Detection of Parasitized Rice Weevils in Wheat Kernels with Near-Infrared Spectroscopy[1]

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Individual kernels of wheat containing immature rice weevils, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), parasitized by *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) could be separated from uninfested kernels and kernels that contained unparasitized weevils by using near-infrared (NIR) spectroscopy. Wheat kernels from cultures of the parasitoid were X-rayed and sorted into uninfested kernels, kernels infested with weevil larvae or pupae, kernels containing parasitoid larvae feeding on host weevils, and kernels containing parasitoid pupae and remains of the host. Kernels were automatically positioned and scanned (400–1700 nm) with the NIR system. Kernels containing parasitoid pupae were completely differentiated from kernels containing weevil pupae as well as all other kernels in this automated system. With the appropriate integration of NIR spectroscopy and seed-sorting instrumentation, kernels containing parasitoid pupae could be automatically collected from cultures of *A. calandrae* during the mass rearing of this beneficial insect.

***Key Words:* biological control; stored products; wheat; parasitoid; Hymenoptera; rice weevil; mass rearing; NIR; near-infrared; detection.**

INTRODUCTION

A complex of parasitoids and predators is associated with the stored-grain ecosystem (Brower, 1990). These beneficial insects exert some level of natural control on most stored-grain pest insect populations. However, the best use of these natural enemies in pest management programs in grain stores is through augmentative release (Brower *et al.,* 1996). Optimal release strategies have not been determined for most of the relevant parasitoids. Small cardboard cards containing parasitized eggs have been used for release of *Tricho-*

gramma spp. (Schöller *et al.,* 1997). However, in most large-scale pilot tests that have been conducted in stores of whole grain, adult parasitoids have been released directly into storage facilities (Flinn *et al.,* 1996). This is particularly true for parasitoids of internal feeders, such as weevils and borers, for which no method currently exists to differentiate parasitized from unparasitized hosts within grain kernels. Such a method could be advantageous in optimizing release mechanisms with regard to the age and stage of the released parasitoid. For example, dispersal of immature parasitoids could be facilitated by addition of grain kernels containing parasitized hosts during bin loading.

The major parasitoids of in-kernel insect pests of whole grain are *Anisopteromalus calandrae* (Howard), *Lariophagous distinguendus* (Foerster), *Pteromalus cerealellae* (Ashmead), and *Choetospila elegans* (Westwood). These pteromalids are routinely reared on their hosts inside whole grain kernels. Within a single culture, there are uninfested kernels, kernels infested with the pest insects (hosts), kernels with hosts that have been attacked by the parasitoid but not parasitized, and kernels with parasitized hosts from which the adult parasitoid will emerge. If grain kernels containing parasitized hosts are to be used as a vehicle to disseminate or release parasites of different developmental stages, an efficient method is needed to ensure that kernels containing unparasitized pest insects are not inadvertently released into the storage. Burks *et al.* (1999) demonstrated that low temperatures prevented emergence of unparasitized larvae of the rice weevil *Sitophilus oryzae* (L.) while not affecting emergence of the parasitoid *A. calandrae.* We have recently demonstrated that near-infrared (NIR) spectroscopy can be used to detect weevil larvae in individual wheat kernels (Dowell *et al.,* 1998). In this study, we demonstrate that the NIR system can also be used to automatically and rapidly differentiate single wheat kernels containing pupae of the rice weevil parasitoid *A. calandrae,* separating these from uninfested kernels and kernels containing weevil larvae or pupae.

MATERIALS AND METHODS

Insects

Stock cultures of the rice weevil were maintained on hard red winter wheat (13.5% moisture content) at 27°C and 55–60% RH. The laboratory strain of *A. calandrae* used in this study was maintained on immature rice weevils by transferring adult wasps to wheat infested with 21-day-old weevil larvae. The laboratory strain of *A. calandrae* has been maintained in culture for >20 years.

Selection of Kernels for NIR Analysis

Wheat kernels were selected from different-aged cultures of *A. calandrae,* X-rayed by the procedures of Throne (1994), and classified into the following categories: uninfested kernels, kernels infested with weevil larvae, kernels infested with weevil pupae, kernels containing parasitoid larvae, and kernels containing parasitoid pupae. Each kernel selected for analysis was uniquely identified and NIR spectra collected within 8 h of the X-ray procedure. Kernels were held for an additional 3 weeks after NIR spectra were obtained to allow all insects to emerge and to confirm the X-ray identification. Two separate trials with kernels obtained from different insect generations were conducted. The total numbers of individual kernels analyzed in each category were: uninfested kernels ($n = 39$), kernels infested with weevil larvae ($n = 28$), kernels infested with weevil pupae ($n = 37$), kernels containing parasitoid larvae ($n = 63$), and kernels containing parasitoid pupae ($n = 40$).

