An analysis of the larval instars of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in northern California black walnut, *Juglans hindsii*, and a new host record for *Hylocurus hirtellus*

Author(s): Paul L. Dallara, Mary L. Flint, and Steven J. Seybold
Source: Pan-Pacific Entomologist, 88(2):248-266. 2012.
Published By: Pacific Coast Entomological Society
DOI: http://dx.doi.org/10.3956/2012-16.1
URL: http://www.bioone.org/doi/full/10.3956/2012-16.1

THE PAN-PACIFIC ENTOMOLOGIST
88(2):248–266, (2012)

An analysis of the larval instars of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in northern California black walnut, *Juglans hindsii*, and a new host record for *Hylocurus hirtellus*

Paul L. Dallara[1], Mary L. Flint[1], & Steven J. Seybold[2]
[1]Department of Entomology, University of California Davis, One Shields Ave., Davis, California 95616
[2]USDA Forest Service-Pacific Southwest Research Station, Chemical Ecology of Forest Insects, 720 Olive Dr., Suite D, Davis, California 95616
e-mail: sjseybold59@gmail.com

Abstract. By measuring and analyzing larval head capsule widths, we determined that a northern California population of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), has three larval instars. We also developed rules to classify *P. juglandis* larval instars. Overlap in the ranges of widths among consecutive instars was addressed by fitting a distribution model to the data and determining limits for head capsule width classes and probabilities of instar misclassification. Growth ratios for laboratory and field samples of *P. juglandis* were consistent with the Brooks-Dyar rule and with results in the literature for other bark and ambrosia beetle species. As a consequence of branch dissection to retrieve larvae of *P. juglandis*, we established that northern California black walnut, *Juglans hindsii* (Jeps.) Jeps. ex R.E. Sm., is a host for the xylophagous "California hardwood bark beetle," *Hylocurus hirtellus* (LeConte) (Coleoptera: Scolytidae), and that *H. hirtellus* is associated with *P. juglandis* beneath the bark of small diameter branches. It appears that in northern California, univoltine *H. hirtellus* is active in flight from early March through early May, with the majority of the adults dispersing in April. The females are the colonizing sex.

Key Words. Bark beetle, California, California hardwood bark beetle, Coleoptera, *Geosmithia morbida*, head capsule, *Hylocurus hirtellus*, instar, *Juglans hindsii*, life history, northern California black walnut, *Pityophthorus juglandis*, Scolytidae, shrub bark beetle, thousand cankers disease, walnut twig beetle.

Introduction

The walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae, *sensu* Wood 2007), is a phloeophagous insect that has recently been associated with the newly described fungus, *Geosmithia morbida* (Kolařik et al. 2011). This insect-fungal complex, also known as thousand cankers disease (TCD), is fatal to walnut trees and is responsible for the gradual deterioration and mortality of several species of black walnuts in the western United States over the past decade (Graves et al. 2009, Seybold et al. 2011, Tisserat et al. 2011). WTB was first collected in New Mexico in 1896 and is considered to be native to the southwestern United States and Mexico (Bright 1981), but the beetle and pathogen have been introduced into Pennsylvania (2011), Tennessee (2010), and Virginia (2011) (Seybold et al. 2011, 2012a). The WTB was first collected in California in 1959 (Bright & Stark 1973), but very little is known of its biology in California or elsewhere (Bright 1981, Wood & Bright 1992). One key aspect missing in the life history of WTB is the number of larval instars.

Insect head capsule width measurements were used originally to classify larval instars of Lepidoptera (Dyar 1890), but the technique has been applied subsequently for many additional insect orders (Daly 1985). Blackman (1915) was the first to report the use of head capsule widths for instar classification in the Scolytidae. He analyzed the larvae of *Pityogenes hopkinsi* Swaine; others have since reported similar studies with numerous other scolytids (Lekander 1968, Balogun 1970, and references therein). In the Scolytidae, the number of larval instars ranges from 2 to 5 (Lekander 1968, Wood 1982). In the few studies addressing larval development of *Pityophthorus*, three instars have been reported for *P. micrographus* L. (Lekander 1968) and *P. orarius* Swaine (Hedlin & Ruth 1970), whereas two instars were reported for *P. confertus* Swaine (Amman et al. 1974).

In most previous studies, classification of larvae into instars has involved plotting a sample of head capsule width measurements in a histogram, followed by recognition of peaks within the distribution as instars. When the distribution of widths does not form discrete groups, decision rules must be developed to assign the instars. Continuous distributions of head capsule widths have been reported frequently in the literature and may be more likely with larger samples (Caltagirone et al. 1983, McClellan & Logan 1994). However, few analyses have used methodically developed classification rules to define instar head capsule width limits for continuous data. Exceptions include several studies of Lepidoptera (Caltagirone et al. 1983, Got 1988, Beaver & Sanderson 1989, McClellan & Logan 1994, Godin et al. 2002), one study of Curculionidae (Panzavolta 2007), and one study of Scolytidae (Logan et al. 1998).

Here we examined larval WTB from northern California black walnut, *Juglans hindsii* (Jeps.) Jeps. ex R.E. Sm., to determine the number of larval instars and to develop an instar classification based on head capsule width. Head capsule widths did not form discrete classes of measurements for each instar, so a distribution model was developed to assign instars to classes and to determine probabilities of instar misclassification.

While dissecting branches of *J. hindsii* for the instar analysis, we also noted the presence of another species of Scolytidae, a xylophagous hardwood bark beetle, *Hylocurus hirtellus* (LeConte) (Fig. 1), which had not been recorded previously from *Juglans* spp. (Wood 1982, Wood & Bright 1992). Known informally as the California hardwood bark beetle or shrub bark beetle (Armitage 1952, Keen 1952, Struble & Hall 1954), *H. hirtellus* is a univoltine species that mines in the hard dry wood of many flowering shrubs and broadleaved trees (Doane et al. 1936, Bright & Stark 1973). Humble et al. (2000) noted from observations in British Columbia that it develops in the dead wood associated with scars on living host plants. This beetle is thought to develop several generations in the same host stem or branch, and has been noted to tunnel occasionally into lead or polyethylene plastic that covered telephone cables (Doane et al. 1936, Armitage 1952, Struble & Hall 1954).

Materials and Methods

Study Sites and Insects. WTB for this project were collected from one site in each of two counties: 1) California: Yolo County (Co.) and 2) California: Alameda Co. (Table 1), located about 100 km apart.

For the Yolo Co. insects, between 1000 and 1500 fertile female WTB were allowed to feed and oviposit in 12 freshly cut and previously uninfested *J. hindsii* branch sections (2–4 cm diameter × 20–25 cm length, Yolo Co., California) in the laboratory in a 19-liter glass carboy from 16 February to 28 March 2010 at ambient

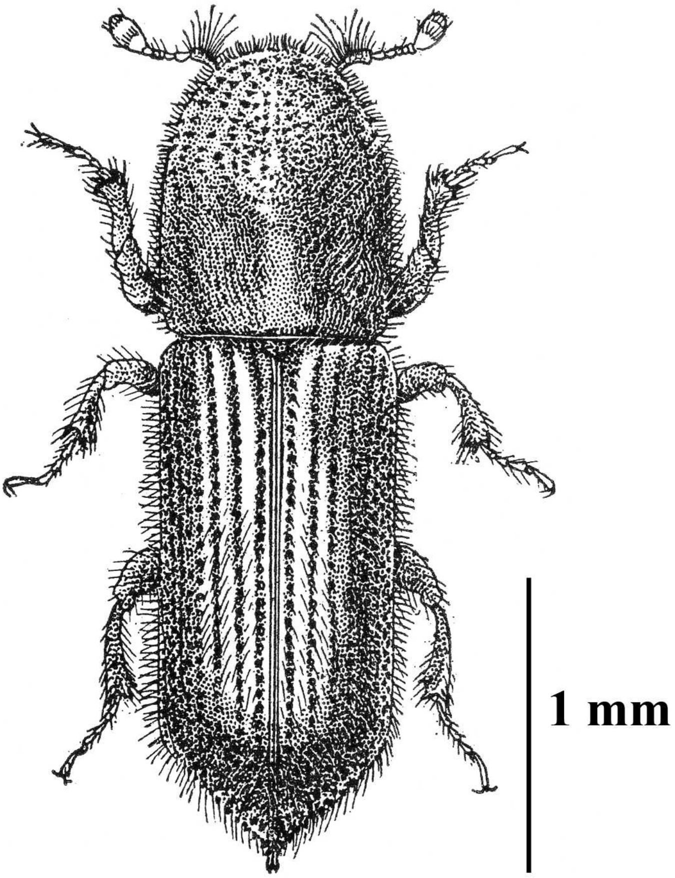

Figure 1. Engraving of the adult "California hardwood bark beetle" or "shrub bark beetle," *Hylocurus hirtellus* (LeConte), drawn by E.C. Van Dyke (Doane et al. 1936). Scale bar in figure = 1 mm.

temperature (approx. 22 to 27 °C). This procedure was part of an analysis of semiochemical production by this species (to be reported elsewhere). On 28 March 2010, branch sections were removed from the carboy and stored at 0 °C. They were dissected over several weeks in January 2011 to retrieve the larvae (n = 277).

