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# THE POTENTIAL OF NEAR-INFRARED SPECTROSCOPY FOR THE RAPID DETECTION OF PESTS IN STORED GRAIN

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by

J. CHAMBERS, C. RIDGWAY, AND C.B. van WYK

Central Science Laboratory (MAFF), London Road, Slough, Berkshire SL3 7HJ

C.W. BAKER

ADAS, Staplake Mount, Starcross, Exeter, Devon EX6 8PE

R.J. BARNES

NIR Systems European Office, Highfield House, Fountain Park,

Roxburgh Way, Maidenhead, Berkshire SL6 3UD

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**Abstract**

Significant progress has been made towards the development of the first rapid method for the detection of insect pests of cereals which are either outside or inside the grain kernels. The method is based on near-infrared spectroscopy (NIR), which is already familiar to the cereal trade for the measurement of moisture and protein. The work has established the optimum conditions for recording the NIR response from samples of cereal grains which are infested with either saw-toothed grain beetles or grain weevils. The method functions with different varieties of cereals at different moisture contents. The NIR technique can distinguish between uninfested kernels and those which contain larval or pupal stages of the grain weevil. The speed of scanning and simplicity of the data analysis mean that the result should be available within a few seconds. This will be of immense value to the cereal trade whether for the analysis of grain samples taken from a lorry, ship or conveyor belt. Presently the sensitivity of the technique is inadequate for trade use but recent developments in hardware will bring major improvements. It will then be possible to assess the feasibility of developing apparatus for the rapid detection of pests and the simultaneous measurement of other parameters including moisture and protein.

## Objectives

The ultimate objective is to assess the feasibility of developing a single piece of apparatus for the rapid detection of pests in cereals and the simultaneous measurement of other parameters including moisture and protein. The work described here involved assessing the importance of factors which influence the spectral response and discovering whether it is possible to develop a robust NIR calibration for insects which will function at population densities of 1 insect/kg or below.

## Introduction

The rapid detection of pests in post-harvest cereals, especially when in transit, is essential to allow informed decisions on quality, value and the need for any control measures. For grain in transit, existing methods rely on sampling, sieving and visual inspection. This is labour intensive, haphazard and largely ineffective: it is unlikely to detect pests at infestation rates of less than 5/kg (Wilkin and Fleurat-Lessard, 1990) whereas densities of more than 1/kg represent a serious potential risk to grain quality (Johnson, 1979). Cereals cannot be guaranteed to be free from live pests. The need to develop an improved method has been indicated in HGCA-funded reviews (Wilkin and Rowlands, 1988; Rowlands *et al*, 1989).

Recently, a significant advance has been reported in removing insects from grain (Wilkin *et al*, 1993). Using a flat-bed reciprocating sieve, samples of wheat and barley of up to 10kg were processed in less than two minutes with almost complete recovery of the adult insects added. However, this still represents only a minuscule proportion of the grain which may be passing on a conveyor at up to 1000 tonnes per hour. Further, it still requires visual inspection to register the presence of insects, and separation from other sievings may hinder this: with grain containing 2% screenings, insects were more difficult to spot in the receiver. Lastly, this method would not be able to detect insects developing within grain kernels.

It should be possible to overcome all these difficulties through the use of a spectroscopic technique (Chambers, 1987). One of the most promising is near-infrared (NIR) spectroscopy. This is already used in the cereal trade for measurement of relatively evenly distributed bulk parameters such as moisture and protein in small milled samples but there has been very little work on the analysis of unevenly distributed trace impurities such as pests. NIR can be used to detect flour mites in animal feed (Wilkin *et al*, 1986) and preliminary studies have shown the potential of NIR to detect stored product beetles in small samples of unmilled wheat (Chambers *et al*, 1992).

The purpose of the present project was to build on these preliminary findings and through the use of new NIR technology, state of the art spectrometers and sophisticated data-handling methods (Barnes *et al*, 1989) to achieve the objectives listed above.

**Materials and Methods**

Except where stated otherwise, the wheat variety used was Mercia conditioned to 15% moisture content and studied without milling. The insects used were *Oryzaephilus surinamensis*, saw-toothed grain beetles, of the Slough Laboratory insecticide susceptible strain, and *Sitophilus granarius*, grain weevils, of the Windsor strain. Adult insects were 2-4 weeks old. No attempt was made to determine their sex. Except where stated otherwise, freshly-killed adult insects were obtained by chilling in a container placed in solid carbon dioxide for ten minutes and then leaving at room temperature for two minutes prior to adding to the cell and scanning. Infested wheat kernels containing either larvae or pupae of *S.granarius* were identified by X-ray inspection. Uninfested kernels for use as control samples were obtained either by this method or (in Experiment 10.1) from the same original source as the wheat used for culturing.

Samples were scanned by reflectance (by transmittance in Experiment 2c only) on an NIR Systems 6500 spectrometer over the wavelength range 400-2500nm. For Experiments 1, 3, 4, 10.1, 11 and 12, samples were held in a round cell of 3.5cm diameter which was rotating, except in Experiment 12. In the other Experiments, samples were held in a transporter cell 20 x 4 x 1cm. The weight of wheat required to fill the transporter cell (about 69g) was measured using one sample of each of the four wheat types studied in Experiment 8.

For Experiments 5 to 9, 10.2 and 12, spectra were recorded with the spectrometer in transport mode and on its back to enable the sample to be scanned horizontally whilst remaining flat. Three horizontal passages of the sample across the spectrometer window were made and the resulting spectra averaged to improve the signal to noise ratio, except for Experiments 8 and 9 where only one was made to minimise the possibility of effects due to sample heating. For Experiments 7 to 11, the cells were backed with aluminium foil (bright side facing the sample). In Experiments 8 to 12, no sample was studied more than once.

Spectral data were subjected to standard normal variate (SNV) transformation and detrending (1100 to 2500nm only) (Barnes *et al*, 1989), using Whitebytes NIR Tools software. This removed scatter, particle size effects and baseline drift, and was used to generate difference spectra. Data handling was undertaken using Infrasoft International NIRS2 calibration software and graphical presentations of the log 1/R spectra were obtained using Sigmaplot Scientific Graph System software. Experiments were as follows:

1. Preliminary tests of optical configuration

Reflectance spectra were recorded using a 3.5cm rotating cup with samples on three layers of glass fibre discs, two white over one blackened with spray paint. Samples were as follows:

a) one sample of wheat with the sample cup covered firstly with a standard clear window then with an etched diffuse window,

b) repeat of the above with the wheat replaced with 51 *O.surinamensis* adults which had been stored in a desiccator over self-indicating silica gel for a week after being killed,

c) one sample of *O.surinamensis* adults placed in the sample cup with their dorsal surface facing the incident radiation, and another with their ventral surface facing the incident radiation (these and all subsequent samples in the rotating cup used the standard clear window),

d) samples of wheat containing ten freshly killed *S.granarius* adults which were either distributed randomly throughout the sample, or evenly spread on the top surface of the wheat facing the incident radiation, or evenly spread at the bottom of the 1cm thick layer of wheat.

2. Preliminary tests with transporter cell

The cell was filled with samples of wheat (69g) to which were added various numbers of freshly killed *S.granarius* adults. As the cell was moved past the beam of incident radiation, scans were made of successive parts of the sample, about three-quarters of the whole, and the scans averaged to give a single spectrum. The number of scans for each passage of the sample was 32 when detection was by reflectance. This was increased to 42 for detection by transmission to counter the increased noise due to the greater spread in pathlengths of the emergent radiation. Three spectra were recorded for each sample at each level of infestation, with thorough shaking of the sample between the spectra.

a) Reflectance spectra were recorded for one wheat sample with 0, 2, 5, 10, 15 and 30 insects, and another with 0, 1, 3, 7, 9, 11, 13 and 20 insects.

b) Reflectance spectra were recorded for a wheat sample with no insects, then with 49 insects all on the surface of the wheat closest to the incident radiation, then with the 49 insects all on the opposite surface of the 1cm thick sample of wheat, furthest away from the incident radiation.

c) Transmission spectra were recorded for one wheat sample with 10 insects, then for another with 0 and 30 insects.

3. Differences between *O.surinamensis* and *S.granarius* adults, dead and live adults, and different developmental stages of *O.surinamensis.*

The following samples were scanned by reflectance in the rotating cup on four layers of glass fibre discs, two white over two black:

a) *S.granarius* adults which had been killed in one sample by dipping in liquid dichloromethane, in another by chilling in a container cooled in solid carbon dioxide and in a third by heating in an oven, then all stored for a week in a desiccator with self-indicating silica gel,

b) repeat of experiment 3a but with *O.surinamensis*,

c) *S.granarius* adults which in one sample were live and in another were freshly killed with carbon dioxide,

d) repeat of experiment 3c but with *O.surinamensis*,

e) two "fresh" samples of *S.granarius* adults, one containing live insects and one insects freshly killed with carbon dioxide, and three "desiccated" samples which had been stored in a desiccator for a week after killing by dichloromethane, carbon dioxide or in an oven (one sample for each method),

f) repeat of experiment 3e but with *O.surinamensis*,

g) live *O.surinamensis* adults in one sample, pupae in another and larvae in a third,

h) two samples of *S.granarius*, one containing live adults and the other adults freshly killed in carbon dioxide, and two similar samples of *O.surinamensis*.

4. Differences between live and freshly killed *O.surinamensis*

Spectra were recorded in the rotating round cell of 3.5cm diameter with samples supported on three layers of glass fibre discs (two white over one black) and the cardboard cell backing which was also blackened. The samples were:

a) Empty cell.

b) Fifty live *O.surinamensis* adults which had been cooled for 20 min in a refrigerator for ease of handling.

c) Fifty *O.surinamensis* adults which had been freshly killed by chilling in a container placed in solid carbon dioxide for 10 min then left to warm for 2 min before addition to the cell.

This procedure was repeated until there was a total of seven samples of each type.

5. Linearity of response to different numbers of *O.surinamensis*

Spectra were recorded using the transporter cell filled with wheat containing 0, 2, 5, 10 and finally 20 freshly killed *O.surinamensis* adults. Five spectra were recorded at each level of infestation. Between each spectrum the contents of the cell were removed, mixed thoroughly and returned to the cell. After the fifth spectrum had been recorded,

further insects were added and the procedure repeated.

6. The effect of aluminium cell backing on the response to insects in wheat

Spectra were recorded using the transporter cell filled with wheat. Samples were as follows:

a) A layer of aluminium foil covering the face of the wheat away from the incident beam (bright side facing wheat).

b) Repeat of (a) but with no aluminium foil backing.

c) Repeat of (b) but with 30 freshly killed *O.surinamensis* adults which were added to the grain sample outside the cell and the mixture shaken before being re-added.

d) Sample (c) with the aluminium backing replaced.

7. The response to insects behind 1cm thickness of grain

Spectra were recorded using the transporter cell filled with wheat and covered on the side away from the incident beam with a layer of clingfilm and then a layer of aluminium foil. Samples were as follows:

a) No insects

b) Fifty freshly killed *O.surinamensis* adults were evenly distributed over the wheat between the clingfilm and the aluminium foil, taking care not to disturb the wheat to minimise spectral variation due to differences in wheat packing. The clingfilm was required to retain the insects at the back of the cell and prevent them from falling through the spaces between the wheat grains.

The insects were then discarded, the wheat was removed from the cell, mixed and repacked into the cell. The procedure was repeated a further six times using fresh clingfilm and fresh insects each time.

8. Variance in response to *O.surinamensis* in wheat

Spectra were recorded using the transporter cell filled with wheat containing 0, 1, 5, 10, 15, 20 and finally 25 freshly killed *O.surinamensis* adults. This procedure was repeated three times for each of four different samples of wheat of variety Mercia (hard wheat) at 13 and 22% moisture content and variety Beaver (soft wheat) at 15 and 24% moisture content, giving a total of 84 samples. Moisture contents were determined by the BS oven method (BS4317: Part 3: 1987). For the infested samples, insects were added to the wheat outside of the cell and the sample was then mixed before being added to the cell and scanned.

9. Origin of response to *O.surinamensis* in wheat

Spectra were recorded using the transporter cell filled with wheat variety Beaver at 15% moisture content containing 0, 5, 10, 15, 20 and finally 25 freshly killed *O.surinamensis* adults. This procedure was repeated twelve times, giving a total of 72 samples. For the infested samples, insects were added to the wheat outside of the cell and the sample was then mixed before being added to the cell and scanned.

10. The response to hidden infestations

*10.1: Preliminary test with round cell*

Spectra were recorded using an aluminium foil-backed round cell of 3.5cm diameter mounted in a standard sample holder and scanned in the transport mode. The spectrometer was oriented on its base with the sample moving vertically across the spectrometer window. Samples were as follows:

a) One 8ml sample of wheat kernels infested internally with *S.granarius* larvae, aged 3.5-4.5 weeks after egg-laying.

b) One 8ml sample of uninfested wheat kernels from the same original source as the infested wheat and stored under the same conditions as the infested wheat.

*10.2: Preliminary test with transporter cell*

Spectra were recorded using the transporter cell backed with aluminium foil. Samples were as follows:

a) One 18g sample of wheat kernels infested internally with *S.granarius* larvae, aged 2.5-3.5 weeks after egg-laying.

b) One 18g sample of uninfested wheat kernels from the same culture as (a).

Each 18g sample gave an almost complete layer of one grain thickness in the transporter cell. The uninfested wheat was separated from the same culture as the infested wheat after X-ray inspection. After the experiment both samples were retained. Adults emerged from the majority of wheat kernels assumed to be infested but no insects emerged from the wheat assumed to be uninfested.

11. Response to hidden infestations at the larval and pupal stage

Spectra were recorded using the round cell with the sample rotated. Samples were as follows:

a) Five 8ml samples with all wheat kernels infested internally with *S.granarius* larvae, aged 3-4 weeks after egg-laying.

b) Five 8ml samples with all uninfested wheat kernels from the same culture as (a).

c) Five 8ml samples with all wheat kernels infested internally with *S.granarius* pupae, aged 4-5 weeks after egg-laying.

d) Three 8ml samples with all uninfested wheat kernels from the same culture as (c).
Kernels containing insects at the required stage were separated from the culture using X-ray inspection and uninfested kernels were separated at the same time. This procedure was completed during one day and the samples were scanned on the following morning having been left at ambient temperature and humidity overnight.

12. Response to hidden infestation for single wheat kernels

Spectra were recorded using the round cell. A black metal annulus of the same external diameter as the internal diameter of the cell and having a shallow central recess was used to hold the sample at the centre of the cell window, such that one side of the kernel was irradiated. The sample was not rotated. Samples were as follows:

a) 7 samples of single wheat kernels infested internally with *S.granarius* larvae, aged 2.5-3.5 weeks after egg-laying.

b) 7 samples of single uninfested wheat kernels from the same culture as (a).

Kernels containing larvae were separated from the culture using X-ray inspection and uninfested kernels were separated at the same time. The samples were left at ambient temperature and humidity for approximately 2 days before being scanned.

**Results**

1. Preliminary tests of optical configuration

In Experiment 1a, log 1/R, first and second derivative spectra all showed differences between the clear and diffuse window in overall intensities but there were no differences in spectral detail. Spectra showed intensity differences between 460 and 680nm which might be due to sample packing artefacts or blocking of radiation by the window. Similar results were obtained in Experiment 1b.

In Experiment 1c, no difference was found between the samples of insects with their dorsal or ventral side uppermost.

There were differences between the spectra recorded in Experiment 1d but nothing which could be correlated with the presence or positioning of insects in the sample.

2. Preliminary tests with transporter cell

There was no obvious difference between the two samples in Experiment 2a, so their data were combined for analysis. Figure 1 shows the log 1/R spectra. Modified partial least squares regression of the spectral data against the number of insects was undertaken and the results are shown in Table 1.

Log 1/R spectra for the samples in Experiment 2b suggest (Figure 2) that the insects below the wheat layer are not detected.

In Experiment 2c with detection by transmission, there were visible differences between the spectra in the 700 to 1500nm region but these did not correlate with insect numbers.

Table 1: Modified partial least squares regressions of reflectance spectral data with scatter correction against number of *S.granarius* adults added to wheat in the transporter cell (Experiment 2a). [a] = respectively: derivative, datapoints used to calculate derivative, and two smoothing factors

| Equation number | Wavelength segments (nm) | Math treatment[a] | Standard Error of Calibration | $r^2$ |
|---|---|---|---|---|
| 1 | 406-1090 & 1110-2490 | 0,0,1,1 | 2.2 | 0.93 |
| 2 | as above | 1,4,4,1 | 1.1 | 0.98 |
| 3 | 1110-2490 | 1,4,4,1 | 1.6 | 0.96 |

3. Tests on differences between *O.surinamensis* and *S.granarius* adults, dead and live adults, and different developmental stages of *O.surinamensis*.

From Experiment 3a, the log 1/R spectra of the *S.granarius* killed in the oven and by chilling in carbon dioxide were similar to each other, whereas that of the insects killed by rinsing in dichloromethane showed larger absorptions at 718, 1492 and 1932nm (the last two possibly being due to water), and less absorption at 1328nm (possibly due to less -C=C-H). In contrast, in Experiment 3b there was no spectral difference between the three methods of killing *O.surinamensis*.

In Experiments 3c and 3d, no differences were observed for either insect species between the log 1/R spectra of the live insects and those which had been freshly killed by chilling with carbon dioxide.

Compared with the fresh *S.granarius* in Experiment 3e, the desiccated sample showed much weaker absorptions in the region 1430-1490nm and at 1930nm (possibly due to less water) but stronger absorptions at 1350, 1688, 1722, 1754 and 2302nm (possibly due to C-H of saturated and unsaturated molecules) (Figure 3). The *O.surinamensis* (Experiment 3f) gave similar results (Figure 4).

