, New Britain	10H	Higashiyama et al. 1984
Lamiinae	*Dectes texanus* LeConte, 1852	Herbaceous	HH	1 year	North America	4, C	Hatchett et al. 1973; Niide et al. 2006
Lamiinae	*Dorcadion pseudopreissi* Breuning, 1962	Herbaceous	HH	2 years	Turkey	C	Kumral et al. 2012
Lamiinae	*Eupogonius pauper* LeConte, 1852	Deciduous and herbaceous	DH	2 years	North America	C	Purrington and Horn 1993
Lamiinae	*Glenea cantor* (Fabricius, 1787)	Deciduous	HH	5 per year	Southern Asia	C	Lu et al. 2011
Lamiinae	Gracisybra flava (Dillion & Dillon)	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Graphisurus fasciatus* (DeGeer, 1775)	Deciduous and coniferous	DH	N/A	North America	1H, C	Gardiner 1970; Haack and Petrice 2009
Lamiinae	*Hestimidus humeralis* Breuning 1939	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Hyperplatys aspersa* (Say, 1824)	Deciduous and herbaceous	DH	1.5 per year	North America	1H, C	Gardiner 1970; Purrington and Horn 1993
Lamiinae	*Leiopus nebulosus* (Linnaeus, 1758)	Deciduous	N/A	1–2 years	Europe, Russia	6	De Viedma et al. 1985
Lamiinae	*Microgoes oculatus* (LeConte, 1862)	Deciduous	DH	2–4 years	North America	1H	Gardiner 1970
Lamiinae	*Moechotypa diphysis* (Pascoe, 1871)	Deciduous and herbaceous	DH	1 year	Japan	13H	Kosaka 2011

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TABLE 7.1 ***(Continued)***

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Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lamiinae	*Monochamus alternatus* Hope, 1842	Coniferous	SH	1–2 years	China, Japan and Korea	10H, 13H, C	Higashiyama et al. 1984; Kosaka and Ogura 1990; Togashi 1989
Lamiinae	*Monochamus carolinensis* (Oliver, 1795)	Coniferous	SH	1–2 years	North America	1, 16H, C	Alya and Hain 1987; Necibi and Linit 1997; Linit 1985
Lamiinae	*Monochamus clamator latus* Casey, 1924	Coniferous	SH	1–2 years	North America	1H	Gardiner 1970
Lamiinae	*Monochamus galloprovincialis* (Oliver, 1795)	Coniferous	SH	1–2 years	Europe	3H, C, 13	Carle 1969; Koutroumpa et al. 2008; Akbulut et al. 2007; Petersen-Silva et al. 2014
Lamiinae	*Monochamus notatus* (Drury, 1773)	Coniferous	SH	2 years	North America	1H, C	Gardiner 1970; Haack and Petrice 2009
Lamiinae	*Monochamus scutellatus* (Say, 1824)	Coniferous	SH	1–2 years	North America	1H, C	Gardiner 1970; Breton et al. 2013
Lamiinae	*Monochamus titillator* (Fabricius, 1775)	Coniferous	SH	up to 3 gen a year	North America	1	Alya and Hain 1987
Lamiinae	*Morimus funereus* (Mulsant, 1862)	Deciduous and coniferous	DH	3–4 years	Europe (IUCN Threatened Species)	12, 9	Dojnov et al. 2012; Ivanovic et al. 2002
Lamiinae	*Musaria affinis affinis* (Harrer, 1784)	Herbaceous	HH	1 year	Europe except north, Russia, Caucasus, Transcaucasia	6	De Viedma et al. 1985
Lamiinae	*Neosciadella brunnipes* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Neosciadella inflexa* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Neosciadella multivittata* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Neosciadella spixi* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014

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Species of Cerambycid Larvae Reared in the Laboratory with Notes on Hosts Utilized, Generation Time, Occurrence, and Artificial Diets Used*

Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lamiinae	*Oberea oculata* (Linnaeus, 1758)	Deciduous	WH	1–3 years	West Palearctic	C	Georgiev et al. 2004
Lamiinae	*Oberea quadricallosa* LeConte, 1874	Deciduous	HH	2–3 years	North America	1H	Gardiner 1970
Lamiinae	*Oberea tripunctata* (Swederus, 1787)	Deciduous and herbaceous	HH	1–3 years	North America	1H	Gardiner 1970
Lamiinae	*Oncideres cingulata* (Say, 1826)	Deciduous	HH	1 year	North America	C	Rice 1995
Lamiinae	*Oopsis velata* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Oopsis zitja* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Oplosia nubila* (LeConte, 1862)	Deciduous	DH	N/A	North America	1H	Gardiner 1970
Lamiinae	*Palame anceps* (Bates, 1864)	Deciduous	WH	N/A	French Guyana, Brazil, Equator	C	Berkov and Tavakilian 1999
Lamiinae	*Palame crassimana* Bates, 1864	Deciduous	WH	N/A	Venezuela, French Guyana, Brazil, Peru, Guyana, Equator, Surinam	C	Berkov and Tavakilian 1999
Lamiinae	*Palame mimetica* Monné, 1985	Deciduous	WH	N/A	Venezuela, French Guyana, Brazil, Surinam	C	Berkov and Tavakilian 1999
Lamiinae	*Paraglenea fortunei* (Saunders, 1853)	Deciduous	HH	N/A	Indochina, continental China, and Taiwan, invasive in Japan	20H	Kitajima and Makihara 2011
Lamiinae	*Phytoecia icterica* (Schaller, 1783)	Herbaceous	HH	1 year	Europe, Turkey, Caucasus, Russia	6	De Viedma et al. 1985
Lamiinae	*Phytoecia rufiventris* Gautier des Cottes, 1870	Herbaceous	HH	1 year	Russia, Mongolia, Korea, China, Taiwan, Japan, Vietnam	22H	Shintani 2011
Lamiinae	*Plagiohammus spinipennis* (Thomson, 1860)	Herbaceous	HH	N/A	Central America, Oceania, South America, introduced into Hawaii	1, C	Harley and Willson 1968; Hadlington and Johnson 1973
Lamiinae	*Plectrodera scalator* (Fabricius, 1792)	Deciduous	WH	2 years	North America	11	M. A. Keena, unpublished data
Lamiinae	*Pogonocherus mixtus* Halderman, 1847	Coniferous and deciduous	DH	1 year	North America	1H	Gardiner 1970

(Continued)

