Detection of infestations by *Cryptolestes ferrugineus* inside wheat kernels using a soft X-ray method

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Karunakaran, C., Jayas, D.S. and White, N.D.G. 2004. **Detection of infestations by *Cryptolestes ferrugineus* inside wheat kernels using a soft X-ray method**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **46**: 7.1-7.9. The Canada Grain Act imposes a zero tolerance for stored-product insects in grain for human consumption. The Berlese funnel method currently used to detect insect infestations in terminal elevators and grain inspection offices is slow and unreliable. The potential of a soft X-ray method to detect infestations caused by *Cryptolestes ferrugineus* (Stephens), the most common stored-grain insect in Canada, was determined in this study. Canada Western Red Spring wheat kernels uninfested and infested by four larval instars, pupae, and adults of *C. ferrugineus* were scanned using soft X-rays at 15 kV potential and 65 μA current. Five hundred sound kernels and 443 kernels infested by different life stages of *C. ferrugineus* were used as the grain samples. Algorithms were developed to extract a total of 57 features using histogram, histogram and shape moments, and textural features using co-occurrence and run length matrix methods. The extracted features were used to identify uninfested and infested kernels using the statistical classifiers and a 4-layer backpropagation neural network (BPNN). More than 75.3, 86.5, and 95.7% sound, kernels infested by larvae, and pupae-adults, respectively, were correctly identified by the parametric classifier, non-parametric classifier, and BPNN using all 57 features. There were no significant differences between the identification percentages of sound kernels by the three classifiers but the parametric classifier and BPNN identified significantly higher percentages of infested kernels. Identification percentages of infested kernels were higher using textural features or all 57 features than using histogram features. **Keywords:** wheat, *Cryptolestes ferrugineus*, X-ray image, insect infestation detection, histogram features, textural features, statistical classifier, neural networks

La Loi canadienne sur les grains prescrit une tolérance zéro en ce qui a trait à la présence d'insectes dans les grains entreposés destinés à la consommation humaine. La méthode de l'entonnoir Berlese couramment utilisée pour détecter les infestations d'insectes dans les élévateurs des terminaux et les bureaux d'inspection des grains est lente et peu fiable. L'objectif de cette étude était de déterminer le potentiel d'une méthode faisant appel aux rayons X doux pour la détection des infestations causées par Cryptolestes ferrugineus (Stephens), l'insecte d'entreposage le plus commun au Canada. Des grains de blé Canada Western Red Spring non infestés ainsi que d'autres infestés par C. ferrugineus à différents stades de croissance (i.e. quatre stades larvaires, chrysalides et adultes) ont été scannés en utilisant des rayons X doux à un potentiel de15 kV et un courant de 65 μA. Cinq cent grains sains et 443 grains infestés ont été utilisés comme échantillons. Des algorithmes ont été développés pour extraire un total de 57 caractéristiques en utilisant un histogramme, des moments d'histogramme et de forme ainsi que des caractéristiques de texture utilisant des méthodes de co-occurence et de matrice de longueur parcourue. Les caractéristiques extraites ont été utilisées pour identifier les grains non-infestés et infestés en utilisant des classificateurs statistiques et un réseau neuronal à quatre couches de propagation arrière (RNPA). Plus de 75,3, 86,5 et 95,7% des grains sains, des grains infestés par des larves et des grains infestés par des chrysalides ou des adultes respectivement ont été correctement identifiés par le classificateur paramétrique, le classificateur non-paramétrique et le RNPA utilisant l'ensemble de ces 57 caractéristiques. Aucunes différences significatives entre les pourcentages d'identification des grains sains par les trois classificateurs n'ont été relevées. En revanche, le classificateur paramétrique et le RNPA ont identifié un pourcentage significativement plus élevé de grains infestés. Les pourcentages d'identification de grains infestés étaient plus élevés en utilisant les caractéristiques de texture ou toutes les 57 caractéristiques qu'en utilisant les caractéristiques d'histogramme. **Mots clés**: blé, Cryptolestes ferrugineus, image rayon X, détection d'infestation d'insectes, caractéristiques histogramme, caractéristiques de texture, classificateur statistique, réseau neuronal.