The results with the different developmental stages of *O.surinamensis* (Experiment 3g) are shown in Figure 5. In the so-called Herschel region of 400-1100nm there were large differences between all three stages in the position of the absorptions. In the 1100-2500nm region the adults and pupae gave absorptions in similar positions although the absorptions from the adults were slightly smaller at 1300nm (possibly due to C-H) and slightly larger at 1440nm (possibly due to water). The larvae showed a similar spectrum in this region apart from a lower absorption at 1174nm, an extra peak at

1626nm and generally larger absorption around 1440 and 1910nm.

In Experiment 3h, the two species gave absorptions in similar positions but *S.granarius* showed more absorption than *O.surinamensis* between 400 and 1100nm, perhaps because the former are darker in colour, and at 1490, 1600-1850 and 2000-2200nm but less absorption at 1300 and 2400-2500nm (Figure 6).

4. Differences between live and freshly killed *O.surinamensis*

The average spectra of the blank, live and freshly killed insects are shown in Figure 7 as log 1/R spectra. The blank samples gave rise to an absorption pattern as expected largely due to water vapour in the air within the cell, shown by the peaks near 1400 and 1900nm. The two spectra for the samples containing insects were noticeably different from the blank spectrum, showing greater absorption extending to higher wavelengths than the two main peaks of the blank. The spectra of the insects were in good agreement with those recorded with this species in Experiment 3. The differences between the spectra of the live and freshly killed insects were small, being mainly slight changes in amplitude not wavelength.

Figure 8 shows the "difference spectra" which are obtained by subtracting the spectrum of the blank samples from each of the spectra in Figure 7. These difference spectra show the spectral effect of the insects themselves and confirm the spectral similarity between the live and freshly killed insects.

5. Linearity of response to different numbers of *O.surinamensis*

For each level of infestation, Figure 9 shows the average of the five spectra from which the average of the five spectra with uninfested grain has been subtracted. The absorption at 2290nm increases with the level of infestation and is probably due to insect lipids. The results of step-wise regression of the SNV transformed data against the number of insects added are shown in Table 2.

Table 2: Step-wise regression of SNV transformed data against number of *O.surinamensis* adults added to wheat in the transporter cell (Experiment 5). [a] = respectively: derivative, datapoints used to calculate derivative, and two smoothing factors

| Equation number | Wavelength segments (nm) | Math treatment[a] | Standard Error of Calibration | $r^2$ |
|---|---|---|---|---|
| 1 | 410-1090 & 1110-2400 | 0,0,1,1 | 3.01 | 0.83 |
| 2 | as above | 1,4,4,1 | 1.84 | 0.94 |

6. The effect of aluminium cell backing on the response to insects in wheat

Figure 10 shows that when the cell has no aluminium foil backing there is little obvious difference between the spectra of the uninfested and infested wheat. Addition of aluminium foil backing to the cell results in greater differences (Figure 11). This is shown more clearly by comparing the absolute size of the peaks in Figures 12 and 13, which show the corresponding difference spectra (wheat with insects - wheat only). The signal to noise ratio is also improved as a result of using the aluminium foil backing. Figures 12 and 13 show two major regions of positive difference for the infested grain between 1400-1404nm and 1876-1888nm; these may be associated with the water content of the insects. Negative differences are evident for the infested grain in the region 1934-1936nm. This region corresponds closely with the wavelength observed for the characteristic combination band of wheat moisture (1930nm, Figures 10 and 11).

7. The response to insects behind 1cm thickness of grain

The addition of insects to the face of the cell away from the incident beam gave rise to a consistent if relatively weak change in NIR response for all seven samples. Figure 14 shows the average of the infested spectra from which the average of the uninfested spectra has been subtracted. The regions of positive difference around 1406nm and 1910nm are probably due to the water content of the insects while those around 1150nm and 1362nm may be due to insect lipids. This is in good general agreement with the spectra recorded previously in Experiment 3 for insects only and Experiment 6 for insects in wheat.

8. Variance in response to *O.surinamensis* in wheat

The best calibration equation found was obtained by carrying out partial least squares (PLS) regression of the SNV transformed data against the number of insects per wheat sample and the results are presented in Table 3 (Equation 1). In an attempt to compensate for significantly decreased wheat densities at the higher moisture contents, constituent values were also presented as the number of insects per 100g wheat (Table 3, Equation 2). For the 84 samples studied, values ranged between 0 and 45.45 insects/100g wheat. A plot of actual constituent values against fitted constituent values for Equation 2 is shown in Figure 15. This gives an estimate of the variance in spectral response at each infestation level and shows that the calibration differentiates reliably between uninfested samples and samples having infestation levels of approximately 0.27 insects/g or higher.

Table 3. Partial least squares regression of SNV transformed data against number of *O.surinamensis* adults: [a] = 2 wheat varieties at 2 moisture contents (n=84) (Experiment 8); [b] = Beaver wheat at 15%mc (n=72) (Experiment 9); [c] = respectively: derivative, datapoints used to calculate derivative, and two smoothing factors.

| Eqn. no. | Constituent range | Wavelength segments (nm) | Math treatment[c] | Standard Error of Calibration | $r^2$ |
|---|---|---|---|---|---|
| 1[a] | 0-25 insects/sample | 410-1090 & 1110-2400 | 2,15,5,1 | 3.89 insects/sample | 0.81 |
| 2[a] | 0-45.45 insects/100g wheat | 410-1090 & 1110-2400 | 2,15,5,1 | 5.98 insects/100g wheat | 0.83 |
| 3[b] | 0-34.63 insects/100g wheat | 410-1090 & 1110-2400 | 0,0,1,1 | 6.75 insects/100g wheat | 0.68 |

Figure 16a shows the loading spectra for the PLS factors which contribute significantly to the calibration performance (factors 4-11 from a total of 11). Bands in the loading spectra indicate wavelengths which are important to the calibration. These correspond closely with negative bands in the second derivative (D2) spectrum of saw-toothed grain beetles only, which is given in Figure 16b (math treatment as for Equation 1; spectrum obtained from Experiment 4).

9. Origin of response to *O.surinamensis* in wheat

As for Experiment 8, the best calibration equation found was obtained by carrying out PLS regression of the SNV transformed data against the infestation level and the results are also included in Table 3 (Equation 3). To allow a comparison of the two experiments, constituent values were again presented as number of insects per 100g wheat and ranged between 0 and 34.63. The calibration performance is significantly inferior to that obtained from Experiment 8.

Alternative calibrations using step-wise regression combined with various math treatments give poorer performance but allow for a simple comparison between wavelengths important to the calibration and wavelengths of bands in the spectrum of the insects only. Five stepwise calibration equations were obtained for which $r^2>0.5$ (0.51-0.58). These are listed in Table 4 (Equations 4-8) together with the wavelengths used by each. A comparable step-wise equation was obtained from Experiment 8 ($r^2=0.63$) and is also included in Table 4 (Equation 9). Only wavelengths positively correlated with infestation level are included and their relative importance within the equation is obtained from a comparison of their coefficients. Corresponding bands at similar wavelengths in the spectrum of saw-toothed grain beetles in the absence of wheat, having the same math

Table 4. Similarity between wavelengths selected for stepwise regressions with *O.surinamensis* in wheat (Experiment 9) and bands observed with insects only: [a] = respectively: derivative, datapoints used to calculate derivative, and two smoothing factors; [b] = intensities of bands are shown as s (strong), m (medium), w (weak).

| Eqn. no. | Scatter correction | Math treatment[a] | Wavelengths selected (nm) | Coefficient (-ve for 2nd derivative) | Presence of band in insect-only spectrum (same math treatment)[b] |
|---|---|---|---|---|---|
| 4 | SNV-detrended | 0,0,1,1 | 1876<br>2068<br>1492<br>1244 | 4660<br>3593<br>2995<br>2849 | Shoulder, (s): $H_2O$<br>Shoulder, (m): PROTEIN<br>Shoulder, (s): PROTEIN<br>Extreme shoulder, (w): LIPID |
| 5 | SNV-detrended | 2,10,10,1 | 1228<br>1468<br>1876 | -25777<br>-4882<br>-3487 | Unconvincing<br>Peak, (m): (-CONH-)<br>Extreme shoulder, (s): $H_2O$ |
| 6 | - | 2,10,10,1 | 2180<br>2060<br>512 | -40147<br>-36321<br>-4652 | Shoulder, (w): PROTEIN<br>Peak, (w): PROTEIN<br>Unconvincing |
| 7 | - | 2,15,5,1 | 2060<br>1892<br>512 | -5280<br>-1373<br>-547 | Peak, (w): PROTEIN<br>Peak, (s): $H_2O$<br>Unconvincing |
| 8 | SNV | 2,15,5,1 | 2052<br>1148<br>512 | -3506<br>-2875<br>-736 | Peak, (w): PROTEIN<br>Shoulder, (w): LIPID<br>Unconvincing |
| 9 | SNV-detrended | 2,10,10,1 | 2060<br>1780 | -10619<br>-7772 | Peak, (w): PROTEIN<br>Peak (2 overlapping), (w): LIPID |

treatment as that used to produce the equation, are also indicated in Table 4. For these bands, the chemical constituent of the insects most likely to be responsible for the absorbance is given. It can be seen that most of the wavelengths selected from the samples of infested wheat can be assigned to bands in the insect-only spectrum.

The relatively weak but well-defined insect band centred at 2050-2060nm appears to be the most consistently useful. It is employed by five of the six equations including the stepwise calibration from Experiment 8 where the samples cover four wheat types (Equation 9). This indicates that it is a feature of the response to insects that is markedly different from the response to wheat irrespective of wheat moisture content and variety. Selection of this band by five of the six calibrations of Experiment 9 shows that this feature of the response to insects is genuine and not an artefact arising from the particular scatter correction method and math treatment used.

The origin of this response is thought to be absorption by the (-CONH-) groups

of insect protein. A band at 2050nm is clearly seen as a well-defined shoulder in the SNV-detrended spectrum of *O.surinamensis*. On plotting the D2(log1/R) spectrum, this band becomes completely resolved (Figure 16b). Osborne and Fearn (1986) list the wavelength 2050nm as a characteristic protein absorbance. Three of the other four protein absorbances listed by Osborne and Fearn also appear to be present in the spectrum of the insects in Figure 16b, and the fourth absorbance, at 1020nm, although not obvious in Figure 16b, is seen in the underivatised spectrum. The band in the insect spectrum at 2170nm is in good agreement with the absorbance at 2180nm which is found in all $\alpha$ polypeptides and proteins. The protein band at around 2050nm is also observed in the underivatised spectrum of *S.granarius* adults (Figure 6) and in those of *O.surinamensis* larvae and pupae (Figure 5). In the spectrum of the larvae, it is seen as a slightly less well-defined shoulder on the water band.

Wavelengths in the region 1876-1892nm, which falls within a major band in the insect spectrum, are selected by three of the six step-wise calibrations. This is likely to be a response to insect moisture. The two major bands in the D2(log1/R) spectrum of saw-toothed grain beetles, at 1900nm and 1414nm, are water absorbances (Figure 16b). The 1900nm insect moisture combination band is at lower wavelength than the characteristic combination band of wheat moisture, which is observed at 1920nm in the D2(log1/R) spectra of the uninfested wheat studied here. This effect is also observed for *O.surinamensis* larvae and pupae and adult *S.granarius* (Experiments 3g and 3h) and is thought to be a consequence of differences between the two matrices in hydrogen bonding effects. The insect moisture combination band is not used when the samples include four different wheat types covering a range of moisture contents (Table 4, Equation 9). This is presumably because changes in this region due to the overlapping grain moisture band mask the detection of insect moisture.

The limit of reliable insect detection of 0.27 insects/g given by the results of Experiment 8 suggests that reliable discrimination between uninfested wheat and wheat containing just one insect is possible with the present hardware if the weight of wheat per scan is reduced to approximately 3g. This possibility is confirmed by the results of a simple discriminant analysis exercise carried on the D2(log1/R) spectra of the 24 samples of this experiment containing either no insects or 25 insects. As the weight of each wheat sample is approximately 70g, the infestation level of 25 insects per sample is close to 1 insect/3g. An almost complete resolution of the two sample groups is obtained by plotting D2 (log1/R) values at one wavelength (2302nm; lipid) against those at a second wavelength (2046nm; protein) (Figure 17; data SNV-detrended and math treatment 2,15,5,1).

10. The response to hidden infestations

With both the round cell (Experiment 10.1) and the transporter cell (Experiment 10.2), there was a clear NIR response to the presence of the hidden infestation. This is illustrated in Figure 18, which shows the spectra of the internally infested and uninfested samples in the transporter cell. Figure 19 shows the corresponding difference spectra. The increased absorbance value at 1934-1936nm for the infested kernels is probably due to the high water content of the larvae. The increased absorbance values at 1138-1140nm and 1320nm may be associated with insect lipids. It is interesting that free *O.surinamensis* larvae did not show a peak in the 1320nm region, although one was seen at 1440nm and was probably due to water (Experiment 3g).

11. Response to hidden infestations at the larval and pupal stage

Figures 20 and 21 show the SNV-detrended log1/R spectra of the samples of wheat kernels infested internally with *S.granarius* larvae and pupae respectively, together with the spectra of the uninfested control samples. The spectral changes due to the presence of hidden pupae are of noticeably greater magnitude than those due to the presence of hidden larvae, but are qualitatively identical. With infestation, increased intensities are observed in the region of the grain moisture band at 1932nm and in the region of the grain starch band at 2092nm, with a decrease in all other major wheat bands. The changes are in agreement with those obtained previously using only two samples of kernels infested with larvae (Experiment 10). Table 5 lists the mean absorbance values at 1932nm and 2092nm, together with the mean changes in absorbance with infestation for the four sets of samples studied in this experiment and shows that in addition to being of greater magnitude the response to hidden pupae is less variable than that to hidden larvae.

Table 5. Effect on mean absorbance values at 1932 and 2092nm of internal infestation by *S.granarius* larvae and pupae (Experiment 11): [a] SNV-detrended; mean+/-standard deviation; [b] Change in mean absorbance value due to presence of insect.

| Sample type | Log1/R[a] | | ΔLog1/R[b] | |
|---|---|---|---|---|
| | 1932nm | 2092nm | 1932nm | 2092nm |
| $Uninfested_{larvae\ expt}$ (n=5) | 0.28+/-0.01 | 0.00+/-0.01 | | |
| $Uninfested_{pupae\ expt}$ (n=3) | 0.26+/-0.01 | 0.01+/-0.01 | | |
| Infested, larvae (n=5) | 0.47+/-0.04 | 0.09+/-0.02 | 0.19 | 0.09 |
| Infested, pupae (n=5) | 0.49+/-0.02 | 0.15+/-0.01 | 0.23 | 0.14 |

It is not yet clear whether the increased water signal is a response to the high moisture content of the insect itself, or whether it is a response to a wheat moisture content which has been increased due to the respiratory actions of the insect within. However, the corresponding moisture combination band in the spectra of freely wandering insects, both larvae and adults, occurs at a slightly lower wavelength than that of wheat moisture (1932nm), at approximately 1910-1917nm (Experiments 3g and 3h; wavelength values refer to underivatised spectra). There is no observable shift to lower wavelength in the water band of infested kernels, suggesting that it is the latter effect that is dominant. The apparent increase in the broad wheat starch band at 2092nm band with infestation might be due to detection of insect protein. Increased absorbance at the two protein bands at 2050nm and 2170nm might be responsible for producing an apparent increase in the broad band at 2092nm. In the light of the argument above for water, however, it is perhaps more likely to be due to products excreted from the insect and which spread into the surrounding kernel. One of the most important of these products is nitrogen, which is excreted chiefly as uric acid. Amide groups (-CONH-) absorb at around 2110nm and could be associated with the uric acid. More subtle changes occur in areas of the spectrum associated with lipids. With infestation, the series of broad overlapping wheat bands between 1130-1300nm displays a slight overall shift to lower wavelength. A similar effect is observed within a second characteristic lipid region, between 2260-2380nm. Increased absorbances are observed at approximately 940nm and 860nm and these can also be assigned to lipids.

The increased response to wheat infested with pupae compared to wheat infested with larvae is also observed at these wavelengths. If the origin of the NIR response is a change in wheat composition as opposed to direct detection of *S.granarius* constituents, then this general effect can be explained simply as being a consequence of the longer time over which the pupae-infested kernels have been exposed to the actions of the developing insect.

12. Response to hidden infestation for single wheat kernels

A discriminant analysis exercise similar to that described in Experiment 9 and again carried out on the D2(log1/R) spectra produces a low but complete resolution of the two sample groups (Figure 22; data SNV-detrended and math treatment 2,20,5,1) and shows that there are detectable differences between the spectra of single infested and uninfested kernels. A scatter plot of the intensity of a lipid band at 2328nm against the intensity of what is thought to be an amide band at 2062nm was found to give the best result. The intensities of both bands increase with infestation. A large variation in the intensity of the wheat moisture combination band is observed between individual kernels

from the same sample group, preventing this from being used. The same exercise carried out on the larger 8ml samples of Experiment 11 gives a similar but greatly enhanced resolution of the larvae-infested and uninfested samples, showing that this detectable response to single infested kernels is genuine.

**Discussion**

1. Preliminary tests of optical configuration

Since the study of insect infestation is a novel application for NIR, it was necessary first to undertake a set of experiments to assess the importance of three factors which might influence the spectra. These were the type of window used to cover the sample in the cup and the orientation and position of the insects within the sample.

Experiments 1a and 1b showed clearly that the use of a diffuse window to cover the rotating cup sample holder brought no advantage to the spectra of either wheat or insects so all subsequent work used the standard clear window.

Some of the radiation incident on the sample is thought to be readily reflected between the various particles comprising a sample before returning to the detector but the extent to which it actually penetrates the wheat grains and insects is not yet known. It seems unlikely that it penetrates either completely. The irregular shape and composition of insects therefore might mean that the spectral response would depend upon their orientation within the sample. Fortunately, Experiment 1c suggests this is not the case for *O.surinamensis* adults when studied in isolation. This is likely to be due to the incident radiation reaching the under surfaces by reflection from the back of the cell. This effect would also occur in samples where grain surfaces could deflect radiation onto insects within the sample and the resulting spectrum would then depend on grain packing rather than insect orientation.

