

“INSECT PEST MANAGEMENT IN SWEET CORN”

(Project VG 97036)

Milestone Number 8

The practicalities, commercial potential and reliability of techniques for mechanically sorting damaged and undamaged cobs in a sweet corn pack-house have been assessed.

- a) Near infra-red equipment has been assessed for its ability to identify damaged and undamaged cobs.**
- b) Commercial suppliers of this equipment have been identified and assessments made on the practicality of incorporation of near infra-red equipment into the sorting line of a sweet corn packing shed.**

Due date:- 30/4/2001.

Summary

Discrimination of the defects of sweet corn cobs (poor grain tip fill, insect damage and presence of the heliothis grub) from clean cobs can be achieved non-invasively by near infra-red spectroscopy. The NIRSystems 6500 scanning spectrometer and the Zeiss MMSI miniature spectrometer (coupled with a high intensity quartz halogen lamp -100 watts) can achieve this discrimination. However, in a practical packing shed operation the relatively rapid and inexpensive Zeiss MMSI spectrometer offers the most promise. Additionally, this unit has no moving parts (monolithic construction) and is compact and less susceptible to vibration and dust compared to the scanning NIRSystems 6500 unit.

Further work needs to be undertaken to improve the signal to noise ratio of the optical configurations. This will involve further experimentation with various optical configurations, integration times for spectral acquisition and light intensities. Consultation with sweet corn packers has indicated the need to acquire spectral data over the first 7 cm of the sweet corn cob tassel end for a full 360 degrees. Our earlier work has shown that transmission mode results in insufficient signal and so reflectance mode is the preferred option.

The next step towards commercialisation would be to build an at-line prototype unit centred on the Zeiss MMS1 photodiode array spectrometer. This unit would need to rotate the individual sweet corn cob so that spectral acquisition occurs over the entire tassel end of the cob. Light intensity must be such as to penetrate to the grain kernels and allow sufficient signal to subsequently reach the detectors without heat damage to the cob.

At this stage the reliability of the technique is untested with regard to performance with new populations. An at-line prototype unit would allow acquisition of large data sets in a packing shed situation to test this reliability / robustness issue.

The following commercial suppliers of NIR equipment have been identified:

Colour Vision Systems Pty. Ltd.
11 Park Street,
Bacchus Marsh,
Victoria Australia 3340

Horticultural Automation Limited
11 Spring Street
Onehunga
PO Box 13516,
Auckland 1132 New Zealand

Mitsui Mining & Smelting Co. Ltd,
Saitama. Japan

Fantec Research Institute
Hamamatsu City
Shizuoka Prefecture. Japan

Currently corn pack houses employ manual sorting of cobs for 'heliothis' damage and a conveyor transport mechanism to a 'tipping' unit for removal of the damaged area. NIRS detection of grub and grub damage could allow further automation of this chain.

Colour Vision Systems (CVS) is the commercialisation party in a HAL supported project area related to NIRS assessment of eating quality of intact fruit. CVS is an Australian based manufacturer of colour and size fruit sorting equipment and are the logical party to involve in the commercialisation of a corn cob sorting technology.

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a) Near infra-red equipment has been assessed for its ability to identify damaged and undamaged cobs, and assessments made on the practicality of incorporation of near infra-red equipment into the sorting line of a sweet corn packing shed.

Near Infra-red Spectroscopy to detect insect larvae(*Helicoverpa armigera* – heliothis) / insect damage in sweet corn cobs

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Introduction

Near infra-red reflectance spectroscopy (NIRS) is a non-destructive procedure that uses optical data rather than wet chemistry methods to analyse both liquid and solid products for chemical composition. Near infra-red reflectance spectroscopy has been used for over twenty years to analyse grain products for protein, oil and moisture (Shenk and Westerhaus 1993). The use of the technique however has been limited to low moisture materials, as water absorbs strongly in the near infra-red (NIR) region of the electromagnetic spectrum of radiation. The advent of powerful personal computers, fibre optics, improved sensor technology and chemometric software packages has allowed this technology to be applied to high moisture materials (such as fresh whole fruit) in an in-line situation, in the last decade.

