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Insect fragments in flour: Relationship to Lesser Grain Borer (Coleoptera: Bostrichidae) infestation level in wheat and rapid detection using near-infrared spectroscopy

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Abstract: The grain milling industry routinely checks wheat flour for insect fragments to determine whether the number found is below the U.S. Food and Drug Administration (FDA) defect action level of 75 fragments/50 g flour. However, the standard chemical extraction method used to detect insect fragments in flour is costly and time-consuming; thus, a rapid detection method is desirable. In addition, little is known about differences in the number of fragments produced from different stages of different insect species. In this study, we determined that wheat infested with a single pre-emergent adult lesser grain borer, *Rhyzopertha dominica* (F.), contributed 28 times and 10 times as many fragments as wheat infested with a single larva or pupa, respectively. Using regression models that we developed from these data, we predicted that 1-kg samples of wheat with more than 20 kernels infested with pre-emergent adult borers would be above the FDA defect action level for insect fragments. Similarly, it would take an infestation level of 380-640 kernels (in a 1-kg sample) containing larvae or pupae to exceed the defect action level. We also determined the accuracy and sensitivity of near-infrared spectroscopy (NIRS) for detecting insect fragments in flour using three different NIR-spectrometers. The number of insect fragments predicted by NIRS was correlated with the actual number of fragments in test samples. NIRS was less precise than the standard flotation method, but it has the advantages that it is rapid, non-destructive, does not require extensive sample preparation, and can be automated for a more sophisticated sampling protocol for flour.

Keywords: Insect fragments, flour, detection, near-infrared spectroscopy

Introduction

The U.S. Food and Drug Administration (FDA) has established a Defect Action Level (DAL) of 75 insect fragments per 50 g of flour as the regulatory standard for quality control (FDA 1997). Immature stages and pre-emergent adults of internal grain feeding insects may not be removed from grain by cleaning before milling; as a result, these stages are the main source of insect fragments in wheat flour (Brader et al. 2002). However, the relationship between internal infestation of wheat kernels and the number of insect fragments produced in flour has not been described well. Sachdeva (1978) reported that wheat infested with granary weevil adults produced 3× more fragments than wheat infested with weevil larvae. The standard method used by the FDA for detection of insect fragments (AOAC 1996) is labor intensive and expensive because it involves milling, extracting, and microscopically examining the number of insect fragments produced (Glaze and Bryce 1994). Thus, development of a fast and reliable alternative method is needed by the milling industry.

In a previous study, we found that a near-infrared (NIR) spectrometer with a spectral range of 400-1700 nm was able to predict accurately whether flour samples contained less than or more than 130 insect fragments (Perez-Mendoza et al. 2003). Although that study had limited success detecting insect fragments at the FDA defect action level, NIR spectrometers with extended wavelength ranges are now available and may improve detection accuracies. In this study, we re-examined this method with instruments that extend the near infrared region tested to 2500 nm.

The objectives of this study were: (1) to characterize the relationship between different levels of internal wheat infestation with larvae, pupae, or pre-emergent adults of the lesser grain borer, *Rhyzopertha dominica* (F.), and the number of insect fragments produced in flour milled from that wheat; and (2) to compare the accuracy of three NIR spectrometers for determining number of insect fragments in the flour produced from the infested wheat. *R. dominica* is a common and destructive pest of stored wheat in the U.S. (Hagstrum et al. 1994) that develops and feeds inside grain kernels and is the main source of insect fragments in wheat flour. Eggs are laid on the surface of grain kernels and, after hatching, the first instars bore into the grain (Elek 1994). Larvae pupate inside the kernels, and adults remain inside the kernels for several days after eclosion (Hagstrum and Flinn 1994). We refer to these newly eclosed adults still in the kernel as pre-emergent adults.