Since the maximum depth of sample from which the radiation returns to the detector may not be the full depth of sample, the position of the insects within the sample might also be important. Experiment 1d was intended to investigate this but the spectral differences between samples of wheat infested with *S.granarius* adults were due to variation in wheat packing rather than position of the insects. Larger samples of wheat would be needed to allow the variation in wheat packing to be averaged out and enable observation of any spectral features due to the insects.

2. Preliminary tests with transporter cell

The need to study larger samples was achieved by changing to the transporter cell, a device which is larger than the rotating cup and is driven slowly past the beam of incident radiation. However, the transporter cell was designed for the analysis of components which are relatively evenly distributed within samples, such as moisture.

Insects in the transporter cell may not have been scanned because the incident beamwidth is less than the full width of the cell and there may be deadspace between adjacent scanned volumes. Since the cell is not stationary, scanned volumes may not be scanned over the whole wavelength range. Further, the averaging of scans into one spectrum for each pass of the cell may dilute the effect of an individual insect seen in a single scanned volume. Despite these problems and the limited number of samples, the regressions between spectral information and the number of insects in Experiment 2a were encouragingly good (Table 1).

However, Experiment 2b showed that insects on the side of a 1cm thick sample furthest away from the incident radiation are unlikely to be detected by reflectance. This suggested that detection of the emergent radiation by transmission might be more useful since it would not need to travel back through the wheat layer.

Unfortunately, the transmission spectra (Experiment 2c) were much more noisy than the equivalent reflectance spectra even though the number of scans during the passage of each sample had been increased from 32 to 42. Since no correlation was observed with insect numbers, subsequent spectra were recorded by reflectance since it was felt that they contained more information than those recorded by transmission. However, it might be worth repeating the transmittance experiment but with incident radiation passing through a slit and detector arrangement better suited to emergent radiation not being parallel to the incident beam.

3. Tests on differences between *O.surinamensis* and *S.granarius* adults, dead and live adults, and different developmental stages of *O.surinamensis*.

In this of experiments a series of comparisons was undertaken between different samples of insects. Since these did not contain wheat, it was possible to scan them by reflectance in the rotating cup without problems.

For the later studies of infested samples, to avoid any problems which might result from movement of live insects during scanning, it was hoped that it would be possible to use dead insects instead. Clearly the method of killing should not alter the spectral response which would have been obtained from live insects.

Experiments 3a and 3b showed that there was no spectral difference for insects of either species between being killed by heating in an oven or by chilling in a container cooled in solid carbon dioxide. Adult *S.granarius* killed by dipping in liquid dichloromethane gave noticeably different spectra from those killed by the other two methods. This is not unexpected since it would almost certainly remove from the surface of the insects some of the lipids which are likely to contribute to their spectral response.

Experiments 3c and 3d showed that insects freshly killed by the carbon dioxide method would be suitable models for live insects.

Desiccated adults of both species gave different spectra from those which were either live or freshly killed by carbon dioxide. The wavelengths suggest that the spectral differences are due to less water in the desiccated samples. After death insects can no longer retain the water which is essential for their survival. It is lost by diffusion and evaporation, a process which is known to take a few days (Chambers *et al*, 1984). The results of Experiments 3e and 3f suggest that desiccated samples will need to be studied as models for insects which have been dead for longer than this.

There are spectral differences between adult, pupal and larval stages of *O.surinamensis* (Experiment 3g) and all will need to be studied in future. The differences are most marked in the visible region but the value of this may be limited by differences in colour of the commodity infested.

Since the differences between the spectra of adult *O.surinamensis* and *S.granarius* seem to be of intensity rather than absorption frequency it would be reasonable to concentrate on one for the present (probably *O.surinamensis* since the various developmental stages are easier to obtain). It may still be necessary to study other species eventually.

4. Differences between live and freshly killed *O.surinamensis*

The results from Experiment 3 with single samples suggested that there was little difference between the spectral effects of live insects and those which had been freshly killed by chilling in solid carbon dioxide. In view of the fundamental importance of this factor to future work in this contract, the experiment was repeated but this time using seven replicate samples of each type to guarantee confidence in the result. It has become clear by doing this that there is indeed no spectral difference between the two types of insect. It has therefore now been proved conclusively that insects freshly killed by this method are suitable models for live insects.

5. Linearity of response to different numbers of *O.surinamensis*

It has been established previously that the NIR response increases with increasing numbers of *O.surinamensis* adults (Chambers *et al*, 1992) but these experiments were only of a preliminary nature and used very high levels of infestation (up to 100 insects) in small samples of wheat of about 5g. The purpose of Experiment 5 in the present study was to repeat this work but with larger samples of wheat (about 69g) and levels of infestation which are more realistic.

The results were encouraging and the regressions of spectral data against numbers of insects were nearly as good as obtained in Experiment 2a for *S.granarius*, which are noticeably larger and therefore likely to be easier to quantify accurately. However, Figure 9 shows that with increasing infestation there is an unexpected decrease

in absorption at 1942nm, which is presumed to be due to moisture. The lack of absorptions in the regions 1400-1430 and 1870-1895nm seen with this species when scanned without wheat suggests that the insects may have dried out.

6. The effect of aluminium cell backing on the response to insects in wheat

The regressions of spectral response against infestation level with both *S.granarius* (Experiment 2a) and *O.surinamensis* (Experiment 5) gave standard errors of calibration of less than two insects. This suggests that the insects are contributing to the spectral response wherever they are located within the transporter cell. However, Experiment 2b suggested that insects on the far side of the cell away from the incident beam of radiation were unlikely to be detected. Since it is essential for practical relevance to be able to scan as large a sample of grain as possible, an experiment was undertaken to establish whether it would be advantageous to cover the back surface of the cell with aluminium foil, which is known to be beneficial for the study of liquids such as milk and tea (R. J. Barnes, personal communication).

Experiment 6 clearly shows that the addition of aluminium foil backing to the transporter cell enhances the NIR response to insects in wheat by reflectance. The aluminium presumably reflects radiation reaching the far side of the cell thereby reducing radiation losses and giving a more complete irradiation of the cell contents. All subsequent experiments studying insects in wheat by reflectance included aluminium foil backing.

7. The response to insects behind 1cm thickness of grain

Experiment 7 shows clearly that insects on the face of a 1cm thick sample of grain away from the incident beam do affect the spectral response when aluminium cell backing is used. Furthermore, the changes in spectral response when insects are added to the cell are similar to the spectra of insects studied without wheat. This confirms that these changes are due to absorption of radiation by the insects and not some other effect such as altered packing of the grain, which would obviously not be specific to insects. This result is particularly important since it proves that cells of this size can be used for further work.

8. Variance in response to *O.surinamensis* in wheat

The purpose of Experiment 8 was to obtain a measure of the variance in the NIR response to adult *O.surinamensis* in wheat by recording spectra from more than one sample at each level of infestation. Four differing wheat types were included to ensure that the results would indicate the performance that might be expected in a trade situation. In addition it was thought that the results of this experiment would provide

information on the possible origins of the observed variance. An estimate of the size of the variance in spectral response at each infestation level is given by the variance in the fitted constituent values (Figure 15). It can be seen that as a consequence of this variance, the calibration only differentiates completely between uninfested samples and samples at approximately 0.27 insects/g or more.

In a separate study (Experiment 9), carried out primarily to investigate the chemical origin of the spectral response to insects in wheat and discussed more fully later, a similar number of infested samples of 15% moisture content Beaver wheat only were investigated. The poor calibration performance obtained from Experiment 9 compared with that in Experiment 8 is surprising, given that the substrate consists of only one wheat variety at one moisture content. This suggests that the major source of variance in the determination of insects is not variability in the nature or composition of the substrate but is likely to be either variability in the position of the insects within the sample or variability in the sample packing as a whole. Another possibility is that a slight variability in the size of the insects has a significant effect. The maximum number of insects added to the cell was the same in Experiments 8 and 9. However, the former included samples of higher moisture content which, because of their lower wheat density, have a higher infestation level when expressed as insects/100g wheat. This is likely to be at least partly the reason for the better calibration performance in Experiment 8.

The detection limit of 0.27 *O.surinamensis*/g is equivalent to a constituent concentration of approximately only 0.01% by weight. The large variance in spectral response and relatively poor calibrations show that the ranges of sample infestation density used in Experiments 8 and 9 are close to the limit of detection of present hardware and methodology.

9. Origin of response to *O.surinamensis* in wheat

Spectral information used in the PLS calibration of Experiment 8 has been related to the spectrum of *O.surinamensis* in the absence of wheat by plotting the loading spectra of the PLS factors important to the calibration equation. A comparison of the band positions of Figures 16a and 16b shows that the majority of wavelengths important to the calibration do correspond to bands in the insect-only spectrum; in D2(log1/R) spectra it is the negative peaks that correspond to band centres in the original underivatised spectrum. This is direct evidence to validate specifically the PLS equation giving the best calibration performance for the samples studied in Experiment 8, and suggests that insect protein, moisture and lipid are responsible for the NIR response, together with the colour difference between the insects and the wheat in the visible region. This finding is in good agreement with the results of Experiment 9, which was undertaken as a more rigorous investigation of the origin of the NIR response to infested

wheat.

In Experiment 9 the approach used was to try and identify wavelengths associated with bands present in the spectrum of *O.surinamensis* which are also selected by stepwise regression as being correlated with insect numbers in samples of infested wheat. The results of this experiment offer convincing evidence to confirm that the NIR response to wheat samples infested with adult *O.surinamensis* arises from NIR absorbing chemical constituents of the insects themselves. Insect protein appears to be the most important contributor, followed by insect moisture.

Protein is a major constituent of insects at all life stages. For example, values for the grain weevil as a percentage of dry body weight range from 47% for pre-pupae to 76% for adults (Singh and Sinha, 1977). The presence of the well-defined protein band at around 2050nm in the spectra of two different life-stages of *O.surinamensis* and also in the spectrum of adult insects of a different species, *S.granarius*, suggests that the developed NIR method would be applicable to all types of pest insect.

It is clear from the calibration performances in Experiments 8 and 9 that a change in methodology and hardware design is required if an NIR insect detection device with the desired sensitivity is to be developed. One approach would be to investigate a reduction in sample size, such that samples containing just one insect can consistently be identified as being infested. As a preliminary step towards evaluating the potential of this approach, a simple discriminant analysis exercise was carried out on the samples of Experiment 9 containing either no insects or 25 insects. The latter samples have an infestation level of about one insect/3g wheat, which is close to the detection limit suggested by the results of Experiment 8. The optimum wavelengths used in the scatter plot of Figure 17 were found by trial and error from the many spectral features that were seen to differ fairly consistently between the uninfested and infested wheat samples. The response to infestation at 2046nm can be explained as detection of the insect protein band centred at 2050nm (Figure 16b) and is agreement with the results of Table 4. Although no wavelengths in the region of 2302nm are listed in the results shown in Table 4, a weak but well-defined band is seen at 2300nm in the spectrum of insects in the absence of wheat (Figure 16b). This suggests that the observed response to infestation at 2302nm is also genuine and is a response to insect lipid.

This encouraging result suggests that further investigation of this approach is justified. Optimisation of detector design and sample cell design is expected to improve performance markedly by ensuring that all the sample is scanned by the instrument such that the variance in response due to insect position within the sample is minimised. For this approach to be successful in a trade situation, the data acquisition and processing steps would also need to be optimised to allow for a statistically significant amount of wheat to be scanned in a realistic time. Recent advances in NIR technology make this a

real possibility. In particular, acousto-optic tuneable filter instruments are now available which can scan a sample in a few milliseconds.

10. The response to hidden infestations

The extent to which radiation of near-infrared wavelengths penetrates into the surface of solid biological samples is still not clear. Obviously there has to be some penetration and subsequent reflectance from within samples. If this were not so, no absorptive interaction could occur and the radiation returning to the detector would carry no useful information about the chemical content of the sample. However, the depth of penetration, remains to be established, even approximately. In the absence of this knowledge, Experiments 10.1 and 10.2 were tried more in optimism than with any real expectation of success. They were undertaken because of the importance of discovering how to detect hidden infestations rapidly.

The results of both experiments were surprisingly encouraging in showing spectral differences between infested and uninfested kernels. These differences might originate from physical or chemical properties of the wheat resulting from the actions of the larvae, rather than from the larvae themselves. This possibility was minimised in Experiment 10.2 by using uninfested kernels selected by X-ray inspection from the same culture as the infested kernels. However, it is likely that feeding, excretory and other actions of the internal larvae will affect the characteristics of infested kernels sufficiently to produce a change in the NIR response of the wheat itself. The ability of NIR to detect a hidden infestation when there are no obvious signs externally is impressive.

11. Response to hidden infestations at the larval and pupal stage

Experiment 11 was carried out to confirm the response to hidden infestation by scanning a larger number of samples and to compare the response between infestations at the larval and pupal stage. The consistency of the changes in the wheat spectrum as a result of infestation confirms that the NIR response to hidden infestation is genuine, reproducible and surprisingly well-defined. It appears that the response arises from changes in the composition of the wheat kernel as a result of the metabolic and excretory actions of the developing insect, rather than from chemical constituents within the insect itself. This would explain the unexpected magnitude of the response, as NIR radiation is known to penetrate solids only weakly and thus is likely to interact mainly with the near-surface region of wheat kernels. The enhanced response to kernels infested with pupae compared to those infested with larvae is perhaps simply a consequence of the longer time over which the wheat has been exposed to the insects in the former case. Moisture and amide-containing compounds expelled by the developing insect and diffusing into the surrounding kernel bulk are likely to be the major origins of the response. In addition,

there is evidence to suggest that the lipid composition of the sample is changed as a result of infestation but to a lesser extent.

For the band centred at 2092nm, increased absorbance with infestation occurs over the wide wavelength range 2020-2160nm. This finding is potentially of great importance, as it suggests that in a commercial situation, measurement at a wavelength which is most useful for the detection of freely wandering adult *O.surinamensis* (2045-2065nm, Experiment 9) might also reveal the presence of internal infestations. This possibility clearly needs to be investigated further, perhaps initially by adding various numbers of internally infested kernels to samples of uninfested wheat and comparing the derived calibration to that obtained for freely wandering adults.

12. Response to hidden infestation for single wheat kernels

Experiment 11 has shown that large and consistent changes occur in the bulk composition of wheat kernels as a result of internal infestation. It was proposed that this effect might make an NIR imaging approach particularly suitable for the detection of infested kernels. Two-dimensional mapping of a wheat sample of one grain thickness would remove the dilution of the response to infested kernels by the uninfested kernels and also remove the variance in response arising from variation in insect position within the sample. NIR imaging hardware is now becoming more readily available, together with powerful image enhancement and analysis software. Experiment 12 was carried out as a preliminary evaluation of the potential of an imaging-based approach, by investigating whether single infested and uninfested kernels could be reliably classified by a simple discriminant analysis of their NIR spectra. Figure 22 shows that this is indeed possible.

Although this needs to be confirmed using many more samples, the ability to differentiate between single infested and uninfested kernels using such a simple form of discriminant analysis is unexpected and very encouraging, particularly when considering the likely variations in individual kernel size and in the age and size of the larvae, and the large observed variation in moisture content between samples of the same type. The same exercise carried out on the larger larvae-infested and uninfested samples of Experiment 11 gives a similar but greatly enhanced resolution of the two sample types, showing that this is a real and reproducible effect. The capability to discriminate between single infested and uninfested kernels using NIR suggests that an imaging based approach for the detection of hidden infestations should be investigated.

**Conclusions**

1. Optimal conditions have been established for the recording of NIR spectral data from samples of whole grain wheat containing insects. Recording spectra of these samples by reflectance is preferable to the use of transmittance. Addition of aluminium foil to the back of the cell enhances the NIR response and allows insects on the far side of a 1cm thick sample of wheat to be detected. Spectral response is independent of the type of window used to cover the sample. The orientation of the insects within the sample does not appear to affect spectral response despite their irregular shape and composition. Adult insects freshly-killed by chilling in carbon dioxide are suitable models for live insects but desiccated insects will need to be studied as models for those which have been dead for longer than a few days (Chambers *et al*, 1994).

2. Studies on insects which would be found external to the grain kernels show that there are spectral differences between adult, pupal and larval stages of the saw-toothed grain beetle, *O.surinamensis*, but there are few differences between adults of *O.surinamensis* and the grain weevil, *S.granarius*. There is convincing evidence that the correlation of infestation in samples of wheat containing *O.surinamensis* adults is due to the insects themselves, with insect protein appearing to be the most important contributor, followed by insect moisture. In a study of two varieties of wheat at two moisture contents with a sufficiently large number of samples to give dependable results, the calibration derived from the spectra recorded under the optimal conditions could only differentiate between uninfested samples and samples with approximately 0.27 insects/g or more. Much simplification of the data handling may be possible: simple discriminant analysis by plotting response at just two wavelengths gave almost complete resolution between these two groups of samples. This is probably close to the limit of detection using present methodology and hardware with samples of this size, about 70g. The major source of variance which limits further improvement is probably variability in the position of the insects within the sample.

3. A most surprising discovery in this project has been that there is a genuine, reproducible and well-defined NIR response to grain kernels infested internally (Chambers *et al*, 1993). The response appears to be due largely to accumulation in the kernel of moisture and amide-containing compounds expelled by the developing insect and involves similar wavelengths to those which will be useful for the detection of insects external to the kernels. Simple discriminant analysis using the response at two wavelengths produced complete resolution of single infested from single uninfested kernels.

4. The work described here has shown that NIR is capable of detecting insects of different species and developmental stages in different varieties of wheat at different moisture contents. The speed of recording is rapid, no more than a few seconds, and discrimination between infested and uninfested samples is achievable with a simple analysis. The main task now is to exploit the substantial and encouraging findings so far by improving the sensitivity of the method so that it will function at infestation levels considerably lower than those used here.

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**References**


Barnes, R.J., Dhanoa, M.S. and Lister, S.J. (1989). Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **43**, 772.

