Chronological Age-Grading of Three Species of Stored-Product Beetles by Near-Infrared Spectroscopy

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INTRODUCTION

- The rice weevil, *Sitophilus oryzae* (L.), the lesser grain borer, *Rhizopertha dominica* (F.), and the red flour beetle, *Tribolium castaneum* (Herbst), are three of the most important pests of stored grain and processed grain products in the U.S.
- Successful management of these pests requires thorough sampling protocols and subsequent decision-making based on predictive population models or expert system analysis.
- Because oviposition by these species is not temporally uniform, the accuracy of predictive models used to manage these species can be improved significantly if the age-structure of the pest population is incorporated.
- However, except for *S. oryzae*, there is no information on methods to determine the chronological or physiological age of these Coleopterans.
- We previously showed that near-infrared spectroscopy (NIRS), a rapid procedure, can be used to determine chronological age of the house fly, a relatively short-lived Dipteran.
- The objectives of this study were to determine if NIRS could be used for determination of chronological age in these three long-lived species of beetles, to determine the role of cuticular lipids in the ability of NIRS to age-grade adult *S. oryzae*, and to determine whether water content in adult weevils varies with age and if NIR wavelengths that are absorbed by water have any effect on the ability to determine age.

METHODS

- A near-infrared spectrometer (DA7000, Perten Instruments) (Fig. 1A) was used to collect spectra from single adults that were placed in a V-shaped trough (Fig. 1B). An 8-mm diameter fiber optic probe illuminated the insect, and a 2-mm diameter optic probe carried reflected energy from the insect to the spectrometer (Fig. 1C).
- We used a physiological age scale, rather than days, in the analysis because the three species live for different lengths of time when maintained at the same temperature. Thus, an insect of age 0 is a newly emerged adult, and an insect of age 1 has reached its maximum life span.
- To test the role of cuticular lipids on NIRS age-grading predictions, weevils with and without cuticular lipids were used to develop NIRS calibration models and these models were validated by predicting the age of another set of weevils.
- Water content on weevils of five different age groups was determined by the oven method.

RESULTS

NIRS AGE-GRADING

- There was a strong relationship between NIRS-predicted age and the actual chronological age of the insects for all the three species (Figs. 2A, 2B, and 2C).
- Confidence limits of the NIRS-predicted ages (± 0.3), were similar in the models developed for each of the three species. Classification accuracies in the validation tests ranged from 82 to 92%.
- Excluding wavelengths associated with H2O from the NIRS models slightly reduced the r2 value, increased the value of confidence limits, and decreased the accuracy of classification from 83% to a 64%.

EFFECTS OF CUTICULAR LIPIDS ON AGE-GRADING

- Although the relationship between NIRS-predicted age and actual age was significant in both unextracted and hexane-extracted weevils, confidence limits were smaller and r2 value was higher for the calibration model developed with unextracted weevils.

EFFECT OF WATER CONTENT

- Water content varied with age but not with sex. Younger weevils tended to have higher water content than older weevils in both sexes (Fig. 4).
- Excluding wavelengths associated with water from the NIRS models slightly reduced the r2 value, increased the value of confidence limits, and decreased the accuracy of classification from 83% to a 64%.
- Our results provide evidence that these compounds have a significant role in NIR absorption and NIR age classification.

SUMMARY

- When life spans are normalized on a scale from 0 to 1, the confidence limits on predicted ages for unsexed adults of each species were about ± 0.3. Thus, younger adults within the first one-third of their life can be easily differentiated from older adults.
- Based on beta coefficients, absorbance regions corresponding to CH3, CH2, and CH groups were the most important for NIRS age-grading in the three species. These methyl groups are common constituents of most insect cuticular and internal lipids.
- Classification accuracies in the validation sets were 79 and 44% from the unextracted and hexane-extracted models, respectively.
- The difference spectrum (Fig. 3) shows higher absorbance at all wavelengths above 900 nm for the unextracted weevils. These wavelengths correspond to lipids.
- Our results provide evidence that these compounds have a significant role in NIR absorption and NIR age classification.