For the Alameda Co. insects, uninfested *J. hindsii* branches were monitored in a native riparian stand of *J. hindsii* and allowed to be colonized by a field population of WTB. These "trap" branches were either unbaited or baited with the WTB aggregation pheromone (Seybold et al. 2012b, c). Fifty branch sections (2–8 cm diameter × 15 cm length) were sampled. About one-half of the 50 branch sections (26) were retrieved monthly (between 9 July and 23 September 2011) from 3 m-long branches that had been cut in Yolo Co. from a healthy tree and then placed in the field in Alameda Co. on 9 April 2011. Sections were collected as pairs from one unbaited and one baited parent branch at each trap station. The other half of the branch sections (24) were retrieved weekly (between 9 July and 17 October 2011) from windfallen branches at the field site (first identified in spring 2011) or branches removed live from trees (spring/summer 2011). Eleven of these natural trap branches

Table 1. Collection of *Pityophthorus juglandis* in California for analyses of larval instars.

Collection Date	Host	County	Locality	GPS Coordinates and Elevation	Comments
16 February 2010[a]	*J. hindsii* × *regia*	Yolo	UC Davis Walnut Collection, Tree #R8T3, Hutchison Drive	38°32′21.40″ N, 121°47′46.40″ W, 21.6 m	Paradox (*J. hindsii* × *regia*) rootstock section from a tree that died July 2009
9 July to 17 October 2011	*J. hindsii*	Alameda	Shadow Cliffs Regional Recreation Area	37°30′1.01″ N, 121°50′15.36″ W, 110 m	*J. hindsii* baited and unbaited branches
10 May to 23 September 2011	*J. hindsii*	Alameda	Shadow Cliffs Regional Recreation Area	37°30′1.01″ N, 121°50′15.36″ W, 110 m	*J. hindsii* cut branches placed in the field on 10 April 2011[b]

[a] These adults were collected for a chemical analysis of the *P. juglandis* aggregation pheromone. They were allowed to emerge in the laboratory from an uncaged log and collected with forceps from the bark surface and cut ends of the log. Once emerged, brood adults and re-emerged parental adults of both sexes were given the opportunity to colonize freshly cut, previously uninfested branches of *Juglans hindsii*. These branches (2–4 cm in diameter) had been removed from live trees and cut into 20–25 cm long sections on 22 February 2010 (Yolo Co., Davis, West Russell Blvd., 38°32′49.20″ N, 121°47′44.40″ W). Males and females were placed separately into branch sections and volatiles were collected for 1 month. Following the volatile entrapment period, the branch sections were frozen on 28 March 2010, and the larval progeny of these adult females were analyzed for head capsule width in January and February 2011. Thus, F_1 larvae from the wild F_0 females were used in the lab for the head capsule analysis.

[b] The branches of *J. hindsii* for this study were collected 7 February 2011 from a live tree, Yolo Co., Davis, 36720 County Road 30, 38°34′41.24″ N, 121°49′41.18″ W. All branches were uninfested when deployed in the field.

were baited with the WTB aggregation pheromone. Following collection in the field, all branch sections were stored for a minimum of 5 days at 0°C prior to dissection to retrieve the larvae (n = 738). The freezing treatment killed all insects in the sections. A maximum of 30 WTB larvae were removed from galleries in each branch section from the Alameda Co. field site.

All larvae were placed in 70% ethanol and head capsule maximum width was measured to the nearest 0.01 mm with a Zeiss Stemi 2000 stereomicroscope (Fisher Scientific, Atlanta, Georgia) equipped with a 50× ocular micrometer. All measurements were performed by PLD.

We investigated the association of the xylophagous bark beetle, *H. hirtellus*, with WTB in *J. hindsii* at the Alameda Co. site by dissecting branch sections, and by monitoring emergence and flight activity of both beetle species. The fifty branch sections noted above were debarked completely and examined for *H. hirtellus* entrances into galleries in the xylem. These galleries were then dissected. Additional branch sections (2–5 cm diameter, 15–30 cm length, n = 44 collected between 7 July 2010 and February 2012) were partially or completely debarked and examined for *H. hirtellus* galleries. To study emergence of *H. hirtellus*, a second set of bark-covered branch sections (2–5 cm diameter, 15–30 cm length, n = 146 collected between July 2010 and October 2011) were placed in chambers constructed of 10 cm diameter ×

30 cm HDPE drain pipe (ADS-Hancor, Findlay, Ohio) with cloth at one end and a clear plastic bag at the opposite end, both affixed by elastic bands. *Hylocurus hirtellus* was also collected during contemporaneous flight studies at the Alameda Co. site. We monitored the flight of WTB (to be reported elsewhere) and other scolytids approximately biweeky from 31 March to 17 October 2011 and 28 February to 1 June 2012, by using traps constructed of clear acrylic sheets (23 × 14 cm) and coated on both sides with Stikem Special (Seabright Industries, Emeryville, California). The number of traps in the field during this time varied from 4 to 15 WTB pheromone-baited/unbaited pairs, and the traps were suspended from tree branches at a height of 3 m and placed a minimum distance of 3 m from the nearest *J. hindsii*.

Mathematical Techniques and Statistical Analyses. Larval instar width classes were determined by modeling the distribution of head capsule widths separately for the laboratory and field samples of *P. juglandis*. A normal distribution of measurements for each instar was assumed (Caltagirone et al. 1983, Beaver & Sanderson 1989, Logan et al. 1998). Frequencies of head capsule widths for both samples were plotted on separate histograms by using width classes of 0.01 mm. All analyses were performed with Microsoft Excel 2008 for Mac; iterative calculations used the Goal Seek routine within Excel.

To produce classification limits and misclassification probabilities, data were fitted to a series of Gaussian curves with nonlinear least squares (NLLS) analysis (McClellan & Logan 1994, Logan et al. 1998). First, visually identified minima among overlapping curves were used as preliminary limits to create subgroups of data for initial estimation of means (c), peak heights (a) and variances (s^2) for each individual distribution. Initial estimates of c, s^2, and a for each curve were substituted in the equation

$$y_i = a_i e^{-b_i(m-c_i)^2} \quad i = 1 \ldots K \tag{1}$$

where K is the number of instars, y_i is the estimated frequency of head capsule width class m for instar i, and $b_i = 1/(2s_i^2)$ (Logan et al. 1998).

Equation 1 was solved iteratively for initial best fit to the data values corresponding to the head capsule width distribution for each instar. This was followed by substitution of the initial best-fit parameters (a, b, and c) for each curve into the equation for the sum of the individual distributions calculated in (**1**), yielding the total distribution

$$h_i = \sum_{i=1}^{K} y_i \tag{2}$$

where h_i is the frequency of head capsule width m for each of the K instars. Equation 2 was then solved iteratively for best fit to the total data set, yielding final estimates for parameters a, b, and c.

An estimate of the number of larvae l_i in each instar, accounting for distribution overlap, was then calculated (Logan et al. 1998):

$$l_i = \frac{a_i \sigma_i \sqrt{2\pi}}{\Delta} \tag{3}$$

where Δ is the measurement interval (0.01 for both laboratory and field samples).

The final best-fit parameter estimates were substituted into the normal distribution equation (**4**) to create a frequency distribution for each of the K instars that accounts for overlap among distribution curves (Logan et al. 1998):

$$f_i = \left(\frac{l_i \Delta}{\sigma_i \sqrt{2\pi}}\right) e^{\frac{-(m-\mu_i)^2}{2\sigma_i{}^2}} \quad i = 1 \ldots K \tag{4}$$

where $\mu_i = c_i$; $\sigma_i = \sqrt{\frac{1}{2b_i}}$

The distribution curves representing individual instars were combined to produce a total distribution for each data set (Yolo Co. and Alameda Co.). Each complete data set was tested for goodness of fit to the normal distribution with a Kolmogorov-Smirnov test; critical values were determined by using an adjusted asymptotic approximation for large samples with an intrinsic hypothesis (Sokol & Rohlf 1995).