Chambers, J. (1987). Recent developments in techniques for the detection of insect pests of stored products. British Crop Protection Council Monograph No.37 "Stored Products Pest Control" ed. T. J. Lawson, 151.

Chambers, J., Cowe, I.A., Van Wyk, C.B., Wilkin, D.R. and Cuthbertson, D.C. (1992). Detection of insects in stored products by NIR. In "Near Infra-red Spectroscopy: Bridging the Gap between Data Analysis and NIR Applications" eds. K.I.Hildrum, T.Isaakson, T.Naes and A.Tandberg. Ellis Horwood, New York, Chapter 31.

Chambers, J., McKevitt, N.J., Stubbs, M.R. (1984). Nuclear magnetic resonance spectroscopy for studying the development and detection of the grain weevil, *Sitophilus granarius*, within wheat kernels. *Bull. Entomol. Res.* **74**: 707-24.

Chambers J, Ridgway C, Baker C W and Barnes R J. (1993). Improving quality by rapid pest detection. *Aspects of Applied Biology* **36**, 1993 Cereals Quality III, 465-470.

Chambers, J., Van Wyk, C.B., Baker, C.W. and Barnes, R.J. (1994). NIR analysis for the detection of insect pests in cereal grains. In "Proceedings of the International Diffuse Spectroscopy Conferences, August 1992", ed. R.A.Taylor. The Council of Near-Infrared Spectroscopy, Gaithersburg, MD USA, 96-100.

Johnson, H.J. (1979). Procedures and sampling intensities in New South Wales grain elevator board system. Research Paper No 90, Division of Marketing and Economics, New South Wales Department of Agriculture.

Osborne, B.G. and Fearn, T. (1986). "Near Infrared Spectroscopy in Food Analysis", Longman, UK, 200pp.

Rowlands, D.G., Pinniger, D.B. and Wilkin, D.R. (1989). The Control of Pests in Stored Cereals. Research Review No 17, 66pp, Home-Grown Cereals Authority, London.

Singh, N.B. and Sinha, R.N. (1977). Carbohydrate, lipid and protein in the developmental stages of *Sitophilus oryzae* and *Sitophilus granarius* (Coleoptera: Curculionidae). *Annals of the Entomological Society of America*, **70**(1), 107.

Wilkin, D.R., Catchpole, D. and Catchpole, S. (1993). The development of a practical method for removing insects from large samples of grain. Project Report No.82, 15pp, Home-Grown Cereals Authority, London.

Wilkin, D.R., Cowe, I.A., Thind, B.B., McNicol, J.W. and Cuthbertson, D.C. (1986). The detection and measurement of mite infestation in animal feed using near

infra-red reflectance. *J. Agric. Sci., Camb.*, **107**, 439.

Wilkin, D.R. and Fleurat-Lessard, F. (1990). The detection of insects in grain using conventional sampling spears. "Proc. 5th Int. Wkg. Conf. on Stored-Product Protection", Bordeaux, France 1990. Eds. F. Fleurat-Lessard and P. Ducom. 1445.

Wilkin, D.R. and Rowlands,D.G. (1988). The Biodeterioration of Stored Cereals. Research Review No 3, 67pp, Home-Grown Cereals Authority, London.

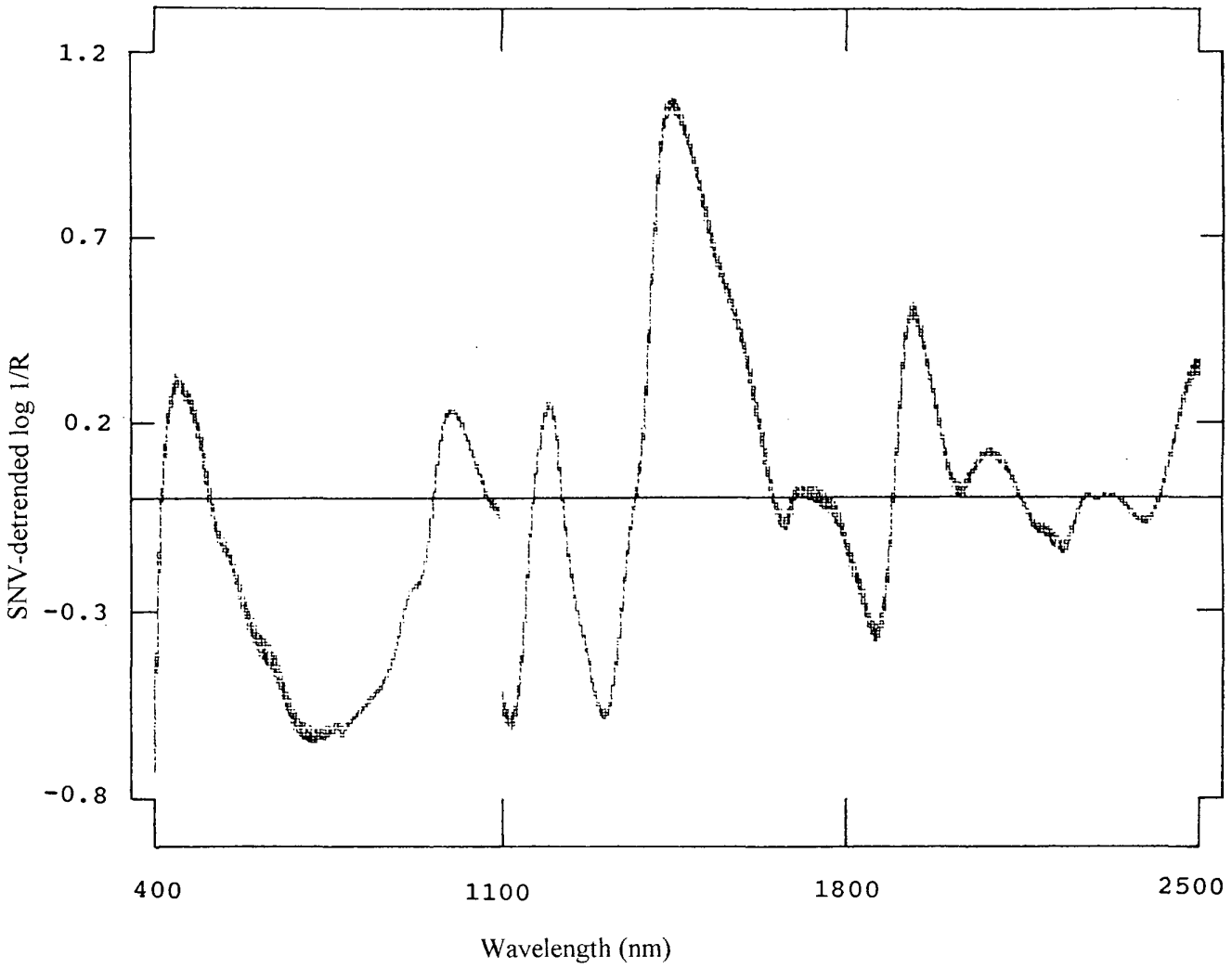


Figure 1: 69g samples of wheat containing up to 30 *S. granarius* adults (Expt. 2a).

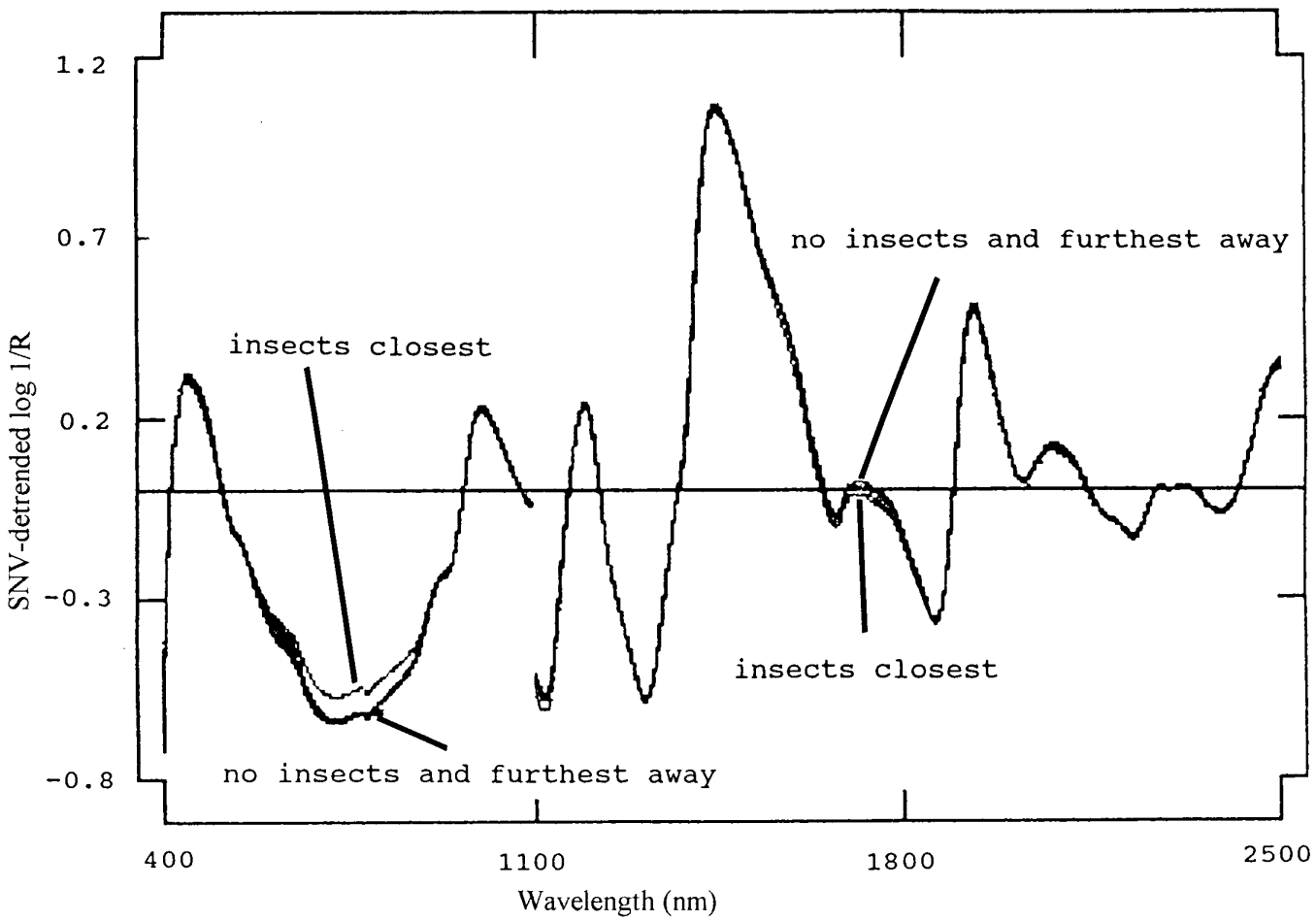


Figure 2: A 69g sample of wheat containing either no insects or 49 *S. granarius* adults on the surface closest to the incident radiation or on the surface furthest away from the incident radiation (Expt. 2b).

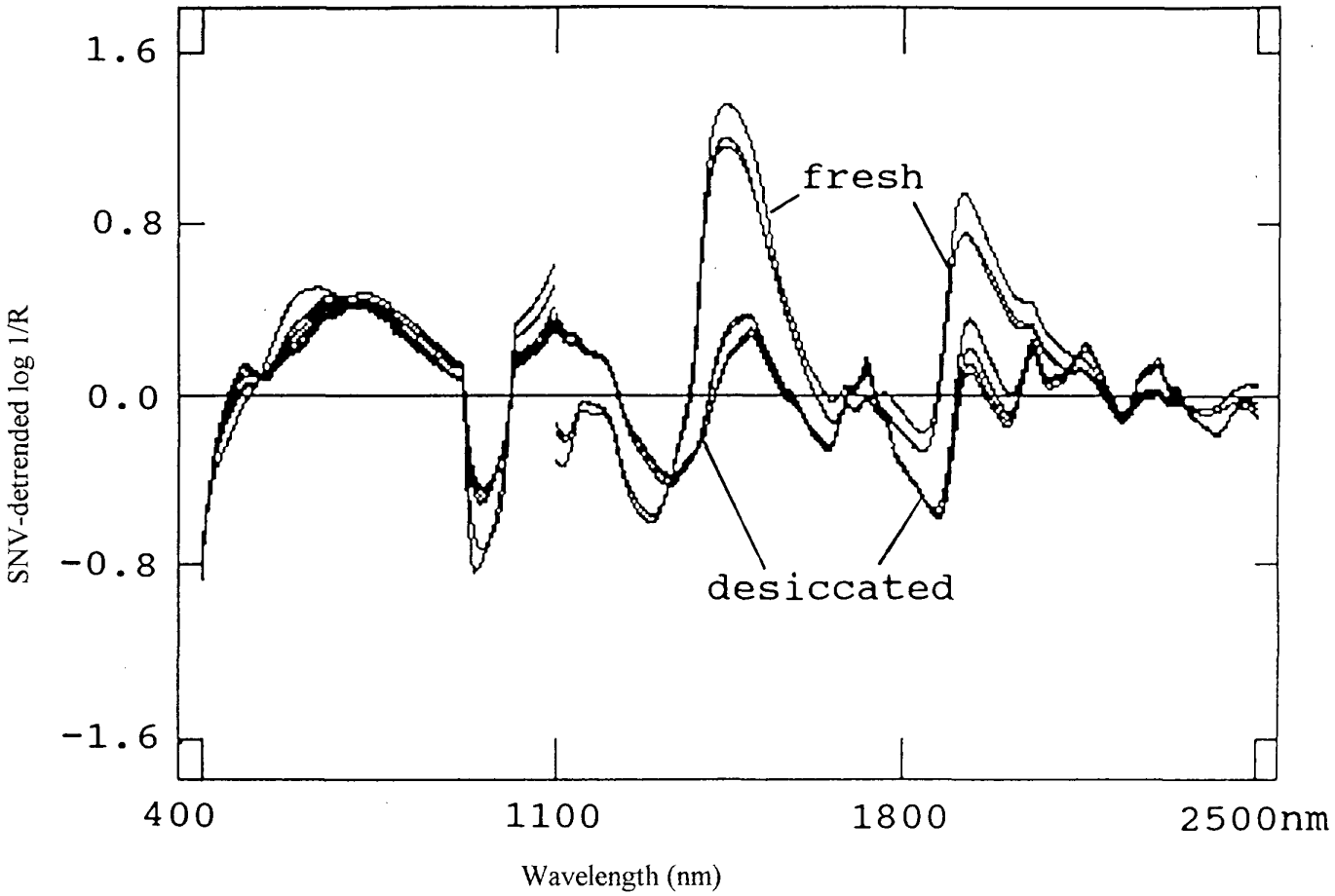


Figure 3: *S. granarius* adults which were live or freshly killed with carbon dioxide ("fresh") compared with those which had been stored for a week after killing ("desiccated") (Expt. 3e).

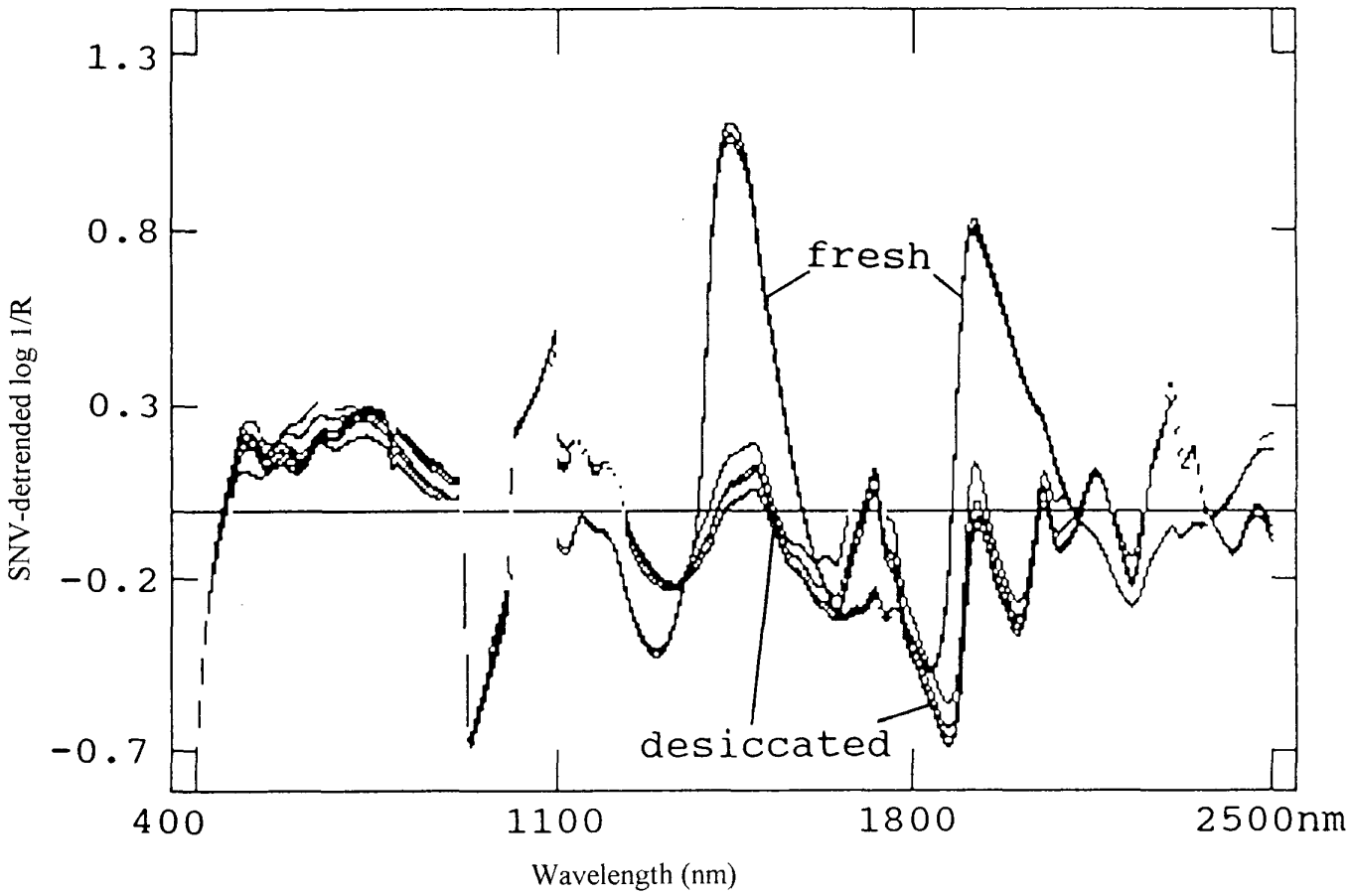


Figure 4: *O. surinamensis* adults which were live or freshly killed with carbon dioxide ("fresh") compared with those which had been stored for a week after killing ("desiccated") (Expt. 3f).