Kernel Analysis with the NIR System

A single kernel characterization system coupled with a diode-array NIR spectrometer (Perten Instruments, Springfield, IL) allowed automated kernel handling and collection of spectra. The system measures visible and NIR absorbance (400–1700 nm) but only the NIR region (700–1700 nm) was analyzed by partial least squares (PLS) regression (Martens and Naes, 1989) because no visible wavelength absorbance difference was expected. Ability to accurately classify kernels was determined by using coefficients of determination (r^2), standard errors of cross validation (SECV), and percentage of kernels correctly classified. PLS beta coefficients used in calibrations and difference spectra of parasitized and weevil-infested kernels indicated wavelength regions used for classifications. Dowell *et al.* (1998) describe the data collection method and analysis procedures.

Two comparisons were made. The first comparison determined if kernels infested with either weevils or parasitized weevils could be differentiated from uninfested kernels. Uninfested kernels and kernels with parasitoid or weevil pupae were used to develop calibrations. All kernels were used in predictions. A second comparison determined if kernels containing parasitoid larvae or pupae (along with remains of the host) could be separated from unparasitized weevil larvae or pupae. A calibration was developed using 4/5 of kernels containing parasitoid or weevil pupae. All kernels were used in predictions. Kernels from each trial were analyzed on different dates but all data were combined for analysis.

RESULTS AND DISCUSSION

Initial analyses determined that uninfested kernels could be separated from kernels infested with either weevils or parasitoids (Table 1). Results showed that 97.4% of uninfested kernels, 100% of kernels with pupae (host or parasitoid), and 90% of kernels with larvae (host or parasitoid) were correctly identified. We used 13 factors in the PLS analysis to classify uninfested versus infested kernels ($n = 116$, $r^2 = 0.90$, SECV = 0.15). The results agree with previous research by Dowell *et al.* (1998) which showed that wheat kernels containing 3rd or 4th instar rice weevil larvae could be detected by NIR with greater than 95% confidence. The misclassified infested kernels likely contained small parasitoid or weevil larvae that did not absorb a threshold amount of NIR radiation.

Subsequent analyses determined that kernels infested with weevil larvae or pupae could be differentiated from kernels with *A. calandrae* pupae (number of factors = 12, $n = 61$, $r^2 = 0.87$, SECV = 0.18). Table 1 shows that 100% of kernels containing parasitoid pupae, weevil pupae, and weevil larvae were correctly classified. However, only 14% of the kernels containing parasitoid larvae were correctly identified. If the detection threshold is changed to correctly classify more parasitoid larvae, then 75% of the kernels containing parasitoid larvae can be detected while correct classifi-

TABLE 1

Use of NIR Spectroscopy to Classify Hard Red Winter Wheat Kernels into Uninfested, Infested with Immature Rice Weevils, or Infested with Immature Rice Weevils Parasitized by *Anisopteromalus calandrae*

| | Kernels correctly classified, % | | | | |
| | Parasitoid | | Weevils (Hosts) | | |
Comparison	Pupae	Larvae	Pupae	Larvae	Uninfested
Uninfested vs infested	100	90	100	90	97.4
Parasitoid vs weevil	100	14	100	100	—
Parasitoid vs weevil[a]	100	75	100	21	—

[a] Same as previous comparison, except that the detection threshold is changed to correctly classify more parasitoids.

cation of parasitoid pupae and weevil pupae remains at 100%. However, 79% of kernels containing only weevil larvae are misclassified with this calibration.

Spectral analysis showed that the greatest differences in NIR spectra between kernels containing weevils or parasitoids occurred in three wavelength ranges, 950–1030, 1130–1200, and 1300–1400 nm (Fig. 1). Functional groups responsible for absorbance in the 950–1030 region are H_2O and second overtones of RNH_2 groups, whereas the longer wavelength regions correspond to C-H overtones and combinations. The C-H overtone regions correspond to chitin absorption regions as reported earlier by Ridgway and Chambers (1996) and Dowell *et al.* (1998). The present data also provide evidence that NIR may be detecting physical or biochemical differences in the composition of chitin or cuticle between the host and parasitoid within the wheat kernels. However, more detailed studies are needed to confirm this observation.