Near infra-red is a small part of the electromagnetic spectrum of radiation (700 - 2500 nm). At one end of this spectrum are the high energy waves such as x-rays and gamma rays, while at the other end of the spectrum are the low energy waves such as micro waves and radio waves. Near infra-red is between the visible and the infra-red regions of the spectrum. The area of the electro-magnetic spectrum of interest is between 700 and 2500 nm and concerns the bending and stretching of electronic bonds (C-H, N-H and O-H). These bonds are involved in most organic compounds, such as sugars, protein, lipids and water. Near infra-red spectroscopy is a secondary method of measurement and so must be calibrated against a primary or reference method, such as a refractometer reading (°Brix). Because of this requirement, the technique is only cost effective with large sample numbers. The calibration may be established in either a quantitative (e.g. linear regression of Brix content) or qualitative (e.g. discrimination between groups) basis.

Ridgway and Chambers (1996) and Chambers and Ridgway (1995) used NIR reflectance spectroscopy to detect external and internal insect (grain weevil) infestation of intact wheat kernels. Large spectral differences were observed between uninfested kernels and kernels infested internally with *Sitophilus granarius* (L) (grain weevil) larvae or pupae, arising from both a changed chemical composition and physical structure. Single uninfested and infested kernels were distinguished by their second derivative (d^2) spectra. For both external and internal infestation there was substantial evidence that insect protein and/or chitin and moisture were being detected. Near infra-red spectroscopy should be useful as a rapid method of detection. Further work by Chambers and Ridgway (1998) with single wheat grain kernels internally infested with pupal stages of the grain weevil showed the possibility of detecting such infestation by measuring just two NIR wavelengths (1202 and 1300 nm). These workers used the 1100-2500 nm region of the spectrum and discriminated infested from uninfested

samples simply by the increased reflectance (decrease in absorbance) of infested kernels due to the increase in specular radiation from the internal cavities (as a result of insect feeding) and from the insect itself.

Materials and Methods

Sweet corn cobs fruit were harvested from two locations in Queensland, namely the Lockyer Valley and the Burdekin irrigation areas. Cobs were selected to include clean cobs, cobs with poor grain tip fill, cobs with grub damage and cobs with grubs present. Sweet corn cobs were transported under dry ice (8-10° C on arrival) and assessed within three days of harvest. Near infra-red spectra were collected from the cob tassel end (first 7 cm of tip) of individual cobs. Spectra were collected from two commercially available research instruments, the NIRSystems 6500 (700 – 2500 nm, remote reflectance probe), and the Perten DA 7000 (700 – 1700 nm, interactance probe), and a purpose built unit based on the Zeiss MMS1 miniature spectrometer (700 – 1050 nm). Various optical configurations were used to gather spectra from the sweet corn cobs (Figs. 1). Integration time for spectral acquisition varied from 100 to 160 milliseconds per cob (four scans per spectra), to maximise the signal to noise for the Zeiss unit. Discriminant equations were developed using partial least squares regression analysis within the WinISI II (vers. 1.02a) chemometric package.

Four experimental runs were undertaken over the season using various spectrophotometers and optical configurations (Table 1 and Fig. 1). In run four, the tip (tassel) of the corn cob was halved longitudinally and presented to the instrument sheath uppermost. In this experiment, spectra were acquired of 80 cobs (20 of each category), of which five were randomly selected from each of the four groups for validation of the discriminant equation (developed on the remaining set). The spectral data was analysed using the discriminate function of the WinISI II vers. 1.02a chemometric software package (Table 2 and 3). In the discriminant analysis, spectral data were pre-treated with regard to derivatives, smoothing and scatter correction.

Results and Discussion

According to Shenk and Westerhous (1993), discriminant analysis is best undertaken with no scatter correction (particle size and scattering of light may assist in sample discrimination) and first derivative to eliminate base line error. A mathematically pre-treatment of first derivative derived over four data points with no smoothing, was found to give the optimum results and subsequently used.

