Variation in Size and Lipid Content of Adult Southern Pine Beetles, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae) in Relation to Season[1]

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ABSTRACT Variations in size, weight, and lipid content of brood adult southern pine beetles, *Dendroctonus frontalis* Zimmermann, were investigated using a spectrophotometric technique to determine total lipid content of individual beetles. Large seasonal variations in these parameters were found with significant differences occurring between months and years. Patterns of variation, however, were not consistent with those reported in previous studies, leading to the conclusion that these variations do not have regular seasonal patterns. Variations in beetle measurements were not related to tree characteristics. Female beetles, on average, were heavier and contained more lipid than male beetles. The spectrophotometric technique used improves on a similar technique recently reported. The technique allowed more extensive measurements, including total lipid content, dry weight, and percent lipid content, to be taken on individual beetles.

KEY WORDS Coleoptera, Scolytidae, *Dendroctonus frontalis,* southern pine beetle, seasonal variation, lipid content, dry weight, total length, pronotal width

Many studies have identified factors influencing bark beetle (Coleoptera: Scolytidae) size and lipid content. Lipids are metabolized during flight, affecting both beetle weight and lipid content (Atkins 1969, Thompson and Bennett 1971). Beetle density and phloem thickness during larval development have been shown to influence emerging adult size and lipid content (Atkins 1975, Amman and Pace 1976, Botterweg 1983, Anderbrant et al. 1985, Slansky and Haack 1986, Sahota et al. 1987). Temperature during larval development also affects emerging adult size and lipid content (Atkins 1967, 1975). In the multivoltine species *Ips paraconfusus* Lanier, seasonal differences in temperature resulted in generations of beetles with significantly different lipid contents (Hagen and Atkins 1975). Seasonal variations in the size and lipid content of another multivoltine species, the southern pine beetle (*Dendroctonus frontalis* Zimmermann), have also been reported (Hedden and Billings 1977, Roberts et al. 1982). These latter studies reported seasonal patterns of variation

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and suggested that size and lipid content reach maxima during favorable conditions in spring and fall. However, these studies were conducted at the same general time (1975-76), and are the only such studies on the southern pine beetle, a serious pest of southern pines (*Pinus* spp.). The question arises as to whether seasonal variations in southern pine beetle size and lipid content are consistent from year to year, or simply a function of the time at which these studies were conducted. Knowledge of regular seasonal variation may lead to better understanding of seasonal patterns in southern pine beetle infestation activity (Thatcher and Pickard 1964, 1967), which may be directly related to beetle size and lipid content (Hedden and Billings 1977).

The objective of this study was to examine southern pine beetle size and lipid content variations and determine if the variation exhibits consistent seasonal patterns and supports previous studies. We hypothesize that regular seasonal patterns of variation in physical parameters do not exist for the southern pine beetle. We employed a sulfophosphovanillin technique to determine total lipid content of individual beetles. A similar method has recently been reported by Kinn et al. (1994). Though similar, our technique offers some improvements and simplifications over their methods.

Materials and Methods

Insects. Insects were collected from southern pine beetle infestations in the Sam Houston, Davy Crockett, and Sabine National Forests in eastern Texas. Two to five loblolly pines (*Pinus taeda* L.) infested with southern pine beetle were selected for sampling each month from June through September of 1990 and from February through November of 1991 (except September). Approximately 2000 cm^2 of infested bark containing pupal and callow adult stage beetles were removed from each tree. These stages were chosen for collection because larval feeding was completed and adult emergence and flight had not yet occurred. Lipid is metabolized during flight and would thus affect overall lipid content (Atkins 1969, Thompson and Bennett 1971).

A total of 55 trees was sampled. Six tree parameters were measured at the time of beetle collection in order to assess possible impact of host tree variation on beetle size and lipid content. Measurements taken were tree height (cm), diameter (DBH, in cm), bark thickness (mm), area of bluestain fungus [*Ophiostoma minus* (Hedg.)] infected phloem (cm^2/100 cm^2), and 1 and 5 year radial growth indices.

