Rapid detection of contaminants in cereals

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Introduction

Cereals such as wheat, rice and maize form one of the most important parts of the World's diet. The cereals industry, however, is presently in a state of change. In the European Union two of the most significant factors are reform of the Common Agricultural Policy, which aims to lower grain prices, and the General Agreement on Tariffs and Trade Agriculture Agreement, which aims to limit expenditure on export subsidies. These changes are forcing the industry to reduce production costs while still meeting the quality specifications demanded by markets. The purpose of this paper is to discuss progress being made to help the cereals industry achieve higher standards by improving the detection of contamination in the harvested crop.

Types of contamination in a bulk of cereal can include other cereal species (for example wild oats), seeds other than cereals (oilseed rape), decayed and damaged grain, rodent pellets, bird droppings, ergot (parasitic fungi of various cereals which form mycotoxins poisonous to humans and animals) and insects. Contamination is rarely found at high levels but it frequently forms part of the specification in contractual agreements for both home and export markets. With increasing competition between suppliers, the detection of contamination can result in rejection. Our work has started by focusing on insects because, if not detected early, they can give rise to subsequent contamination of rapidly increasing severity.

Insect infestation in harvested cereals is caused largely by a series of beetle species which have become adapted to the conditions in stores. The insects are typically small, are either translucent or dark in colour, depending on life stage, and tend to hide in cracks and crevices. These factors make them difficult to detect. Significant advances have been made in the detection of insects in static bulks of cereals by the development of traps and attractant lures. This approach is very useful for monitoring static bulks in stores but it has limitations because it takes several days and is not effective at low temperatures, when insects move less, or for hidden infestations of insects which develop within grain kernels.

By far the majority of cereal is traded at least once during its passage from the farm on which it was grown, through one or more stores, at point of export or import, to arrival at its destination, whether intended for human or animal consumption. At busy times, traders may have to deal with grain flows in excess of 1000 tonnes each hour, constantly having to make quick judgements about its quality on which large sums of money depend. In the absence of any better alternative, assessment of contamination is presently made largely by eye using experienced inspectors. To improve this position, we are trying to develop the first rapid method for detecting contaminants in bulks of cereal on the move. This would allow grain quality to be improved by helping sources of contamination to be identified and avoided in the future. By revealing more accurately which bulks are infested with insects, it would reduce the prophylactic use of pesticides and minimise the spread of insect resistance to pesticides. Other advantages would result from avoiding the

inevitable subjectivity of human assessors, providing a constant performance standard, being cheaper than an experienced inspector and examining a greater proportion of the bulk. Our target is to detect infestations of one insect per kilogram of cereal at a speed which does not interfere with the normal flow of grain at a point of inspection.

The first indication that near infrared (NIR) might be useful for pest detection came from a study in which it was shown that NIR could be used to quantify levels of mite infestation in pig feed down to about 10^5 mites kg^{-1}.[1] Our first work with insects showed the ability of NIR to detect adults of all four species examined in samples of about 5 g of unmilled wheat, even though they formed less than 1% of the sample by weight and volume and were unevenly distributed.[2] We then showed that samples of about 69 g of unmilled wheat infested with up to 30 adult grain weevils (*Sitophilus granarius*) gave encouraging partial least squares regressions of SNV-detrended spectra with standard errors of calibration between one and two insects.[3] Similar results were obtained with adults of the saw-toothed grain beetle (*Oryzaephilus surinamensis*), these being even smaller than the grain weevils.[4] However, while there is good evidence to believe that the NIR response is due to the insects not an artefact, the sensitivity of the method is inadequate for practical use since the limit of detection is no better than about 270 insects kg^{-1}.[5]

In exploring how to improve the sensitivity substantially, we have exploited an unexpected discovery that there were clear spectral differences between a sample of uninfested wheat and another in which the kernels contained larvae of the grain weevil developing invisible to the naked eye.[4] Subsequently, it was found that single uninfested wheat kernels and single wheat kernels infested internally with grain weevil larvae might be distinguished from each other by their scatter-corrected, second derivative log $1/R$ spectra.[5] We have now undertaken a detailed study of the NIR reflectance response to infestation in single kernels, using many more samples, insects at an advanced stage of development and with the kernel orientation controlled. The aim was to identify a single measurement wavelength which could be used to detect internal infestation from raw log $1/R$ data without scatter correction procedures. Such a wavelength might then be used in developing a simple filter-based NIR imaging system for the rapid and non-destructive detection of internal infestation in unmilled cereals.