TABLE 7.1 ***(Continued)***

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Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lamiinae	*Pogonocherus penicillatus* LeConte in Agassiz, 1850	Coniferous	DH	1 year	North America	1H	Gardiner 1970
Lamiinae	*Psacothea hilaris* Pascoe, 1857	Deciduous	WH	1–2 years	Asia, invasive in Europe	14H	Fukaya and Honda 1992
Lamiinae	*Psenocerus supernotatus* (Say, 1823)	Herbaceous and deciduous	DH	N/A	North America	C	Purrington and Horn 1993
Lamiinae	*Saperda calcarata* Say, 1824	Deciduous	WH	2–5 years	North America	1H	Gardiner 1970
Lamiinae	*Saperda discoidea* Fabricius, 1798	Deciduous	WH	1 year	North America	1H	Gardiner 1970
Lamiinae	*Saperda populnea* (Linnaeus, 1758)	Deciduous	WH	2 years	Europe	C	Georgiev et al. 2004
Lamiinae	*Saperda similis* Laicharting, 1784	Deciduous	WH	2–3 years	Europe, Near East	C	Georgiev et al. 2004
Lamiinae	*Saperda vestita* Say, 1824	Deciduous	WH	2–3 years	North America	1H	Gardiner 1970
Lamiinae	*Sormida cinerea* Dillon & Dillon 1952	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Sormida maculicollis* (Thomson 1865)	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Sybra alternans* (Wiedemann, 1825)	Deciduous	N/A	N/A	Southeast Asia, Introduced to United States (Hawaii, California and Florida)	9H	Stein and Haraguchi 1984
Lamiinae	*Sybromimus obliquatus* Breuning 1940	Deciduous	WH	1 year	Oceania (Fiji)	C	Waqa-Sakiti et al. 2014
Lamiinae	*Tetraopes tetrophthalmus* (Forster, 1771)	Herbaceous	HH	1 year	North America	1H	Gardiner 1970
Lamiinae	*Urgleptes querci* (Fitch, 1858)	Deciduous and coniferous	DH	N/A	North America	C	Purrington and Horn 1993
Lepturinae	*Acmaeops proteus* (Kirby in Richardson, 1837)	Coniferous	DH	2–3 years	North America	1H	Gardiner 1970
Lepturinae	*Anastrangalia sanguinolenta* (Linnaeus, 1761)	Coniferous	WH/DH	2–3 years	Europe, Turkey, Caucasus, Transcaucasia	6	De Viedma et al. 1985; Notario et al. 1993
Lepturinae	*Anastrangalia sanguinea* (LeConte, 1859)	Coniferous	DH	1+ years	North America	1H	Gardiner 1970

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TABLE 7.1 ***(Continued)***

Species of Cerambycid Larvae Reared in the Laboratory with Notes on Hosts Utilized, Generation Time, Occurrence, and Artificial Diets Used*

Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lepturinae	*Anthophylax attenuatus* (Haldeman, 1847)	Deciduous	DH	N/A	North America	1H	Gardiner 1970
Lepturinae	*Anthophylax cyaneus* (Haldeman, 1847)	Deciduous	N/A	N/A	North America	1H	Gardiner 1970
Lepturinae	*Centrodera decolorata* (Harris, 1838)	Deciduous	DH	N/A	North America	1H	Gardiner 1970
Lepturinae	*Evodinus monticola* (Randall, 1838)	Coniferous	N/A	N/A	North America	1H	Gardiner 1970
Lepturinae	*Grammoptera subargentata* (Kirby in Richardson, 1837)	Deciduous	N/A	N/A	North America	1H	Gardiner 1970
Lepturinae	*Judolia montivagans montivagans* (Couper, 1864)	Coniferous and deciduous	DH	2 years	North America	1H	Gardiner 1970
Lepturinae	*Paracorymbia stragulata* (Germar, 1824)	Coniferous	DH	2 years	Europe	12H, 6, 8	Iglesias et al. 1989; De Viedma et al. 1985; Notario et al. 1993
Lepturinae	*Pidonia ruficollis* (Say, 1824)	Deciduous	N/A	N/A	North America	1H	Gardiner 1970
Lepturinae	*Rhagium bifasciatum* (Linnaeus, 1758)	Coniferous and deciduous	DH	2–3 years	Europe except NE, Caucasus, Transcaucasia, Turkey	6	De Viedma et al. 1985; Notario et al. 1993
Lepturinae	*Rhagium inquisitor* (Linnaeus, 1758)	Coniferous and deciduous	DH	2 years	Europe and Northern Asia (excluding China), North America	12H, 6, 8	Iglesias et al. 1989; De Viedma et al. 1985; Notario et al. 1993
Lepturinae	*Stictoleptura scutellata* (Fabricius, 1781)	Deciduous	DH	2–3 years	Europe except north, North Africa, Turkey, Caucasus, Transcaucasia, North Iran	6	De Viedma et al. 1985
Lepturinae	*Stictoleptura canadensis* (Oliver, 1795)	Coniferous	DH	N/A	North America	1H	Gardiner 1970
Lepturinae	*Stictoleptura fontenayi* (Mulsant, 1839)	Coniferous and deciduous	DH	2–3 years	Africa, Europe	6	De Viedma et al. 1985
Lepturinae	*Stictoleptura rubra* (Linnaeus, 1758)	Coniferous	DH	2–3 years	Europe, Russia, North Africa	6	De Viedma et al. 1985; Notario et al. 1993

(Continued)

TABLE 7.1 ***(Continued)***

Species of Cerambycid Larvae Reared in the Laboratory with Notes on Hosts Utilized, Generation Time, Occurrence, and Artificial Diets Used*

Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Lepturinae	*Trachysida mutabilis* (Newman, 1841)	Coniferous and deciduous	DH	N/A	North America	1H	Gardiner 1970
Lepturinae	*Trigonarthris minnesotana* (Casey, 1913)	Ceciduous	DH	N/A	North America	1H	Gardiner 1970
Lepturinae	*Typocerus velutinus* (Olivier, 1795)	Deciduous	DH	1+ years	North America	1H	Gardiner 1970
Parandrinae	*Neandra marginicollis marginicollis* Schaeffer, 1929	Deciduous and coniferous	DH	2–3 years	North America, introduced into Europe	1H	Gardiner 1970
Prioninae	*Ergates faber* (Linnaeus, 1761)	Coniferous	DH	3+ years	Central and South Europe, North Africa	12H, 6, 8	Iglesias et al. 1989; De Viedma et al. 1985; Notario and Baragaño 1978; Notario et al. 1993
Prioninae	*Orthosoma brunneum* (Forster, 1771)	Deciduous and coniferous	DH	2–3 years	North America	1H	Gardiner 1970
Prioninae	*Prionoplus reticularis* White, 1843	Coniferous and deciduous	DH	2+ years	New Zealand	21H	Rogers et al. 2002
Prioninae	*Prionus imbricornis* (Linnaeus, 1767)	Deciduous	WH	3–5 years	North America	4H	Payne et al. 1975
Spondylidinae	*Arhopalus ferus* (Mulsant, 1839)	Coniferous	DH	2–4 years	Europe, North Africa, Northern Asia, New Zealand	6	Notario et al. 1993
Spondylidinae	*Arhopalus foveicollis* Halderman, 1847	Coniferous	DH	N/A	Eastern North America	1H	Gardiner 1970
Spondylidinae	*Arhopalus rusticus* Linnaeus, 1758	Coniferous	WH	2 years	North America, Northern and Central Europe, Siberia, Korea, Mongolia, Japan and the Northern China	12H,21H, 6, 8	Iglesias et al. 1989; Rogers et al. 2002; De Viedma et al. 1985

(Continued)

TABLE 7.1 ***(Continued)***

Species of Cerambycid Larvae Reared in the Laboratory with Notes on Hosts Utilized, Generation Time, Occurrence, and Artificial Diets Used*

Subfamily	Species	Host Type	Host Condition Code	Generation	Occurrence	Diet	Diet/Rearing References
Spondylidinae	*Arhopalus syriacus* Reitter, 1895	Coniferous	DH	2–3 years	Mediterranean region, Canary Islands	3H, 12H, 6, 8	Carle 1969; Iglesias et al. 1989; De Viedma et al. 1985
Spondylidinae	*Asemum striatum* Linnaeus, 1758	Coniferous	WH	1–3 years	Europe, Siberia, Korea, Mongolia, Japan and Sakhalin Island	1H	Gardiner 1970
Spondylidinae	*Atimia confusa* (Say, 1826)	Coniferous	DH	1–2 years	North America	1H, 12H	Gardiner 1970, Iglesias et al. 1989
Spondylidinae	*Spondylis buprestoides* Linnaeus, 1758	Coniferous	DH	2–3 years	Europe	6	De Viedma et al. 1985; Notario et al. 1993
Spondylidinae	*Tetropium cinnamopterum* Kirby, 1837	Coniferous	SH	N/A	North America	1H	Gardiner 1970

* Specie names were verified using the Integrated Taxonomic Information System (http://www.itis.gov) or Cerambycidae of the World (http://cerambycidae.org/search). Other information on the species was obtained from Craighead (1923), Jeniš (2001), Linsley (1961, 1962a, 1962b, 1963, 1964), Linsley and Chemsak (1972, 1976, 1984, 1995, 1997), http://wiki.bugwood.org, http://www.cerambyx.uochb.cz/, http://www.cerambycoidea.com, or links to online references given in the previously mentioned Web sites. Host condition codes were assigned based on the system presented in Hanks (1999) where HH = healthy host, SH = stressed host, WH = weakened host, and DH = dead host. Diet numbers are assigned based on the diets in Tables 7.2 (numbers only) and 7.3 (numbers followed by an H), and C = reared in host material. N/A = information not available.

