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Abstract

Our recent work on applications of near-infrared spectroscopy (NIRS) to entomological problems is reviewed. 1. Using an automated NIRS system scanning individual wheat kernels at the rate of 15 per minute, we were able to differentiate between uninfested kernels and kernels infested with late-instar larvae of *Rhyzopertha dominica*, *Sitophilus oryzae*, or *Sitotroga cerealella*. The ability to rapidly scan individual kernels indicates potential for automated segregation of infested kernels from bulk grain. 2. The automated NIRS system was used to differentiate uninfested wheat kernels, kernels infested with rice weevils, and kernels that contained rice weevils that were parasitised by *Anisopteromalus calandrae*. Being able to distinguish kernels containing parasitoids would be useful for quality control in commercial insectaries that rear biological control agents, and would be useful for research on natural enemies. 3. The NIRS system was used to segregate samples of transgenic maize that contain low and high levels of the protein avidin. Avidin is toxic to a number of insect pests, and transgenic maize that contains a level of avidin that is toxic to insect pests of stored grain has been developed. 4. A manual NIRS system was used to quantify insect fragments in flour. Although the sensitivity is not sufficient to detect insect fragments at the U.S. Food and Drug Administration defect action level (75 fragments per 50 grams of flour), the technique is accurate at an action level of 130 fragments per 50 grams of flour and could be useful for prescreening large numbers of flour samples to select samples for more expensive and labour-intensive chemical analyses. 5. A manual NIRS system was used to identify stored-product insect pests to species level. We were able to identify insects to genus with greater than 95% accuracy and to identify insects as being primary or secondary pests with greater than 99% accuracy. Ability to identify insects to species depended on the genus. This technology could be useful to pest managers who may not be familiar with insect taxonomy. 6. The manual NIRS system was used to determine chronological age of two primary pests and one secondary pest of stored products.

Keywords: Near-infrared spectroscopy; Insect detection; Biological control; Taxonomy; Insect fragments; Age-grading; transgenic maize; Avidin

Introduction

Near-infrared spectroscopy (NIRS) has been used to measure quality traits in grain, including moisture, oil, and protein content (Williams and Norris, 2001). The use of NIRS for entomological applications has included determining level of external and internal insect infestation in bulk wheat samples (Ridgway and Chambers, 1996) and detection of internal insects in individual wheat kernels (Ridgway and Chambers, 1996; Ghaedian and Wehling, 1997; Ridgway et al., 1999). NIRS is a useful tool for applications such as these because the method is rapid and nondestructive, little or no sample preparation is required, and the technology can be automated. We report here on a wide range of entomological applications of NIRS conducted in our laboratory, including detection of internal insect infestations in individual wheat kernels, detection of parasitoids of internally infesting insect pests of stored grain, segregation of transgenic maize kernels with low and high levels of the biopesticide avidin, quantification of insect fragments in flour, identification of insects to species level, and chronological age-grading of stored-product beetle pests.

Detection of internal insects

Current inspection procedures in the grain industry detect only external insects. However, the larval stages of many of the most serious pests of grain occur inside the kernels. The presence of these internal insects can result in insect fragments in milled products and, if the grain is stored before processing, these insects can emerge and reproduce causing quality losses. Internal insect populations are not currently assessed when grain is sampled because of the difficulty and expense of available detection methods for internal insects [see Dowell et al. (1998) for a review of detection methods]. A rapid, automated method for sampling these internal insects is desirable because of the seriousness of this problem.

We used a diode-array, near-infrared spectrometer integrated with a single kernel characterisation system (Perten Instruments, Springfield, IL, USA) to automatically collect spectra from individual wheat kernels that were infested with different stages of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Dowell et al., 1998). We also tested the effect of wheat class (hard red winter, soft red winter, hard red spring, soft white, and hard white), protein content (11.3 to 16.2%), and moisture content (10 to 13.2%) on the ability of NIRS to detect internal insects. We used X-rays to measure the size (length × width) of larvae in the kernels,

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and then NIRS to predict the size of larvae in the same kernels.

Moisture content, protein content, and wheat class had little effect on the ability of NIRS to detect internal insect infestations. The minimum size of insects that could be detected by NIRS varied with species. We could detect *R. dominica* as small as 1.1 mm^2 with 95% confidence, whereas minimum detectable size for *S. oryzae* was 2.0 mm^2 and for *S. cerealella* was 2.7 mm^2. Kernels containing rice weevil pupae or adults were classified as being infested with 100% accuracy, and accuracy decreased as insects became smaller – 4th instars, 95% accuracy; 3rd instars, 82% accuracy; 2nd instars, 24% accuracy; and 1st instars, 10% accuracy. The ability of NIRS to classify kernels containing insects may have been partly due to the presence of chitin in the cuticle because wavelengths at which absorbance peaks of pure cuticle samples occurred were similar to those wavelengths that were most important for differentiation of uninfested and infested samples.