Materials and methods

Insects

Kernels infested with lesser grain borers were obtained from a laboratory strain reared on whole kernel, hard red winter wheat, *Triticum aestivum* L. Insect cultures were started by placing 200 unsexed adults into 200 g of wheat, adjusted to 13.5% moisture content by adding distilled water as needed, in 800 mL glass jars capped with screen/filter paper lids. Jars were held in a rearing chamber at 30±1°C and 70±5% r.h. with a 12:12 L:D photoperiod. All founding adults were removed by sieving after 7 d. After 21 days, kernels containing larvae, pupae, or pre-emergent adults were detected by x-ray analysis (Throne 1994). Infested wheat kernels were placed in aluminum dishes which were placed in a mechanical convection oven (Precision Scientific Inc., Chicago, IL) maintained at 130°C for 30 min to kill the insects. After cooling at room temperature, the desired number of infested kernels with each life stage was added to batches of uninfested wheat to complete 100 g samples. Samples were conditioned to 15% moisture content for 1 wk before milling.

Levels of Insect Infestation

Ten levels of infestation were tested to determine the fragment contribution of each stage of insect development (Table 1). The level of infestation was adjusted according to the insect stage and the expected fragment contribution based on a preliminary study (data not shown). The infestation levels used produced flour samples with fragment counts below and above the FDA defect action level.

Milling

Individual wheat samples were milled on a Brabender Quadrumat Sr. mill (type 12-10-N87, C.W. Brabender Instruments, Hackensack, NJ). Temperature of the rolls was maintained at 31.1-32.2°C during milling. The milling efficiency (% flour yield) of this mill was around 60%; therefore, milling produced flour samples of about 60 grams each.

Determination of Insect Fragment Counts Using the Standard Flotation Method

The standard flotation method used by the FDA (AOAC 1996) was used to determine the number of fragments produced in the flour samples. This method was scaled up to collect

insect fragments in 60 ± 5 g of flour samples (Perez-Mendoza et al. 2003). Fragments in five replicates of each infestation level for each insect stage were determined.

Detection of Insect Fragments Using Near-Infrared Spectroscopy (NIRS)

Three near-infrared spectrometers were used to collect spectral data from the wheat flour samples containing varying levels of insect infestation.

Perten Diode Array 7000

The DA 7000 (Perten Instruments Inc., Springfield, IL) collects absorbance spectra over a range of 400 to 1690 nm. Each flour sample was poured into a 12.5-cm-diameter sample ring above the 12.5-cm-diameter fixed sample viewing area. The thickness of the flour sample was about 1.2 cm. The light beam comes from below the sample viewing area and penetrates the flour sample. Each spectrum saved was an average of 15 spectra collected in about three seconds.

Cognis-QTA™ FT-NIR

The QTA™ system (Cognis, Cincinnati, OH) collects spectra in the 830 to 2500 nm wavelength range. Flour samples were placed directly in a rotating cup. Two replicates were collected for each flour sample. The instrument was set to automatically average the spectra of 100 scans into one spectrum for each sample being scanned. Prior to development of the calibration model, the resulting spectra from the two replicates of each sample were averaged yielding one spectrum that represented that specific sample.

Foss NIR Systems 6500

The Foss NIR Systems 6500 (Foss NIRSystems, Silver Spring, MD) scanning monochromator collects spectra from 400 to 2500 nm. The instrument has a sample transport device that allows the sample in a quarter cup to be moved vertically past the light source. Flour samples were poured and leveled in the quarter cup and secured flush using a white board material before placing the sample in the transport device. Each sample was scanned 64 times, and the data were saved as a single spectrum.

Statistical Analysis

Equations describing the relationship between infestation level and the number of insect fragments produced in flour recovered with the standard flotation method were fit to the data using TableCurve 2D (SYSTAT Software Inc. 2002). We used 95% prediction limits of the fitted equations to determine the maximum number of infested kernels that could be milled and still be below the FDA defect action level, which was calculated as the lowest number of infested kernels whose upper 95% prediction limit was below 90 fragments in 60 g of flour (= 75 fragments in 50 g of flour).