Infested bark was returned to the laboratory and placed in ventilated rearing cans fitted with glass jars to collect emerging beetles (Cooper and Stephen 1978). The laboratory was held at 24° C while beetles emerged. Beetles were collected approximately every 12 h as they emerged and immediately frozen at -60° C until processing.

Beetle sex and size were determined using a stereomicroscope fitted with an ocular micrometer. Total length (from the frons to the end of the abdomen) and pronotal width (at the widest point) were measured to the nearest 0.05 mm on approximately equal numbers of male and female beetles each month. Due to variation in availability of suitable sample trees and brood emergence, the number of beetles measured varied each month from 50 to 195.

After measuring, beetles were placed in individual test tubes (12 × 75 mm) and lyophilized at -50° C for 24 h. After drying, beetles were immediately weighed (to prevent moisture adsorption) to the nearest ± 0.01 mg (Sartorius balance, Model #2434) and then analyzed for lipid content.

Lipid analysis. Lipid content was determined using a spectrophotometric technique for measuring blood lipids (Goldsworthy et al. 1972) as modified by Bloem and Duffey (1990). The technique measures small quantities (minimum = 10 μg) of total lipids (Barnes and Blackstock 1973), enabling the method to be used on small invertebrates. Individual beetles were thoroughly ground with a glass rod in 1.0 ml of a 2:1 chloroform/methanol solution to remove lipids (Barnes and Blackstock 1973). Beetles were allowed to stand 48 h in airtight (to prevent evaporation) glass tubes to ensure complete lipid extraction. A 0.5 ml aliquot of this solution was then removed with a 1 ml capacity glass tuberculin syringe fitted with a #25 gauge hypodermic needle, thereby leaving the beetle solids suspended in the remaining 0.5 ml of solution. This step was necessary because preliminary analysis revealed that pieces of exoskeleton were charred by sulfuric acid during the subsequent analysis, creating contaminants that interfered with the lipid reagents. Similar methods (Botterweg 1983, Kinn et al. 1994) used intact beetles without lipid extraction with polar solvents, a technique we found to be unsatisfactory. We believe that the extraction of lipids from the beetles before the addition of sulfuric acid prevents possible interference with lipid reagents later in the assay and is a significant improvement to the technique as recently reported (Kinn et al. 1994). The amount of lipid determined to be in the 0.5 ml aliquot was doubled at the end of the assay to obtain an estimate of the original amount of total lipid in the beetle. The 0.5 ml aliquots were heated in test tubes at 40° C in a dry bath until the liquid solution evaporated, leaving the extracted lipids as residue. One milliliter of concentrated sulfuric acid was added to the residue and heated at 100° C for 15 minutes. After cooling to room temperature, 3.0 ml of 13 mM vanillin in 85% phosphoric acid was added, mixed, and incubated at room temperature. After 1 h the absorbance of the solution was read against a blank on a spectrophotometer at 546 nm. The blank was created by taking a clean (no lipids) 0.5 ml aliquot of the chloroform/methanol solution through the entire assay for each group of beetles assayed daily. The absorbance was converted into a lipid concentration with a precision of ± 5 μg using a standard curve developed with linoleic acid, a major component of southern pine beetle lipid composition (Hodges and Barras 1974). The standard curve was developed by dissolving 0.5 mg of linoleic acid in 0.1 ml of 2:1 chloroform/methanol. Portions of this solution were then diluted to make standards of 500, 250, 125, 62.5, and 31.25 μg/0.1 ml. Standards were evaporated at 40° C in a dry bath then assayed as above. The assay of each beetle was conducted in new, disposable test tubes to prevent contamination from soap residue. Approximately 1500 beetles were analyzed.