Although the method was unable to detect eggs or 1st or 2nd instars accurately, NIRS can be used for rapid and automated detection of 3rd instar or older insects in wheat kernels. These are the insect stages that contribute most to insect fragments in processed goods and cause the greatest amount of feeding damage to kernels. Thus, a high-speed sorter with a near-infrared detector could be useful for cleaning bulk grain of infested kernels. Analysis time for hidden insects is dramatically reduced by the use of NIRS instrumentation, so more sophisticated and thorough sampling protocols for the estimation of population size of pests in stored grain are possible using NIRS.

Detection of parasitoids in wheat kernels

Populations of primary pests of grain, such as *S. oryzae*, can be reduced by natural enemies. Parasitoids such as *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) lay eggs on a weevil larva inside a grain kernel, and the parasitoid larva develops within the grain. Currently, only adult wasps are used for augmentative releases because of the difficulty in differentiating between parasitised and unparasitised hosts inside grain kernels. However, adults are subject to damage during handling, and they may leave the release area. Thus, release of immature parasitoids inside kernels would be preferable. Ability to detect parasitoids inside kernels also would aid in quality assurance in biological control production facilities.

We investigated the ability of NIRS to detect *A. calandrae* developing on *S. oryzae* in wheat kernels using the same NIRS system that was used for the insect detection study described above (Baker et al., 1999). We found that the system worked best for parasitoid detection when the spectra were analysed twice. We first analysed the spectra to determine which kernels were uninfested and which kernels contained insects—either weevils or parasitoids developing on weevils. We then analysed the spectra using a different calibration to differentiate kernels that contained weevils from those that contained parasitoids developing on weevils.

In the first analysis, 97% of uninfested kernels, 90% of kernels containing weevil or parasitoid larvae, and 100% of kernels containing weevil or parasitoid pupae were correctly classified. In the second analysis, 100% of the kernels that contained weevil larvae or pupae or parasitoid pupae were correctly classified, but only 14% of kernels that contained parasitoid larvae were correctly classified. We intentionally adjusted our criteria for determining whether a kernel contains a parasitoid pupa to ensure that no kernels containing weevils were classified as parasitised. This resulted in a higher rate of misclassification of kernels containing parasitoid larvae. The advantage to this procedure is that we can select a sample of kernels that contains only parasitoid pupae and older parasitoid larvae, but no weevils. Thus, we ensure that every kernel in the release contains a parasitoid, that the age of the parasitoids is synchronised, and that none of the kernels contains a weevil. This is the ideal situation for an augmentative parasitoid release.

Detection of biopesticidal avidin in transgenic maize

Avidin is toxic to most insects against which it has been tested (Kramer et al., 2000), and a gene that encodes the protein avidin has been incorporated into maize (Hood et al., 1997). About 50% of these transgenic kernels do not contain avidin because of male sterility. Thus, for some uses, it is desirable to segregate kernels containing avidin from those that don't. The standard method for determining avidin concentration in grain is by ELISA, which is time-consuming, expensive, and destructive. So, we investigated whether we could use NIRS to segregate kernels with low and high levels of avidin (Kramer et al., 2000).

Single maize kernels were manually placed on the quartz window of a fibre-optic bundle attached to a DA7000 spectrometer (Perten Instruments, Springfield, IL, USA). We were able to classify kernels containing more than 60 ppm avidin with 92% accuracy and kernels containing less than 60 ppm avidin with 77% accuracy. The amount of avidin required to kill most insects tested is around 100 ppm (Kramer et al., 2000). Thus, NIRS is useful for segregating maize into samples containing low or high levels of avidin.

Quantification of insect fragments in flour

Insect fragments in processed commodities are an indicator of poor quality of the product. Thus, the U.S. Food and Drug Administration has set the defect action level for insect fragments in flour at 75 per 50 g of flour. The standard method used to extract and quantify these fragments is time-consuming and expensive (AOAC, 1997). Thus, we investigated whether NIRS could be used to quantify insect fragments in flour (Perez-Mendoza et al., 2003).

Flour samples containing from 0 to 300 *S. oryzae* fragments were prepared. We analysed the samples by placing the flour above a quartz window and illuminating the sample from below. We used a DA7000 spectrometer to analyse the data (Perten Instruments, Springfield, IL, USA).