NIR spectra were analyzed by partial least squares (PLS) regression (Martens and Naes 1989) using PLSPlus/IQ software (Galactic Industries 2003). Calibration models for each insect stage infesting the grain (larvae, pupae, or pre-emergent adults) and for each NIR spectrometer were developed using PLS (9 individual models). Five test samples of each insect-stage infestation level were scanned (n = 150). Finally, a calibration model that included combined data from the three insect stages (larvae + pupae + adults) was developed. The relationship between NIRS-predicted number of insect fragments and actual number of fragments in the flour samples (as estimated by the flotation method) was determined by using TableCurve 2D (SYSTAT Software Inc. 2002). We used inverse prediction to estimate the actual number of fragments in a sample based on NIRS estimates, including 95% confidence limits on the estimates. We used these confidence limits from the inverse predictions to determine the maximum number of fragments that could be present in flour based on NIRS predictions and still be below the FDA defect action level, which was calculated as the lowest number of actual fragments whose upper 95% confidence limit was below 90 fragments in 60 g of flour (= 75 fragments in 50 g of flour).

Results

Effects of Insect Infestation Level on Fragment Counts

Larvae: Larvae produced the fewest fragments (Table 1). The predicted number of insect fragments produced by a single wheat kernel infested with one larva was 0.6 [95% confidence limits (CL) = 0.23 – 0.93; 95% prediction limits (PL) = 0 – 27.2]. The number of insect fragments increased with increasing infestation levels. This relationship was described by the equation (SE's in parentheses):

$$y = 0.5775\ (\pm 0.175)^{*}\ x^{1.126\ (\pm 0.0627)} \qquad \text{Equation 1}$$

where y = number of fragments and x = number of infested kernels (r^2 = 0.95) (Fig. 1A). Based on the 95% prediction limits for this equation, the maximum number of infested wheat kernels with larvae that millers can accept to produce flour that meets the FDA Defect Action Level is 64 infested kernels in 100 g of wheat (95% PL = 35.3 – 89.8 fragments in 60 g of flour).

Fragments contributed by pupae: Fragment counts in flour prepared from wheat samples infested with different numbers of kernels infested with pupae were intermediate between those produced by wheat samples infested with larvae or adults (Table 1). The predicted number of insect fragments produced from a single wheat kernel infested with one pupa was 1.6 (95% CL = 0.90 – 2.4; 95% PL = 0 – 22.1). The relationship between the number of kernels infested with pupae and the number of insect fragments produced in flour was described by the equation:

$$y = 1.646\ (\pm 0.372)^{*}\ x^{1.025\ (\pm 0.0568)} \qquad \text{Equation 2}$$

where y = number of fragments and x = number of infested kernels (r^2 = 0.95) (Fig. 1B). Based on the 95% prediction limits for this equation, the maximum number of infested wheat kernels with pupae that millers can accept to produce flour that meets the FDA Defect Action Level is 38 infested kernels in 100 g of wheat (95% PL = 47.7 – 89.2 fragments in 60 g of flour).

Table 1. Mean (± SEM, n = 5) number of insect fragments recovered in flour from wheat samples (100 g) infested with three stages and different infestation levels of the lesser grain borer, *Rhyzopertha dominica*.

Larvae		Pupae		Pre-emergent adults	
No. of infested kernels	No. of fragments	No. of infested kernels	No. of fragments	No. of infested kernels	No. of fragments
0	0 ± 0	0	0 ± 0	0	0 ± 0
10	8.6 ± 1.1	5	10.6 ± 1.0	1	14.0 ± 1.6
20	12.0 ± 1.8	10	17.6 ± 2.0	2	38.2 ± 2.4
40	28.8 ± 2.8	15	25.4 ± 3.9	3	65.8 ± 4.9
60	66.8 ± 3.6	20	37.6 ± 2.2	4	94.6 ± 6.4
80	78.8 ± 7.7	30	50.2 ± 3.8	5	135.4 ± 6.0
100	110.0 ± 8.5	40	71.8 ± 7.3	6	167.4 ± 5.8
120	126.2 ± 9.7	50	97.8 ± 7.3	7	201.2 ± 3.9
140	144.2 ± 6.2	60	100.6 ± 3.7	8	225.4 ± 11.0
160	178.0 ± 8.3	70	131.6 ± 8.7	9	276.8 ± 8.5