INTRODUCTION

Canada is known for its high quality grains in international markets because of its strict grading policies. The Canada Grain Act (1975) imposes a zero tolerance for stored-product insects in grain for human consumption. Grain destined for domestic and export markets is inspected to determine the presence of insects that reduce the grain quality and consequently the economic value. If a live stored-product insect is detected in the incoming grain at elevators (grain handling facilities), the grain is termed infested and is not accepted until fumigated to kill the insects.

In spite of a zero tolerance for stored product insects, infestations are reported in primary and terminal elevators and significant monetary losses can occur (White 1995). The Berlese funnel method is used in terminal elevators and Canadian Grain Commission offices to detect infestations in grain samples but this method is slow and unreliable in detecting many insect species. It takes 5 to 6 h to determine the presence of insects and extracts 49 to 79% of the adults and only 30% of the larvae of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Smith 1977; Minkevich et al. 2002). During this time the grain would have been loaded into bins for storage or ships for export. Hence, if the infestation is due to an early developmental stage of the insect, the chances of reaching a misleading conclusion are high. Also, the efficiency of insect detection by the Berlese funnel depends on grain moisture, temperature, and insect developmental stages (Smith 1977). The failure to detect infestations leads to manifestation of infestation and cross contamination of stored-grain in the elevators. For example, wheat graded as No.1 and stored for six months in a terminal elevator was found to be infested (Sinha 1992, cited by Muir 1997). Therefore, detection of low levels of infestation in the incoming grain at the elevators and frequent monitoring of grain within the elevator will help maintain Canada's reputation for supplying high quality grains.

Sieving is a simple and fast method to detect insects feeding outside the grain kernels. Other methods such as crack-flotation, sound detection, carbon dioxide measurement, uric acid measurement, near-infrared spectroscopy, and nuclear magnetic resonance spectroscopy have been investigated for their potential to detect insect infestations in grain samples (Chambers et al. 1984; Hagstrum et al. 1990; Pedersen 1992; Dowell et al. 1999). Not all these methods are efficient in detecting infestations; some can detect infestations only of live insects; some can detect external feeders; some are time consuming or expensive; and some are indirect.

Dead and hidden (immature stages developing within the grain kernels) insects are the potential sources of contamination in grain products particularly insect body parts in grain flour (Pedersen 1992). However, the present grading system has no limitation on the presence of dead insects, as there is no objective method available to detect the presence of dead insects in grain. Therefore, there is a need for an objective test method that is rapid, accurate, and detects live and dead insects in grain. The potential uses of the method are: detection of insect infestations for grain grading; and identification of species, insect stages, and extent of infestation to guide grain management such as grain cleaning and fumigation.

A soft X-ray method has been used in different studies to determine its potential to detect insect infestations in grain samples. This is the only non-destructive and direct method that can detect insect infestations inside grain kernels (Milner et al. 1952; Stermer 1972; Schatzki and Fine 1988; Haff and Slaughter 1999; Karunakaran et al. 2000, 2003a, 2003b). Extensive work has been reported on the use of X-rays to detect infestations due to internal grain feeders, the granary weevil, *Sitophilus granarius* L., the rice weevil, *Sitophilus oryzae* L., the maize weevil, *Sitophilus zeamais* Mots., and the Angoumois grain moth, *Sitotroga cerealella* (Olivier) in grain kernels by visual examination of the X-ray radiographs (Milner et al. 1950; Schatzki and Fine 1988; Keagy and Schatzki 1991). Only a few studies have used image processing algorithms to identify the insect infested wheat kernels using digital images of kernels (Keagy and Schatzki 1993; Haff and Slaughter 1999; Karunakaran et al. 2000, 2003a, 2003b). The soft X-ray method can identify whether the insects inside the grain kernels are alive or dead (Karunakaran et al. 2003a) and can be used in "real-time".