Experimental

General

Insects used were *S. granarius*, Windsor strain. Wheat used for culturing was variety Mercia. Infested samples consisted of single wheat kernels containing pupae, which were identified to developmental stage by x-ray inspection. Uninfested kernels for use as control samples were also obtained by this method at the same time and from the same culture.

Preparation of samples

Twenty replicate samples, each consisting of a single infested kernel, were obtained together with twenty replicate samples each consisting of a single uninfested kernel. All samples were stored at ambient temperature and humidity for approximately one day before scanning. Immediately prior to scanning it was confirmed by visual inspection that the outer surface of each infested kernel remained intact, free from any signs of the commencement of insect emergence.

NIR spectroscopy and data analysis

Samples were scanned by reflectance on an NIRSystems 6500 spectrometer over the wavelength range 400–2500 nm at 2 nm intervals. Single kernels were held tight to the inner face of a standard round sample cell of 3.5 cm diameter by means of a black metal annulus. The annulus

had a shallow central recess to hold the sample, such that one side of the kernel was irradiated. For both sample types, 10 kernels were oriented such that the kernel crease faced towards the spectrometer optics (crease up) and the remaining 10 such that the kernel crease faced away from the spectrometer optics (crease down). To allow for any change in ambient temperature or humidity during the experiment, or for any instrument drift, uninfested and infested samples were scanned alternately. Also, the first 10 samples were scanned crease up, the second 10 crease down, the third 10 crease up and the final 10 crease down. Each sample was rotated during scanning. Data handling was undertaken using Infrasoft International NIRS2 calibration software. Where stated, standard normal variate (SNV) transformation and detrending,[6] using Whitebytes NIR Tools software, were employed to remove scatter, particle size effects and baseline drift from raw log 1/*R* spectra. Figures were obtained using Sigmaplot Windows.

Results and discussion

All spectra are shown from 1100–2500 nm only, as in the region 400–1100 nm they consist of a broad featureless band centred in the visible. Log 1/*R* spectra of the infested and uninfested kernels are shown in Figure 1. Gross differences in absorbance arise from differences in kernel size between samples. To allow observation of the spectral changes due to infestation, the average raw log 1/*R* spectra of the two sample types were produced (Figure 2). It can be seen that the main feature of the NIR response to infestation in a single kernel is a large general decrease in absorbance (increase in reflectance) at all wavelengths. This observation is in agreement with the response to internal infestation observed in the case of larger samples containing many kernels (Ridgway and Chambers, in preparation). Such an effect is thought to be a consequence of an increase in the concentration of refractive index discontinuities within the sample, in particular air–particle interfaces.[7] It appears likely that specular reflection from the surface of the internal cavity of the infested kernel (produced by insect feeding) is being detected, possibly together with specular reflection from the surface of the insect itself.

The spectra of Figure 2 suggest that, as a result of such changes in the physical structure of the kernel with infestation, any one of a wide range of measurement wavelengths might be suitable for incorporation into a detection system based on imaging. In this study however, gross variation in absorbance levels between kernels of different size produces the need for a second (reference)

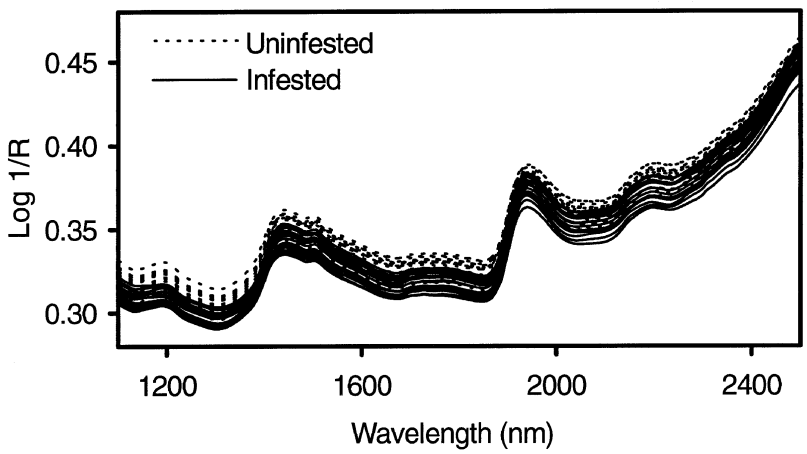

Figure 1. Individual log 1/*R* spectra of uninfested and infested kernels.