Data analyses. Five variables were measured in this study: dry weight (in μg); total lipid content (in μg); the percentage of dry weight lipid content, which is the ratio of total lipid content to dry weight multiplied by 100 (hereafter referred to as percent lipid content); total body length (in mm); and maximum pronotal width (in mm).

Percentage data were normalized with arcsine transformation before analysis. Reported values represent re-transformed data. Data summarization and statistical analyses were conducted using the Statistical Analysis System (SAS Institute 1985, 1989). A three-factor (month of collection, sample tree, and sex) analysis of variance was conducted using the general linear models procedure (PROC GLM) and Type III sum of squares due to uneven sample sizes. The error term associated with mean values is standard error of the mean (SEM). Tree parameters were analyzed with linear regressions (PROC REG) using the five beetle measurements as dependent variables.

Results

Percent lipid content. Beetle percent lipid content varied significantly between the sexes by month (an interaction, $P < 0.007$). Both sexes contained the highest percentage of lipid in June, July, October, and November of 1991 (Fig. 1). Lowest percent lipid occurred in August of 1990 and 1991. The sexes were different in average percent lipid content in May and June of 1991. Otherwise differences between the monthly averages by sex were not statistically significant. Percent lipid content varied significantly between years as beetles collected in June and July of 1991 contained a higher average lipid content than beetles collected in June and July of 1990.

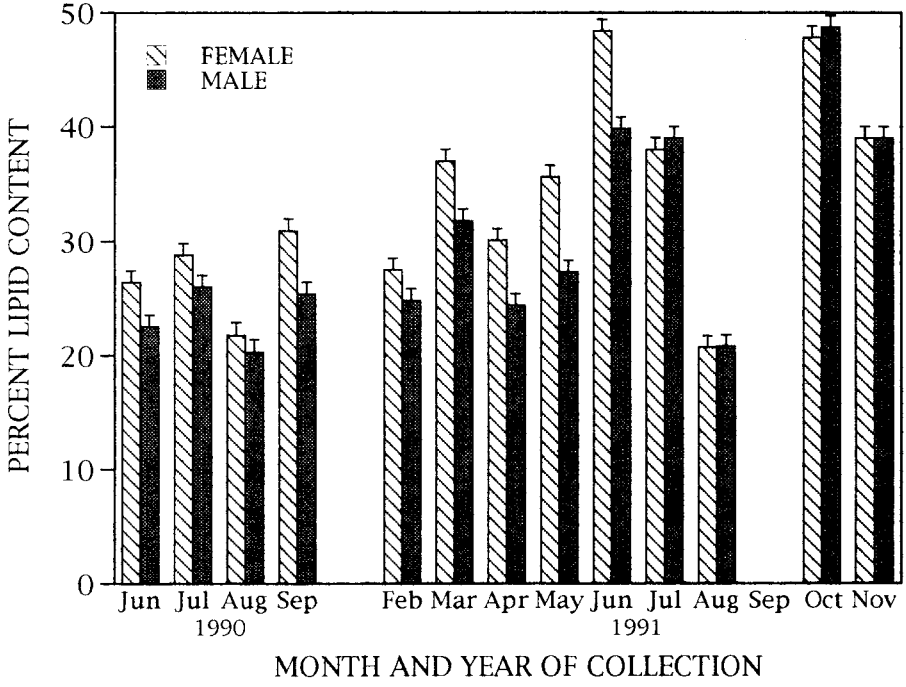

Fig. 1. Seasonal variation in the average percent lipid content of male and female brood adult southern pine beetles. Error bars represent 1 SEM.

Total lipid content and dry weight. The sexes differed significantly by month (an interaction) in both total lipid content ($P < 0.009$) and dry weight ($P < 0.003$). Females were consistently heavier and contained more total lipid than males in all months studied. Total lipid content was highest in March, June, October, and November of 1991 for both sexes (Fig. 2). Females contained significantly more total lipid than the males in March, May, and June of 1991. Total lipid content was lowest in August of 1991 for both sexes. Dry weight for both sexes was highest in August and September of 1990 and March and November of 1991 (Fig. 3), and lowest in August of 1991. Females were significantly heavier than males only in March and November of 1991.