Fragments contributed by pre-emergent adults: Fragment counts for flour prepared from wheat samples infested with different numbers of kernels infested with pre-emergent adults were the highest compared with those produced in wheat samples infested with larvae or pupae (Table 1). The mean number of insect fragments produced by an individual infested wheat kernel containing one adult was 14.0 ± 1.6 (predicted number of insect fragments = 16.7; 95% CL = 13.8 – 19.6; 95% PL = 0 – 42.8). The relationship between the levels of internal wheat infestation with adults and the number of insect fragments produced in flour was described by the equation:

$$y = 16.69\ (\pm 1.43)^{*}\ x^{1.273\ (\pm 0.0430)} \qquad \text{Equation 3}$$

where y = number of fragments and x = number of infested kernels (r^2 = 0.98) (Fig. 1C). Based on the 95% prediction limits for this equation, the maximum number of wheat kernels infested by newly eclosed adults that millers can accept to produce flour that meets the FDA Defect Action Level is 2 infested kernels in 100 g of wheat (95% PL = 14.0 – 66.7 fragments in 60 g of flour).

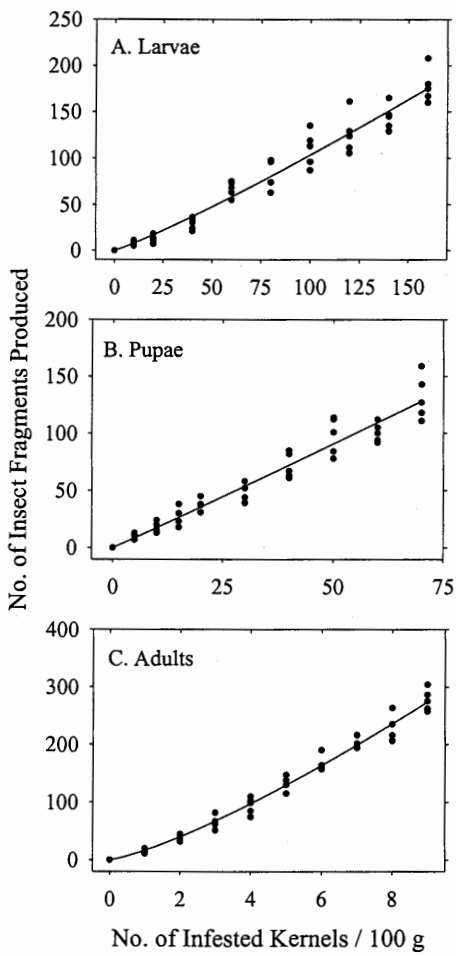

Fig. 1. Relationship between number of infested kernels/100 g of wheat with larvae (A), pupae (B), and pre-emergent adults (C) and number of insect fragments detected in milled flour samples by using the standard flotation method. Solid lines are from equations 1, 2, and 3.

Prediction of Insect Fragments in Flour by NIRS: *Cognis-QTA™ FT-NIR:* NIR spectra generated with this spectrometer correlated well with the actual number of insect fragments present in flour samples produced from wheat infested with larvae, pupae, or newly eclosed adults of the lesser grain borer (Table 2, Fig. 2). Based on inverse predictions, a 60-g flour sample that had a maximum of 56, 59, 44, or 45 insect fragments, based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be below the FDA defect action level of 75 fragments in 50 g of flour (upper 95% confidence limit would be below 90 fragments in 60 g of flour). Samples with 125, 116, 145, or 136 insect fragments or more based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be above the FDA defect action level of 75 fragments in 50 g of flour (lower 95% confidence limit would be above 90 fragments in 60 g of flour). It follows that one would not be able to determine whether the number of insect fragments in a sample was above or below the action level when NIRS predicts 57 – 124, 60 – 115, 45 – 144, or 46 – 135 insect fragments in 60-g samples containing larvae, pupae, pre-emergent adults, or all stages combined, respectively.

Table 2. Equations describing the relationship between the number of insect fragments present in flour and the number of insect fragments predicted by using NIRS.