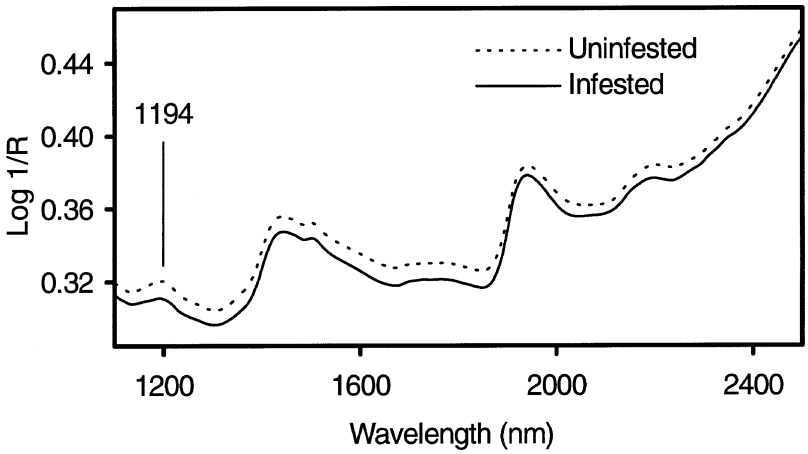

Figure 2. Average spectra for uninfested and infested kernels.

wavelength. Use of a reference wavelength to remove the physical component of the response to infestation, limits the present study to detection of changes in chemical composition.

Evidence for a changed chemical composition with infestation can also be seen in Figure 2. In particular, what is thought to be a starch band at 1194 nm (Reference 8: 1202 nm) is much less pronounced in the average raw log 1/*R* spectrum of the infested kernels. Figure 3 shows the corresponding SNV-detrended average spectra. Here, effects due to changes in the physical properties of the samples are removed and spectral differences arise solely from differences in chemical composition. Figure 3 confirms the band at 1194 nm to be a suitable candidate for use as a measurement wavelength. It also suggests that 1304 nm may be useful as a reference wavelength: as well as correcting for kernel size, the difference [log 1/*R* (1194) – log 1/*R* (1304)] will give a measure of the height of the band at 1194 nm.

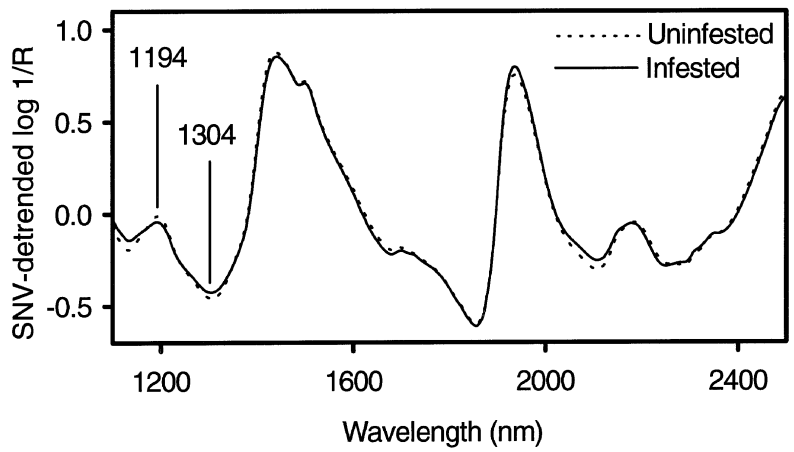

Figure 3. SNV-detrended average spectra for uninfested and infested kernels.