TABLE 7.2

Diet Ingredients (in grams, unless specified) Required to Make One Liter of Each of 12 Artificial Diets for Cerambycid Larvae that Contain No Host Material

Ingredient	Harley and Willson 1968	Galford 1969	Galford 1969	Hatchett et al. 1973	Payne et al. 1975	Notario and Baragaño 1978	Galford 1985	Iglesias et al. 1989	Ivanovic et al. 2002	Dubois et al. 2002	Keena 2005	Dojnov et al. 2012	Petersen-Silva et al. 2014
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Agar	24.3	24.6	25.9	26.8	23.5	40.9	21.1	37.0	8.6	35.4	21.8	62.0	30.0
Lecithin	1.2	0.6	–	–	–	–	–	–	–	–	–	–	–
Alphacel (cellulose)	51.3	246.0	226.7	22.3	144.1	20.5	198.0	52.9		176.4	121.5		160.0
corn meal	–	–	–	–	–	–	–	–	107.5	–	–	–	–
Palenta	–	–	–	–	–	–	–	–	–	–	–	620.3	–
Wheat germ	–	15.4	97.2	52.1	28.2	–	79.2	–	–	–	52.9	–	–
Soybean powder	–	–	–	–	–	–	–	–	–	60.0	–	–	50.0
Soybean protein	–	12.3	–	–	–	–	–	–	–	–	–	–	–
Sucrose	11.7	12.3	–	40.2	32.8	25.6	6.6	66.1	21.5	25.3	15.6	155.1	20.0
Dextrose	11.7	–	–	–	–	–	–	–	–	–	–	–	–
Fructose	–	6.2	–	–	–	–	–	–	–	–	–	–	–
Glucose	–	6.2	–	–	–	15.3	–	39.7	–	–	–	–	–
Potato starch	11.7		–	–	–	–	–	–	–	25.3	–	–	20.0
Cholesterol	0.5	0.6	–	–	–	–	0.1	–	0.1	1.5	0.9	–	–
Linoleic acid	0.5	–	–	–	–	–	–	–	–	–	–	–	–
Soybean oil (mL)	–	–	–	–	–	–	–	–	–	5.9	–	–	4.0
Wheat germ oil (mL)	–	3.1	–	7.7	–	–	2.6	–	–		4.6	–	–
10% potassium hydroxide (mL)	9.7	–	–	2.7	–	–	–	–	–	–	–	–	–
Aureomycin	–	–	–	0.3	–	–	–	–	–	–	–	–	–
Benzoic acid	–	–	–	–	–	1.0	–	2.6	–	–	–	–	–
Chloramphenicol	–	–	–	–	–	–	0.4		–	–	–	–	–
Ethyl alcohol 95%	12.1	–	–	9.1	–	–	4.0	13.2	–	–	–	6.2	3.0
Formaldehyde 10% (mL)	–	–	–	–	7.5	–	–	–	–	–	–	–	–

(Continued)

TABLE 7.2 ***(Continued)***

Diet Ingredients (in grams, unless specified) Required to Make One Liter of Each of 12 Artificial Diets for Cerambycid Larvae that Contain No Host Material

Ingredient	Harley and Willson 1968	Galford 1969	Galford 1969	Hatchett et al. 1973	Payne et al. 1975	Notario and Baragaño 1978	Galford 1985	Iglesias et al. 1989	Ivanovic et al. 2002	Dubois et al. 2002	Keena 2005	Dojnov et al. 2012	Petersen-Silva et al. 2014
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Formaldehyde 40% (mL)	–	–	–	0.9	–	–	–	–	–	–	–	–	–
Methyl paraben	1.1	0.8	0.8	1.6	1.9	–	1.3	2.6	2.2	2.5	1.6	1.2	–
Potassium sorbate	–	–	–	2.4	–	–	–	–	–	–	–	–	–
Propionic acid (mL)	–	–	–	–	–	–	–	–	–	1.7	–	–	1.0
Sodium propionate	–	–	–	–	–	–	–	–	–	–	1.2	–	–
Sorbic acid	1.5	1.5	1.6	–	1.9	0.5	1.3	–	–	2.5	1.6	–	–
Brewer's yeast	–	30.8	–	–	–	30.7	–	79.4	–	45.6	–	155.1	40.0
Torula yeast	–	–	–	–	–	–	13.2	–	–	–	28.1	–	–
Casein	23.3	–	–	37.5	32.8	12.3	6.6	31.7	–	15.2	9.4	–	–
L-Methionine	–	–	–	–	–	–	–	2.6	–	–	–	–	–
Ascorbic acid	–	–	–	–	3.8	4.1	–	10.6	–	2.5	–	–	–
Choline chloride	–	–	–	–	0.9	–	–	–	–		–	–	–
Citric acid	–	–	–	–	–	–	–	–	–	2.0	–	–	1.0
USDA Vitamin pre mix 26862	–	–	–	–	–	–	–	–	–	–	4.4	–	–
Vanderzant's vitamin mix (mL)	19.4	–	–	–	9.4	20.5	–	52.9	–	–	–	–	–
Vitamin B	–	0.1	–	–	–	–	–	–	–	–	–	–	–
Vitamin diet fortification mix	–	9.2	–	10.7	–	–	3.3	–	–	–	–	–	–
Wesson's salt mixture	3.5	15.4	–	10.7	9.4	10.2	2.0	26.5	–	4.6	2.8	–	–
Water (mL)	816.7	615.1	647.8	774.7	703.9	818.9	660.2	582.0	860.1	653.6	685.3	–	671.0

Note: Numbers 1–13 indicate diet number. Dashes indicate the ingredient was not part of that diet.

need to be processed or heated to denature enzymes and reduce unpalatable components (Cohen 2003). These ingredients can also be of variable quality among vendors or from the same vendor, so monitoring quality of the product and tracking quality control measures for insect colonies can help identify new problems before they have a negative effect. For example, *Anoplophora glabripennis* larvae will develop a gut obstruction if the cellulose fibers are too short (M. A. Keena, unpublished data), so purchasing from a company that has tested their product for use with insects is best.

