DETERMINING BOLL WEEVIL AGE WITH CUTICULAR HYDROCARBON PROFILES

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Abstract

Cuticular hydrocarbons were extracted from the surface of boll weevils of different ages, from the day of eclosion through 49 days of age. Gas chromatography revealed 13 components that change in amount with age. The presence of three components, *n*-nonacosane, 15-&13-methylhentriacontane, and an unidentified compound consisting of 29 carbons with either two or three double bonds, and/or containing a ring moiety, is diagnostic of boll weevils <2 days old. Principal components (PC) analysis produced an eigenvector that explains 66.8% of the variance, and linear regression of principal components (PC) factor scores on age revealed a relationship that may prove useful in estimating the age of field captured boll weevils.

Introduction

One of the most valuable sampling tools available to boll weevil researchers is the pheromone trap. This tool is more versatile, in terms of the potential information it can provide, than its counterparts available for most other insects because it is baited with an aggregation pheromone that attracts both sexes. On the other hand, it has proven difficult to tap the full research potential of the boll weevil pheromone trap, because interpretation of trap captures is usually problematic. Part of the problem stems from seasonal, day to day, and site to site factors which generate fluctuations in trap captures independent of weevil distribution and population levels. Beyond this, however, we generally do not know which segment of the population is being sampled on any given day. It is extremely unlikely that pheromone trap captures represent a random sample of the weevils in a nearby population. In other words, different segments of a population may be more or less subject to capture at a given time because of differences in behavior associated with differences in physiology, dietary history, age, reproductive status, mating status, and phenology of the host crop.

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A void exists in our understanding of boll weevil flight behavior and the way in which that behavior influences weevil dispersal. Movement and dispersal behavior of the boll weevil has a seasonal component that is only vaguely understood, and yet an ability to predict timing and extent of weevil movement is essential to more efficient management of this pest. There is general agreement that interfield movement increases near the end of the growing season (e.g., Fenton and Dunnam 1928, Hopkins et al. 1971), but factors generating such movement are not clearly defined. Deteriorating food supply and lack of suitable squares for oviposition as the crop matures and weevil densities increase may be important factors initiating late season dispersal of weevils (Fenton and Dunnam 1928, Guerra 1986). In addition, there may be an intrinsic period of dispersal activity. In many insects, migratory behavior coincides with the prereproductive period or a period of reproductive diapause, a pattern designated the oogenesis-flight syndrome (Johnson 1969, Sappington and Showers 1992). Consistent with this idea in the case of the boll weevil, peaks in flight activity sometimes appear to coincide with the emergence of generations (Isley 1926, Hopkins et al. 1971). Although boll weevils undergo a period of reproductive dormancy in the non-host season, it is not clear if this state is directly related to dispersal activity. In tethered flight experiments, weevils with chorionated eggs tended to make flights of shorter duration than weevils with less developed ovaries (Rankin et al. 1994). However, the synthesis and uptake of vitellogenin, the major yolk protein precursor in most insects including boll weevil (Sappington and Raikhel 1998), were not correlated with flight behavior (Rankin et al. 1994), and the same hormone (juvenile hormone) induces both flight and oogenesis in this insect (Rankin and Burchsted 1992). Thus, the oogenesis flight syndrome may not be an appropriate descriptor of weevil dispersal behavior. Efforts to understand this difficult but extremely important aspect of boll weevil ecology will be facilitated to the extent that we can improve our ability to interpret pheromone trap data.

Both the study of boll weevil dispersal behavior and efforts to monitor and interpret dispersal activity with pheromone traps could be aided if the age structure of the source population and of the weevils being sampled by the traps could be assessed. Combined with dissection data, such information would provide insights into the timing of dispersal behavior within the weevil life cycle. Conventional wisdom is that very young weevils look “red” compared to older more “brown” weevils. However, our experience has shown that color is an unreliable indicator of age, and offers little precision in age estimates (e.g., a “brown” [old] weevil could be from 5 days to 5 months old).

Cuticular hydrocarbons are present on the surface of all insects, serving primarily as a barrier to water loss, though they may also function as pheromones and a defensive barrier

against microorganisms (Blomquist et al. 1987). Profiles of the hydrocarbon components change with age in many arthropods (Sappington and Taylor 1990b), including other coleopterans (Peschke 1987), and provide a potential clock for determining the age of individuals. In this paper, we report progress in developing a method for determining boll weevil age based on age-related changes in cuticular hydrocarbon profiles.