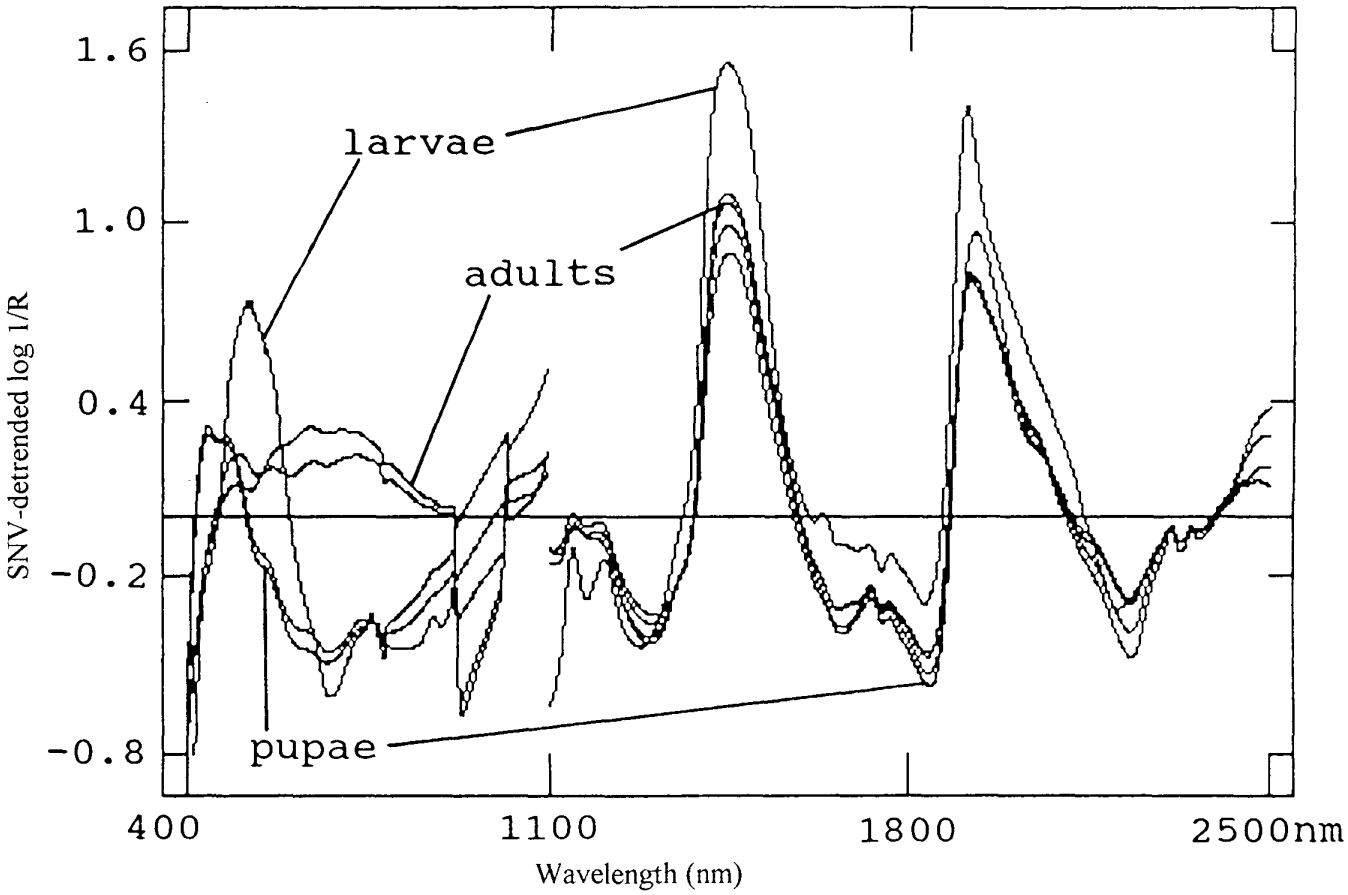


Figure 5: Different developmental stages of *O. surinamensis* (Expt. 3g).

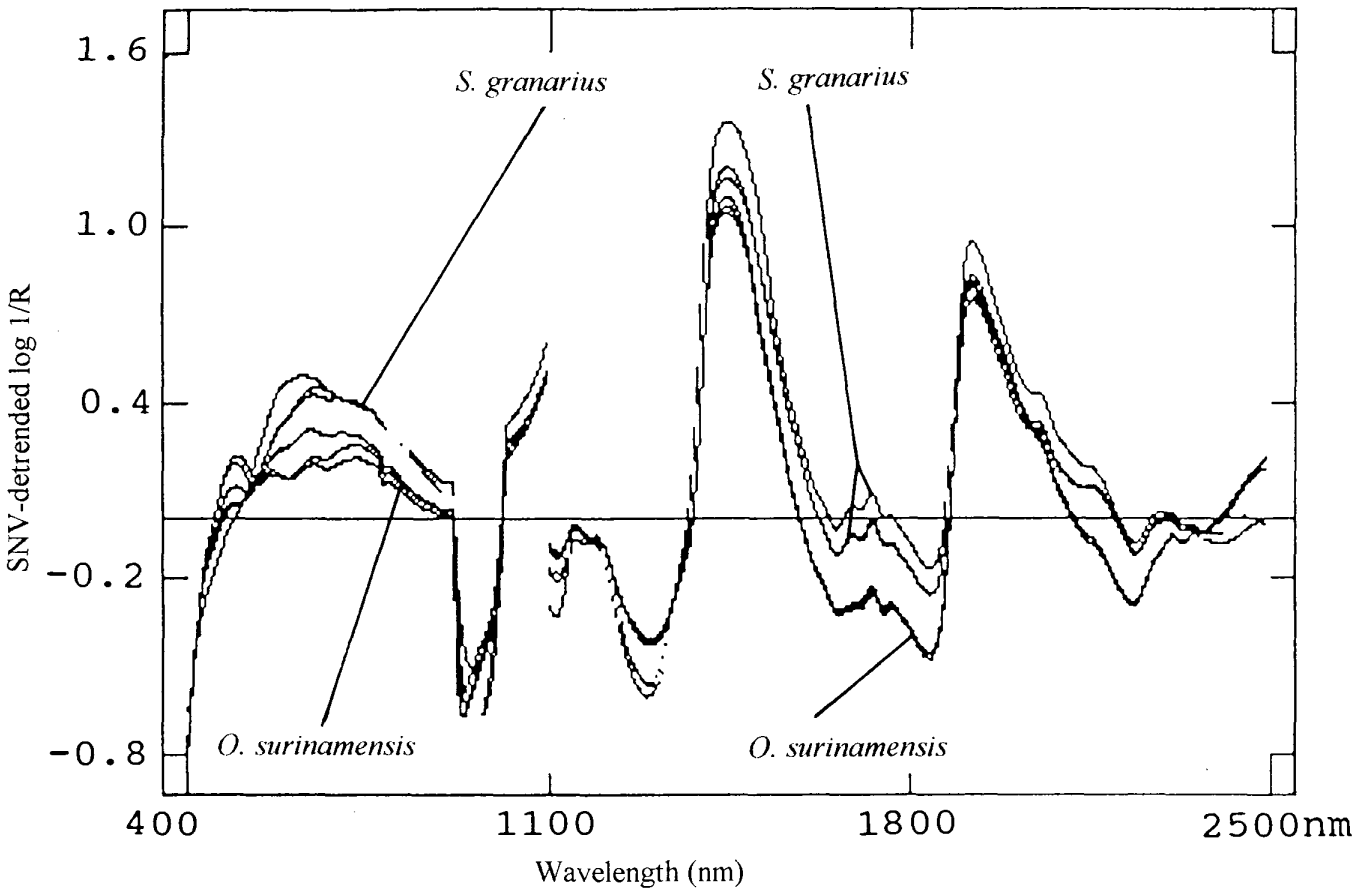


Figure 6: *S. granarius* adults compared with *O. surinamensis* adults (Expt. 3h).

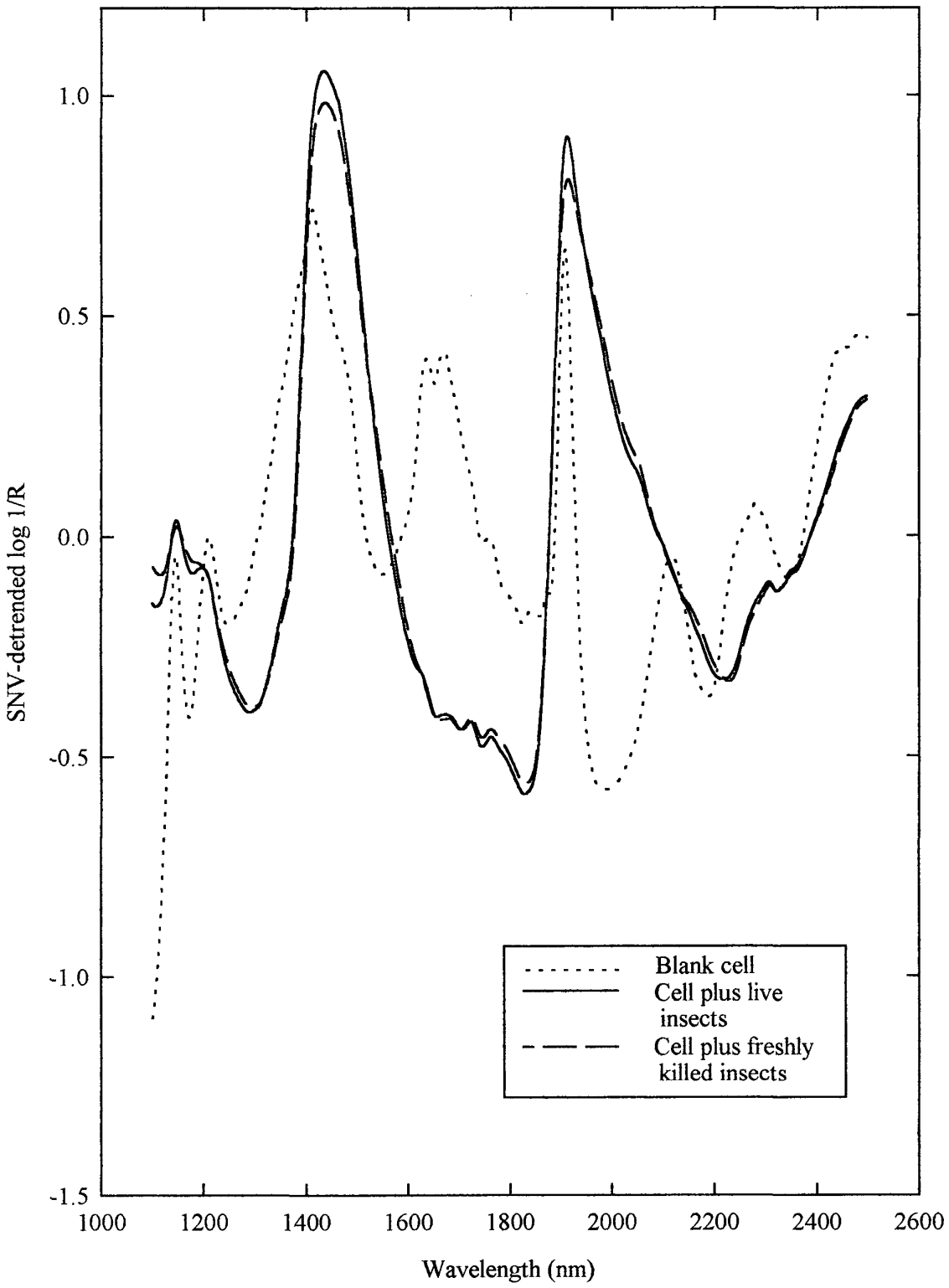


Figure 7: Spectra of live and freshly killed *O. surinamensis* compared with the spectrum of the blank cell (Expt. 4).

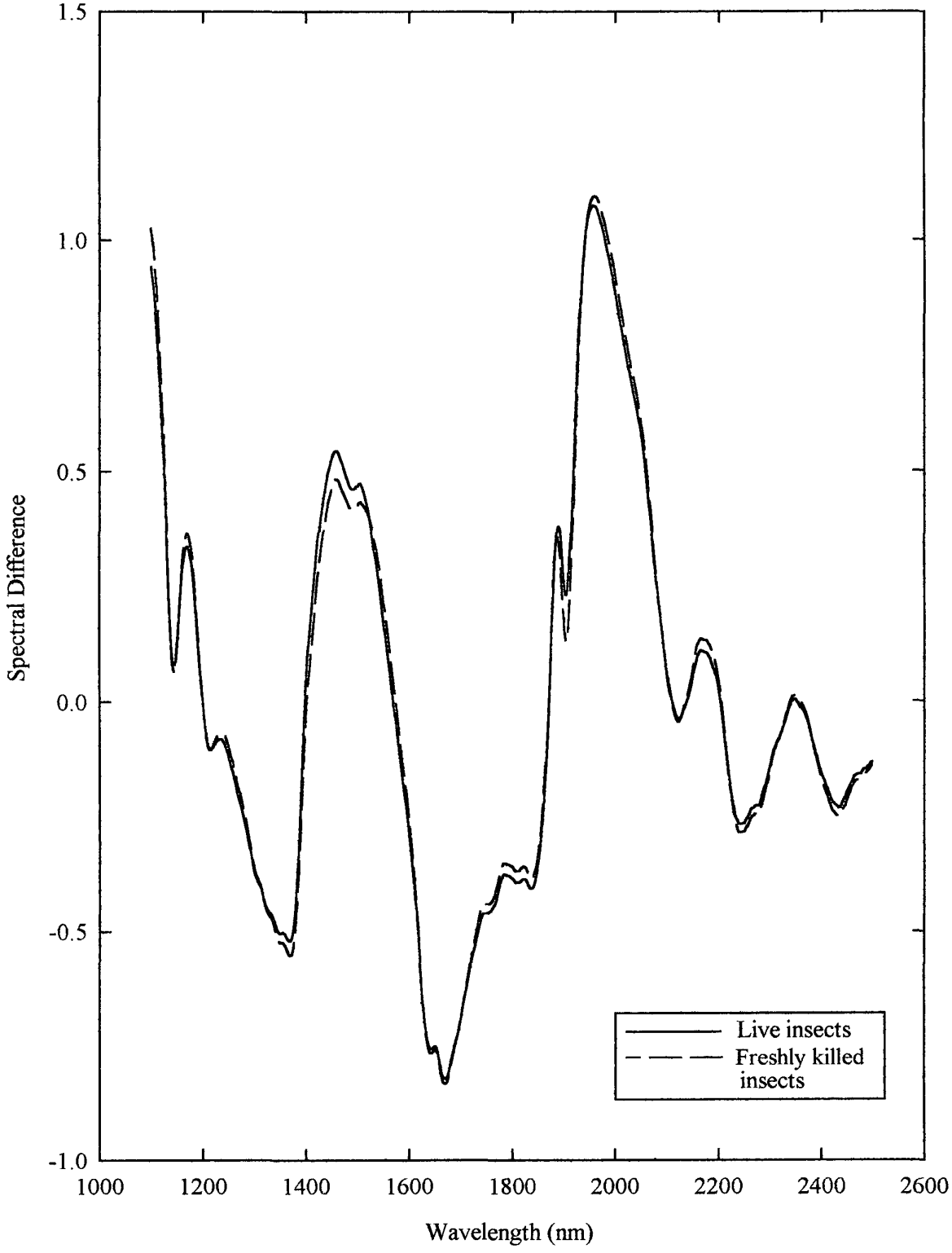


Figure 8: Spectra of live and freshly killed *O. surinamensis* from which the spectrum of the blank has been subtracted (Expt. 4).