All but five of the diets contain casein, which provides protein containing all but three of the essential amino acids in high quantities and well-balanced proportions (Reinecke 1985; Cohen 2003). Sucrose or various monosaccharaides are added to all but one of the diets because they are important as building materials and fuels, and they may also be phagostimulants. Yeast is added to eight of the diets and provides a good source of protein as well as vitamins and minerals. Only four diets (3, 9, 12, 13) do not include both Wesson salt mixture (a mineral mixture) and vitamins. There are some indications that more work on the dietary requirements of cerambycids with regard to particular vitamins and minerals is needed. For example, unlike *Lymantria dispar* (a caterpillar that feeds on leaves), *A. glabripennis* (wood feeder) larvae do not require higher levels of available iron in the diet. In fact, not adding any iron in the salt mix improves development of these larvae (Keena 2005). Additionally, ensuring that the vitamin A in the diet is either released slowly (incorporated in starch beadlets) or is maintained in the diet over time improves *A. glabripennis* larval development and reduces the number of diet changes needed (M. A. Keena, unpublished data).

Protective agents are added to all the diets to prevent microbial contamination, oxidation, or other means of destruction of essential nutrients. The function of these agents is antibacterial (e.g., Aureomycin or Chloramphenicol), antifungal (e.g., sorbic acid, methyl paraben, propionic acid, and formaldehyde) or antioxidant (e.g., ascorbic acid). Although cerambycids seem to be fairly tolerant of many of these compounds, it is always a fine balance between incorporating enough protective ingredients to preserve the diet but not so much that the insect is adversely affected. Some of these ingredients are also considered dangerous for human health (e.g., formaldehyde) and safer alternatives may need to be investigated. If problems with microbial contamination arise and modifications to the diet are needed, Cohen (2003) provided information on the protective agents. For more information on how to deal with microbial contaminants and how to identify which ones are present, see the control of pathogens and microbial contaminants in insect rearing section in *Advances and Challenges in Insect Rearing* (King and Leppla 1984).

7.2.1.2 Diets Containing Pulverized Host Material

The earliest artificial cerambycid diets containing host material were two developed in 1969 (Carle 1969; Wollerman et al. 1969) and a diet originally developed in 1965 for rearing spruce budworm that was modified a year later (Gardiner 1970). Twenty additional diets have been developed, often based on previously published diets (Table 7.3). Five of the listed diets use commercially available formulations, with minor modifications, originally developed for *Bombyx mori* (10H, 13H, and 14H), phytophagus insects (22H), and *Vanessa cardui* (16H, Table 7.3). Nine of these diets (2H, 4H, 5H, 7H, 14H, 16H, 17H, 19H, and 21H) have been used to rear only one species, and only one (1H) has been evaluated for rearing larvae from six different cerambycid subfamilies. These artificial diets have been used to rear larvae that have multiple generations per year, a single generation per year, and those that take multiple years to complete their development. The larvae successfully reared on these diets come from all host types and conditions.

The ingredients and amounts required to make one liter batches of each of the 23 artificial diets containing host material are given in Table 7.3. The host material to be incorporated into the diet is first dried and then pulverized before being used. The dried pulverized host material added to these diets averages 14% by weight (range 1–40%). All of these diets (except 13H, 14H, 22H, and 23H) are made by first bringing an agar and water solution to a boil, mixing in the remaining ingredients either while boiling or at different points in the cooling process, pouring or spooning the diet into containers, and then letting the diet cool and dry for varying amounts of time before use.

About half of these diets have components added that specifically provide a nitrogen source (casein or peptone), carbohydrates (sucrose or potato starch), vitamins, and minerals (via mixtures), and four have lipids added (cholesterol or oils). Thirteen of these diets have cellulose added as a bulking agent,

TABLE 7.3

Diet Ingredients (in grams, unless specified) Required to Make One Liter of Each of the 23 Artificial Diets for Cerambycid Larvae that Contain Dried, Pulverized Host Material

Ingredient	Gardiner 1970	Wollerman et al. 1969	Carle 1969	Payne et al. 1975	Akutsu et al. 1980	Baragaño Galán et al. 1981	Murakoshi and Aono 1981	Cannon and Robinson 1982	Stein and Haraguchi 1984	Higashiyama et al. 1984	Boldt 1987	Iglesias et al. 1989
	1H	2H	3H	4H	5H	6H	7H	8H	9H	10H	11H	12H
Water (mL)	797.4	666.1	779.2	684.0	745.7	687.5	710.7	516.4	851.4	727.3	816.7	572.1
Agar	24.3	22.2	37.1	22.8	33.6	27.5	17.8	34.4	49.9	31.1	24.3	28.6
Lecithin	–	–	–	–	–	–	–	–	–	–	1.2	–
Alphacel (cellulose)	4.9	88.8	33.4	91.2	67.1	–	40.9	129.1	0.7	–	–	–
Powdered host material	48.9	106.6	111.3	77.1	134.2	60.5	63.5	129.1	10.1	103.9	51.3	207.7
Soybean powder	–	–	–	–	–	–	102.8	44.8	–	–	–	–
Corn meal	–	–	–	–	–	60.5	–	–	–	–	–	–
Wheat germ	29.3	26.6	–	27.4	–	121.0	–	39.6	4.4	–	–	116.3
Sucrose	34.2	31.1	–	31.9	–	–	–	–	5.1	20.8	11.7	0.0
Dextrose	–	–	–	–	–	–	–	–	–	–	11.7	–
Glucose	–	–	–	–	–	–	–	–	–	–	–	–
Potato starch	–	–	–	–	–	–	–	–	–	10.4	11.7	–
Casein	34.2	31.1	–	31.9	–	–	–	–	5.1	–	23.3	–
Ascorbic acid	3.9	3.6	–	3.6	–	1.7	–	44.8	–	–	–	1.7
Choline chloride	1.0	0.9	–	0.9	–	–	–	5.2	0.1	–	–	–
Citric acid	–	–	–	–	–	–	–	3.4	–	–	–	–
Vitamin mix (see reference for mix)	9.8	8.9	14.8	9.1	–	–	7.4	–	51.1	–	19.4	–
Wesson's salt mixture	9.8	8.9	–	9.1	–	–	8.9	–	1.5	–	3.5	–
Bacto-peptone	–	–	–	–	–	–	–	10.3	–	–	–	–
Peptone	–	–	–	–	–	–	–		2.0	–	–	–
Cholesterol	–	–	–	–	–	–	–	13.8	–	–	0.5	–
Linoleic acid	–	–	–	–	–	–	–	–	–	–	0.5	–
Soybean oil (mL)	–	–	–	–	–	–	–	–	–	–	–	–

(Continued)

TABLE 7.3 ***(Continued)***

Diet Ingredients (in grams, unless specified) Required to Make One Liter of Each of the 23 Artificial Diets for Cerambycid Larvae that Contain Dried, Pulverized Host Material