NIR spectrometer	Stage	Wavelength range used in model (nm)	n	Equation Parameters [a]		r^2
				a ± SE	b ± SE	
Cognis-QTA	Larvae	1055 - 2500	47	12.54 ± 3.97	0.8684 ± 0.0402	0.91
	Pupae		48	5.644 ± 3.26	0.9087 ± 0.0487	0.88
	Adults		50	13.73 ± 5.84	0.9016 ± 0.0384	0.92
	All[b]		146	14.10 ± 2.84	0.8478 ± 0.0261	0.88
Perten 7000	Larvae	550 - 1700	48	6.152 ± 3.82	0.9224 ± 0.0387	0.93
	Pupae		46	7.514 ± 4.15	0.8649 ± 0.0612	0.82
	Adults		49	9.722 ± 6.40	0.9241 ± 0.0417	0.91
	All[b]		141	14.25 ± 3.78	0.8171 ± 0.0348	0.80
Foss 6500	Larvae	650 - 2250	46	11.47 ± 5.01	0.8818 ± 0.0497	0.88
	Pupae		48	7.881 ± 3.74	0.8473 ± 0.0571	0.83
	Adults		48	31.29 ± 9.52	0.7742 ± 0.0622	0.77
	All[b]		145	51.22 ± 4.87	0.3933 ± 0.0438	0.36

[a] Relationship is $y = a + bx$, where y is NIRS-predicted number of fragments and x is actual number of fragments.
[b] Larvae + Pupae + Adults.

Perten Diode Array 7000: NIR spectra generated with this spectrometer correlated well with the actual number of insect fragments present in flour samples (Table 2, Fig. 3). Based on inverse predictions, a 60-g flour sample that had a maximum of 56, 51, 39, or 29 insect fragments based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be below the FDA defect action level of 75 fragments in 50 g of flour (upper 95% confidence limit would be below 90 fragments in 60 g of flour). Samples with 122, 121, 147, or 147 insect fragments or more based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be above the FDA defect action level of 75 fragments in 50 g of flour (lower 95% confidence limit would be

above 90 fragments in 60 g of flour). It follows that one would not be able to determine whether the number of insect fragments in a sample was above or below the action level when NIRS predicts 57 – 121, 52 – 120, 40 – 146, or 30 – 146 insect fragments in 60-g samples containing larvae, pupae, pre-emergent adults, or all stages combined, respectively.

Foss NIR Systems 6500: NIR spectra generated with this spectrometer correlated well with the actual number of insect fragments present in the flour samples (Table 2, Fig. 4). Based on inverse predictions, a 60-g flour sample that had a maximum of 49, 51, 19, or 8 insect fragments based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be below the FDA defect action level of 75 fragments in 50 g of flour (upper 95% confidence limit would be below 90 fragments in 60 g of flour). Samples with 132, 119, 181, or 165 insect fragments or more based on NIRS predictions for larvae, pupae, pre-emergent adults, or all stages combined, respectively, would be above the FDA defect action level of 75 fragments in 50 g of flour (lower 95% confidence limit would be above 90 fragments in 60 g of flour). It follows that one would not be able to determine whether the number of insect fragments in a sample was above or below the action level when NIRS predicts 50 – 131, 52 – 118, 20 – 180, or 9 – 164 insect fragments in 60-g samples containing larvae, pupae, pre-emergent adults, or all stages combined, respectively.