Classification rules (head capsule width limits for each instar) were based on the 3-parameter model (**2**). The head capsule widths corresponding to the intersection of adjacent curves were determined by solving iteratively for the minimum ordinate value common to each pair of overlapping curves from (**4**). Misclassification probabilities, defined as the distribution tail areas on either side of these curve intersections (McClellan & Logan 1994), were then determined by calculating normal deviates for each curve. For practical application to new samples of *P. juglandis* larvae, width limits were adjusted up and down to the nearest 0.01 mm and misclassification probabilities recalculated.

Larval growth rates were estimated for each data set by determining the ratios of successive instar head capsule mean values; the latter from the total distribution NLLS model. The natural logarithm of each mean was plotted against instar number to evaluate fit to the Brooks-Dyar rule (Daly 1985, Logan et al. 1998). The significance of each regression line slope was tested with one-way ANOVA as in Logan et al. (1998).

Voucher Specimens and Nomenclature. The identification of *H. hirtellus* was verified by D.E. Bright (C.P. Gillette Museum of Arthropod Diversity, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado). Voucher specimens of the adults were placed in the above collection and at the California Academy of Sciences, San Francisco, California. In this paper, we have elected to use the original nomenclature for bark and ambrosia beetles (Coleoptera: Scolytidae) based on the argument presented in Wood (2007) and a more extensive treatment of the issue developed by D.E. Bright (personal communication), which is to be published in his third supplement to the world catalog of the Scolytidae and Platypodidae. In essence, morphological and fossil evidence of adult scolytids support the family-level treatment, whereas similarity in scolytid and curculionid larval morphology supports a subfamily placement. Because this issue is not entirely resolved, we prefer to take the more conservative approach of using the original nomenclature.

Results

WTB Larval Instar Analysis. We examined 1015 WTB larvae that had developed in *J. hindsii* in Yolo Co. (277 specimens, laboratory sample) and in Alameda Co. (738 specimens, field sample). Larval head capsule widths across both samples ranged from 0.18 mm to 0.46 mm (Figs. 2 and 3). Overlap among all pairs of adjacent instar head capsule width distributions was evident for both samples of larvae. For each total distribution, the three peaks were interpreted as separate instars and two between-distribution minima were selected for the purpose of initial parameter estimation.

Within each sample, NLLS fitting of Eq. 1 (three parameters) and Eq. 2 (nine parameters) yielded individual instar head capsule width distributions and a combined instar distribution accounting for overlap (Figs. 2 and 3). Each calculated total distribution fit the data well (Yolo Co. adjusted $r^2 = 0.878$ with 9 parameters; Alameda Co. adjusted $r^2 = 0.941$ with 9 parameters). Neither total distribution of sample data differed significantly from the total distribution defined by the respective sample parameters (Kolmogorov-Smirnov test, Yolo Co.: $g = 0.026$, $P > 0.05$, critical value $g_{0.05} = 0.052$; Alameda Co.: $g = 0.015$, $P > 0.05$, critical value $g_{0.05} = 0.032$). With this 3-parameter model, overlap between distributions increased with head capsule width in both samples, and maximum probability of misclassification was 3.90%. The analyses of both Yolo and Alameda Co. samples produced similar means, classification limits, and misclassification probabilities (Tables 2–5). A 2-parameter model, based on only means and variances (Logan et al. 1998), was also developed for each sample. This resulted in classification rules that were functionally identical to the 3-parameter model; misclassification probabilities (not shown) also exhibited no practical differences.

Adjustment of width limits for each instar (Table 5) to the nearest upper and lower 0.01 mm yielded a classification in which each instar was represented by a

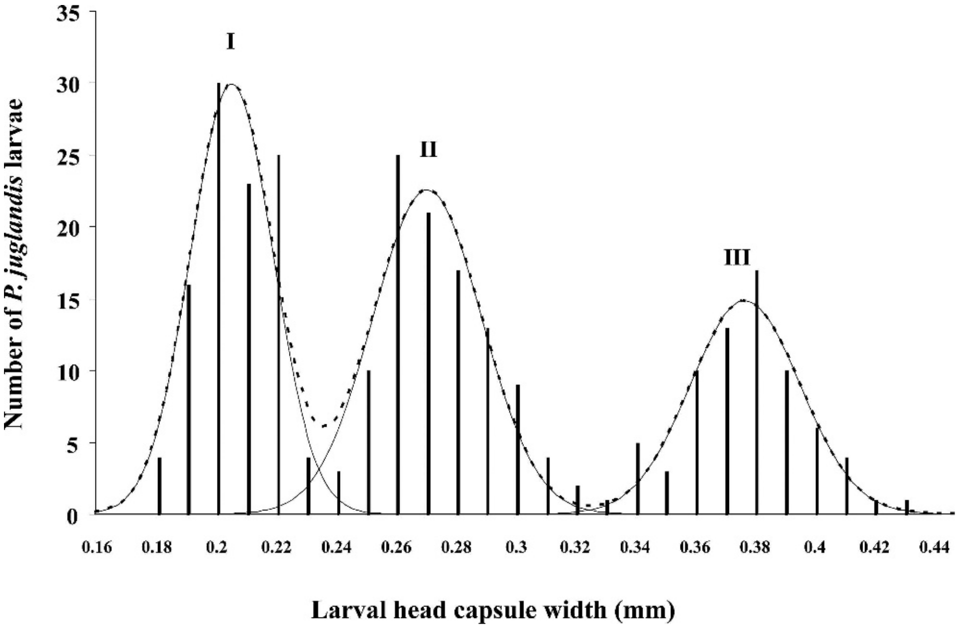

Figure 2. *Pityophthorus juglandis* larval head capsule width distribution (Yolo Co. sample). Lines represent predicted distributions calculated from data; histograms indicate raw data. Dashed line is total distribution. Branches infested in the laboratory (females only), Yolo Co., California, 2010, $n = 277$ larvae.

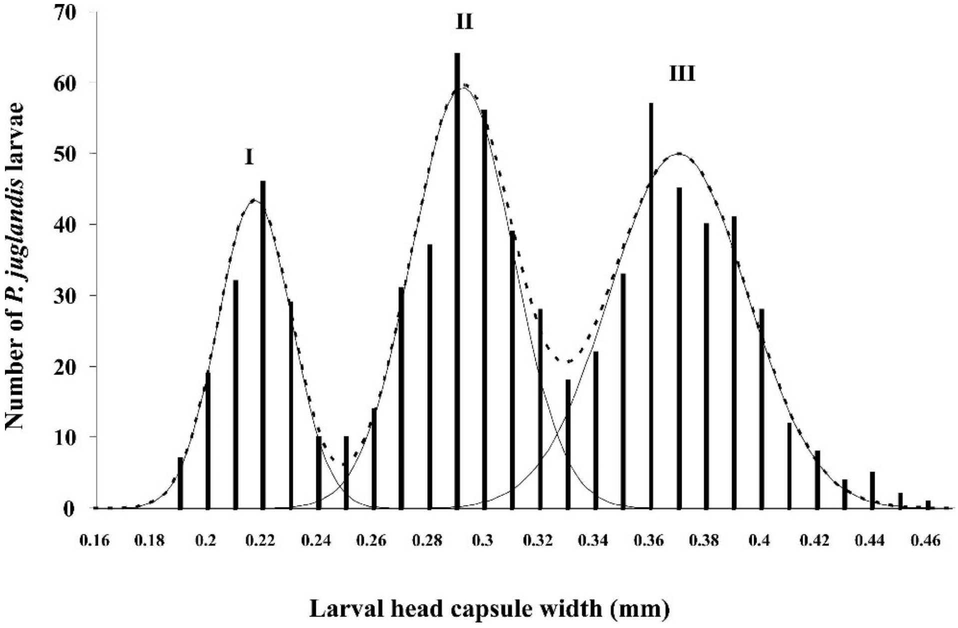

Figure 3. *Pityophthorus juglandis* larval head capsule width distribution (Alameda Co. sample). Lines represent predicted distributions calculated from data; histograms indicate raw data. Dashed line is total distribution. Branches infested in the field (males and females), Shadow Cliffs Regional Recreation Area, Alameda Co., California 2011, $n = 738$ larvae.

Table 2. Estimated final values for normal distribution functions fitted[a] to larval *Pityophthorus juglandis* head capsule width data, Yolo Co., California, 2010.