Ingredient	Gardiner 1970	Wollerman et al. 1969	Carle 1969	Payne et al. 1975	Akutsu et al. 1980	Baragaño Galán et al. 1981	Murakoshi and Aono 1981	Cannon and Robinson 1982	Stein and Haraguchi 1984	Higashiyama et al. 1984	Boldt 1987	Iglesias et al. 1989
	1H	2H	3H	4H	5H	6H	7H	8H	9H	10H	11H	12H
10% potassium hydroxide (mL)	–	–	–	–	–	–	–	–	–	–	9.7	–
Acetic acid (25%)		–	–	–	–	–	–	–	1.5	–	–	–
Aureomycin	0.3	–	–	–	–	–	–	–	0.1	–	–	–
Chloramphenicol	–	–	–	–	–	–	–	–	–	0.01	–	–
Pyruvic acid	–	–	–	–	–	–	–	–	–	0.5	–	–
Benzoic acid	–	–	0.7	–	–	2.8	–	–	–	–	–	2.9
Butyl paraben	–	1.8	–	–	–	–	–	–	–	–	–	–
Ethyl alcohol 95% (mL	–	–	–	–	–	5.5	–	–	11.2	–	12.1	35.8
Formaldehyde 10% (mL)	–	–	–	7.3	–	–	–	–	0.6	–	–	–
Formaldehyde 37% (mL)	0.5	–	–	–	–	–	–	13.8	–	–	–	–
Methyl paraben	1.5	1.8	1.1	1.8	2.2	2.8		1.7	1.2	2.1	1.1	3.6
Potassium hydroxide	–	–	–	–	–	–	–	–	2.2	–	–	–
Potassium sorbate	–	–	–	–	–	–	–	–	–	–	–	–
Propionic acid (mL)	–	–	–	–	–	–	–	3.4	–	–	–	–
Propylene glycol (mL)	–	–	–	–	–	–	–	–	–	–	–	–
Sorbic acid	–	1.8	–	1.8	2.2	–	48.2	–	1.6	–	1.5	–
Bacto yeast extract	–	–	–	–	–	–	–	10.3	–	–	–	–
Dry yeast (see reference for type)	–	–	22.3	–	15.0	30.2	–	–	–	–	–	31.5
Dry mix Insecta F-II, Nihon Nosan Co.	–	–	–	–	–	–	–	–	–	–	–	–
Dry commercial diet for *Bombyx mori*	–	–	–	–	–	–	–	–	–	103.9	–	–
Dry commercial diet for *Vanessa cardui*	–	–	–	–	–	–	–	–	–	–	–	–

(Continued)

TABLE 7.3 ***(Continued)***

Diet Ingredients (in grams, unless specified) Required to Make One Liter of Each of the 23 Artificial Diets for Cerambycid Larvae that Contain Dried, Pulverized Host Material

Ingredient	Kosaka and Ogura 1990	Fukaya and Honda 1992	Zhang and Hu 1993	Necibi and Linit 1997	Yoon et al. 1997	Lee and Lo 1998	Zhao et al. 1999	Kitajima 1999	Rogers et al. 2002	Palanti et al. 2010	Shintani 2011
	13H	**14H**	**15H**	**16H**	**17H**	**18H**	**19H**	**20H**	**21H**	**22H**	**23H**
Water (mL)	375.0	–	604.7	374.6	842.1	541.5	499.9	480.8	697.9	518.2	375.0
Agar	–	–	10.1	–	–	36.1	35.0	25.7	32.5	–	–
Lecithin	–	–	–	–	–	–	–	–	–	–	–
Alphacel (cellulose)	–	–	100.8	–	–	–	70.0	109.0	73.0	45.5	–
Powdered host material	250.0	–	141.1	249.8	140.4	415.2	200.0	96.2	48.7	172.7	156.3
Soybean powder	–	–	40.3	–	–	–	60.0	–	–	–	–
Corn meal	–	–	–	–	–	–	–	–	–	–	–
Wheat germ	–	–	–	–	–	–	–	–	24.3	–	–
Sucrose	–	–	25.2	–	–	–	25.1	88.1	20.3	–	–
Dextrose	–	–	–	–	–	–	–	–	–	–	–
Glucose	–	–	–	–	–	–	–	–	12.2	–	–
Potato starch	–	–	–	–	–	–	25.1	32.8		145.5	–
Casein	–	–	45.4	–	–	–	15.0	98.4	24.3	–	–
Ascorbic acid	–	–	–	–	–	–	3.0	4.4	3.5	–	–
Choline chloride	–	–	1.0	–	–	–	–	–	–	–	–
Citric acid	–	–	4.0	–	–	–	2.0	4.3	–	–	–
Vitamin mix (see reference for mix)	–	–	10.1	–	–	–	–	–	16.2	–	–
Wesson's salt mixture	–	–	10.1	–	–	–	4.5	6.5	8.1	–	–
Bacto-peptone	–	–	–	–	–	–	–	–	–	–	–
Peptone	–	–	–	–	–	–	–	–	–	–	–
Cholesterol	–	–	–	–	–	–	1.5	1.0		–	–

(Continued)

TABLE 7.3 ***(Continued)***

Diet Ingredients (in grams, unless specified) Required to Make One Liter of Each of the 23 Artificial Diets for Cerambycid Larvae that Contain Dried, Pulverized Host Material

Ingredient	Kosaka and Ogura 1990	Fukaya and Honda 1992	Zhang and Hu 1993	Necibi and Linit 1997	Yoon et al. 1997	Lee and Lo 1998	Zhao et al. 1999	Kitajima 1999	Rogers et al. 2002	Palanti et al. 2010	Shintani 2011
	13H	14H	15H	16H	17H	18H	19H	20H	21H	22H	23H
Linoleic acid	–	–	–	–	–	–	–	–	–	–	–
Soybean oil (mL)	–	–	–	–	–	–	7.0	6.7	–	–	–
10% potassium hydroxide (mL)	–	–	2.0	–	–	–	–	–	–	–	–
Acetic acid (25%)	–	–	–	–	–	–	–	–	–	–	–
Aureomycin	–	–	–	–	–	–	–	–	–	–	–
Chloramphenicol	–	–	–	–	–	–	–	–	–	–	–
Pyruvic acid	–	–	–	–	–	–	–	–	–	–	–
Benzoic acid	–	–	1.2	–	–	–	–	–	–	–	–
Butyl paraben	–	–	–	–	–	–	–	–	–	–	–
Ethyl alcohol 95% (mL)	–	–	–	–	–	–	–	–	12.1	–	–
Formaldehyde 10% (mL)	–	–	–	–	–	–	–	–	–	–	–
Formaldehyde 37% (mL)	–	–	–	–	7.0	–	–	–	–	–	–
Methyl paraben	–	–	2.0	–	2.1	3.6	2.5	1.0	1.1	–	–
Potassium hydroxide	–	–	–	–	–	–	–	–	–	–	–
Potassium sorbate	–	–	–	1.0	–	–	–	–	–	–	–
Propionic acid (mL)	–	–	–	–	–	–	2.0	–	–	–	–
Propylene glycol (mL)	–	–	–	–	7.0	–	–	–	–	–	–
Sorbic acid	–	–	2.0	–	1.4	3.6	2.5	1.0	1.4	–	–
Bacto yeast extract	–	–	–	–	–	–	–	–	–	–	–
Dry yeast (see reference for type)	40.0	–	–	40.0	–	–	45.0	44.2	24.3	118.2	–
Dry mix Insecta F-II, Nihon Nosan Co	–	–	–	–	–	–	–	–	–	–	468.8
Dry commercial diet for *Bombyx mori*	335.0	1000.0	–	–	–	–	–	–	–	–	–
Dry commercial diet for *Vanessa cardui*	–	–	–	334.7	–	–	–	–	–	–	–

Numbers 1–13 indicate diet number. Dashes indicate the ingredient was not part of that diet.

and almost all have protective agents added—with methyl paraben being the most common. Twelve of the diets contain some crude ground or powdered plant products (from wheat, corn, or soybeans), which provide additional bulk and other essential components; 11 have yeast added, which provides protein, vitamins, and minerals. Thus, most do not rely solely on the host material for vital nutrients. Instead, the host material may provide some unidentified critical elements or more likely phagostimulants needed to get the larvae to establish and develop normally.