Materials and Methods

Collection of Boll Weevils

Infested squares were collected from areas of regrowth or volunteer cotton in the Lower Rio Grande Valley of Texas in December 1998 and maintained at room temperature. Pupae were harvested from the infested squares and placed on moist vermiculite in glass petri plates. Through all stages of rearing, weevils were isolated from plastic materials, because phthalate from the plastic can contaminate the insect's surface hydrocarbons, making interpretation of gas chromatograms more difficult. Petri plates containing pupae were maintained at room temperature, and checked daily for eclosion. Newly eclosed weevils were sexed by the tergal notch method (Agee 1964), as described by Sappington and Spurgeon (2000), and placed in cartons separated by sex and date of emergence. Adult weevils were maintained at a constant 29.4ºC (85ºF), and a 13:11 (L:D) photoperiod. They were fed fresh squares daily at a rate of 1 square per 5 weevils.

Hydrocarbon Extraction

Weevils selected for extraction at a given age were removed from the rearing cartons, placed in new cartons segregated by sex, and killed by freezing at -80ºC for at least 30 min. Weevils were placed individually in small glass vials and swirled gently for 2 min in 1 ml of hexane to remove the surface hydrocarbons. The hexane was evaporated under a stream of nitrogen, and the samples were stored at room temperature. Hydrocarbons from 5 weevils of each sex were extracted for ages of 0 through 14, 21, 28, 35, 42, and 49 days.

Gas Chromatography and Mass Spectroscopy (GC-MS)

Samples were reconstituted in 200 µl hexane with 2 µg each of *n*-octacosane (C28) and *n*-tetratriacontane (C34) as internal standards. Five µl of samples were injected with a 5:1 split at 250ºC into a Hewlett Packard 6890 series gas chromatograph (GC) equipped with an autosampler. Hydrocarbons were separated on a 25 m x 0.32 mm (length x i.d.) Ultra 1 methyl siloxane (Hewlett Packard, Palo Alto, CA) capillary column. The column temperature started at 70º for 1 min, then was ramped to 280ºC at 20º/min where it was held for 15 min. Peaks were detected with a flame ionization detector and data were compiled and integrated with Hewlett Packard GC System software. Hydrocarbon profiles of individual weevils were compared by matching peak retention times and using the internal standards as landmarks. Areas under the peaks were quantified as ng per weevil based on the areas under the internal standard peaks.

We have begun to chemically identify the components of the boll weevil hydrocarbon mixture. Representative samples (1 µl from 15 µl) were analyzed in total as previously described (Nelson et al. 1994) by capillary GC-MS on a Hewlett Packard quadrupole system equipped with an autosampler and a temperature and pressure programmable cool on-column injection port. The injection port was connected to a 1-m retention gap connected to a 12-m x 0.2-mm capillary column of cross-linked dimethyl silicone Ultra 1 siloxane (Hewlett Packard, Palo Alto, CA). The column temperature was programmed from 150 to 320ºC at 4º/min. and then held at 320ºC for 5 min.

Results and Discussion

Several hydrocarbon components were observed to either increase (Fig. 1) or decrease (Fig. 2) in quantity with increasing age of weevils. Analyses of GC-MS data disclosed the identities of some of these hydrocarbons and provided clues for others (Table 1). Complete identification will require further GC-MS after derivatization of the unknown compounds. In general, the components are fairly large molecules, with the first eluting hydrocarbon of detectable quantity being *n*-pentacosane, a straight chain hydrocarbon of 25 carbons.

Three compounds appear to be diagnostic of weevils 2 days old or less (Fig. 2): *n*-nonacosane (straight chain 29 carbons), 15-&13-methylhentriacontane (31-C backbone with one methyl branch), and an unidentified compound consisting of a 29-C backbone with either two or three double bonds, or more likely, with a ring moiety. One unidentified compound, possibly a 31-C chain with a double bond, may be diagnostic of weevils more than 2 weeks old (Fig. 1C). However, it was present in very low abundance, and not all weevils $\geq$21 days old yielded detectable quantities. Other components tended to increase gradually with age.

Principal components were calculated from the hydrocarbon data using a correlation matrix, and the first two eigenvalues were > 1, indicating they were significant. The first eigenvector represents an axis contrasting peaks that tend to increase with age (Fig. 1) with peaks that tend to decrease (Fig. 2), and accounts for 66.8% of the variance. The second eigenvector appears to be a general quantity axis, but is difficult to interpret. Factor scores were generated from the eigenvector coefficients along the first axis for ages 2, 7, 14, 21, 28, 35, 42, and 49 days, and the means were subjected to regression analysis. The regressions indicated a significant ($F = 48.4$, df = 7, $P = 0.0004$, $r^2 = 0.89$) negative relationship between factor scores and age (Fig. 3).