Instar	Number	Scaling Factor	Mean (mm)	Standard Deviation (mm)
1	103[b]	30.0[c]	0.206	0.0137
2	103	22.6	0.271	0.0181
3	68.5	14.9	0.377	0.0184

[a] Nonlinear least squares (NLLS) analysis of 277 larval head capsules.
[b] Number = l_i from Equation 3.
[c] Scaling factor = a_i from Equation 3.

Table 3. Estimates of limits, Brooks-Dyar ratios, and probabilities of instar misclassification (3-parameter model) for larval *Pityophthorus juglandis* head capsule width data, Yolo Co., California, 2010.

	Width (mm)		Brooks-Dyar ratio	Probability of Misclassification		
Instar (i)	Lower	Upper	($i : i - 1$)	i as $i - 1$	i as $i + 1$	Total (i as $i \pm 1$)
1	—	0.235	—	—	0.0165	0.0165
2	0.235	0.325	1.32	0.0231	0.00146	0.0246
3	0.325	—	1.39	0.00232	—	0.00232

Table 4. Estimated final values for normal distribution functions fitted[a] to larval *Pityophthorus juglandis* head capsule width data, Alameda Co., California, 2011.

Instar	Number	Scaling Factor	Mean (mm)	Standard Deviation (mm)
1	145[b]	43.4[c]	0.218	0.0133
2	272	59.2	0.293	0.0183
3	310	49.9	0.371	0.0248

[a] Nonlinear least squares (NLLS) analysis of 738 larval head capsules.
[b] Number = l_i from Equation 3.
[c] Scaling factor = a_i from Equation 3.

Table 5. Estimates of limits, Brooks-Dyar ratios, and probabilities of instar misclassification (3-parameter model) for larval *Pityophthorus juglandis* head capsule width data, Alameda Co., California, 2011.

Instar (i)	Width (mm)		Brooks-Dyar ratio	Probability of Misclassification		
	Lower	Upper	($i : i - 1$)	i as $i - 1$	i as $i + 1$	Total (i as $i \pm 1$)
1	—	0.249	—	—	0.0108	0.0108
2	0.249	0.327	1.34	0.00764	0.0314	0.0390
3	0.327	—	1.27	0.0386	—	0.0386

Table 6. Proposed limits and probabilities of instar misclassification for practical classification of larval *Pityophthorus juglandis* based on 3-parameter modeling[a].

Instar (i)	Width (mm)		Probability of Misclassification		
	Lower	Upper	i as $i - 1$	i as $i + 1$	Total
1	—	0.24	—	0.00084	0.00084
2	0.25	0.32	0.0092	0.022	0.031
3	0.33	—	0.020	—	0.020

[a] Data for this analysis were from field-reared larvae collected in Alameda Co.

non-overlapping set of measures (Table 6) and reduced most misclassification probabilities (cf. Table 5 with Table 6).

Growth ratios among Yolo and Alameda Co. samples ranged from 1.27 to 1.39. Regression analysis of ln-transformed means produced linear trends and significant slopes for each sample (Yolo Co.: F = 238.4, df = 1,1; $P = 0.041$; Alameda Co.: F = 375.3, df = 1,1; $P = 0.033$) (Fig. 4). This is consistent with a geometric growth rate, and suggests that all three WTB larval instars indicated by the model were represented in each sample (Dyar 1890, Daly 1985). In addition to WTB larvae, Yolo Co. branch sections yielded WTB eggs, pupae, and parental adults, whereas Alameda Co. yielded eggs, pupae, and parental and brood adults. Thus, two lines of evidence support the critical assumption that all larval instars were present in both samples (Daly 1985).

The "split" peak of the third-instar distribution of the Alameda Co. data was investigated with further NLLS modeling to test the hypothesis that two distributions may better fit the third-instar larval data. This resulted in an improved best-fit model (adjusted $r^2 = 0.952$ with 12 parameters describing the total distribution). The two new peaks had means of 0.361 mm (SD = 0.0130) and 0.392 mm (SD = 0.0131). Final estimates of parameters for the first and second instars were essentially unchanged, as were classification rules for all larvae.

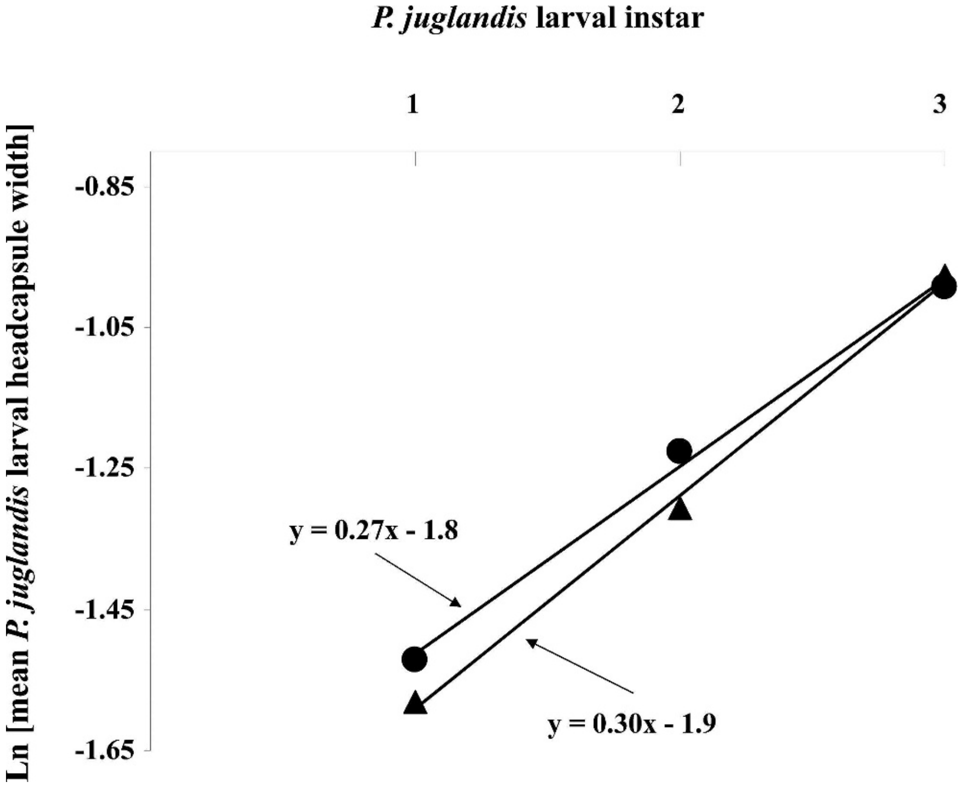

Figure 4. Relationship between larval instar of *Pityophthorus juglandis* and natural logarithm of larval head capsule mean width. A linear relationship satisfies the Brooks-Dyar rule. Larvae were from: 1) Branches infested in the laboratory (females only), Yolo Co., California, 2010, $n = 277$ (triangles, $y = 0.30x - 1.9$, slope of line was significant, $P < 0.05$, one-way ANOVA) and 2) Branches infested in the field (males and females), Shadow Cliffs Regional Recreation Area, Alameda Co., California 2011, $n = 738$ (circles, $y = 0.27x - 1.8$, slope of line was significant, $P < 0.05$, one-way ANOVA).

Attempted two-distribution NLLS modeling of the Alameda Co. second-instar data did not produce any improvement in the overall model.

While standard deviations increased in proportion to means for the Alameda Co. data (Table 4), yielding a linear relationship with a significant slope ($F = 249.6$, df = 1,1; $P = 0.040$; $r^2 = 0.996$), the slope of the regression line for Yolo Co. data was not significant ($F = 2.1$, df = 1,1; $P = 0.385$; $r^2 = 0.677$). In the latter data set, the standard deviation for the third instar was almost identical to that of the second instar (Table 2).

Colonization, Emergence, and Flight Activity of Hylocurus hirteilus. In 2010 and 2011, adult *H. hirtellus* were observed in the xylem of *J. hindsii* branch sections collected from the Alameda Co. site. One branch section (4cm diam, 30cm length) from an unbaited, windthrown branch was collected on 7 July 2010, placed in an emergence cage, and yielded one adult specimen of *H. hirtellus*, which emerged between 7 July and 14 August 2010. Adult WTB also emerged contemporaneously from this branch. Two branch sections (2 and 3 cm diam. × 30 cm lengths) from unbaited, dying *J. hindsii* branches were collected on 24 July 2011 and were partially dissected. These branch sections contained WTB adults and larvae in the phloem, in addition to four separate adult galleries of *H. hirtellus* in the xylem. Two of the galleries each contained two adult *H. hirtellus* (one of each sex); two of the galleries each contained one adult (females only). One of the galleries inhabited by a male and female also contained two eggs in separate niches along the parental gallery. The galleries consisted of one to three arms, and the arm length ranged from 2 to 10 mm. The longest gallery formed an unbranched chord through the branch xylem, narrowly skirting the pith. We did not observe any larval mines, larvae, or pupae of *H. hirtellus* in these branches. After partial dissection, each branch was placed in an emergence chamber. Five adult *H. hirtellus* (1 male, 3 females, 1 undetermined) emerged from these branches between 5 June and 13 July, 2012.