Therefore, the objectives of this study were:

1. to determine the efficiency of the soft X-ray method to detect infestations due to *C. ferrugineus* in Canada Western Red Spring wheat kernels; and
2. to determine the best classifier for the identification of kernels infested by *C. ferrugineus.*

MATERIALS and METHODS

Grain samples

Canada Western Red Spring (CWRS) wheat used in this study was clean and had no visible mechanical damage. The moisture content of the grain was elevated to approximately 15% (wet basis). Five hundred sound kernels were used for uninfested sample and infested kernels were prepared by artificial implantation of insect eggs in the germ area of the kernels. Adults of *C. ferrugineus* were allowed to feed on wheat flour for 24 to 48 h at 30°C and 70% relative humidity (r.h.). The culture was then removed and passed through sieve no. 40 (425 μm aperture) and no. 60 (250 μm aperture) to separate the adults and eggs, respectively, from the wheat flour. A hole was made on the germ of the kernel using a needle (26 gauge) and an insect egg was implanted into the kernel using a single hair brush. Each single kernel was then placed in a gelatin capsule and incubated continuously under controlled conditions (30°C and 70% r.h.) and was removed on days 4, 8, 11, 15, 22, and 27 for taking the X-ray images to show infestations by the four larval, pupal, and adult stages. Five hundred and fifty kernels were thus prepared for the infested grain sample.

X-ray images

The X-ray imaging system consisted of i) Lixi fluoroscope (Model: LX-85708, Lixi Inc., Downers Grove, IL) which produced soft X-rays and real time images; ii) a coupled charge device (CCD) black and white camera (Sony XC-75/75CE) which captured the real time images; iii) black and white monitor which displayed the real time images; iv) an image digitizer (Dazzle digital video creator, Dazzle Multimedia Inc., Fremont, CA) which converted the video images into digital images, and v) a personal computer (5300 series, Compaq Computer Corporation, Houston, TX) which was used to store and process the digital images. The X-ray detection system is 25 mm in diameter and had a resolution of 62.5 μm. Grain kernels were placed manually on cellophane wrap on the sample platform with the kernel crease facing down and single kernels were X-rayed at 15 kV potential and 65 μA current for 3 to 5 s. The X-ray images were digitized as 8-bit images at a resolution of 60 pixels/mm and saved as gray scale images.

Feature extraction

The gray values of all digitized images were in the range 25 to 252. The gray value of the cellophane wrap (background) itself taken at different times during the experiments had a consistent and constant value of 252. Hence, kernels were segmented from the background by the simple thresholding method (Gonzalez and Woods 1998). For comparing grain kernels of different sizes and orientation, a normalized histogram (histogram values divided by the grain kernel area) was obtained for each kernel and was grouped into 23 groups (Karunakaran et al. 2000). The first 22 groups contained the total number of pixels of the grain kernel with 10 gray levels beginning from gray level 251. The last bin included all pixels with gray values less than 31. Other histogram features extracted were: total gray value, mean gray value, standard deviation of the gray levels, area, and histogram moments of order 2 to 6 (Gonzalez and Woods 1998). Shape features of the grain kernels were extracted using the first invariant moments of binary images. The gray value distributions were determined using the first four invariant moments and textural properties of gray level images (Galloway

1975; Haralick 1979; Unser 1986; Majumdar and Jayas 2000). The textural properties extracted using the co-occurrence and run length matrices were determined for the maximum gray levels of 32 (each pixel gray value was divided by 8) and for 0, 45, 90, and 135° directions at a distance, d=1. A total of 16 textural features which were invariant to size, translation, and orientation were determined for the grain kernels (Majumdar and Jayas 2000).

Grain kernel identification

A total of 57 features extracted for each kernel were used by the statistical classifiers and artificial neural network to identify uninfested and infested grain kernels (SAS 1990; Neuroshell 2, version 4.0, Ward Systems Group, Frederick, MD). The DISCRIM procedure of the parametric and non-parametric classifiers was used for identification of uninfested and infested kernels (SAS 1990). The backpropagation network is widely used for quality inspection and classification of agricultural products and a 4-layer BPNN gave the highest classification percentages of cereal grains (Jayas et al. 2000; Paliwal et al. 2001). Therefore, a 4-layer BPNN was chosen to determine the classification accuracies and the results were compared with the statistical classifiers.