7.2.1.3 Larval Handling Methods and Holding Conditions

The artificial diets are poured into, scooped and pressed into, or cut into chunks and then placed into a variety of containers for rearing the larvae. Container size is often changed as larvae grow. Some larvae can be reared in plastic petri dishes, cups, or tubes for their whole developmental period, while others must be transferred to glass containers when they are larger so that they will not chew their way out. Most cerambycid larvae must be grown individually or they will mutilate or cannibalize each other. There are only two published cases where larvae are reared in groups for all (Cannon and Robinson 1982) or part (Rogers et al. 2002) of their development but, in each case, some mortality due to the grouping occurred. Each time the larvae are placed in the diet (at initial setup or after a diet change), they are either inserted into a hole made in the diet that is just large enough to accommodate them (Figures 7.1 and 7.2) or dropped into a tight space between a diet cube and the side of the container. There is only one case in which surface-sterilized eggs are used to initially infest the diet (Kosaka and Ogura 1990).

The artificial diet generally has to be changed one or more times while larvae are growing. About half the diets used for cerambycids have to be changed monthly, but the range of intervals between changes goes from three days to eight months. If larvae become contaminated during rearing or before establishment in the diet, they can be surface sterilized. Two methods for larval surface sterilization have been published: a quick dip (10s) in a 5% bleach solution (Dubois et al. 2002, M. A. Keena, unpublished data) or dipping in 3% formalin (Higashiyama et al. 1984), both being followed by multiple rinses in distilled water and then allowing the larvae to dry before inserting them in the diet.

FIGURE 7.1 *Anoplophora glabripennis* rearing container showing a wedge cut out and newly hatched larva in the grove. The wedge will be placed back over the larva before the lid is secured. (Courtesy of M. Keena.)

FIGURE 7.2 *Anoplophora glabripennis* rearing container for larger larvae with a hole cut in the center using the apple corer shown. The large larva is placed head down in the hole, which is just larger than its body width. (Courtesy of M. Keena.)

The majority of larvae reared in an artificial diet are held at temperatures between 23°C and 27°C; the larvae of two species were held at 20°C (Payne et al. 1975; Rogers et al. 2002), and the species reared on three other diets are held at 28°C (Palanti et al. 2010), 29°C (Alya and Hain 1987), and 30°C (Galford 1969), respectively. The optimal temperature (maximum growth rate without increased mortality) for species where the effects of temperature on development have been studied is between 25°C and 30°C. For example, the developmental rate of *A. glabripennis* larvae increases linearly between 10°C and 30°C; at higher temperatures, the larvae are adversely affected and most will die (Keena and Moore 2010). Tolerance to higher and lower temperatures can also be greatly affected by relative humidity (Linsley 1959). Generally, there is better survival at higher humidities. When humidity of the environmental chamber where cerambycid larvae are held is controlled, relative humidities between 50% and 70% are commonly used for rearing them. However, the moisture content of cerambycid diets for those where it has been evaluated varies between 50% and 65% (Galford 1969; Wollerman et al. 1969), and most diets are dried for a period of time before use. Diets with a high moisture content may reduce first instar larval establishment and survival in some species. Variability in moisture needs of larvae is to be expected due to the wide range of host plant conditions they inhabit, from living hosts to processed lumber to decaying wood. Further evaluation of differences in larval resistance to desiccation could improve our understanding of the role moisture plays in larval survival and development and improve laboratory rearing.

Although cerambycid larvae spend their time inside host plant tissues, some have been shown to respond to photoperiod changes. For example, larvae of *Xylotrechus pyrrhoderus* (Ashihara 1982) and *Psacothea hilaris* (Shintani et al. 1996) held at 25°C will proceed to pupation under long-day conditions, but under short-day conditions, they will undergo a few supernumerary molts and stop feeding; they will not pupate until undergoing a chill followed by increased temperatures and long-day conditions. Photoperiod has also been shown to affect larval development and pupation timing in three other species of cerambycids (Honda et al. 1981; Ueda and Enda 1995; Shintani 2011). About half the larvae reared in an artificial diet are held in the dark, and the other half are held in containers that let in some light (Figure 7.3), and these are exposed to various light cycles (14L:10D and 16L:8D being the most common). When developing a rearing system for cerambycids, some consideration should be given to the photoperiod that larvae are exposed to because it can impact metamorphosis timing.

Although not all cerambycid larvae require a chill period to proceed to pupation, chilling the larvae once they have reached the weight at which they would normally pupate can speed pupation and synchronize adult emergence (Gardiner 1970; Hanks et al. 1991; Keena 2005; Keena and Moore 2010). This also can be beneficial for stockpiling larvae and building colonies. Methods for rearing about half the species

FIGURE 7.3 Opaque containers with holes in the side that are used to hold *Anoplophora glabripennis* larvae in diet containers during rearing. (Courtesy of M. Keena.)

on an artificial diet call for a larval chill period of one to six months in length. The temperatures used for chill range from 1°C–10°C and, in most cases, the larvae are moved straight from the rearing temperature to the chill temperature. In three cases, the holding temperatures for the larvae are ramped down and then back up after the chill period. For example, *Paraglenea fortunei* larvae are held at 15°C for 10 days both before and after a 96-day chill period at 10°C (Kitajima and Makihara 2011). The proportion of larvae that require chilling is often high in the first generation in the laboratory but can quickly decline as they adapt to the rearing conditions (Hatchett et al. 1973, M. A. Keena, unpublished data). However, chilling larvae before they reach the right size can pose problems. For example, if *A. glabripennis* larvae are chilled when they are too small (after only 6–9 weeks at 25°C), they will resume feeding and growth after the chill and then require a second chill before pupation (Keena 2005). Chilling prepupae at temperatures close to 0°C can delay development after chill in one species (Hanks et al. 1991). In *A. glabripennis*, providing the larvae with drier than normal diet can reduce or eliminate the need for a larval chill period (M. A. Keena, unpublished data).

7.2.1.4 Preventing Mite Infestation of Artificial Diets

There are many grain and cheese mites that commonly infest stored food products that can get into artificial diets and cause problems. They proliferate under high moisture conditions and are often found in conjunction with fungal growth. When the infestation is severe, the surface of the diet will appear to have dust on it and, on closer inspection, the dust will be moving. The mites are 0.3–0.6 mm in length, pale or whitish in color, with spines on both body and legs. They have eight legs (except in the first larval stage when they have only six legs).

Prevention is the best way to avoid dealing with the mites. Good sanitation of the facilities and rearing containers is important because any diet crumbs or residue will attract mites and provide them with a place to become established. Work surfaces should be regularly disinfected with antimicrobial cleaners, floors should be mopped (not swept because that sends potential contaminants into the air), and clean protective clothing and gloves used. Work done with host plant material should be kept separate from work with artificial diets because it can be a source of fungal and mite contamination. Keeping diet ingredients in tight, dry containers and/or in a freezer is also important to prevent contamination. Mites do not survive below ~50% relative humidity or in a freezer. To prevent artificial diets with larvae in them from becoming

contaminated, use either rearing or holding containers with tight fitting lids (no holes) or filter paper inside of screw-on lids if there are air holes. Once the diet becomes contaminated, there are no good ways to get rid of the mites that will not harm the larvae, so it is best to just dispose of the containers unopened.

7.2.2 Larval Rearing Using Cut Host Material

Rearing larvae exclusively on host material may be advantageous in some cases by eliminating challenges associated with artificial diets, such as requiring a long time to develop a diet on which larvae will develop normally, labor-intensive making of the diet and transferring larvae to fresh diets periodically, possibly changing the biology or behavior of the insects as they adapt to the laboratory, and requiring relatively clean conditions to keep the diet from becoming contaminated. Although using cut host material to rear larvae can overcome many of these problems, it has some of its own, including the selection of host material in optimal condition for the normal development of larvae (some cerambycids cannot complete development in cut host material), fungal and mite contamination of host material, desiccation of host material, predators and parasitoids that may be present if host material is naturally infested, and the need to obtain fresh host material under less than ideal weather conditions if rearing year round.