Size measurements. Interaction terms for size measurements were insignificant ($P > 0.05$). Average beetle total length varied significantly by month ($P < 0.001$) but not overall between sexes ($P > 0.6$). Beetles were longest in March and November of 1991 and shortest in June of 1990 and August and October of 1991 (Fig. 4). Average pronotal width also varied significantly between months ($P < 0.001$). Beetles in general were widest in February, March, and November of 1991 and smallest in June of 1990 and August and October of 1991 (Fig. 4). Pronotal width did not vary overall between the sexes ($P > 0.08$).

Tree characteristics. All interaction terms involving sample trees were insignificant for each of the five variables ($P > 0.05$). However, between-tree differences were significant for each variable of beetle measurement ($P < 0.001$). However, additional analyses, including both linear and non-linear regression,

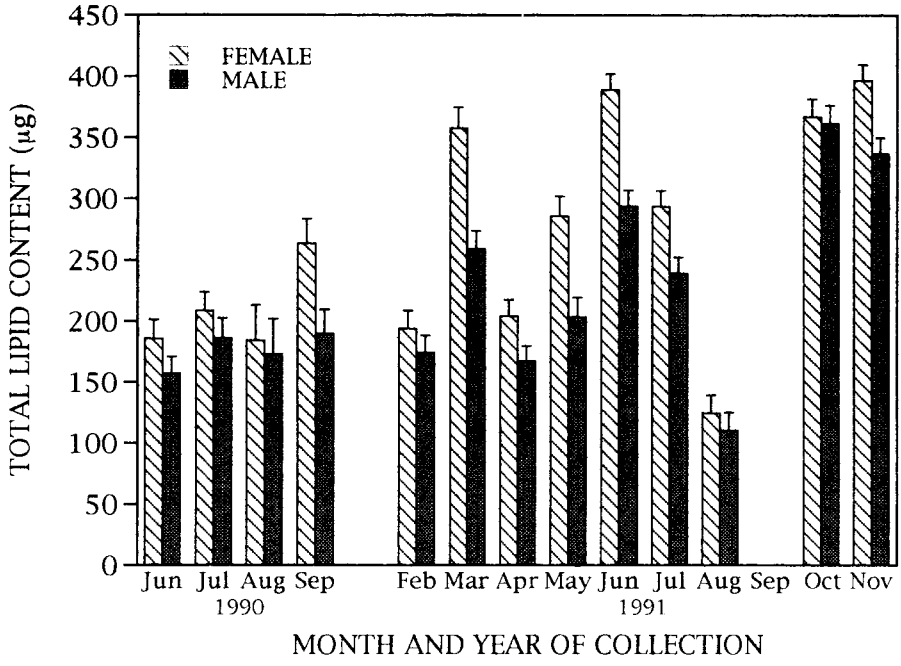

Fig. 2. Seasonal variation in the average total lipid content (µg) of male and female brood adult southern pine beetles. Error bars represent 1 SEM.