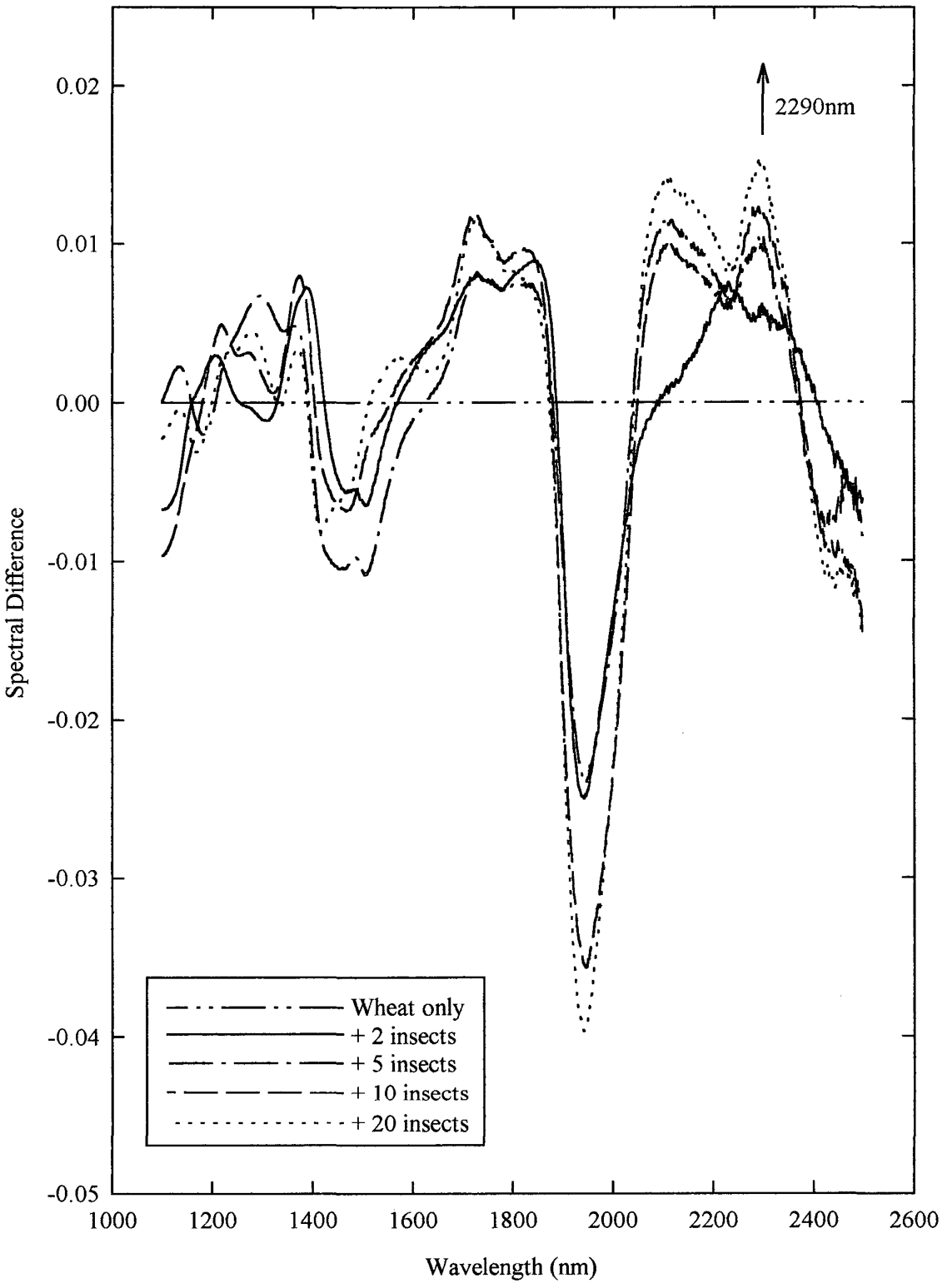


Figure 9: Spectra of wheat with various numbers of *O. surinamensis* from which the spectrum of uninfested wheat has been subtracted (Expt. 5).

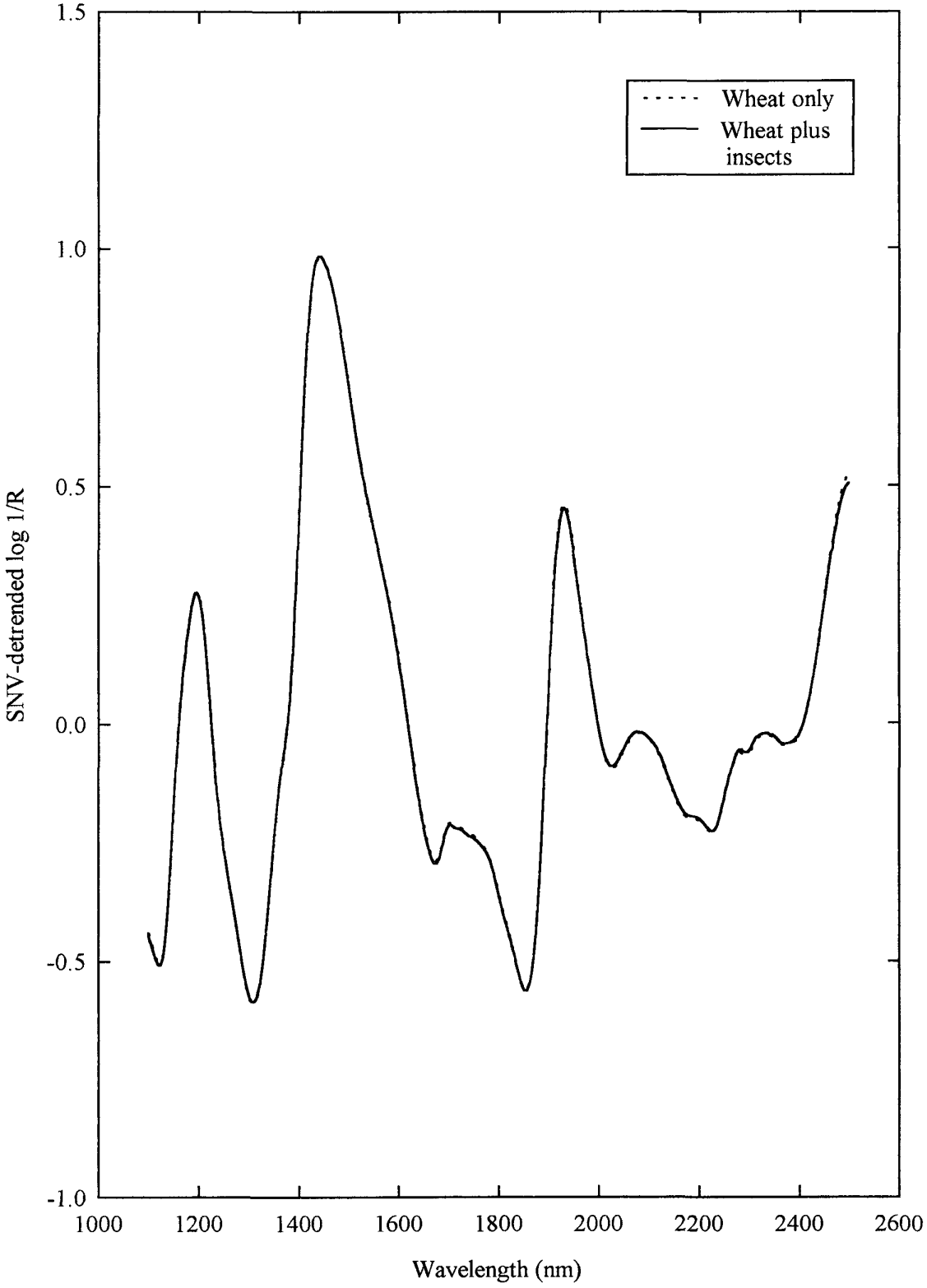


Figure 10: Wheat containing either no insects (Expt. 6b) or 30 freshly killed *O. surinamensis* (Expt. 6c) with no aluminium foil backing.

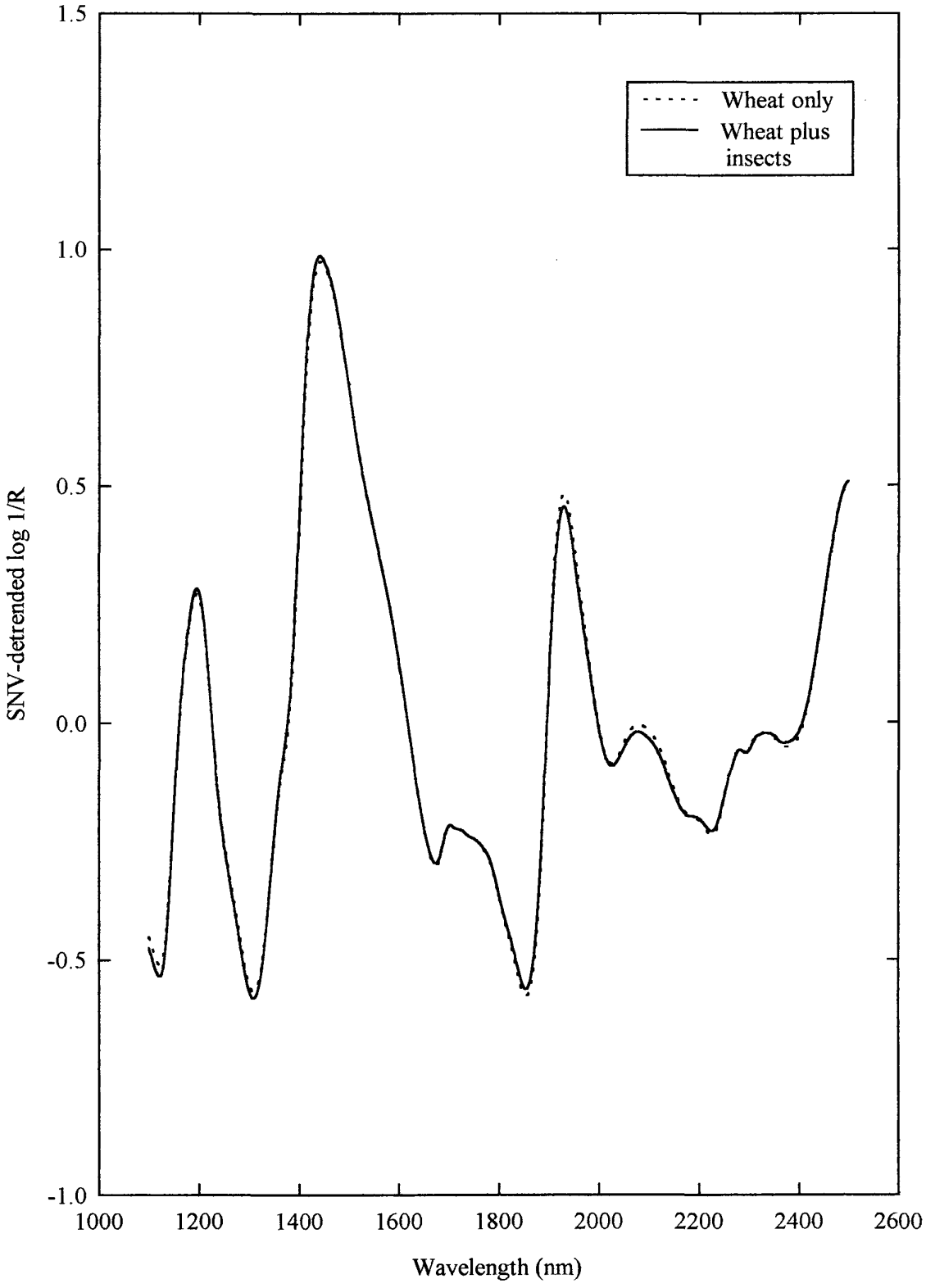


Figure 11: Wheat containing either no insects (Expt. 6a) or 30 freshly killed *O. surinamensis* (Expt. 6d) with aluminium foil backing.

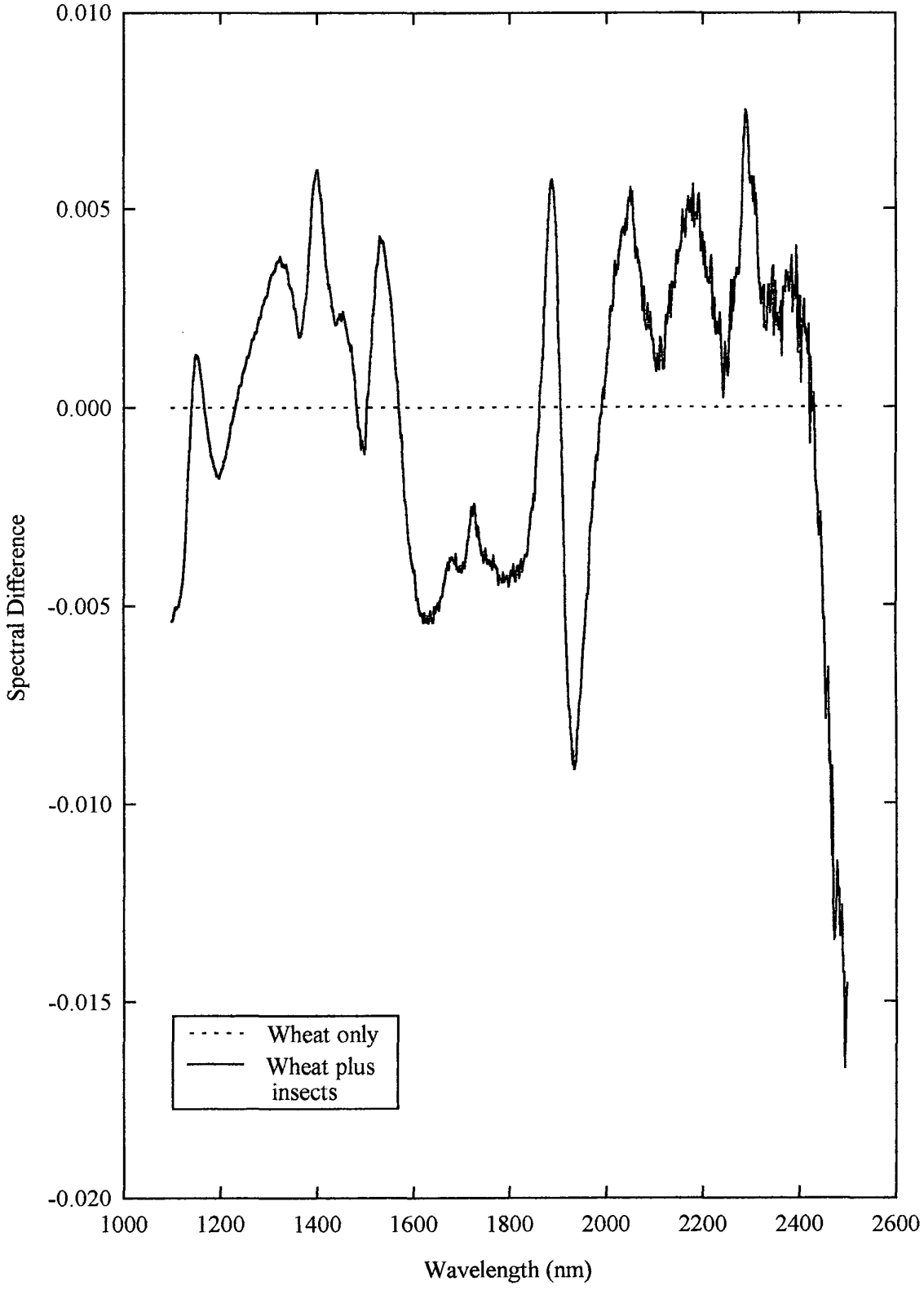


Figure 12: Spectra of infested and uninfested wheat from which the spectrum of uninfested wheat has been subtracted, no aluminium foil backing (Expt. 6).

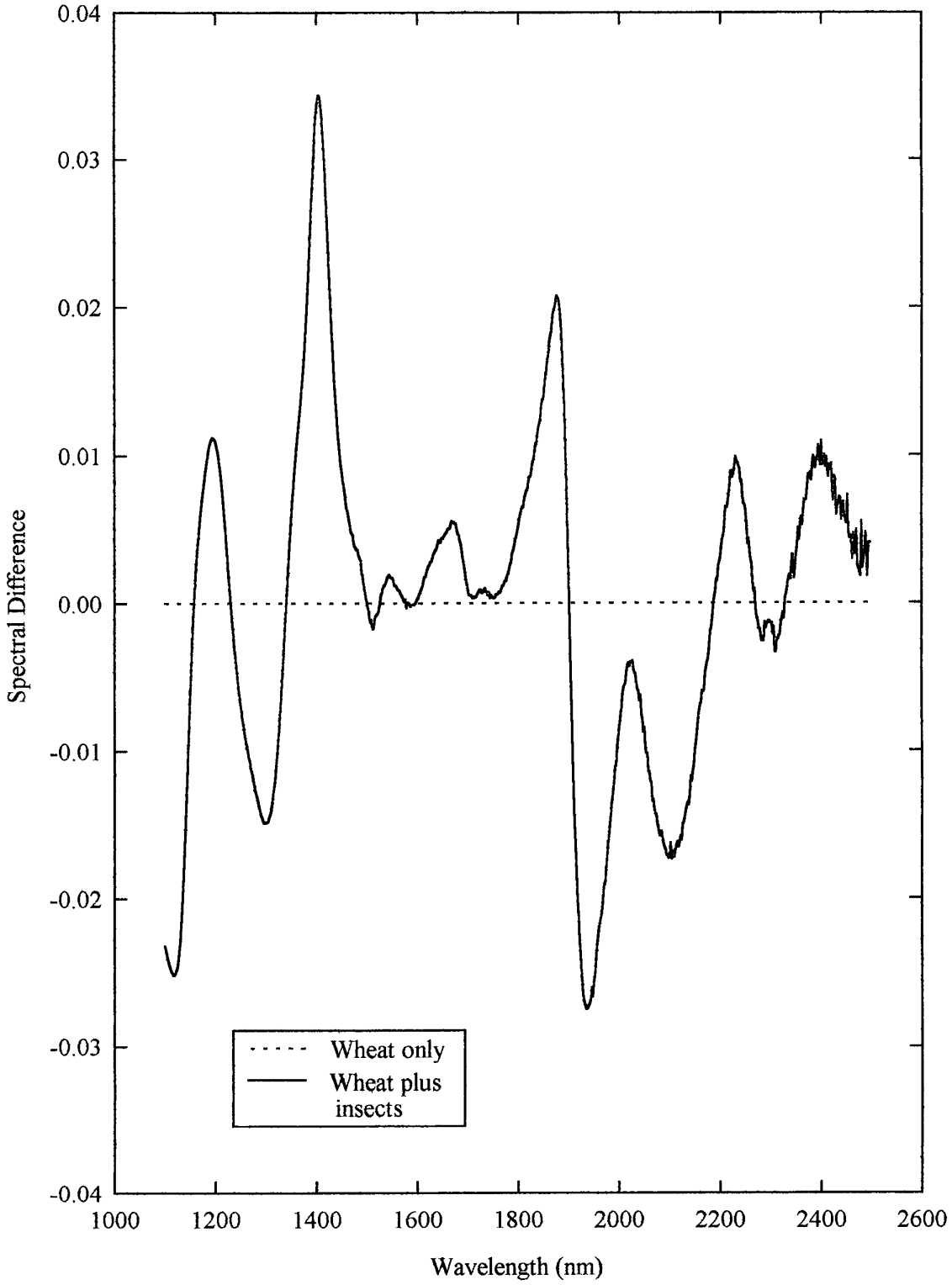


Figure 13: Spectra of infested and uninfested wheat from which the spectrum of uninfested wheat has been subtracted, with aluminium foil backing (Expt. 6).