In 2011, 24 specimens of *H. hirtellus* (11 males and 13 females) were flight-trapped at the Alameda Co. site. The majority of the flight-trapped insects (17 specimens) were captured between 2 and 28 April; the range of flight occurred between 2 April and 24 May. In 2012, 47 specimens of *H. hirtellus* (31 males and 16 females) were flight-trapped. One male specimen was collected in flight between 28 February and 8 March, but the majority of the *H. hirtellus* (34 specimens) were captured between 28 March and 28 April; the range of flight occurred between 28 February and 18 May. WTB adults were also detected contemporaneously on these traps during both years.

We measured the lengths (from anterior edge of the pronotum to the tip of the elytra, with the same technique described above for larval WTB) of intact specimens of *H. hirtellus* dissected from *J. hindsii* and caught on flight traps. Female lengths ranged from 2.38 to 3.16 mm (n = 27; mean ± SE = 2.85 ± 0.168 mm); male lengths ranged from 2.36 to 3.14 mm (n = 39; mean ± SE = 2.72 ± 0.156 mm).

Record. CALIFORNIA: Alameda Co., Shadow Cliffs Regional Recreation Area, 37°39′54.00″ N, 121°50′20.40″ W, elev. 120 m. VII-24-2011, coll. P.L. Dallara, ex: xylem of a 2 cm diameter *Juglans hindsii* branch section infested with *Pityophthorus juglandis*.

Discussion

For WTB, we report three larval instars, and this situation concurs with about half of the 52 examples in the literature (Table 7). Accurate determination of instar number by head capsule width analysis requires samples of larvae that contain all instar classes and histograms with distinguishable peaks in the distribution of measurements (Gaines & Campbell 1935, Lekander 1968). For most scolytids, the probability that all instar classes are present increases with the number of galleries that are sampled and with the presence of viable eggs and pupae in the galleries with the larvae. Assuming a normal distribution of measures within each instar, production of histograms with distinguishable peaks is dependent on sample size; head capsule width variability within an instar; selection of measurement intervals; and the recognition of subgroups within peaks (e.g., larval sexual dimorphism). Caution is also warranted when counts of larvae within instars are reported, particularly when instar distributions overlap. Within the Scolytidae, our study and that of *Dendroctonus ponderosae* Hopkins larval instars (Logan et al. 1998) use methodically developed classification rules for definition of the subgroups.

In addition to the similarity in the number of larval instars relative to other Scolytidae, the range of WTB larval growth ratios (1.27–1.39) (Tables 3 and 5) also concurs with published data from other Scolytidae [e.g., 1.28–1.43 for *D. ponderosae*

Table 7. Larval instars of bark and ambrosia beetles by tribe and species.

Tribe	Species	Number of Instars	Reference
Hylastini	*Hylurgops palliatus* (Gyllenhal)	4	Lekander (1968)
	Hylurgops rugipennis (Mannerheim)	4	Reid (1955)
	Hylastes brunneus Erichson	4	Lekander (1968)
Hylesinini	*Hylesinus californicus* (Swaine)	3	Langor & Hergert (1993)
	Hylesinus fraxini (Panzer) = *H. varius* (F.)	3	Lekander (1968)
Tomicini	*Chaetoptelius vestitus* (Mulsant & Rey)[a]	5	Russo (1926)
	Hylurgopinus rufipes (Eichhoff)	5	Becker (1939), Kaston & Riggs (1937)
	Dendroctonus frontalis Zimmerman	4	Mizell & Nebeker (1979)
	Dendroctonus micans (Kugelann)	5	Gøhrn et al. (1954)
	Dendroctonus ponderosae Hopkins	4	Reid (1962), Logan et al. (1998)
	Dendroctonus pseudotsugae Hopkins[b]	4	Vité & Rudinsky (1957)
	Dendroctonus simplex LeConte	4	Prebble (1933), Langor & Raske (1987)
	Tomicus minor (Hartig)	4	Lekander (1968), Fernández et al. (1999)
	Tomicus piniperda (L.)	4	Lekander (1968)
Polygraphini	*Polygraphus poligraphus* (L.)	3	Lekander (1959)
Scolytini	*Ernoporus tiliae* (Panzer)	2	Lekander (1968)
	Scolytus intricatus (Ratzeburg)	5 or 6	Yates (1984)
	Scolytus tsugae (Swaine)	4	McMullen & Atkins (1959)
	Scolytus unispinosus LeConte	4	McMullen & Atkins (1962)
Crypturgini	*Crypturgus cinereus* (Herbst)	2	Lekander (1968)
Dryocoetini	*Dryocoetes autographus* (Ratzeburg)	3	Lekander (1968)
Ipini	*Pityogenes chalcographus* (L.)	3	Lekander (1968)
	Pityogenes hopkinsi Swaine[d]	5	Blackman (1915)
	Pityogenes knechteli Swaine[c]	4	Reid (1955)
	Pityogenes quadridens (Herbst)	3	Lekander (1968)
	Pityokteines (*Ips*) *curvidens* (Germar)	3	Hierholzer (1954)
	Pityokteines sparsus (LeConte)	3	Prebble (1933), Hosking & Knight (1976)
	Orthotomicus laricis (Fabricius)	3	Lekander (1968)
	Orthotomicus proximus (Eichhoff)	3	Lekander (1968)
	Orthotomicus suturalis (Gyllenhal)	3	Lekander (1968)
	Orthotomicus vicinus (LeConte)	3	Reid (1955)
	Ips amitinus (Eichhoff)	3	Witrylak (2008)
	Ips avulsus (Eichhoff)	3	Wilkinson (1963)
	Ips calligraphus (Germar)	3	Wood & Stark (1964), Wilkinson (1963)
	Ips cembrae (Heer)	3	Balogun (1970)
	Ips grandicollis (Eichhoff)	3	Wilkinson (1963)
	Ips latidens (LeConte)	3	Miller & Borden (1985)

Table 7. Continued.

Tribe	Species	Number of Instars	Reference
	Ips (*Pseudips*) *mexicanus* (Hopkins)	4	Smith et al. (2009)
	Ips perroti Swaine	3	Reid (1955)
	Ips pini (Say)	3	Prebble (1933), Thomas (1961), Reid (1955)
	Ips sexdentatus (Boerner)	3	Lekander (1968)
	Ips typographus (L.)	3	Lekander (1968)
Xyloterini	*Trypodendron lineatum* (Olivier)[e]	2 or 4	Hadorn (1933), Novák (1960), Lekander (1968)
Xyleborini	*Xylosandrus germanus* (Blandford)	3	Weber & McPherson (1983)
	Xyleborus cryptographus (Ratzeburg)	3	Lekander (1968)
Cryphalini	*Cryphalus abietis* (Ratzeburg) = *C. asperatus* (Gyllenhall)	2	Lekander (1968)
Corthylini	*Conophthorus* sp.	2	Herdy & Thomas (1961)
	Pityophthorus confertus Swaine	2	Amman et al. (1974)
	Pityophthorus juglandis Blackman	3	This study
	Pityophthorus micrographus (L.)	3	Lekander (1968)
	Pityophthorus orarius Bright	3	Hedlin & Ruth (1970)

[a] Wood (1982) suggested that reports of five larval instars for the Scolytidae should be verified before acceptance. Organization of tribes, genera, and species based on Wood (1982).

[b] Bedard (1933) suggested that this species had five instars.

[c] Reid (1955) noted that his data could indicate four or five instars. However, the potential for sexual dimorphism in this species (see text) suggests that *P. knechteli* may have three instars.

[d] Lekander (1968) believed that this estimate was an error and hypothesized that *P. hopkinsi* had three instars.

[e] Hadorn (1933) determined that there were four instars, whereas Novák (1960) and Lekander (1968) reported two instars.