The sample was split into two subsets as training and independent testing sets and classification accuracies were determined by randomly selecting the training and testing sets three times. Four-fifths of each infested kernel types and uninfested sample was used for training and the remaining one-fifth as the independent test set. The average of the three trials was calculated as the mean classification accuracy. ‘Classification accuracy’ is the percentage of kernels correctly identified as belonging to a specific class. ‘Misclassification’ is the classification of a particular class as other classes. ‘False positives’, is classification of a sound kernel as infested and ‘false negatives’, is identification of an infested kernel as uninfested. The classification percentages were first determined using the histogram and textural features independently and were then compared with the classification accuracies determined using all 57 features. The significant differences between the histogram groups of different classes were determined using Duncan’s multiple range test at the 5% significance level. The significant differences between the classification percentages obtained using different classifiers were compared using the F-test at the 5% significance level. The most significant features from the 57 feature model were selected by the statistical classifier and BPNN.

RESULTS and DISCUSSION

Infestation classes and extracted features

Of the 550 kernels infested with *C. ferrugineus* eggs, only 443 eggs developed into larvae, matured into adults, and produced continual infestation in the wheat kernels. The mortality of *C. ferrugineus* eggs at 30°C and 70% r.h. is 10% when wheat flour with 5% germ, by mass, is used as the diet (Bishop 1959). The damage due to handling of the eggs during the implantation process and the whole-wheat kernel as the food source, which does shorten the life span of *C. ferrugineus* compared to a diet of ground wheat (White and Bell 1994), might have caused higher mortality (19.5%) in this study. Moreover, completely sound wheat kernels are unsuitable for the development of newly hatched *C. ferrugineus* larvae (Ashby 1961) but typical mechanically harvested wheat has damage to over 25% of the germs (White and Bell 1990).

There was a total of 500 X-ray images of uninfested kernels, 1645 images of kernels infested by all four instars (one set of 127 kernels was missed during scanning for infestation due to second instar), and 886 images of kernels infested by pupae and adults. The X-ray images of a sound kernel and a kernel infested by different life stages of *C. ferrugineus* are shown in Fig. 1. Infestations were characterised in the germ area of the kernels by a small bright spot during second and third instar stages and by a bright region after the third instar stage of the insect. The infestations due to *C. ferrugineus* were evident after the third instar larval stage from the X-ray images. The fourth instar larvae and pupae inside the germ area of wheat kernels were identified by visual examination of X-ray images of the infested kernels (Fig. 1). Some larvae were seen lying outside the wheat kernels inside the gelatin capsules when kernels were taken out for imaging. Not all adults matured and exited from the kernels on day 27. Some matured earlier and some later than day 27.

A high percentage of misclassifications occurred when the kernels infested by four instars, pupae, and adults were considered as six separate classes. Hence, all kernels infested by the four instar stages were grouped into ‘Larvae’ class, and kernels infested by the pupae and adults were grouped into ‘Pupae-Adults’ class. The sound kernels were classed as ‘Uninfested’.

Figure 2 shows the normalized histogram groups of sound and infested wheat kernels. The consumption of germ by *C. ferrugineus* decreased the density of the germ area. The infestation area increased with the developmental stages of *C. ferrugineus* and allowed better penetration of X-rays and appeared as brighter regions in the X-ray images. This resulted in first five histogram group values significantly lower and values of 6 to 23 groups significantly higher for the infested kernel classes than the uninfested kernels. A *Cryptolestes ferrugineus* larvae consumes the entire germ when it reaches the pupal stage (Sinha and Watters 1985). Total gray value that represents the density of wheat kernels is shown in Fig. 3. Kernel mass depends on the density. The less dense regions (infested regions) have lighter shades of gray with high gray values. The infested kernels have less grain kernel mass than the sound kernels and hence had significantly higher total gray values. The adults feeding on the germ (after development was completed) resulted in a significantly higher total gray value of ‘Pupae-Adults’ class than the ‘Larvae’ class.