Larvae from 45 species, 10 cerambycines and 35 lamiines, have been reared on cut host material (Table 7.1). Those reared on naturally infested wood brought into the laboratory include species that are twig girdlers or pruners (Rice 1995; Clarke and Zamalloa 2009), ones that infest turf grass roots (Kumral et al. 2012) or herbaceous plants (Niide et al. 2006), and those that prefer recently cut or dead wood (Tyson 1966; Purrington and Horn 1993; Berkov and Tavakilian 1999; Georgiev et al. 2004; Haack and Petrice 2009; Waqa-Sakiti et al. 2014). Generally, the infested material is held in a cage, tube, or other type of enclosure, either under controlled laboratory conditions or outdoors in a sheltered area (Figures 7.4 through 7.6). Several *Monochamus* and one *Aerenicopsis* species have been reared by exposing recently cut (usually partially dried) bolts/steams of the preferred host to ovipositing females and then letting the eggs hatch and larvae complete development in the bolts (Linit 1985; Anbutsu and Togashi 1997;

FIGURE 7.4 Sheltered outdoor adult emergence cage containing *Eucalyptus* bolts infested with *Phoracantha*. (Courtesy of L. Hanks.)

FIGURE 7.5 Cardboard tubes often used to hold infested wood until adults emerge. The lids have clear cups that let in light that attracts the adults and makes removing them easier. (Courtesy of M. Keena.)

FIGURE 7.6 Garbage barrels with wire screens in the lids used to either hold infested wood and contain adults that emerge until the can be removed or to hold large oviposition bolts and mating pairs when natural infestation of host material is desired.

Zhang and Linit 1998; Palmer et al. 2000; Akbulut et al. 2007; Koutroumpa et al. 2008; Breton et al. 2013). Species that prefer stressed or minimally weakened hosts have been reared by inserting larvae either into cut bolts/twigs (Figure 7.7) (Hanks et al. 1993; Shibata 1995; Bancroft et al. 2002; Bybee et al. 2004; Lu et al. 2011) or into potted trees in a greenhouse (Figure 7.8) (Ludwig et al. 2002; Morewood et al. 2005). Larvae are inserted into notches or under bark flaps (a hole or depression may be cut into the wood under it), and then the bark is held in place with plastic wrap and tape until the larvae establish. The bolts used

FIGURE 7.7 Bolt showing site of larval insertion after the larva has established and begun pushing out wood shavings and frass. (Courtesy of D. Long.)

FIGURE 7.8 Potted tree in a quarantine greenhouse that has had *Anoplophora glabripennis* larvae inserted. (Courtesy of D. Long.)

for insertion or oviposition have either one or both ends sealed with hot paraffin wax to slow desiccation (Figure 7.9). If only one end is sealed, the other end is put in either damp sand or a damp sand–peat mixture to help maintain the wood moisture content. Both these bolts and naturally infested host material are often misted with water one or more times a week to help maintain moisture levels. Two species have also been reared by providing larvae with fresh twigs of a preferred host in a glass tube and changing the twigs weekly (Adachi 1994; Bancroft et al. 2002). The cut host material for larval rearing is held at temperatures

FIGURE 7.9 Bolts that have had larvae inserted. Both ends have been dipped in hot paraffin to seal them and prevent moisture loss. (Courtesy of M. Keena.)

between 20°C and 30°C, most often at 25°C. The humidity, when controlled, is generally 70–80% RH and the light:dark (L:D) cycles used include 12L:12D, 14L:10D, and 16L:8D.

7.2.2.1 Preventing Fungal Infestation of Cut Host Material

Fungal spores present on cut host material can germinate and compromise rearing operations by degrading the host material and potentially harming the insects themselves. Sealing the ends of woody material with hot paraffin (which is already used to prevent moisture loss) can help prevent fungal growth on the exposed tissue. Cut twigs without leaves used for *Anoplophora glabripennis* food are treated with ultraviolet light for 30 minutes, and the bolts used for oviposition are stood on end and treated with ultraviolet light for 60 minutes to kill bacteria and fungi that are on the surface. This has eliminated entomophagus fungal infections of the adults and greatly reduced fungal infestation of the oviposition bolts (M. A. Keena, unpublished data). Alternative surface sterilization methods may be possible (e.g., wiping them with ethanol or dusting them with sulfur), but their efficacy and effects on the beetle have not been documented.

7.3 Prepupal and Pupal Handling Methods

Prepupae and pupae are delicate and can be easily damaged, so handling usually is kept to a minimum. Many rearing protocols for cerambycids indicate that larvae are allowed to pupate in the diet or host material; some are removed after a few days, but most remain in the diet until emergence as an adult. There are at least three reasons why removing these stages from artificial diets may be advantageous: exact timing of pupation or adult emergence is needed, diet changes disrupt pupal chamber formation, or pupation or adult emergence does not proceed normally in the diet. When prepupae or pupae are removed from the diet, they usually are placed in a container with moistened paper (e.g., filter paper, paper towel) or vermiculite to help maintain moisture and to provide a rough surface on which to pupate (Hatchett et al. 1973; Galford 1985; Kosaka and Ogura 1990; Keena 2005; Shintani 2011). Determining when larvae reach the right stage to remove them from the diet is not difficult. For example, once *A. glabripennis* larvae have reached full size and cease feeding, they form a pupal chamber (wider area than the normal tunnels) in the diet or a trough open to the top and then begin to show signs they are becoming prepupae. As larvae become prepupae, the body segments shorten, a section near the head becomes translucent and, just before pupation, they become flaccid (Figure 7.10).

FIGURE 7.10 *Anoplophora glabripennis* prepupa on top of the diet showing the translucent section just behind the head and the shrinkage as evidenced by the narrower body rings. (Courtesy of M. Keena.)

FIGURE 7.11 *Anoplophora glabripennis* pupae in 50-mL containers with two pinholes in the lid and a piece of paper towel to wick moisture. The tubes are placed on grates over water to maintain high humidity. (Courtesy of M. Keena.)

Prepupae can be carefully moved to the holding container as shown in Figure 7.11. This holding container is a 50-mL centrifuge tube with two pin holes in the lid and a piece of paper towel along one side—the end of which sticks out under the lid to act as a wick. The tubes are then placed in an opaque box with a thin layer of water and a grate on the bottom to hold the tubes above the water (keeping the humidity near 90% RH). This keeps the prepupae and pupae moist but not wet, which is critical to the successful shedding of the larval and pupal skins. For species that are held in the diet, the diet provides this critical moisture.

7.4 Adult Handling Methods and Oviposition Substrates

7.4.1 Teneral Adults and Maturation Feeding

Teneral adults generally require several days to sclerotize before they are ready to feed. Rearing methods where the pupae are taken off the diet indicate that new adults are held in the dark without food during this time (e.g., Keena 2005). This period of time is when the newly enclosed adult would be in the pupal chamber within the host before it starts chewing out. A recent study on *A. glabripennis* has indicated that the period of time before they start chewing will vary with holding temperature (Sánchez and Keena 2013), and it likely will also vary with species. Males usually emerge before females and can be distinguished from the females by their longer antennae.