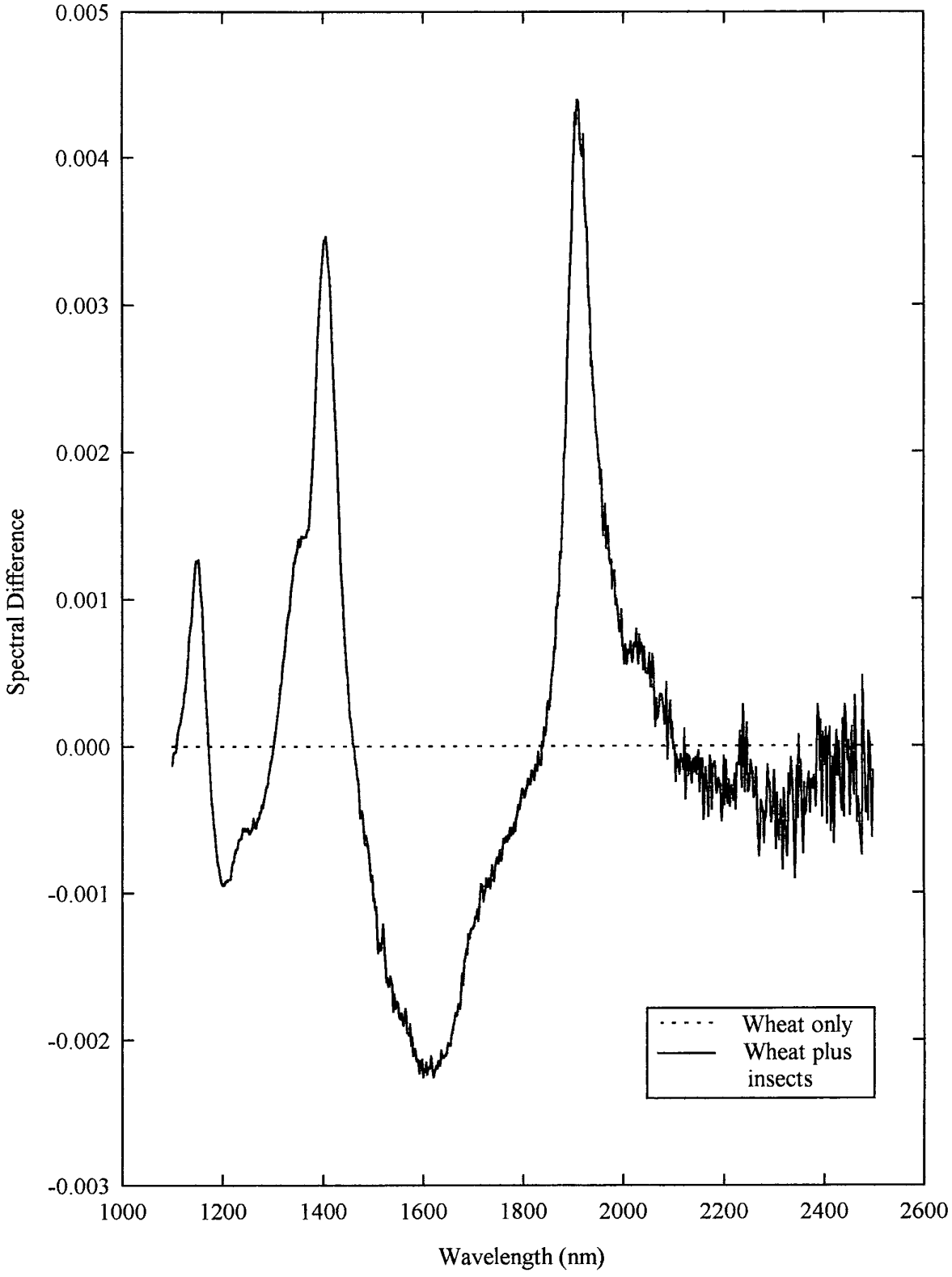


Figure 14: Average spectrum of wheat infested at face of cell away from incident beam from which average spectrum of uninfested wheat has been subtracted (Expt. 7).

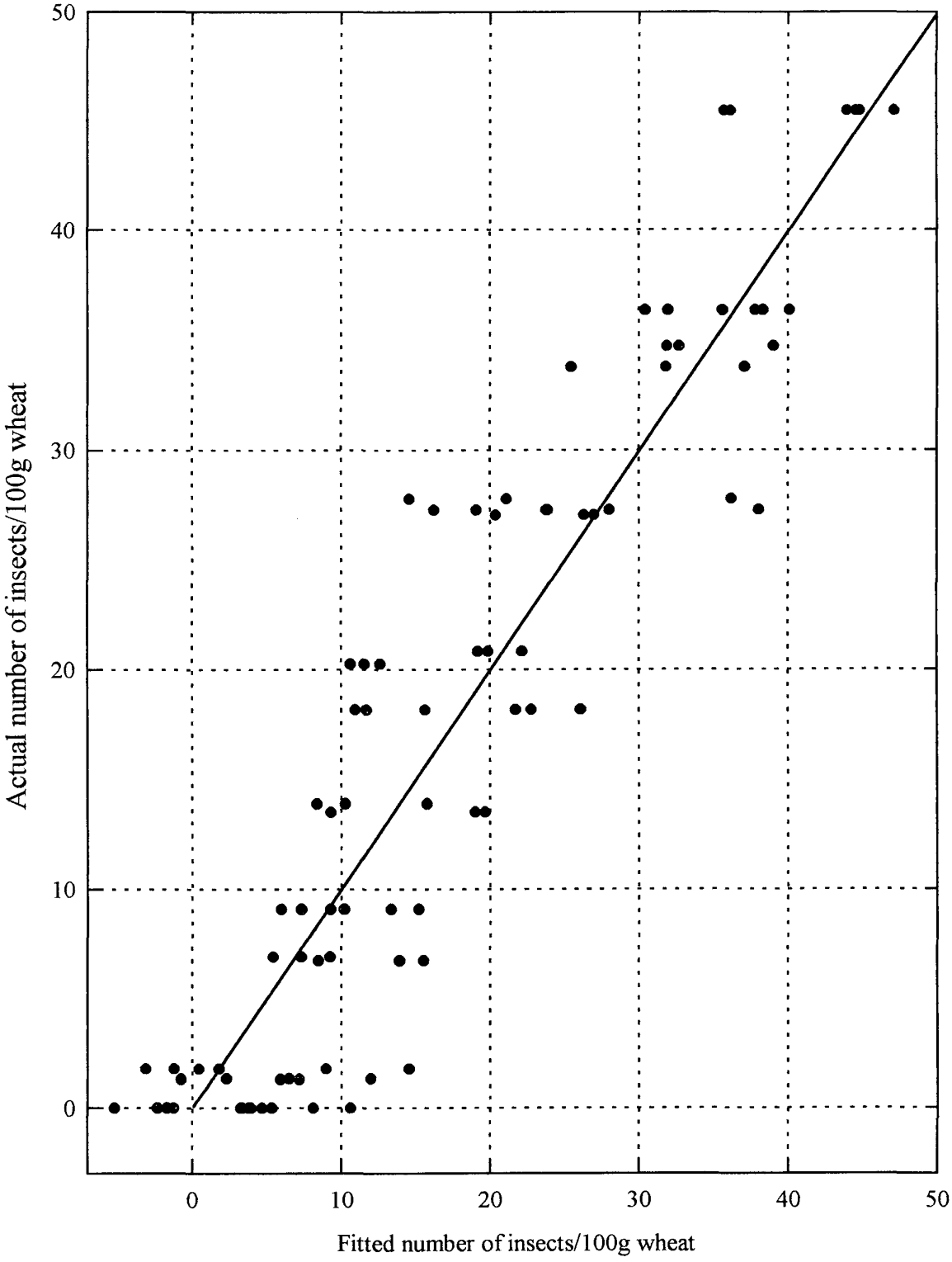


Figure 15: Variance in response to *O. surinamensis* in wheat at different levels of infestation. Samples include Mercia variety wheat at 13 and 22% mc and Beaver variety wheat at 15 and 24%mc (Expt. 8).

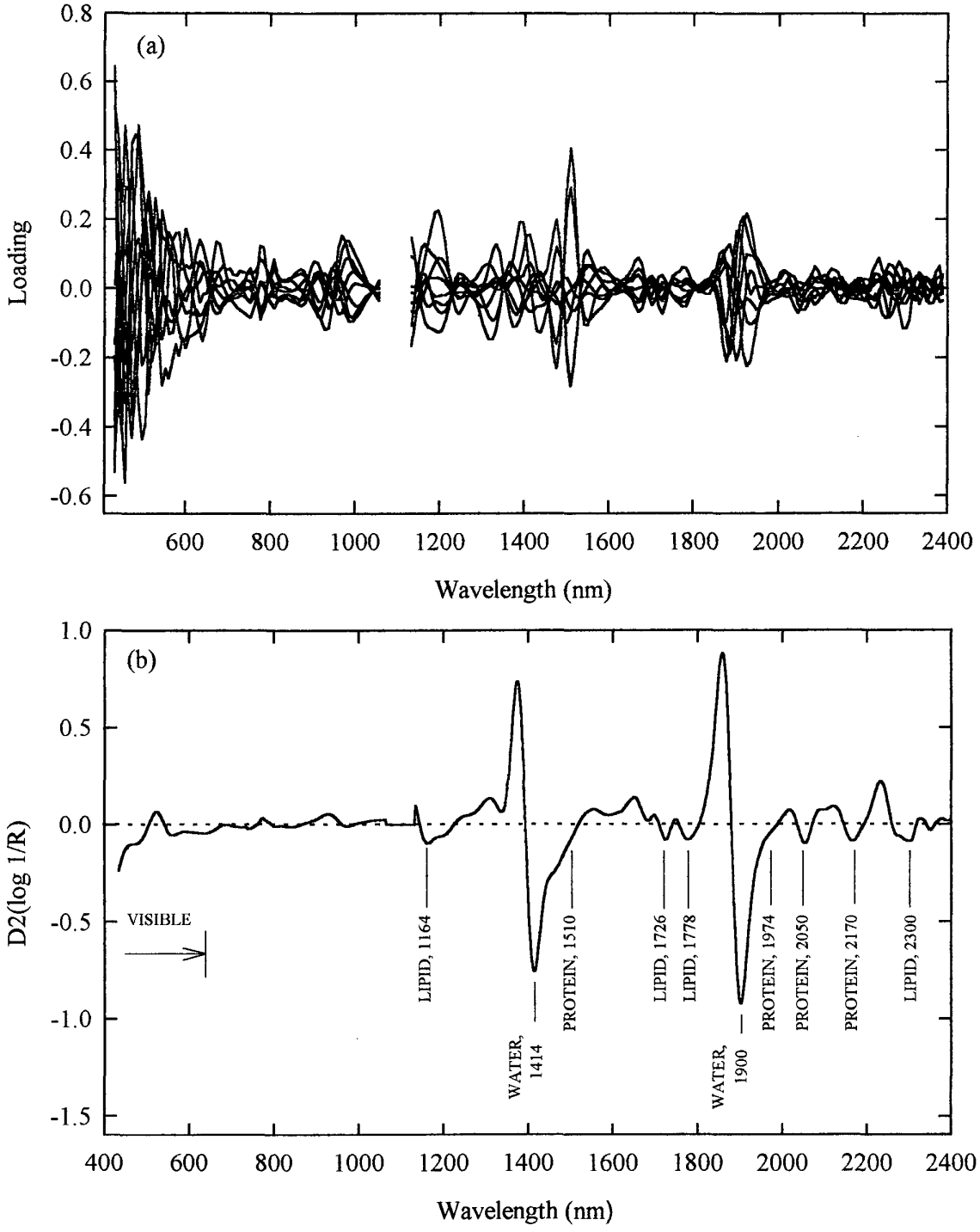


Figure 16: (a) Loading spectra of PLS factors from Equation 2 (Expt. 8); (b) D2(log 1/R) spectrum of freshly killed *O. surinamensis* (Expt. 4), math treatment as for Equation 2.

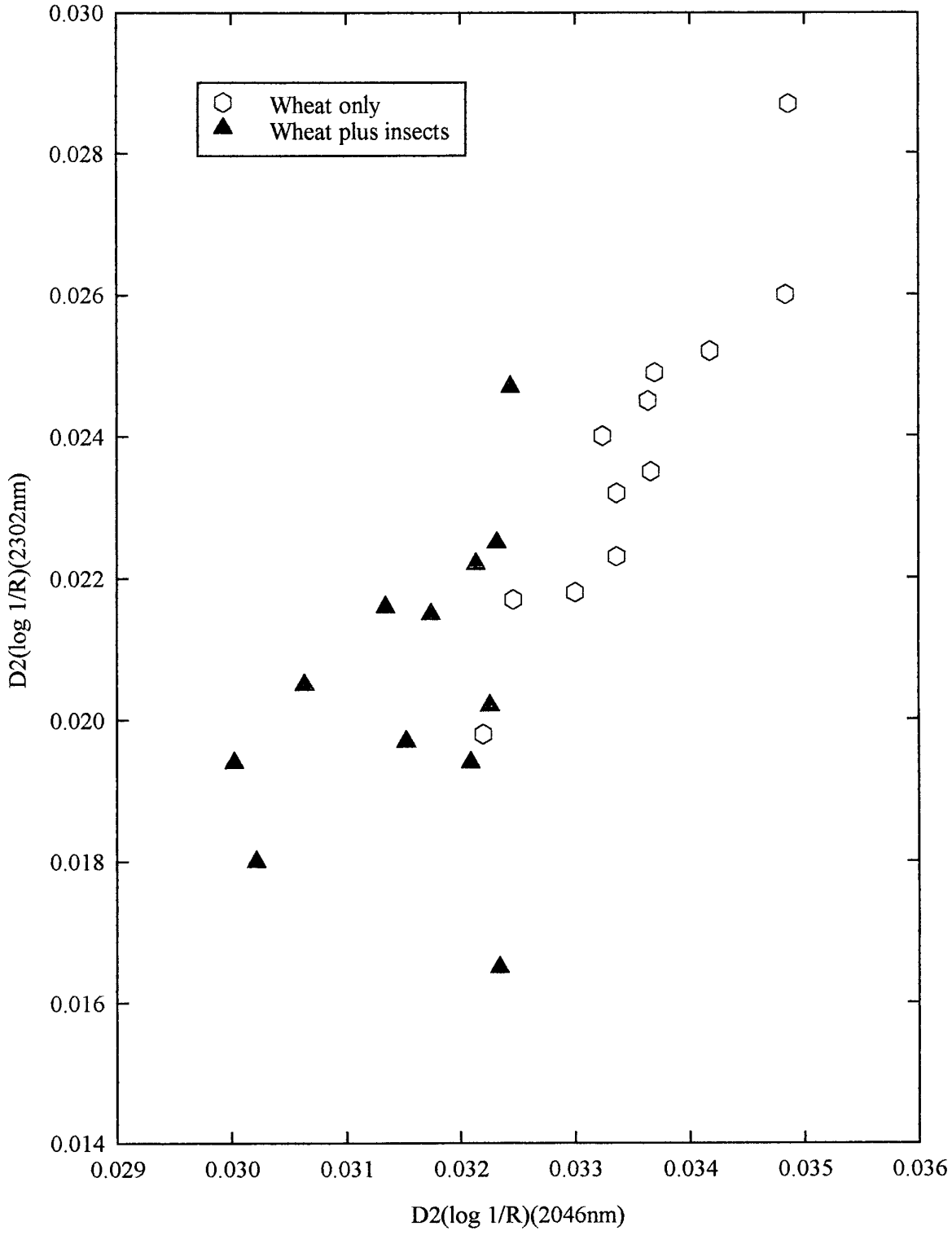


Figure 17: Absorbance at 2302nm versus absorbance at 2046nm for uninfested wheat and wheat infested at approx. 1 *O. surinamensis*/3g (Expt. 9).