Histogram features

The classification percentages of uninfested and infested kernels were determined using 23 histogram group values and area of kernels as input features by the statistical classifiers and BPNN. The classification percentages of the training sets were higher than the independent test sets determined by all classifiers. The mean classification accuracies by the parametric method for the independent test sets were: 69.0% (uninfested), 63.8% (larvae), and 71.5% (pupae-adults). The parametric method identified 28.3 and 2.7% of false positives as infested by larvae and pupae-adults and 18.7 and 17.5% of kernels infested by larvae as uninfested and infested by pupae-adults, respectively. Misclassification of kernels infested by pupae-adults was 24.7 and 3.8% as infested by larvae and uninfested, respectively.

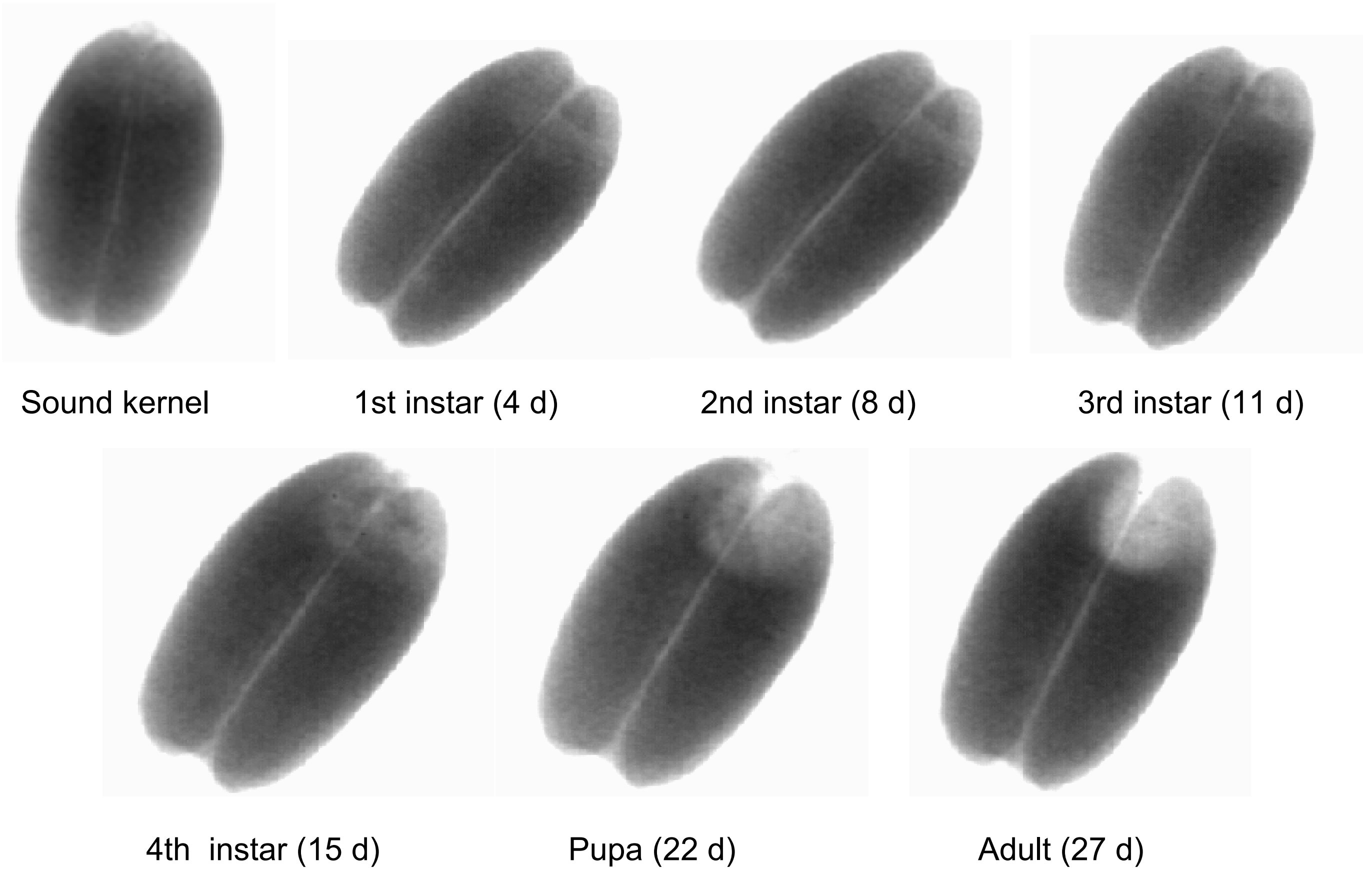

Fig. 1. X-ray images of a sound kernel and a kernel infested by different life stages of *Cryptolestes ferrugineus*.