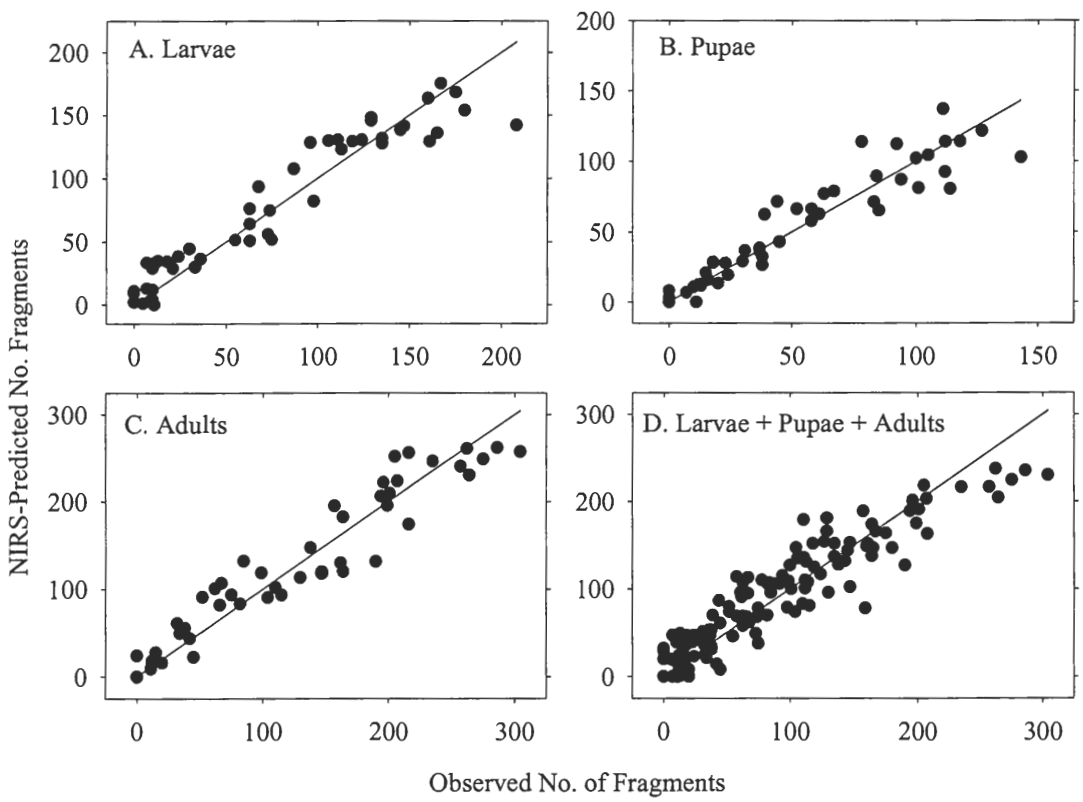

Fig. 2. Relationship between NIRS-predicted and observed number of insect fragments in flour samples produced from wheat infested with larvae (A), pupae (B), pre-emergent adults (C), and the three life stages combined (D). Calibration models generated with the Cognis-QTA NIR-instrument. Reference line shows perfect correlation.

Discussion

Wheat kernels infested with a single pre-emergent adult contributed about 28× and 10× as many fragments as wheat kernels infested with a single larva or pupa, respectively. This may be due to the fact that the larval and pupal exoskeletons are weakly sclerotized, compared to the adult stage, and only the most heavily sclerotized structures of their bodies are able to resist the milling process or the hydrochloric acid used for the standard flotation method.

The number of insect fragments detected with the standard flotation method was directly proportional to the infestation level, similar to results found by Harris et al. (1952) and Atui et al. (2002). Contrary to our results, Brader et al. (2002) found no strong correlation between the fragment counts and the level of infestation by late instar larvae of granary weevil, *Sitophilus granarius* (L.), perhaps because of their sampling protocol, which included subsampling. They infested batches of 250 g of wheat with 0 to 60 infested kernels and prepared sub-samples of 50 g, which they assumed would have homogeneous distributions of 0 to 12 infested kernels. Russell (1988) showed that the insect distribution in sub-samples taken from the same grain sample is not homogeneous. In the case of insect fragments, Brader et al. (2002) showed that both false positive and negative counts occurred in results from three laboratories with the standard flotation method. The standard flotation method requires highly trained technicians in microanalytical entomology to recognize insect fragments in flour (Kurtz and McCormack 1965).