(Logan et al. 1998); 1.32–1.40 for *Hylesinus californicus* (Swaine) (Langor & Hergert 1993); 1.32–1.37 for *Ips* (*Pseudips*) *mexicanus* (Hopkins) (Smith et al. 2009); 1.27–1.40 for *Ips pini* (Say) (Prebble 1933, Reid 1955, Thomas 1961); 1.25–1.44 for *Pityokteines sparsus* (LeConte) (Prebble 1933, Hosking & Knight 1976); 1.36–1.45 for *Pityophthorus confertus* Swaine (Amman et al. 1974); 1.19 for *Pityophthorus micrographus* L. (Lekander 1968); and 1.24–1.31 for *Scolytus unispinosus* LeConte (McMullen & Atkins 1962)]. When we estimated the larval growth ratios for *Pityophthorus orarius* Bright from published histograms (Hedlin & Ruth 1970), they also fall into the ranges listed above for other species.

NLLS modeling of the Alameda Co. WTB third-instar larval distribution yielded two Gaussian distributions centered on similar means. If we assume that the Brooks-Dyar rule (geometric growth) pertains in this case, the improved fit is evidence for two subgroups within the third instar and not a fourth instar. The presence of two subgroups in the third instar may be an indication of WTB larval sexual dimorphism; a similar late-instar split peak in the cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), was shown to represent separate sexes (Hoxie & Wellso 1974). Furthermore, Becker (1939) hypothesized that larval sexual dimorphism explained an unusual head capsule width distribution in the final and penultimate instars of *Hylurgopinus rufipes* (Eich.) (Scolytidae). Inspection of published histograms (Prebble 1933, Reid 1955, Thomas 1961) also reveals that sexual dimorphism may be present in the final larval instars of *I. pini*, *Pityogenes knechteli* Swaine, and *Hylurgops rugipennis* (*pinifex*) (Mannerheim) (all Scolytidae). With *P. knechteli*, a re-evaluation of instar number under the hypothesis of larval sexual dimorphism would result in 3 instead of the 4–5 instars suggested by Reid (1955). Furthermore, if this species had three instars, then the Brooks-Dyar rule would be satisfied.

If sexual dimorphism were present in the WTB third-instar larvae in the Alameda Co. sample, the unexpectedly small variance about the mean head capsule width from the Yolo Co. sample could indicate the presence of a single sex in this instar (or in all instars of the sample). Since the Yolo Co. sample was derived from parental females alone colonizing the branches, we hypothesize that the progeny may have been exclusively female. We assumed that the female parents in the case of this laboratory study had been mated by their siblings prior to emergence or by mates prior to re-emergence. Future research should investigate the artificial colonization of branches by females alone to determine the sex ratio of the emerging brood adults. It would be interesting if only female progeny are produced under these circumstances.

As we consider the general methods that we have used in this study, we note that Kishi (1971) argued that head capsule measurement for the determination of larval instars is less reliable than counting larval exuviae in galleries. However in our dissections, complete series of larval exuviae were difficult to find and rarely observed. This was likely due to the consumption or breakdown of exuviae by detritivores, which are ubiquitous in the subcortical galleries. The fragility and fleeting presence of the exuviae is likely to be an obstacle to using this method for nearly all species of Scolytidae.

Daly (1985) argued that accurate determination of instar number requires evaluation of both laboratory- and field-reared larval head capsule measures. Our results from modeling laboratory-reared larvae (Yolo Co.) were quite similar to those from field-reared larvae (Alameda Co.). Means for corresponding instars fell within 1 SD (Tables 2 and 4) and growth rates appeared to have comparable slopes (Fig. 4). This was somewhat unexpected, as the Alameda Co. larvae developed in a much more heterogeneous environment than the Yolo Co. larvae. Field conditions included varying bark quality, microclimate, seasonal effects, and probably greater genetic variability of parents, all factors that would be expected to influence developmental rates and head capsule size (Kaston & Riggs 1937, Mizell & Nebeker 1979, Logan et al. 1998).

Although the results from both of our samples were similar, we elected to use only the Alameda Co. sample for the three-parameter practical classification. We reasoned that future workers would encounter primarily field-reared larvae from galleries containing both male and female parents. Furthermore, we had a two-fold larger sample of WTB larvae from Alameda than Yolo Co. Given the recent expansion of WTB geographic and host range (Seybold et al. 2011, 2012a), development of circumstantial site- and host-specific instar classification rules would be advisable to augment life history studies and provide information affecting management decisions for other regions and hosts.

Colonization, Emergence, and Flight Activity of Hylocurus hirtellus. Our survey established the first records of colonization and development in *Juglans* spp. by *H. hirtellus*; helped to define the flight activity of the adult from early March to early May in northern California; and documented that the female is the first to colonize the xylem of *Juglans*. The caaracterization of this species as univoltine (Bright & Stark 1973) was supported directly by our observations of the initiation of breeding in *J. hindsil* in July 2011 followed by emergence of adults (likely brood adults) in June and July 2012, and indirectly by our data on the relatively narrow spring window of flight and by the likely re-emergence of a parent adult in July/August 2010 from a windfallen branch section of *J. hindsii* that had been colonized earlier in the year. We have also documented the colonization by *H. hirtellus* in the same 2–5 cm diameter branch sections of *J. hindsii* as WTB. Our measurements of the body lengths of specimens of *H. hirtellus* collected in Alameda Co. agree with those of Bright (1976) (2.4 to 3.0 mm). Furthermore, a series of 34 specimens (16 male, 17 female, 1 undetermined) collected in northern California (Merced, Santa Clara, and Santa Cruz Cos.) and Oregon (Multnomah Co.) were borrowed from the J. Gordon Edwards Museum of Entomology (San Jose State University), and these adults ranged in body length from 2.56 to 3.12 mm. Female lengths ranged from 2.70 to 3.12 mm ($n = 15$; mean $\pm$ SE = 2.90 $\pm$ 0.127 mm); male lengths ranged from 2.56 to 2.96 mm ($n = 16$; mean $\pm$ SE = 2.78 $\pm$ 0.132 mm). Given our results from newly collected Alameda Co. specimens and museum specimens, the length measurements for *H. hirtellus* reported by Wood (1982) (1.4 to 1.8 mm) are inaccurate.

The relative abundance of *H. hirtellus* at our field site in coastal northern California is congruent with the published distribution in the state (Bright & Stark 1973). It was noted in the literature as early as the late 1800s as "injurious" to California bay-laurel, *Umbellularia californica* (Hook. & Arn.) Nutt. (Packard 1890), which is a distinctively Pacific coast tree species. Subsequent historic northern California collection localities in the USDA Forest Service Hopkins U.S. System Index (HUSSI) include San Francisco (1913), Berkeley (1914), Palo Alto (1918), San Jose (1920 and 1927), and Arcata (1951). Beyond California, records for *H. hirtellus* are from British Columbia, Washington, Oregon, and Baja California (Wood & Bright 1992, Bright & Skidmore 1997). Wood & Bright (1992) listed the following hosts for *H. hirtellus*: *Alnus* sp., *Arbutus menzeisii*, *Ceanothus* sp., *Myrica* sp., *Populus* sp., *Quercus* sp., *Rhamnus* sp., *Rhus* sp., *Salix* sp., and *Umbellularia* sp. An additional host listed in HUSSI is *Ulmus campestris* (record #16152d). Chamberlain (1958) lists bigleaf maple, *Acer macrophyllum* Pursh, as a host; Humble et al. (2000) also listed *Populus* sp. and *Salix scouleriana* as hosts from which adults were reared in British Columbia. The genera *Alnus, Populus, Quercus, Rhus, Salix*, and *Umbellularia* are represented at the Alameda Co. site, with *Populus* and *Salix* being perhaps the most abundant. Since *H. hirtellus* has been documented as polyphagous on many hardwood trees and shrubs, its use of walnut as a host is not surprising.

Observations on the biology and taxonomy of *H. hirtellus* are relatively rare; no new reports were documented in the second supplement to the world catalog (Bright & Skidmore 2002). More research needs to be conducted on its interactions beneath the bark with *P. juglandis*, and in particular the synchrony of colonization of *J. hindsii* by both species of Scolytidae. The potential for *H. hirtellus* to vector *G. morbida* is also of interest, though preliminary data from our lab with another woodboring scolytid, *Xyleborinus saxeseni* Ratz., suggests that as a group, woodborers may not carry the pathogen of thousand cankers disease.