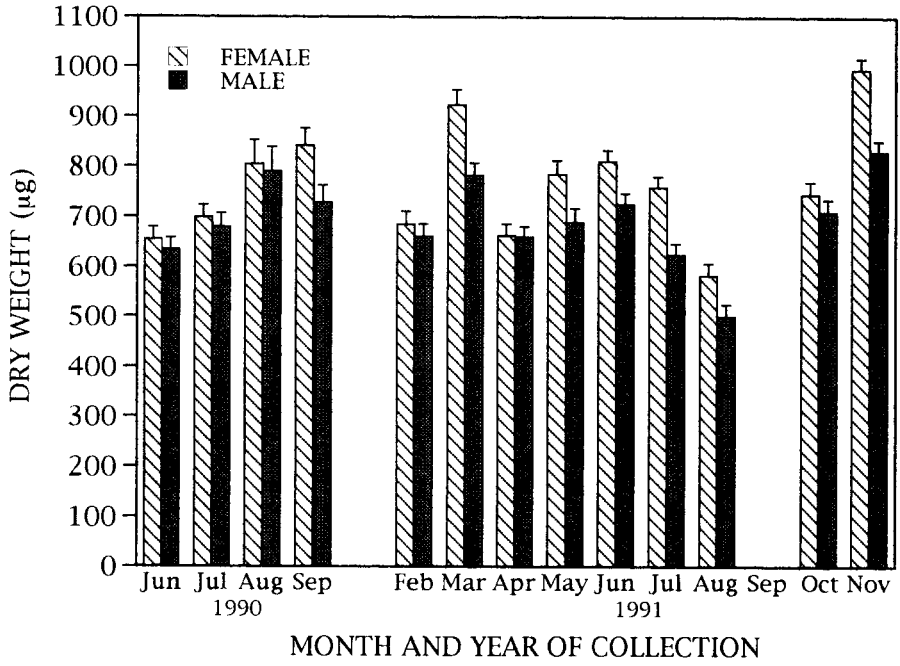

Fig. 3. Seasonal variation in the average dry weight (µg) of male and female brood adult southern pine beetles. Error bars represent 1 SEM.

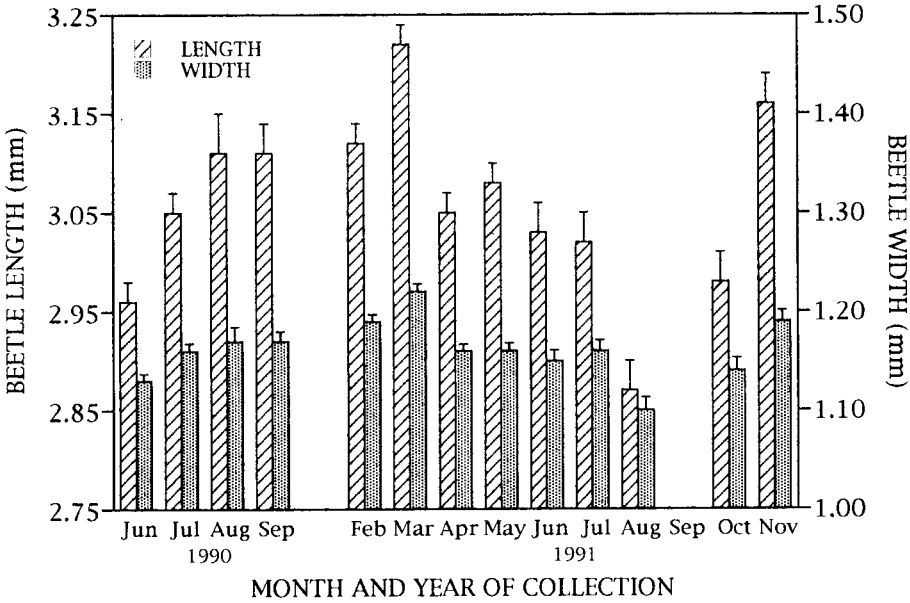

Fig. 4. Seasonal variation in average total length and pronotal width (both in mm) of brood adult southern pine beetles. Error bars represent 1 SEM.

failed to relate variations in beetle measurements to variations in the six measured tree parameters ($P > 0.05$).