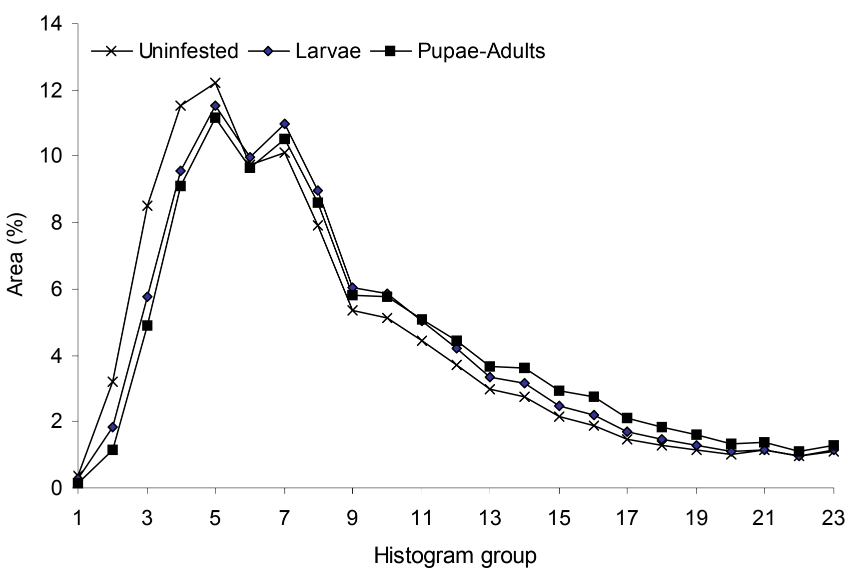

Fig. 2. Histogram groups for wheat kernels uninfested and infested by *Cryptolestes ferrugineus.* (Uninfested: 500 kernels; Larvae: 1645 kernels; Pupae-Adults: 886 kernels)

The non-parametric classifier correctly identified 82.7, 57.9, and 67.3% respectively, as belonging to 'Uninfested', 'Larvae', and 'Pupae-Adults' classes from the independent test sets. The false positives were 15.7 and 1.7% as infested by larvae and pupae-adults, respectively. The classifier misclassified 29.9% as uninfested and 12.2% as infested by pupae-adults for kernels infested larvae and 8.8% as uninfested and 23.8% as infested for kernels infested by pupae-adults.

The mean classification accuracies by the trained BPNN for independent test sets were 55.0% (uninfested), 56.7% (larvae), and 78.5% (pupae-adults). The trained BPNN classified 42 and 3% of false positives as infested by larvae and pupae-adults, respectively. The false negatives were 15.4 and 0.5% for kernels infested by larvae and pupae-adults. The BPNN misidentified 27.9% of kernels infested by larvae as infested by pupae-adults and 21.0% of kernels infested by pupae-adults as infested by larvae.

Figure 4 compares the correct identification percentages of true positives and true negatives irrespective of the classes by the statistical classifiers and BPPN. There was no significant

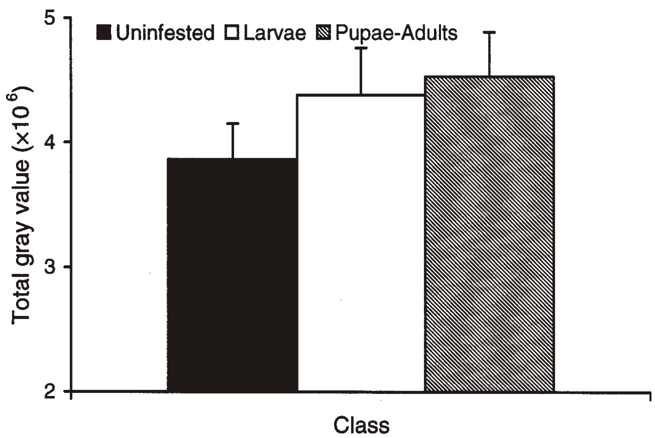

Fig. 3. Total gray values (mean ± SD) of wheat kernels uninfested and infested by *Cryptolestes ferrugineus*. (Uninfested: 500 kernels; Larvae: 1645 kernels; Pupae-Adults: 886 kernels, infested regions have lighter shades of gray with high gray values.)