Some feeding is a prerequisite to egg maturation and oviposition for most cerambycids (Linsley 1959). The length of the maturation feeding ranges from a few days to more than a month and varies among species (e.g., Hadlington and Johnson 1973; Koutroumpa et al. 2008; Lu et al. 2011) and with temperature (Keena 2006). During this period, various protocols indicate the adults are either held singly or in groups (usually by sex) (Figure 7.12). Possible food sources for adults include honeybee pollen, 10% honey solution, 30% sucrose solution, thin bark on twigs or branches, leaves, petioles, needles, cones, sap, fruit chunks, blocks of artificial diet (Gardiner 1970), and roots. A source of water is also often added when the moisture content of the host material is too low, such as moist filter paper on the container bottom, moist cotton or sand, or misting the containers periodically. If twigs or branches without leaves are used, they generally are changed weekly, and foliage and other food sources that can mold or dry out quickly are changed more frequently. Choice of food source is based on the individual species' natural food preferences or from information on closely related species if their biology is not known.

FIGURE 7.12 Wide mouth jars used for holding single adults during maturation feeding on top and larger mating jars below. (Courtesy of M. Keena.)

7.4.2 Mating and Oviposition Substrates

Cerambycid species belonging to the subfamilies Prioninae, Spondylidinae, and primitive Cerambycinae mate at night or late in the day, while those from other subfamilies mate during bright sunlight (Linsley 1959). Ensuring that adults have mated may be more difficult in the night mating species, but males may still be in the mate guarding mounted position, which makes the pairing obvious. Because male competition for females is often violent, resulting in mutilation, most species are mated in single pairs. In a few species, group cages of 5 pairs (Fukaya and Honda 1992), 10 pairs (Hanks et al. 1993), 20 pairs (Akbulut et al. 2007; Lu et al. 2011), 25 pairs (Linit 1985), or 200 individuals (Hadlington and Johnston 1973) are used. The pairs are housed in glass jars, screen cages, organdy bags, or plastic cages of varying sizes. The mating containers are held outdoors, in greenhouses, at room temperature, or in environmental chambers set at 18–28°C and 40–80% RH. Choice of temperature and humidity for holding pairs can be critical. At lower temperatures within the 10–30°C range, adults generally live longer but fecundity will be reduced. In *A. glabripennis*, for example, the optimum temperature for survival is 18°C, but maximum fecundity occurs at about 24°C and no oviposition occurs at <10°C and >35°C (Keena 2006). If humidity is too low, at either higher or lower temperatures in particular, and adequate sources of moisture are not available, adult survival will be reduced.

In addition to food and water sources, an oviposition substrate is added. Oviposition in cerambycids occurs on host surfaces, in bark/wood cracks, in the soil, or under the bark in specially prepared egg niches (Linsley 1959). Species that oviposit on surfaces or in host cracks are either supplied with host material (or a host surrogate, Hatchett et al. 1973) to oviposit on or some other cracks to use. Cracks or tight places provided for oviposition include rolled paper (García-Ruiz and Pérez-Moreno 2012); 1–2 cm wide cotton fabric wrapped in a spiral (with gaps between wraps) around a host bolt/stem (Galford 1969; Wollerman et al. 1969; Boldt 1987); tightly rolled leaves (Fukaya and Honda 1992); polyethylene sheeting cut in strips, stacked, folded, and stapled together and placed so that the folds are available to the female (Hanks et al. 1993); or tight places, such as between a damp filter paper and the cage bottom (Gardiner 1970; Shibata 1995). A freshly cut bolt of host material with both ends dipped in paraffin wax (to retain moisture) generally is provided as an oviposition substrate to species that oviposit in specially prepared egg niches. The bolts are changed weekly. An example of a mating container setup used for *A. glabripennis* is shown in Figure 7.13. These bolts subsequently will have to have the bark carefully peeled off to retrieve eggs or newly hatched larvae depending on how long the bolt is held (Figure 7.14).

FIGURE 7.13 *Anoplophora glabripennis* mating pair in a jar with maple twigs for food and a bolt for oviposition. (Courtesy of M. Keena.)

FIGURE 7.14 Bolt with some of the bark peeled back to show the *Anoplophora glabripennis* eggs laid underneath. (Courtesy of M. Keena.)

7.5 Egg Handling and Stockpiling Methods

Once eggs are extracted from the oviposition substrate, they can be held for hatch either with or without surface disinfection. Four surface disinfecting methods are listed in the published literature: dipped in 70% ethanol for 10 seconds, in 0.05% benzalkonium chloride for 5 minutes, and washed three times with distilled water (Kosaka and Ogura 1990); dipped in 70% ethanol, then three to five times in 1% methanol, and washed four times with distilled water (Zhao et al. 1999); dipped in 1% sodium hydroxide solution for 20 seconds, soaked in 10% formalin for 10 minutes, and then washed three times with distilled water (Kitajima 2003); and dipped in 4% bleach solution for 20 seconds, soaked in 70% ethanol for 12 seconds, and then washed with distilled water (Akutsu et al. 1980). Generally, eggs are held in groups in petri dishes or other plastic or glass containers with or without damp filter paper in the bottom. In one case, the filter paper had to be wet with Ringer's solution to prevent egg rupturing (Wollerman et al. 1969). *A. glabripennis* eggs also can be held singly with no disinfection, as in Figure 7.15, to prevent newly hatched larvae from mutilating each other (Keena and Moore 2010). In some cases, the eggs are not removed from the oviposition substrate. Instead, they are held until the larvae hatch and then they are retrieved.

Eggs can be held at similar temperatures to those used for rearing larvae or holding adults if hatch is desired immediately. If hatch needs to be delayed, synchronized, or egg stockpiling is desired, cerambycid eggs can be held at lower temperatures. Egg hatch can be delayed by holding eggs or the oviposition substrate with eggs at 15–20°C (Hanks et al. 1993; Keena 2006; Shintani 2011), or egg hatch can be prevented by holding them at 5–10°C for up to 80 days without substantial mortality (Keena 2005, 2006). The humidity in the holding location must be maintained at >80% RH to ensure egg survival when holding them for longer periods of time.

7.6 Developing Rearing Methods for Additional Species

When no published method for rearing a species of cerambycid exists, begin by assembling all available knowledge of the biology and hosts used by the species of interest. Next search for methods or diets that exist for other species (Table 7.1) that are either most closely related (e.g., same genus) or that inhabit

FIGURE 7.15 Section of a 24-well plate showing *Anoplophora glabripennis* eggs and newly hatched larvae. Well plates are held on grates over water in boxes to maintain near 100% RH. (Courtesy of M. Keena.)

similar hosts that are in the same condition (e.g., weakened or dead hosts). If there are no likely candidates based on these two criteria or there is insufficient knowledge about the species you wish to rear, the best diets to try initially are those that have been evaluated for use with a number of species from several different subfamilies.

Start by using the most commonly utilized rearing methods and environmental conditions as documented in this chapter. Another good source of information and ideas are the researchers that have already successfully developed methods and diets for cerambycids. The two most common problem areas for rearing cerambycids are diet moisture content and environmental conditions not being favorable for pupation. Using a drier diet (too much moisture can have adverse effects on establishment and development) and mimicking a natural overwintering environment (temperature and light cycle) are potential ways to avoid these problems. Start with a diet that has 40–50% moisture content if working with a larva that develops in a live, stressed, or moist decaying host and with a drier diet (e.g., 30%) if the larva develops in a weakened or dry, dead host. To determine the best environmental conditions to use, obtain the climatic data for the native habitat and take the modulating effect of the wood environment into account (add at least 2°C). Average weekly temperatures in the summer will work for many species as the larval rearing temperature. Larvae generally survive chill well and progress to pupation if chilled at 5–10°C for one to three months. The correct photoperiods to use will depend on the timing of pupation and the light cycle in the native habitat, both during larval rearing and chill. Be aware that in all rearing method and diet development programs, it may take several years to fully evaluate and modify existing methods to adequately rear a new species.

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