McKibben and Robbins (1986) reported preliminary results in which boll weevil cuticular hydrocarbon profiles were used to distinguish local populations, but did not present details of their. They also indicated that overwintered weevils could be distinguished from mid-season weevils, but again provided no details. Our goal is to be able to take a captured weevil from the field, score its hydrocarbons, and estimate its age from relationships such as that illustrated in Fig. 3. Our results suggest we will be able to estimate weevil age using this method, but the precision of this method has not been determined. The hydrocarbon profiles of another set of weevils extracted at the same ages as those reported here are being generated, and will be used to determine the precision of our age estimates. We suspect predictions will be more accurate at the youngest ages and at the oldest ages where differences are most extreme.

Numerous factors that potentially can generate variation in cuticular hydrocarbon profiles must be assessed in the boll weevil before age determination can be approached with conviction. Preliminary analyses indicate that profiles of females and males differ, with quantities of components on females being consistently 20-30% greater than on males (T.W.S., unpublished data). However, there do not appear to be sex-related differences in general trends of component-change with age. Thus, it may be possible to improve the precision of age estimates by analyzing the sexes separately. Other factors known to contribute to hydrocarbon variation in other insects include size (Anderbrant et al. 1985), genetic variation (Sappington and Taylor 1990a, Ferveur and Jallon 1996), diet (Espelie et al. 1991, Schal et al. 1994), and environmental conditions such as temperature (Peschke 1987, Sappington and Taylor 1990b). In addition to testing the effects of such factors on boll weevil cuticular hydrocarbon profiles, we plan to expand the range of ages tested to include overwintered weevils.

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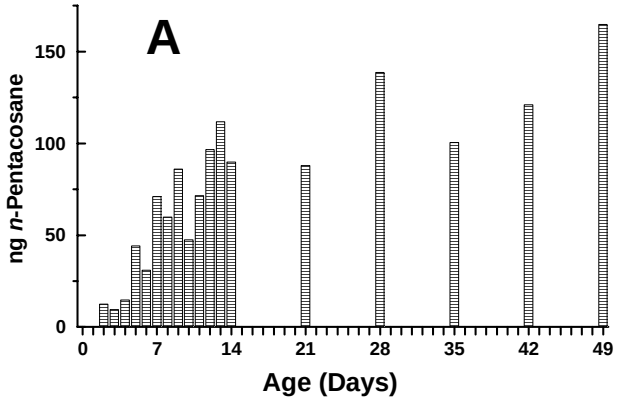
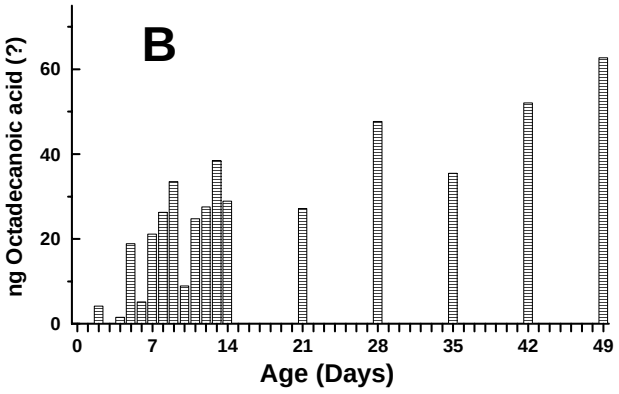
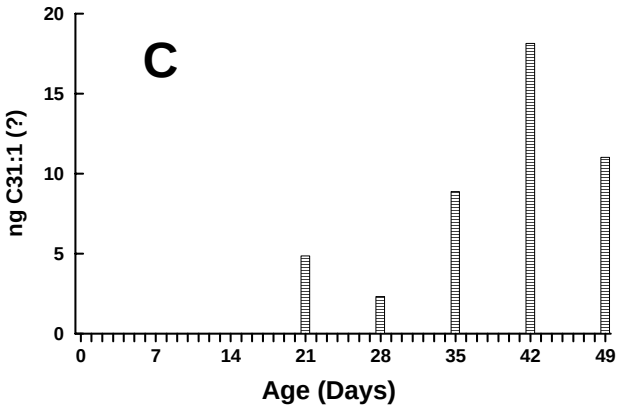
Table 1. Retention times of components of boll weevil cuticular hydrocarbons that change with age.