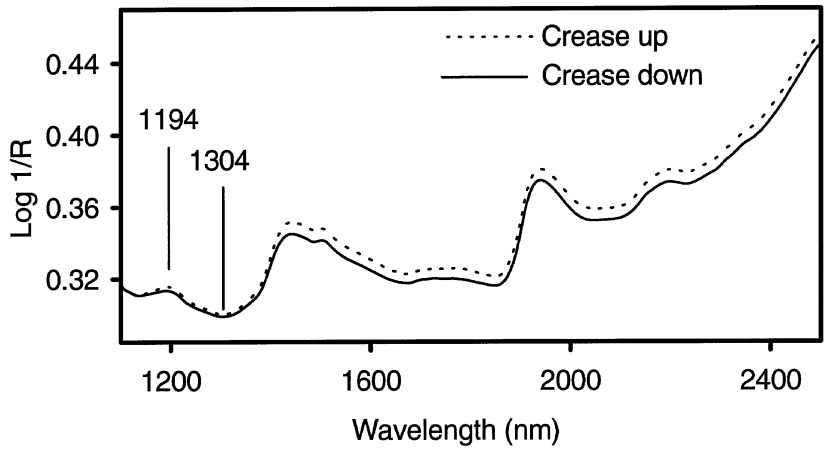

Figure 4. Average spectra for kernels crease up and kernels crease down.

In choosing a reference wavelength, a further consideration is the effect of kernel orientation. Figure 4 shows the average raw log 1/*R* spectrum of the 20 samples placed crease up in the sample cell (infested and uninfested combined), together with the average spectrum of the 20 samples placed crease down. This shows that the change in absorbance with orientation at 1304 nm is similar in direction and magnitude to the change at the proposed measurement wavelength 1194 nm, confirming 1304 nm to be a suitable candidate for the reference wavelength.

Figure 5 gives [log 1/*R* (1194) – log 1/*R* (1304)] for each of the 40 samples studied. A small but complete resolution of the two sample types, infested and uninfested, is obtained. This result shows that measurement of absorbance values at the above two wavelengths, without any form of scatter correction, can be used to detect the presence of internal infestation in single wheat kernels. Detection is successful irrespective of kernel orientation, although it can be seen that resolution

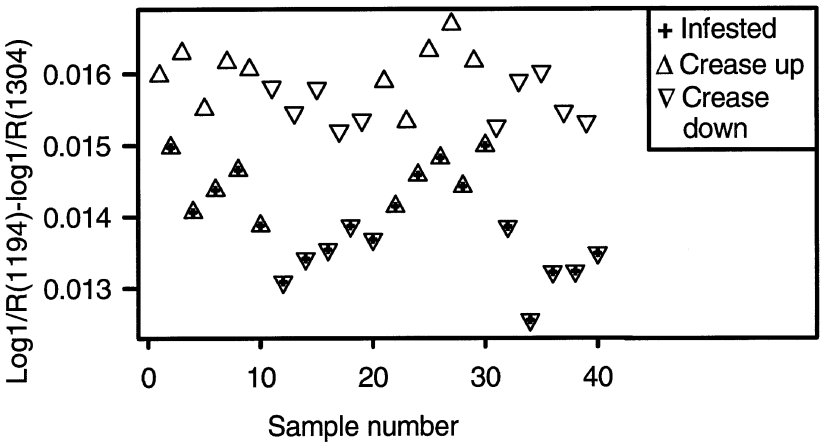

Figure 5. Log 1/*R* (1194) – log 1/*R* (1304) for uninfested and infested kernels.

is greater when the kernels are placed crease down. The origin of this response to infestation is likely to be wheat starch, lost as a consequence of insect feeding.

Conclusions

Infestation in post harvest cereals, even by insects developing within grain kernels, causes changes in NIR response. These changes are small and limit the sensitivity of the technique using conventional NIR methods. However, work presented here with single grain kernels internally infested with pupal stages of the grain weevil shows that it is possible to detect infestation by measurement at just two wavelengths. This suggests that it should be possible to develop a method of detecting infestation of the required sensitivity by an approach based on imaging. This would simultaneously be capable of detecting the other contaminants which are typically found in cereals.

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