Acknowledgments

We dedicate this manuscript to the late Richard L. Penrose. ''Dick'' was a good friend and colleague, whose enthusiasm for the detection of invasive bark beetles and woodborers was contagious and stimulating to those around him. From 2004 through 2008, Dick had a big impact on the direction of research in our labs by helping us shift focus from the chemical ecology of native forest insects to using the tools of chemical ecology to detect invading species in urban and peri-urban forest environments. He had a strong, positive influence on the research activities of postdoctoral associates, Jana C. Lee and Deguang Liu, who worked with us on invading elm and pine bark beetles. Dick also assisted with countless identifications of new bark beetles and longhorned beetles. Due to his ever perceptive ''nose'' for new problems, from 2003–2007 Dick did some focused early ''modern'' collecting of walnut twig beetles in California. Both from funnel trap catches in his annual CAPS surveys and through rearing of infested walnut, Dick's efforts presaged the current bloom in research on walnut twig beetle, which includes the discoveries reported in this paper. We thank A.D. Graves (USDA Forest Service, Albuquerque, NM) for assistance with preparing the Yolo Co. WTB samples, and S.M. Hishinuma (UC Davis, Department of Entomology, Davis, California) and R.C. Venette (USDA Forest Service, St. Paul, Minnesota) for critical reviews of the manuscript. S.M. Hishinuma and J.A. Tillman assisted with the graphics for this paper. Funding for this work was provided by the USDA Forest Service, Pacific Southwest Research Station, and by grants from the USDA Forest Service, Washington Office Forest Health Protection and USDA APHIS CPHST administered through cooperative agreements #10-CA-11272172-055 and 10-JV-11272172-092 between the USDA FS PSW Station and the UC Davis Department of Entomology. The East Bay Regional Parks District allowed the use of park properties for collection and field trials. We thank J. Honda for facilitating the loan of the specimens of *H. hirtellus* fron San Jose State University and D. E. Bright for determination of *H. hirtellus*.

Literature Cited

Amman, A. G., S. L. Amman & G. D. Amman. 1974. Development of *Pityophthorus confertus. Environmental Entomology* 3:562–562.

Armitage, H. M. 1952. Bureau of Entomology Report. 33rd Annual Report, State of California Department of Agriculture, Sacramento, California, 41(4):218.

Balogun, R. A. 1970. The life-history and habits of the larch bark beetle, *Ips cembrae* (Coleoptera: Scolytidae), in the north-east of Scotland. *The Canadian Entomologist* 102:226–239.

Beaver, R. J. & J. P. Sanderson. 1989. Classifying instars of the navel orangeworm (Lepidoptera: Pyralidae) based on observed head capsule widths. *Journal of Economic Entomology* 82:716–720.

Becker, W. B. 1939. Larval development of the native elm bark beetle, *Hylurgopinus rufipes* (Eich.), in Massachusetts. *Journal of Economic Entomology* 32:112–120.

Bedard, W. D. 1933. Number of larval instars of *Dendroctonus pseudotsugae* Hopk. *Journal of Economic Entomology* 26:1128–1134.

Blackman, M. W. 1915. Observations on the life history and habits of *Pityogenes hopkinsi* Swaine. *New York State College Forestry Technical Bulletin* 16:11–66.

Bright, D. E., Jr. 1976. *The Insects and Arachnids of Canada. Part 2: The Bark Beetles of Canada and Alaska, Coleoptera: Scolytidae*. Canada Department of Agriculture, Ottawa, Ontario, Canada, Publication 1576, 241 pp.

Bright, D. E., Jr. 1981. Taxonomic monograph of the genus *Pityophthorus* Eichhoff in North and Central America. *Memoirs of the Entomological Society of Canada* No. 118, 378 pp.

Bright, D. E., Jr. & R. E. Skidmore. 1997. A Catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 1 (1990–1994). NRC Research Press, Ottawa, Canada, 368 pp.

Bright, D. E., Jr. & R. E. Skidmore. 2002. A Catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 2 (1995–1999). NRC Research Press, Ottawa, Canada, 523 pp.

Bright, D. E., Jr. & R. W. Stark. 1973. The Bark and Ambrosia Beetles of California, Coleoptera: Scolytidae and Platypodidae. Bulletin of the California Insect Survey, Volume 16, University of California Press, Berkeley, California, U.S.A., 169 pp.

Caltagirone, L. E., W. Getz & D. W. Meals. 1983. Head capsule width as an index of age in larvae of navel orangeworm, *Amyelois stansitella* (Lepidoptera: Pyralidae). *Environmental Entomology* 12:219–221.

Chamberlain, W. J. 1958. The Scolytoidea of the Northwest: Oregon, Washington, Idaho, and British Columbia, Oregon State College Press, Corvallis, Oregon, 208 pp.

Daly, H. V. 1985. Insect morphometrics. *Annual Review of Entomology* 30:415–438.

Doane, R. W., E. C. Van Dyke, W. J. Chamberlain & H. E. Burke. 1936. *Forest Insects*. McGraw-Hill Book Co., Inc., New York, 463 pp.

Dyar, H. G. 1890. The number of molts of lepidopterous larvae. *Psyche* 5:420–422.

Fernández, M. M. F., J. A. P. Alonso & J. M. S. Costas. 1999. Oviposition and development of the immature stages of *Tomicus minor* (Coleoptera, Scolytidae). *Agricultural and Forest Entomology* 1:97–102.

Gaines, J. C. & F. L. Campbell. 1935. Dyar's rule as related to the instars of the corn earworm *Heliothis obsoleta* (Fab.), collected in the field. *Annals of the Entomological Society of America* 28:445–461.

Godin, J., P. Maltais & S. Gaudet. 2002. Head capsule width as an instar indicator for larvae of the cranberry fruitworm (Lepidoptera: Pyralidae) in southeastern New Brunswick. *Journal of Economic Entomology* 95:1308–1313.

Gøhrn, V., H. A. Henriksen & B. Bejer-Petersen. 1954. Jagttagelser over *Hylesinus* (*Dendroctonus*) *micans*. *Forstlige Forsogsvaesen Danmark* 21(4):383–483.

Got, B. 1988. Determination of instar of the European corn borer (Lepidoptera: Pyralidae) based on a distribution model of head capsule widths. *Annals of the Entomological Society of America* 81:91–98.

Graves, A. D., T. W. Coleman, M. L. Flint & S. J. Seybold. 2009. Walnut twig beetle and thousand cankers disease: Field identification guide, UC-IPM Website Publication, 2 pp. Available from http://www.ipm.ucdavis.edu/PDF/MISC/thousand_cankers_field_guide.pdf (accessed 25 July, 2012).

Hadorn, C. 1933. Recherches sur la morphologie, les stades evolutifs et l'hivernage du bostryche lisere (*Xyloterus lineatus* Oliv.). *Beiheft zu den Zeitschriften des Schweizerischen Forstvereins* 11:1–120.

Hedlin, A. F. & D. S. Ruth. 1970. A Douglas-fir twig mining beetle, *Pityophthorus orarius* (Coleoptera: Scolytidae). *The Canadian Entomologist* 102:105–108.

Herdy, H. & J. B. Thomas. 1961. The seasonal development of a species of *Conophthorus* (Hopkins) (Coleoptera: Scolytidae) in the shoots of jack pine, *Pinus banksiana* (Lamb.), in Ontario. *The Canadian Entomologist* 93:936–940.

Hierholzer, O. 1954. Die Massenvermehreung der krummzähnigen Tannenborkenkäfer in Württemberg-Hehenzollern von 1947–1950, pp. 329–384. *In*: G. Wellenstein (Ed.). *Die grosse Borkenkäferkalamität in Sudwestdeutschland 1944–1951*. Ebener, Ulm, Germany.

Hosking, G. P. & F. B. Knight. 1976. Investigations on the life history and habits of *Pityokteines sparsus* (Coleoptera: Scolytidae). Life Sciences and Agriculture Experiment Station, University of Maine at Orono, Technical Bulletin 81, 37 pp.

Hoxie, R. P. & S. G. Wellso. 1974. Cereal leaf beetle instars and sex, defined by larval head capsule widths. *Annals of the Entomological Society of America* 67:183–186.

Humble, L. M., N. N. Winchester & R. A. Ring. 2000. The potentially rare and endangered terrestrial arthropods in British Columbia: Revisiting British Columbia's Biodiversity. Proceedings of a Conference on the Biology and Management of Species and Habitats at Risk, Kamloops, B. C., 15–19 February 1999, Volume 1, B.C. Ministry of Environment, Lands and Parks, Victoria, B.C. and University College of the Cariboo, Kamloops, B.C., L. M. Darling (Ed.), 490 pp., 101–108 pp.

Kaston, B. J. & D. S. Riggs. 1937. Studies on the larvae of the native elm bark beetle. *Journal of Economic Entomology* 30:98–108.