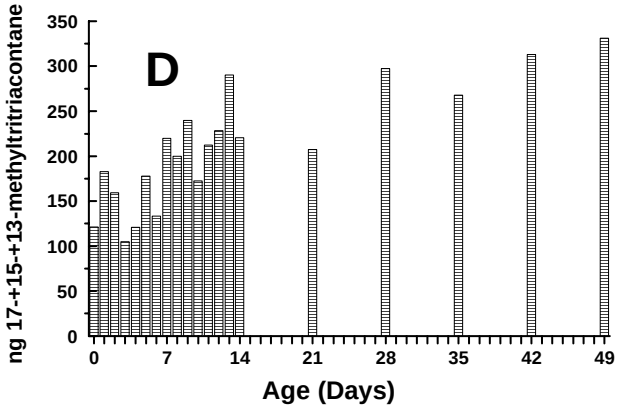
Retention Time (min)[a]	Component
8.90	n-Pentacosane
11.90	Octadecanoic acid (?)
12.69	*n*-Heptacosane
13.85	C29:2+C29:3, possibly containing ring moiety
13.98	C29:1
14.21	*n*-Nonacosane
15.35	C31:1 (?)
15.40	15-&13-Methylhentriacontane
16.53	17-&15-&13-Methyltritriacontane
16.67	15,19-Dimethyltritriacontane
16.78	Unknown, m/z 222, 490
17.39	Unknown, m/z 319, 337
18.11	Unknown, m/z 335, 363

[a]Retention times are approximate, and were observed under the conditions described for the first GC separation parameters.