In summary, NIR spectroscopy can be used to differentiate among individual wheat kernels that are uninfested, those infested with weevil larvae or pupae, or those that contain a parasitoid pupa. With our current system, we could not accurately differentiate between kernels containing weevil larvae and those containing weevil larvae being parasitized by *A. calandrae* larvae. The inability to differentiate larval parasitoids from host larvae may not be a big disadvantage, however, because of the relatively short duration of the larval stage of this ectoparasitoid compared with the much longer duration of the pupal stage (Ghani and Sweetman, 1955).
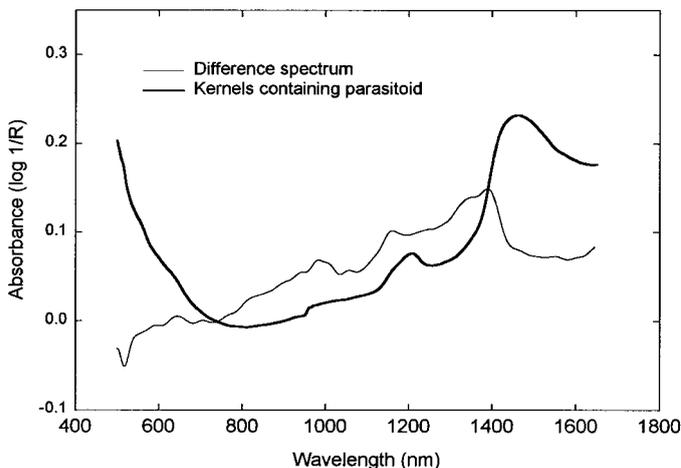
The NIR system used in this research can scan kernels at a rate of about 2/s. However, technology exists that can sort seeds at speeds to 1000 kernels/s. Adaptation of NIR to this type of instrumentation would provide insectaries that mass rear beneficial insects with a rapid means of collecting large numbers of immature parasitoids of the same stage for shipping and for use in augmentative release programs. The unique characteristics of NIR could also be used for quality control by determining percentage parasitization within mass cultures and to stage classify cultures to ensure synchronous or asynchronous release patterns.

REFERENCES

Brower, J. H. 1990. Pests of stored products. *In* "Classical Biological Control in the Southern United States" (D. H. Habeck, F. D. Bennett, and J. H. Frank, Eds.), pp. 113–122. South. Coop. Series, Bulletin No. 355.

Brower, J. H., Smith, L., Vail, P. V., and Flinn, P. W. 1996. Biological control. *In* "Integrated Management of Insects in Stored Products" (B. Subramanyam and D. W. Hagstrum, Eds.), pp. 223–286. Dekker, New York.

Burks, C. S., Hagstrum, D. W., and Baker, J. E. 1999. Selection of cold injury treatments to facilitate release of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) reared on the rice weevil (Coleoptera: Curculionidae). *J. Econ. Entomol.* **92,** 473–479.

Dowell, F. E., Throne, J. E., and Baker, J. E. 1998. Automated nondestructive detection of internal insect infestation of wheat kernels using near-infrared reflectance spectroscopy. *J. Econ. Entomol.* **91,** 899–904.

Flinn, P. W., Hagstrum, D. W., and McGaughey, W. H. 1996. Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. *Environ. Entomol.* **25,** 505–511.

Ghani, M. A., and Sweetman, H. L. 1955. Ecological studies on the granary weevil parasite, *Aplastomorpha calandrae* (Howard). *Biologia* **1,** 115–139.

Martens, H., and Naes, T. 1989. "Multivariate Calibrations." Wiley, Guildford.

Ridgway, C., and Chambers, J. 1996. Detection of external and internal insect infestation in wheat by near-infrared reflectance spectroscopy. *J. Sci. Food Agric.* **71,** 251–264.

Schöller, M., Prozell, S., Al-Kirshi, A.-G., and Reichmuth, Ch. 1997. Towards biological control as a major component of integrated pest management in stored product protection. *J. Stored Prod. Res.* **33,** 81–97.

Throne, J. E. 1994. Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. *Environ. Entomol.* **23,** 1459–1471.

FIG. 1. NIR spectrum of a hard red winter wheat kernel containing a parasitized weevil and the difference spectrum resulting from a spectrum of a kernel containing a parasitized weevil subtracted from a spectrum of a kernel containing an unparasitized weevil.