Keen, F. P. 1952. *Insect Enemies of Western Forests*. USDA Misc. Publ. No. 273, 280 pp.

Kishi, Y. 1971. Reconsideration of the method to measure the larval instars by use of the frequency distribution of head capsule widths and lengths. *The Canadian Entomologist* 103:1011–1015.

Kolařik, M., E. Freeland, C. Utley & N. Tisserat. 2011. *Geosmithia morbida* sp. nov., a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on *Juglans* in U.S.A. *Mycologia* 103:325–332.

Langor, D. W. & C. R. Hergert. 1993. Life history, behavior, and mortality of the western ash bark beetle, *Hylesinus californicus* (Swaine) (Coleoptera: Scolytidae), in southern Alberta. *The Canadian Entomologist* 125:801–814.

Langor, D. W. & A. G. Raske. 1987. Reproduction and development of the eastern larch beetle *Dendroctonus simplex* LeConte (Coleoptera: Scolytidae), in Newfoundland. *The Canadian Entomologist* 119:985–992.

Lekander, A. B. 1959. Der doppelaugige Fichtenbastkäfer *Polygraphus poligraphus* L. Ein Beitrag zur kenntnis seiner Morphologie, anatomie, Biologie, und Bekämpfung. *Meddleander Fran Statens Skogsforskningsinstitut* 48(9):1–127.

Lekander, A. B. 1968. The number of larval instars in some bark beetle species. *Entomologisk Tidskrift* 89:25–34.

Logan, J. A., B. J. Bentz, J. C. Vandygriff & D. L. Turner. 1998. General program for determining instar distributions from headcapsule widths: Example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. *Environmental Entomology* 27:555–563.

McClellan, Q. C. & J. A. Logan. 1994. Instar determination for the gypsy moth (Lepidoptera: Lymantriidae) based on the frequency distribution of head capsule widths. *Environmental Entomology* 23:248–253.

McMullen, L. H. & M. D. Atkins. 1959. Life-history and habits of *Scolytus tsugae* (Swaine) (Coleoptera: Scolytidae) in the interior of British Columbia. *The Canadian Entomologist* 91:416–426.

McMullen, L. H. & M. D. Atkins. 1962. The life history and habits of *Scolytus unispinosus* LeConte (Coleoptera: Scolytidae) in the interior of British Columbia. *The Canadian Entomologist* 94:17–25.

Miller, D. R. & J. H. Borden. 1985. Life history and biology of *Ips latidens* (LeConte) (Coleoptera: Scolytidae). *The Canadian Entomologist* 117:859–871.

Mizell, R. & T. E. Nebeker. 1979. Number of instars of the southern pine beetle (Coleoptera: Scolytidae) and some comparisons of head capsule widths. *Annals of the Entomological Society of America* 72:313–316.

Novák, V. 1960. Dřevokaz čárkovaný a boj proti němu [*Trypodendron lineatum* and its control]. Státní Zemèdělské Nakladatelstvi, Prague, Czechoslovakia, *Lesnické Aktuality* 1, 129 pp.

Packard, A. S. 1890. *Insects Injurious to Forest and Shade Trees*. USDA, Fifth Report of the United States Entomological Commission, Bulletin No. 7, Washington, D.C., 670 pp.

Panzavolta, T. 2007. Instar determination for *Pissodes castaneus* (Coleoptera: Curculionidae) using head capsule widths and lengths. *Environmental Entomology* 36:1054–1058.

Prebble, M. L. 1933. The larval development of three bark-beetles. *The Canadian Entomologist* 65:145–150.

Reid, R. W. 1955. The bark beetle complex associated with lodgepole pine slash in Alberta. *The Canadian Entomologist* 87:311–323.

Reid, R. W. 1962. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the East Kootenay region of British Columbia. I. Life cycle, brood development and flight periods. *The Canadian Entomologist* 94:531–538.

Russo, G. 1926. Contirbuto alla conoscenza degli Scolytidi. Studio morfo-biologico del *Chaetoptelius vestitus* (Muls. & Rey) Fuchs e dei suoi simbionti. *Bolletino del Laboratorio di Zoologica Generale e Agraria R. Scuola Sup. Agric. Portici* 19:103–260.

Seybold, S. J., D. Haugen, J. O'Brien & A. D. Graves. 2011. Thousand cankers disease. USDA Forest Service, Northeastern Area State and Private Forestry Pest Alert, NA-PR-02-10, May 2010, reprinted August 2010 and October 2011, 2 pp., http://www.na.fs.fed.us/pubs/detail.cfm?id=5225 (accessed 25 July, 2012).

Seybold, S. J., T. W. Coleman, P. L. Dallara, N. L. Dart, A. D. Graves, L. Pederson & S.-E. Spichiger. 2012a. Recent collecting reveals new state records and the extremes in the distribution of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in the United States. *The Pan-Pacific Entomologist* 88:377–380.

Seybold, S. J., P. L. Dallara, S. M. Hishinuma & M. L. Flint. 2012b. Detecting and identifying the walnut twig beetle: Monitoring guidelines for the invasive vector of thousand cankers disease of walnut, University of California Agriculture and Natural Resources, Statewide Integrated Pest Management Program, 11 pp., http://www.ipm.ucdavis.edu/PMG/menu.thousandcankers.html (accessed 25 July 2012).

Seybold, S. J., P. L. Dallara, L. J. Nelson, A. D. Graves, S. M. Hishinuma & R. Gries. 2012c. Methods of monitoring and controlling the walnut twig beetle, *Pityophthorus juglandis*. Patent application filed with the U.S. Patent and Trademark Office, U.S. Dept. of Commerce, 13 July, 2012, U.S. Patent Application No. 13/548.319, 38 pp. + 7 Figs.

Smith, G. D., A. L. Carroll & B. S. Lindgren. 2009. Life history of a secondary bark beetle, *Pseudips mexicanus* (Coleoptera: Curculionidae: Scolytinae), in lodgepole pine in British Columbia. *The Canadian Entomologist* 141:56–69.

Sokal, R. R. & F. J. Rohlf. 1995. *Biometry.* 3rd ed. W. H. Freeman, New York, 887 pp.

Struble, G. R. & R. C. Hall. 1954. Telephone cables invaded by shrub bark beetle in Pacific coastal region. *Journal of Economic Entomology* 47:933–934.

Thomas, J. B. 1961. The life history of *Ips pini* (Say) (Coleoptera: Scolytidae). *The Canadian Entomologist* 93:384–390.

Tisserat, N., W. Cranshaw, M. Putnam, J. Pscheidt, C. A. Leslie, M. Murray, J. Hoffman, Y. Barkely, K. Alexander & S. J. Seybold. 2011. Thousand cankers disease is widespread on black walnut, *Juglans nigra*, in the western United States. Published online only. *Plant Health Progr*. (30 June 2011) doi:10.1094/PHP-2011-0630-01-BR.

Vité, J. P. & J. A. Rudinsky. 1957. Contribution toward a study of Douglas-fir beetle development. *Forest Science* 3:2.

Weber, B. C. & J. E. McPherson. 1983. Life history of the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* 76:455–462.

Wilkinson, R. C. 1963. Larval instars and head capsule morphology in three southeastern *Ips* bark beetles. *The Florida Entomologist* 46:19–22.

Witrylak, M. 2008. Studies of the biology, ecology, phenology, and economic importance *of Ips amitinus* (Eichhoff) (Coleoptera: Scolytidae) in experimental forests of Krynica (Beskid Sadecki, southern Poland). *Acta Scientiarum Polonorum: Silvarum Colendarum Ratio et Industria Lignaria* 7:75–92.

Wood, D. L. & R. W. Stark. 1968. The life history of *Ips calligraphus* (Coleoptera: Scolytidae) with notes on its biology in California. *The Canadian Entomologist* 100:145–151.

Wood, S. L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* No. 6.

Wood, S. L. 2007. *Bark and Ambrosia Beetles of South America (Coleoptera, Scolytidae)*. Brigham Young University, M.L. Bean Life Science Museum, Provo, Utah, 900 pp.

Wood, S. L. & D. E. Bright, Jr. 1992. A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index, Volume B. *Great Basin Naturalist Memoirs* No. 13, 1553 pp.

Yates, M. G. 1984. The biology of the oak bark beetle, *Scolytus intricatus* (Ratzeburg) (Coleoptera: Scolytidae), in southern England. *Bulletin of Entomological Research* 74:569–579.

Received 7 Mar 2012; Accepted 7 June 2012 by F. W. Shockley; Publication date 14 Sep 2012.