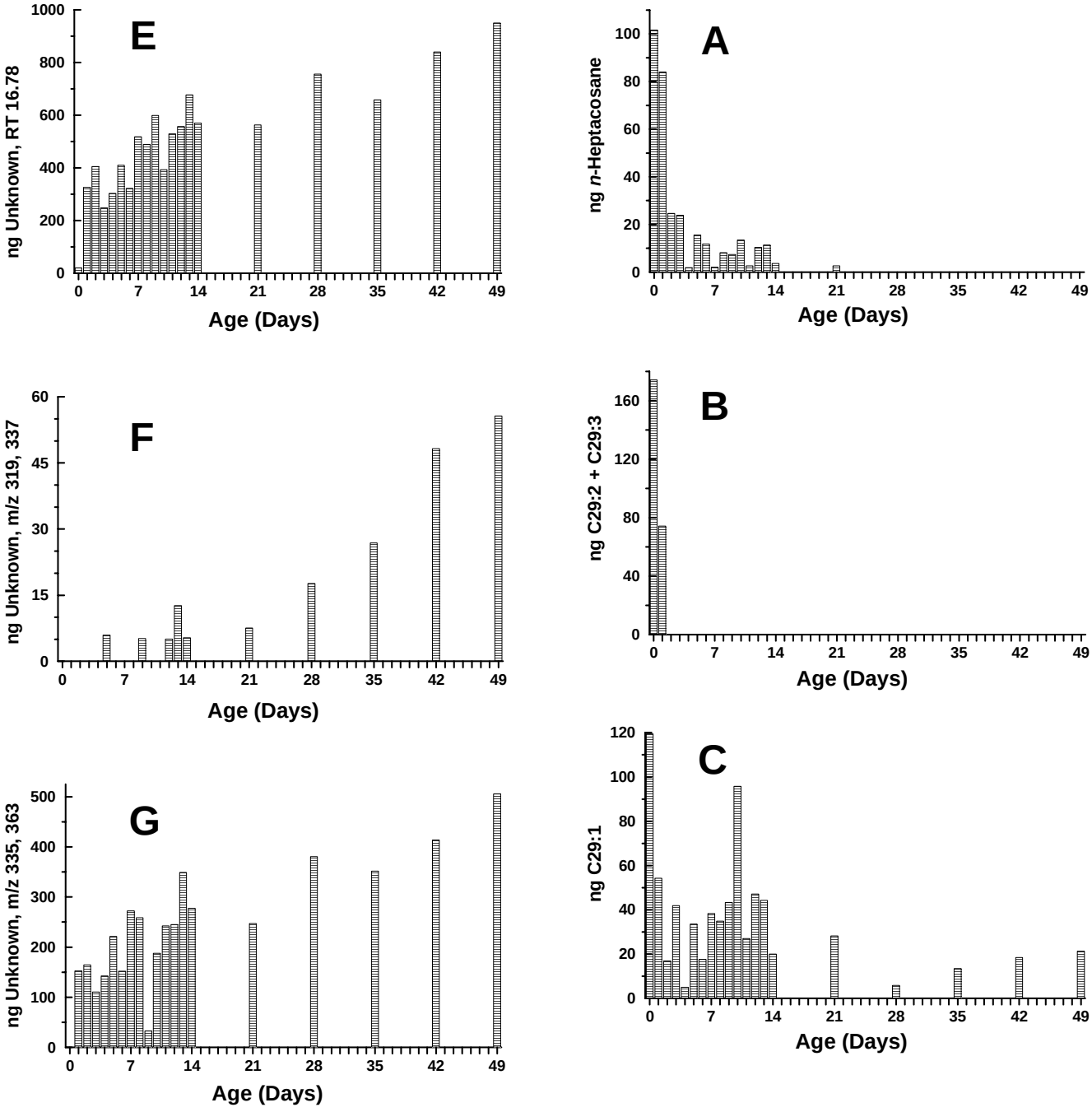


Figure 1. Changes in quantities of boll weevil cuticular hydrocarbon components that increased with age. (Bars represent the means of 10 individuals, 5 of each sex.)

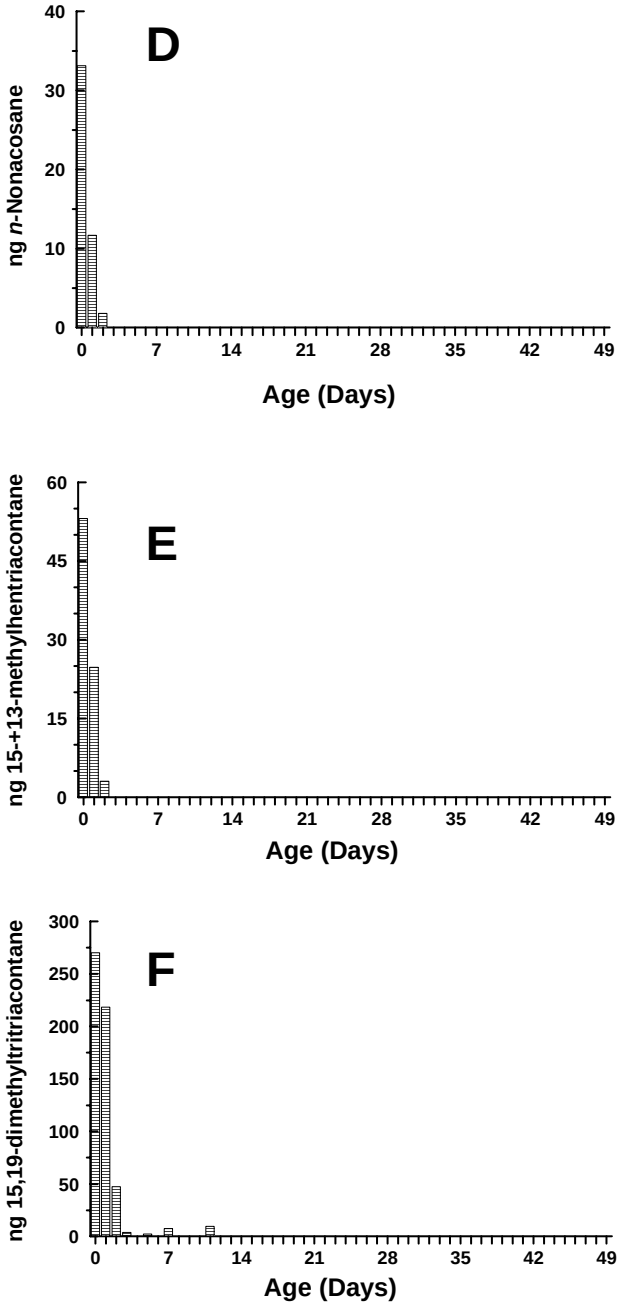


Figure 2. Changes in quantities of boll weevil cuticular hydrocarbon components that decreased with age. (Bars represent the means of 10 individuals, 5 of each sex.)

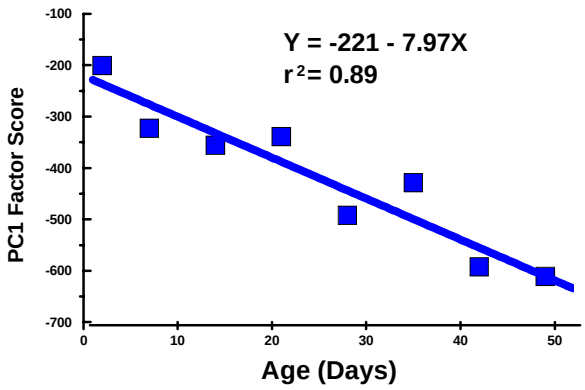


Figure 3. Linear regression of mean principal component-1 (PC1) factor scores on boll weevil age. PC1 contrasts cuticular hydrocarbon components that tend to decrease with age to those that tend to increase with age.