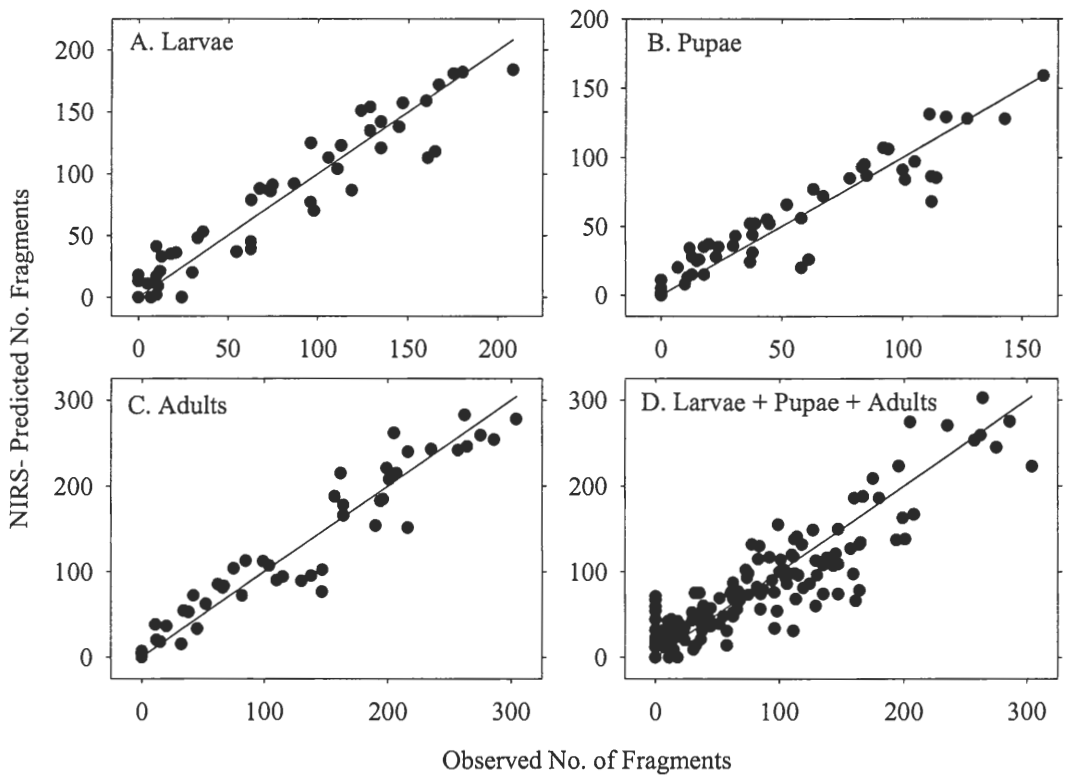

Fig. 3. Relationship between NIRS-predicted and observed number of insect fragments in flour samples produced from wheat infested with larvae (A), pupae (B), pre-emergent adults (C), and the three life stages combined (D). Calibration models generated with the Perten 7000 NIR-instrument. Reference line shows perfect correlation.