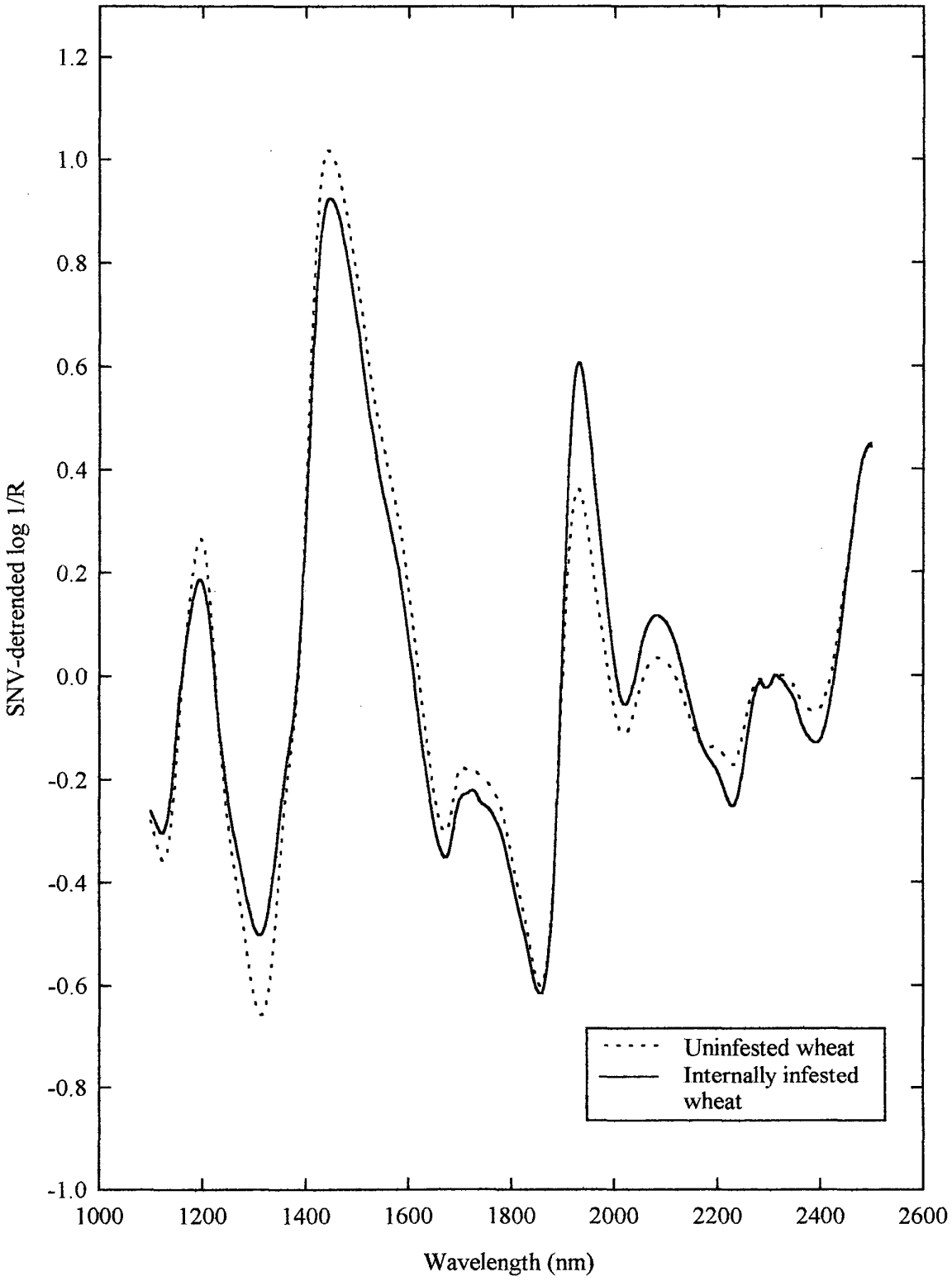


Figure 18: Internally infested wheat or uninfested wheat in the transporter cell (Expt. 10.2).

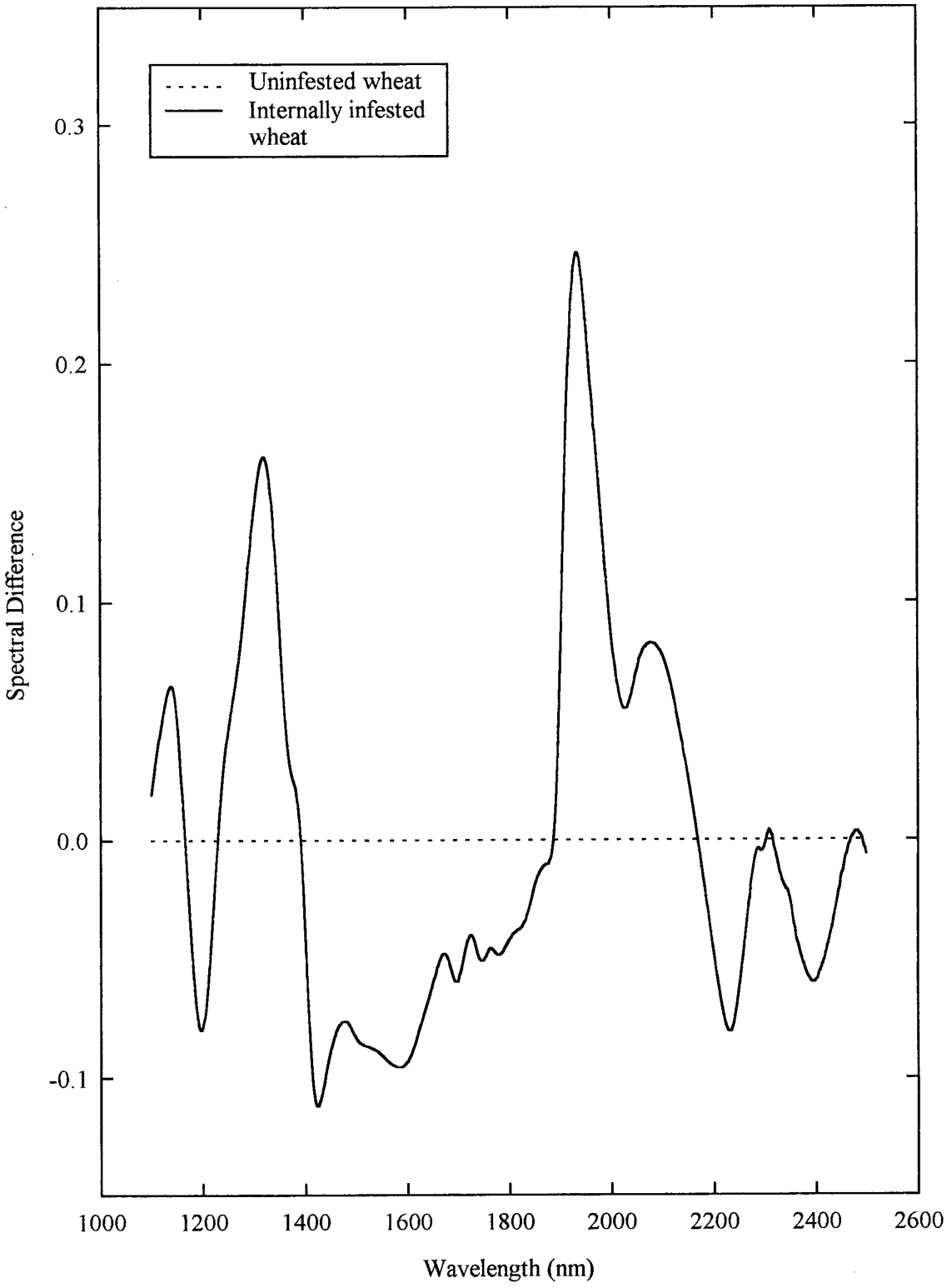


Figure 19: Spectrum of internally infested wheat from which spectrum of uninfested wheat has been subtracted (Expt. 10.2).

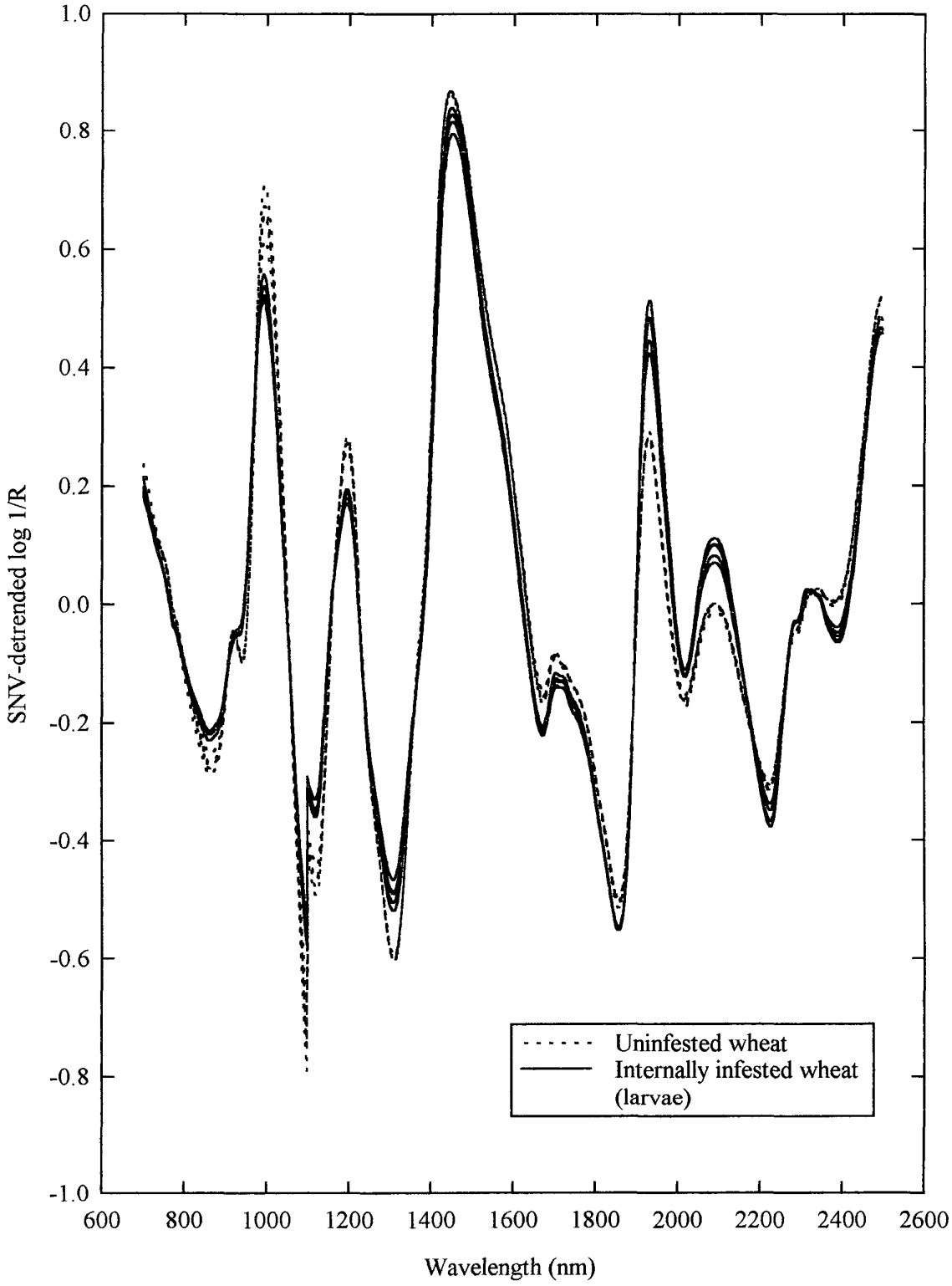


Figure 20: Spectra of uninfested wheat and wheat internally infested with larvae (Expt. 11).

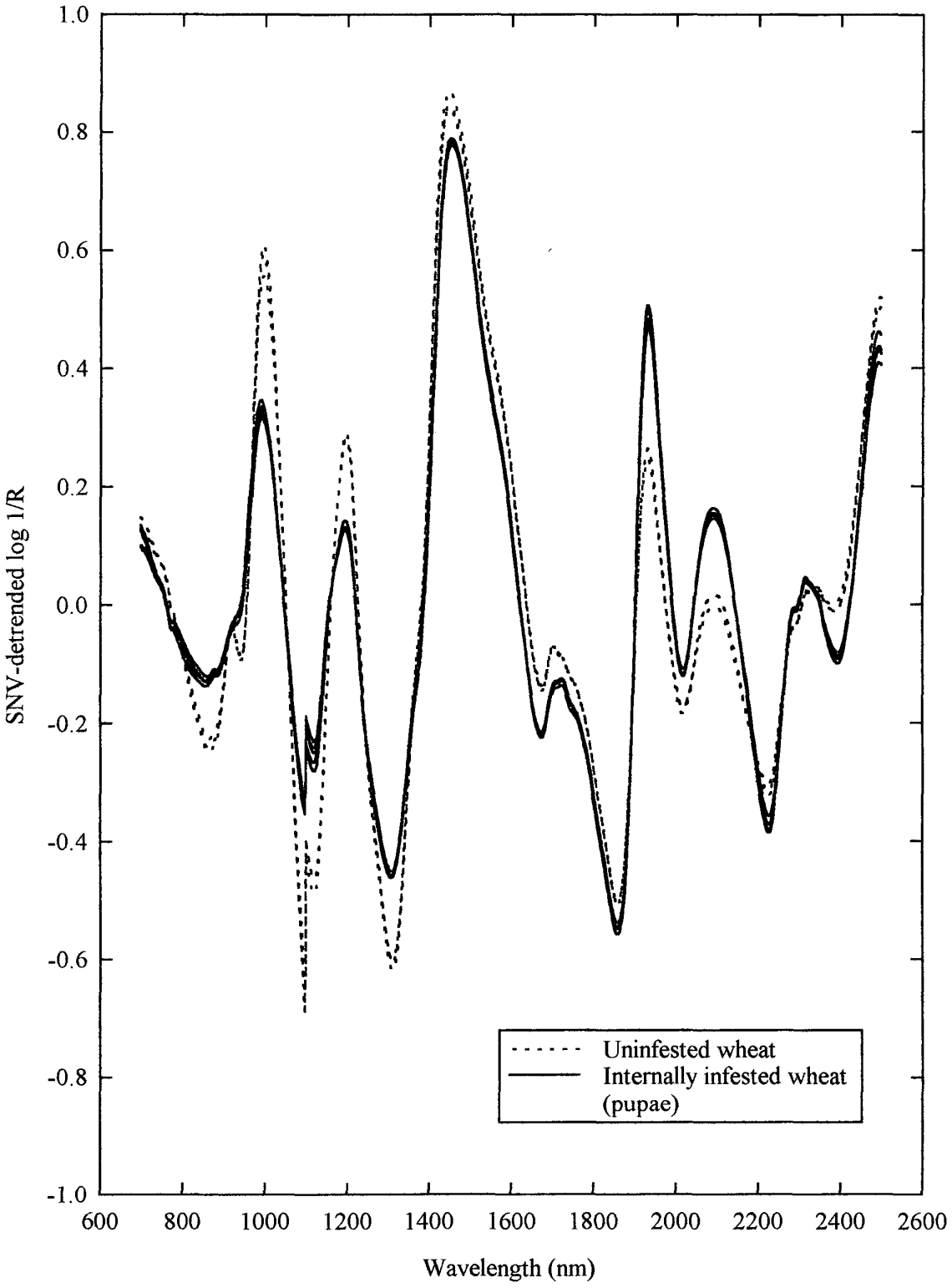


Figure 21: Spectra of uninfested wheat and wheat internally infested with pupae (Expt. 11).

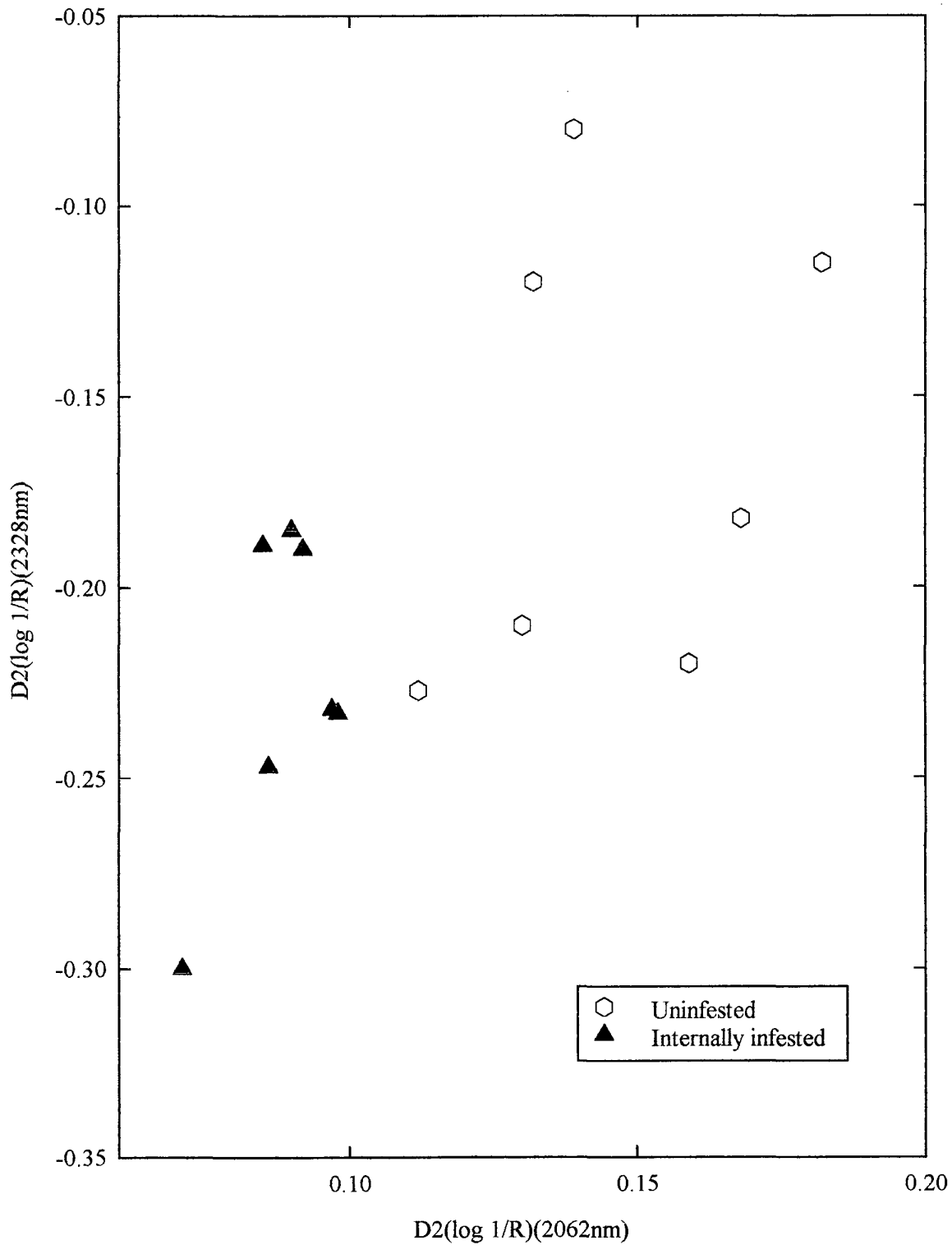


Figure 22: Absorbance at 2328nm versus absorbance at 2062nm for uninfested single wheat kernels and single wheat kernels internally infested with larvae (Expt. 12).