The Perten DA 7000 spectrophotometer was unable to discriminate between groups although it operates with a high intensity tungsten halogen lamp (42 watt) and covers an area of the electromagnetic spectrum from 700-1700 nm, using both silicon (Si) and (InGaAs) photodiodes. This unit was operated in the interactance mode (Fig. 1(a)) and as such, the area viewed by the probe was relatively small (e.g. less than 10% of the area viewed by the remote reflectance fibre optic probe of the NIRSystems 6500). The bifurcated fibre optic bundle carries incident light down the outside bundle and the reflected light from the sample back to the detectors through the centre fibres. However, the high intensity light and interactance mode should have resulted in good light penetration of the sample. The poor result cannot be explained except by the fact that the instrument was on loan for a short time period and was possibly not set up optimally with regard to integration time. Possibly, the interactance mode

was not the ideal configuration, or gathering information over a very small area of sample with the potential to miss the localised defect.

The experimental run utilising the Zeiss MMSI spectrophotometer in a side and tip presentation of the sweet corn cobs (Table 1 and Fig. 1(b &c)), again showed an inability to discriminate between the various groups of sweet corn cobs (clean, damaged, poor grain tip fill and grub presence). Both these optical configurations utilised the transmission mode. Because of the difficulty in sealing the shroud containing the fibre optic bundle (carrying transmitted light to the detector array), the poor result could be best explained by the intrusion of excessive specular radiation. Also most of the light reaching the detector would have arisen from the sheath with little useful information regarding the actual defect.

The third experimental run utilised a high intensity quartz halogen car lamp (100 watt) with a parabolic reflector to deliver light to the object, operated in the transmission mode (Fig. 1e). Again poor discrimination occurred and this could be attributed to the transmission mode reducing the proportion of the signal containing defect information reaching the detectors. In the fourth experiment, discrimination was achieved using the same configuration but reduced sample thickness.

In the fourth experimental run, good discrimination also was achieved (Table 2 and 3, Fig.1 (d & f)) with the NIRSystems 6500. In this run, the transmission mode was utilised but only half of the tip of the cob was viewed. The slightly better results of the 6500 could be attributed to the operation of the remote reflectance fibre optic probe in a light proof box (no ambient light adding to the signal) and the general precision of the instrument compared to the photodiode array of the Zeiss. Discriminant analysis for the 6500 as reported in Table 2 and 3, was carried out on the full spectrum (700-2300 nm). However, separate analysis also was undertaken on 700-1100 nm (as with the Zeiss) and 1100-2300 nm areas of the spectrum. These results demonstrated the region of 700-1100 nm gave better results than the 1100-2300 nm region alone.

In a plot of the first derivative data obtained from both the Zeiss and 6500 instruments (experiment 4) the areas of the spectrum showing most divergence, occurred around 960 and 1030 nm. These areas could be attributed to water and protein, respectively

Clean cobs were always distinguished (by both instruments) from damaged ones. However, there was some confusion with poor tip fill cobs sometimes misdiagnosed as insect damaged. Also, the discriminant equation could not always differentiate between damaged and damaged with grub.

With both the Zeiss and 6500 (experiment 4), in distinguishing the groups and various combinations of the groups (Table 2 and 3) on no occasion was a defect cob (ie. damaged, poor tip fill or grub present) included in the clean group.

Table 1 Discriminant analysis of the spectral data obtained from the various instruments and configurations, using the chemometric package WinISI vers. 1.02a. Inability to distinguish between groups occurred when <20% correctly identified.

| Experiment | | No. of spectra | Instrument | Optical configuration | Groups distinguished |
|------------|----------|----------------|-----------------------------|--|----------------------|
| No. | Date | | | | |
| 1 | 18/09/98 | 198 | Perten DA7000 | Interactance (42 watt) | No |
| 2 | 24/11/98 | 410 | Zeiss MMSI | side –transmission (50 watt) | No |
| | | 410 | Zeiss MMSI | tip –transmission (50 watt) | No |
| 3 | 25/11/98 | 106 | Zeiss MMSI (700-1100 nm) | large lamp – transmission (100 watt) | No |
| 4 | 25/02/99 | 80 | 6500 (700-2300 nm) | Reflectance (75 watt) | Yes |
| | | 80 | Zeiss MMSI | Half cob tip – transmission (100 watt) | Yes |