Discussion

Hedden and Billings (1977) suggested that variation in southern pine beetle lipid content exhibits a seasonal pattern, with high lipid content in spring and fall and low content in summer and winter. However, this and another study (Roberts et al. 1982) do not support this idea. Hedden and Billings (1977) reported high average lipid content occurring in April, June, September, and October. Roberts et al. (1982) reported high beetle lipid content in May, October, December, and January, while we found high lipid content in June, July, October, and November (Fig. 1). Though October is common to all, accumulated data from the three studies report high seasonal averages in nine months of the year. Large pronotal widths were previously found in April, May, June, October, and December (Hedden and Billings 1977, Roberts et al. 1982), while we found beetles were largest in February, March, and November (Fig. 4). Large beetles have thus been found in eight months of the year. The number of months in which high average lipid contents and pronotal widths have been found and differences in seasonal patterns between studies show that no regularity exists in these measurements, thus supporting our hypothesis that southern pine beetle physical parameters do not vary regularly by season.

While not dismissing the influence of temperature during development on beetle size and lipid content (Atkins 1967, 1975, Hagen and Atkins 1975), studies indicate that bark beetles are influenced by factors other than temperature than can result in size and lipid content variations. Although Hedden and Billings (1977) and this study found no relationship between tree parameters and variation in beetle measurements, unmeasured factors such as seasonal differences in host condition or phenology during larval feeding, especially phloem nutrition or thickness (Amman and Pace 1976, Slansky and Haack 1986), might be important. Other factors known to be important in scolytids include variation in levels of nematode infection (Hagen and Atkins 1975), the effects of larval density (Atkins 1975, Amman and Pace 1976, Botterweg 1983, Anderbrant et al. 1985, Sahota et al. 1987), and associated microorganisms (Bridges 1983). The southern pine beetle is associated with three species of fungi (Barras and Perry 1972, Barras and Taylor 1973, Bridges and Perry 1987, Harrington and Zambino 1990). One species, the blue staining fungus *Ophiostoma minus* [=*Ceratocystis minor* (Hedg.) Hunt], has been shown as detrimental to beetle development (Barras 1970), although this study found no relationship between beetle size and lipid content fluctuations and varying levels of blue stain infection in sample trees. The two other fungal species associated with southern pine beetle are beneficial symbionts (Bridges and Perry 1985). Bridges (1983) reported differences in mass of southern pine beetle females carrying different combinations of these two fungi. Mutualistic fungi in some scolytid species do impart nutritional benefits (Baker and Norris 1968, Norris et al. 1969). Different combinations of southern pine beetle symbiotic fungi might result in various nutritional opportunities during larval stages, resulting in adult beetles of different size and/or lipid content. Future

investigations into the influence of symbiotic fungi on southern pine beetle size and lipid content are certainly warranted.

In a study of lipid dynamics during the southern pine beetle life cycle, Barras and Hodges (1974) showed that the dry weight of southern pine beetle eggs are 78% lipid. Because changes in lipid composition occur in some scolytid females during egg-laying (Penner and Barlow 1972, Nijholt and Sahota 1974), it is not unreasonable to suggest that female beetles with more total micrograms (μg) of lipid, regardless of overall percentage of lipid content, may have the ability to produce more eggs than females with less lipid. This illustrates additional advantages of the technique used to determine beetle lipid content. It enables two measures of lipid content to be taken on individual beetles, both absolute or total lipid content and percentage of total body weight that is soluble lipid. Which measurement may be a better indication of reproductive or dispersal potential and more important to overall beetle biology is as yet unknown.

Lipid content has been linked to bark beetle flight and dispersal behavior (Atkins 1966, 1969, Thompson and Bennett 1971, Slansky and Haack 1986, Jactel 1993, Kinn et al. 1994). Although a relationship has not been firmly established for the southern pine beetle, it has been hypothesized that seasonal increases in size and lipid content are correlated with increased activity in new infestations and infestation growth (Hedden and Billings 1977). Because size and lipid content have been linked to reproductive effort (Reid 1962, Amman 1972, Clarke et al. 1979) and emergence, re-emergence, and survival (Safranyik 1976, Botterweg 1982, 1983, Anderbrant 1988) in other species, these parameters may play an important role in southern pine beetle reproduction and population dynamics.

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