More than 90% of samples that contained more than 75 fragments per 50 g of flour were correctly classified as having more than 75 fragments. However, less than 40% of samples that contained less than 75 fragments were correctly classified as containing less than 75 fragments. The method is more accurate using a defect action level of 130 fragments. In this case, more than 90% of samples that contain more than 130 fragments are correctly classified, and more than

80% of samples that contain less than 130 fragments are correctly classified. Although the standard method for fragment detection is more sensitive than NIRS, the standard method is expensive and time-consuming. Thus, the NIRS method may be useful for prescreening a large number of samples, and then conducting chemical analyses on only selected samples.

Insect identification

Beetle pests of stored products can be difficult for the storage manager to identify because most of these species are small, similar in colour, and lack distinctive features. Correct identification of insects is critical to making pest-management decisions. Thus, we investigated whether NIRS could be used to identify 11 species of adult stored-product beetle pests (Dowell et al., 1999).

Insects were manually placed in a black V-shaped trough and illuminated. We used a DA7000 spectrometer (Perten Instruments, Springfield, IL) for the analyses. Data were analysed by using a back-propagation neural network (NeuralWare, 1995).

Classification accuracy was over 99% when identifying insects as primary or secondary pests (primary pests can damage intact kernels of grain; secondary pests can't damage intact kernels of grain), and over 95% when identifying insects to genus level. Insects could not be reliably identified to species when all 11 species were included in the analyses. However, some species could be reliably separated from others in the same genus or family. The bostrichids, *R. dominica* and *Prostephanus truncatus*, could be differentiated with 100% accuracy; *Cryptolestes ferrugineus* and *C. pusillus* could be differentiated with 90% accuracy; *Sitophilus granarius*, *S. oryzae*, and *S. zeamais* could be differentiated with 83% accuracy; *Tribolium castaneum* and *Tribolium confusum* could be differentiated with 80% accuracy; whereas, *Oryzaephilus mercator* and *Oryzaephilus surinamensis* could not be differentiated (55% accuracy). Although we were unable to correctly identify some of these insects to species, we could correctly identify them to genus and as primary or secondary pests, which is probably sufficient for making most pest-management decisions. NIRS would be a useful tool for differentiating some species within a genus that are difficult to identify, such as differentiating *C. ferrugineus* from *C. pusillus*.

Chronological age-grading of stored-product beetles

Knowing the age of insects can be important in making pest-management decisions. For example, many female stored-product beetles lay most of their eggs during the first third of their lives. Thus, if a sample of insects contains mostly old insects, then no control action may be required. However, if a sample of insects contains mostly young insects, then that population is of prime reproductive age and may quickly reach damaging levels. Current methods of age-grading stored-product beetles, such as examination of ovaries (e.g. Scholz et al., 1998), can be time-consuming and must be done in the laboratory by trained personnel. We examined the potential of NIRS for age-grading *R. dominica*, *S. oryzae*, and *T. castaneum* (unpublished data). We previously showed that NIRS was more accurate than the standard age-grading method used for flies (Perez-Mendoza et al., 2002).

We used a DA7000 spectrometer (Perten Instruments, Springfield, IL, USA) for the analyses. Insects were manually placed in a black V-shaped trough and illuminated. We used a physiological age scale from 0 to 1, rather than days, to normalise the data in the analyses because the three species live for different lengths of time when reared at the same temperature (at 20•C, *R. dominica* lived 216 days, *S. oryzae* lived 103 days, and *T. castaneum* lived 227 days). Thus, an insect of age 0 is a newly emerged adult, and an insect of age 1 is nearly dead from old age.

The sex of the beetles did not affect confidence limits on predicted ages of the beetles. However, confidence limits on age predictions for all three species were about 0.3. These are broad confidence limits, but the method allows us to differentiate insects in the first third of their lives from insects in the last third of their lives. Reproductive rates for these two age groups are quite different, with young beetles being at their most fecund and the old beetles laying few or no eggs. Thus, NIRS provides a quick method for determining reproductive potential of these beetles to aid in making pest-management decisions.

Conclusions

We have developed several novel applications of NIRS to entomological problems associated with stored products, including use of NIRS to measure a physiological process such as insect ageing. Several of these applications have current potential for commercial use. Although NIRS is not always as accurate or as sensitive as chemical or chromatographic analytical techniques, NIRS does have the advantage of being rapid, relatively inexpensive, and non-destructive. However, one of its most beneficial advantages is its portability and potential for incorporation into automated processing. With newer and more powerful NIRS instrumentation in development, it is very likely that the number of applications for NIRS in entomological research will continue to increase.

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