Table 2. Discriminant analysis of sweet corn cobs into 4 groups using partial least squares regression (WinISI II vers. 1.02a).

| Groups | Instrument | Diagnosis (out of 5) | Correct (of diagnosed) | Incorrect diagnosis |
|-------------------------|------------|-------------------------|---------------------------|------------------------|
| 1 Tip fill (T) | 6500 | 5 | 3 | 1D, 1G |
| | Zeiss | 5 | 4 | 1G |
| 2 Clean (C) | 6500 | 5 | 5 | |
| | Zeiss | 6 | 5 | 1T |
| 3 Damaged (D) | 6500 | 5 | 3 | 2T |
| | Zeiss | 3 | 2 | 1G |
| 4 Damaged + grub (G) | 6500 | 5 | 4 | 1D |
| | Zeiss | 6 | 3 | 3D |

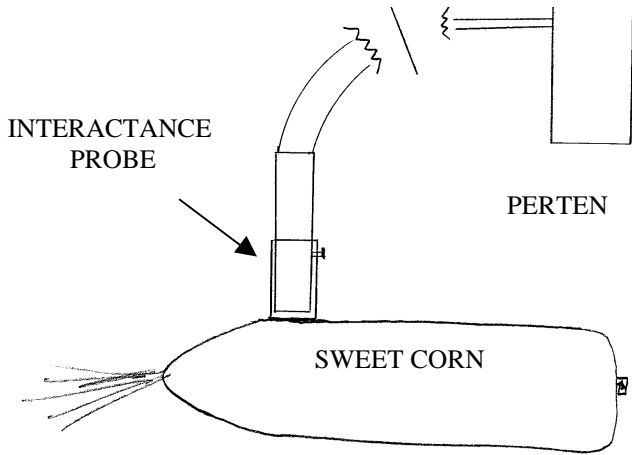
The discrimination was undertaken on four groups of sweet corn cobs with 15 samples in each calibration set and 5 samples in each validation set. The number of samples from the validation set (Diagnosis column) correctly assessed is given under the heading ‘Correct out of diagnosis’ and the misdiagnosed in the final column.

Table 3. Discriminant analysis of sweet corn cobs into 2 groups, using partial least squares regression (WinISI II vers. 1.02a) analysis. Data of Table 2 (recatergorised).

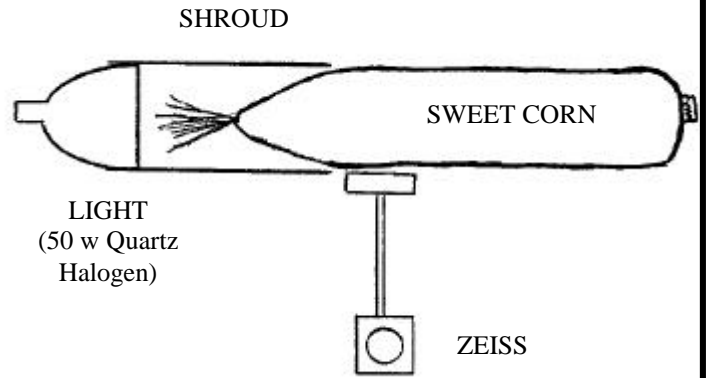
| Groups | Instrument | No. in calibration set | No. in Validation set | Diagnosis | Correct | Incorrect |
|--------------------------------------|-------------------|-------------------------------|------------------------------|------------------|----------------------|------------------|
| 1. Clean vs Damaged | 6500 | 30 | 5C,5D | 10 | 10 (5C,5D) | |
| | Zeiss | 30 | 5C,5D | 10 | 10 (5C,5D) | |
| 2. Clean vs Damaged with grub | 6500 | 30 | 5C,5G | 10 | 10 (5C,5G) | |
| | Zeiss | 30 | 5C,5G | 10 | 10 (5C,5G) | |
| 3. Clean vs Tip Fill | 6500 | 30 | 5C,5T | 10 | 10 (5C,5T) | |
| | Zeiss | 30 | 5C,5T | 10 | 9 (4C,5T) | 1 T |