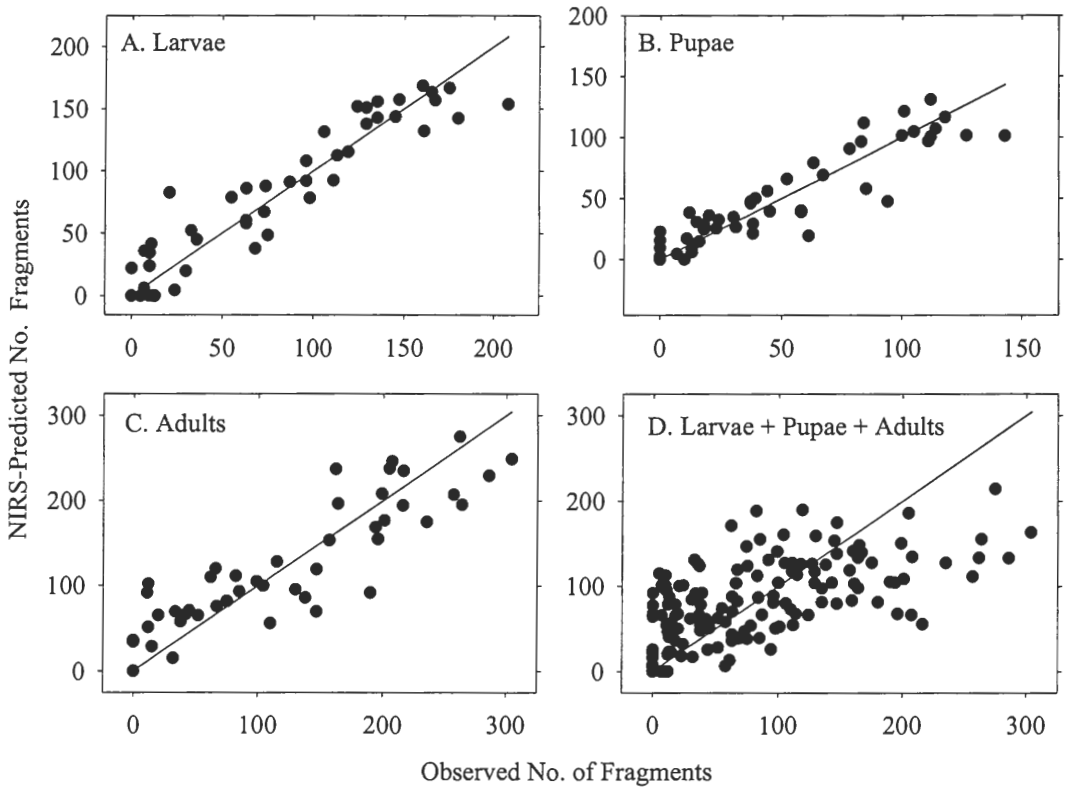

Fig. 4. Relationship between NIRS-predicted and observed number of insect fragments in flour samples produced from wheat infested with larvae (A), pupae (B), pre-emergent adults (C), and the three life stages combined (D). Calibration models generated with the Foss 6500 NIR-instrument. Reference line shows perfect correlation.

We developed equations describing the relationships between the number of insect fragments produced and the level of insect infestation for each lesser grain borer life stage. These equations will be useful in predicting the maximum level of internal infestation that can be accepted by millers to produce flour with insect fragment counts below the FDA defect action level. If the grain is mainly infested with pupae and larvae, the level of allowable maximum infestation fluctuates from 380 to 640 infested kernels/kg, respectively. But when the grain is internally infested primarily with pre-emergent adults, the level of maximum infestation is reduced to less than 20 infested kernels/kg. However, this last scenario may be less likely to occur in the milling industry because kernels infested internally with pupae and pre-emergent adults are weak and are more easily broken open by using impact machines, and then exposed insects may be removed by screens or aspiration (Sachdeva 1978, Mills and Pedersen 1992, Brader 1997). On the other hand, kernels containing insects in the early stages of development may not be broken open by the impact machines because they have not been sufficiently weakened by the insect (Sachdeva 1978, Mills and Pedersen 1992). As a result, several researchers have reported that most of the insect fragments present in flour are produced by the immature stages of the internal feeding insects. Our equations will be useful in sampling programs to determine how many insect fragments would be expected to be produced in flour milled from a sample of wheat, based on the number of internal insects of each stage detected in that sample of wheat.

NIR spectra generated using the three spectrometers were correlated with the actual number of insect fragments present in flour samples prepared from wheat infested with larvae, pupae, or pre-emergent adults. The QTA spectrometer gave the best estimates of insect fragment levels in flour samples in our tests. For the model combining data for all life stages, the QTA spectrometer would not be able to determine whether fragment counts were above or below the defect action level when actual fragments in a 60-g sample were between 46 and 135; similar levels for the Perten and Foss spectrometers were 30 – 146 and 9 – 164. The QTA spectrometer may have yielded the best predictions of the number of fragments in samples compared with the calibration models generated with the other two spectrometers because the QTA spectrometer continuously mixed the flour sample while collecting spectra whereas the other spectrometers sampled a static sample. In addition, the QTA and Perten spectrometers collect spectra for the whole sample, while the Foss spectrometer collects spectra for only a portion of the sample. Although the spectrometers are not highly accurate in determining number of insect fragments in flour samples, they provide results very quickly and could be used to screen a large number of flour samples. When the spectrometers indicate that either small numbers or large numbers of fragments are present, the standard flotation method would not need to be used. If the spectrometers are not able to determine whether the number of insect fragments is above or below the defect action level, then more samples could be processed quickly using NIRS or the standard flotation method could be used as a follow-up test.

Both the standard flotation method and the NIRS methods can detect and quantify the number of insect fragments produced by the lesser grain borer in wheat flour. The flotation method is more precise, but it is destructive, time consuming, expensive, and requires highly trained technicians. In contrast, although NIRS is less precise, it is rapid, non-destructive, does not require extensive sample preparation, and could easily be automated for a more sophisticated sampling protocol for large flour bulks.

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