difference among the three classifiers for the identification of uninfested kernels. The BPNN and parametric classifier identified significantly higher percentages of infested kernels by larvae than the non-parametric classifier. When the infestations were due to pupae-adults of *C. ferrugineus,* the statistical classifiers and BPNN identified the same percentage of infested kernels.

Textural features

The 16 textual features extracted using the co-occurrence and run length matrix methods were used as input features for classification. All classifiers gave higher classification percentages of individual classes, and lower false negatives and false positives for the training sets than the independent test sets. The mean classification accuracies by the parametric

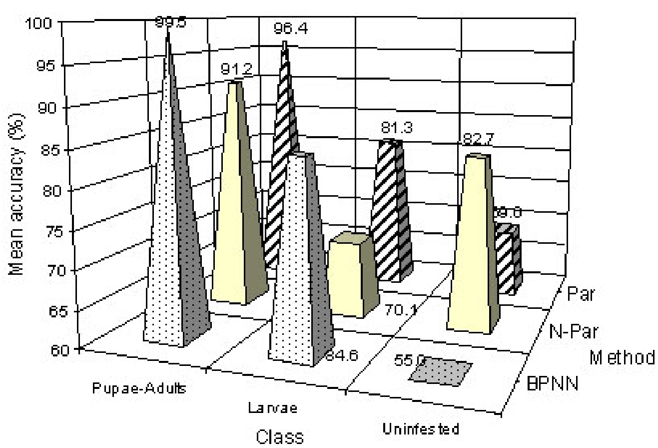

Fig. 4. Comparison of classification percentages of wheat kernels uninfested and infested by *Cryptolestes ferrugineus* using histogram features by the statistical classifiers and BPNN. (Par: Parametric method, N-Par: Non-parametric method; BPNN: Backpropagation neural network).

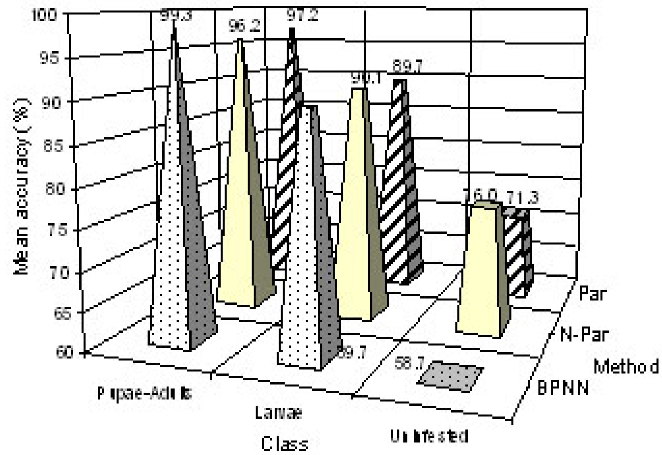

Fig. 5. Comparison of classification percentages of wheat kernels uninfested and infested by *Cryptolestes ferrugineus* using textural features by the statistical classifiers and BPNN. (Par: Parametric method, N-Par: Non-parametric method; BPNN:

classifier were 71.3% (uninfested), 63.7% (larvae), and 71.7% (pupae-adults) for the independent test sets. The false positives by the parametric classifier were 23% as infested by larvae and 5.7% as infested by pupae-adults. The false negatives were 10.3 and 2.8% for kernels infested by larvae and pupae-adults, respectively. About 26% of kernels infested by larvae and pupae-adults were misidentified as infested by the other infested class.