| | | | | | | |
|--|-------|----|-----------|----|-------------------------|-------|
| 4. Clean vs Tip fill, damaged, grub | 6500 | 45 | 15TDG,5C | 20 | 20 (15TDG,5C) | |
| | Zeiss | 45 | 15TDG,5C | 20 | 18 (15TDG,3C) | 2 TDG |
| 5. Tip Fill & Clean vs Damage & damage + grub | 6500 | 60 | 10DG,10CT | 20 | 17 (10DG,7CT) | 3 DG |
| | Zeiss | 60 | 10DG,10CT | 20 | 19 (10DG,9CT) | 1 DG |
| 6. Clean vs Damaged & Damaged + Grub | 6500 | 45 | 5C,10DG | 15 | 15 (10DG,5C) | |
| | Zeiss | 45 | 5C,10DG | 15 | 14 (11DG,4C) | 1 DG |

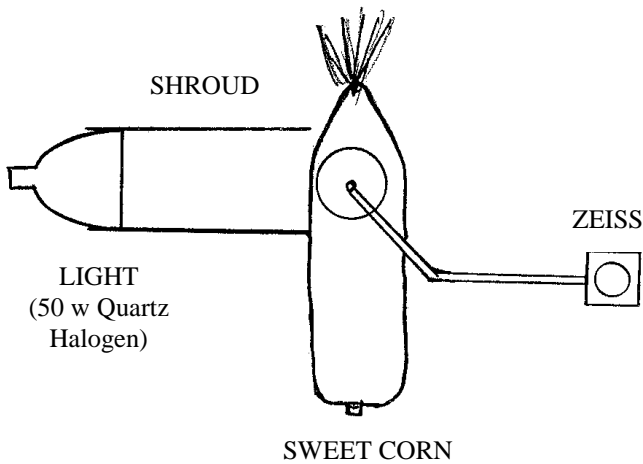
1a. PERTEN INTERACTANCE (Experiment 1)



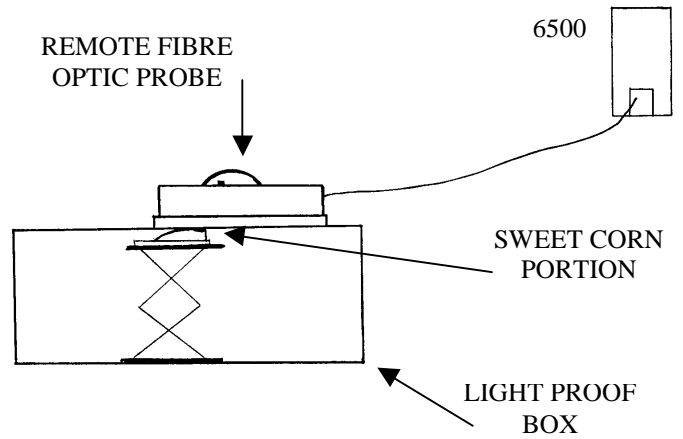
1b. ZEISS MMSI – TRANSMISSION (Experiment 2A)



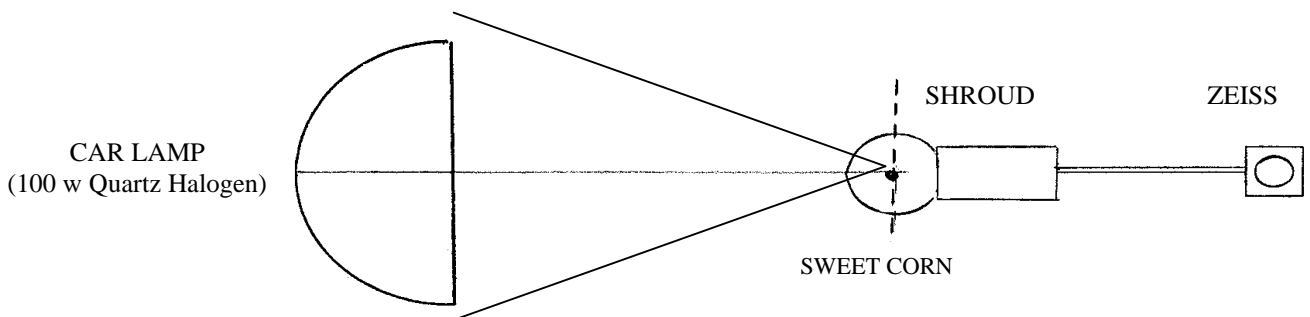
1c. ZEISS MMSI TRANSMISSION (Experiment 2B)



1d. 6500 – REFLECTANCE (Experiment 4A)



1e. ZEISS MMSI – TRANSMISSION (Experiment 3)



1f. ZEISS MMSI – TRANSMISSION (Experiment 4B)

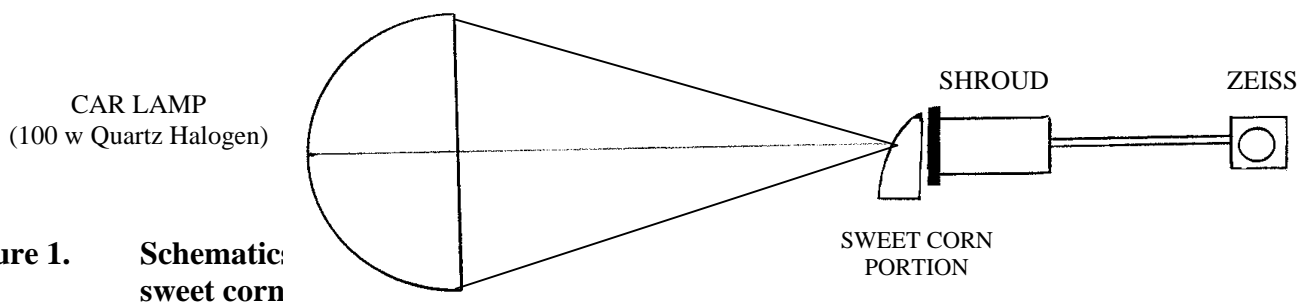


Figure 1. Schematic: sweet corn

Discrimination of the defects of sweet corn cobs (poor grain tip fill, insect damage and presence of the heliothis grub) from clean cobs can be achieved non-invasively by near infrared spectroscopy. The NIRSystems 6500 scanning spectrometer and the Zeiss MMSI miniature spectrometer (coupled with a high intensity quartz halogen lamp -100 watt) can achieve this discrimination. However, in a practical packing shed operation the relatively rapid and inexpensive Zeiss MMSI spectrometer offers the most promise. Further work needs to be undertaken to improve the signal to noise ratio of the optical configurations. This will involve further experimenting with optical configurations, integration times for spectral acquisition and light intensities.

The next step towards commercialisation would be to build an at-line prototype unit centred on the Zeiss MMS1 photodiode array spectrometer. This unit would need to rotate the individual sweet corn cob so that spectral acquisition occurs over the entire tassel end of the cob. Light intensity must be such as to penetrate to the grain kernels and allow sufficient signal to subsequently reach the detectors without heat damage to the cob.

An at-line prototype unit would allow acquisition of large data sets in a packing shed situation.

Acknowledgments

We thank Perten Australia for the loan of the DA7000 unit. The project was funded by the Horticultural Research and Development Corporation (VG97036).

References

Chambers, J. and Ridgway, C. (1995) *Near Infrared Spectroscopy: The Future Waves*. Eds. A.M.C. Davies and Phil Williams, NIR Publications, Chichester, UK, 484-489.

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.

Ridgway, C. and Chambers, J. (1998) Detection of insects inside wheat kernels by NIR imaging. *J. Near Infrared Spectrosc.* **6**, 115-119.

Shenk, J.S. and Westerhaus, M.O. (1993) *In:NIRS Handbook, Infrasoft International*, marketed by NIRSystems, Inc. Silver Springs, MD, USA, p. 14).

b) Commercial suppliers of this equipment have been identified.

The following commercial suppliers of NIR equipment have been identified:

Colour Vision Systems Pty. Ltd.
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Bacchus Marsh,
Victoria Australia 3340

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11 Spring Street
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PO Box 13516,
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