The classification percentages by the non-parametric classifier for the independent test sets were 76.0% (uninfested), 64.3% (larvae), 72.0% (pupae-adults) and the false positives were 20.7 and 3.3% as infested by larvae and pupae-adults, respectively. The classifier misclassified 9.9 and 25.8% of kernels infested by larvae as uninfested and infested by pupae-adults, respectively. The false negatives were 3.8% for kernels infested by pupae-adults and 24.4% of kernels were misidentified as infested by larvae.

The mean classification accuracies by the trained BPNN for the independent test sets were 58.7% (uninfested), 52.8% (larvae), and 77.2% (pupae-adults) and the false positives were 38.7 and 2.7% as infested by larvae and pupae-adults, respectively. The false negatives were 10.3 and 0.7% for infested kernels by larvae and pupae-adults, respectively.

Figure 5 shows the identification percentages of true positives and true negatives determined by the statistical classifiers and BPNN using the textural features. The parametric classifier, non-parametric classifier, and BPNN identified the same percentages of sound kernels and kernels infested by *C. ferrugineus* larvae. When kernels were infested by pupae-adults, the BPNN and parametric classifier identified a significantly higher percentage of infested kernels than the non-parametric classifier.

Combined features

The classification percentages of different classes determined by the parametric classifier, non-parametric classifier, and BPNN using combined feature sets (all 57 features) for the independent

Table 1. Classification accuracies of uninfested and kernels infested by *Cryptolestes ferrugineus* using parametric classifier, non-parametric classifier, and BPNN using all 57 features for the independent test sets.

Class to→ from↓	Uninfested (%)	Larvae (%)	Pupae-Adults (%)
Unifested			
Parametric	**75.3 ± 9.0**	21.3 ± 10.2	3.3 ± 3.2
Non-parameteric	**83.3 ± 7.8**	14.3 ± 5.5	2.3 ± 3.2
BPNN	**75.5 ± 6.0**	22.3 ± 3.8	2.0 ± 2.6
Larvae			
Parametric	7.2 ± 0.1	**74.3 ± 2.3**	18.5 ± 2.4
Non-parameteric	13.5 ± 1.1	**72.6 ± 1.6**	13.9 ± 0.7
BPNN	10.4 ± 2.0	**64.3 ± 3.6**	25.3 ± 3.7
Pupae-Adults			
Parametric	2.0 ± 1.7	22.2 ± 2.5	**75.8 ± 2.0**
Non-parameteric	4.3 ± 1.0	34.0 ± 3.9	**61.7 ± 4.7**
BPNN	1.2 ± 1.6	17.5 ± 4.4	**81.3 ± 3.4**

test sets are shown in Table 1. The parametric classifier correctly identified 75.3% of sound and 74.3 and 75.8% of kernels infested by larvae and pupae-adults, respectively. The false positives were 21.3 and 3.3% as infested by larvae and pupae-adults, and false negatives were 7.2 and 2.0% for kernels infested by larvae and pupae-adults, respectively.

The mean classification accuracies by the non-parametric classifier for the independent test sets were 83.3% (uninfested), 72.6% (larvae), and 61.7% (pupae-adults). The false positives were 14.3 and 2.3% as infested by larvae and pupae-adults, respectively. The false negatives were 13.5 and 4.3% respectively, when kernels infested by larvae and pupae-adults were classified.

Table 2. Classification accuracies of CWRS wheat kernels uninfested and infested by *Cryptolestes ferrugineus* using 57 features by the 4-layer BPNN (first instar kernels no included, test set).

Class to→	Uninfested		Larvae		Pupae-Adults	
from↓	No.	%	No.	%	No.	%
Unifested						
set 1	85	**85.0**	15	15.0	0	0.0
set 2	71	**71.0**	27	27.0	2	2.0
set 3	83	**83.0**	17	17.0	0	0.0
mean		**79.7 ± 7.6**		19.7 ± 6.4		0.7 ± 1.2
Larvae						
set 1	28	9.3	150	**50.0**	122	40.7
set 2	44	14.7	165	**55.0**	91	30.3
set 3	39	13.0	157	**52.3**	104	34.7
mean		12.3 ± 2.7		**52.4 ± 2.5**		35.2 ± 5.2
Pupae-Adults						
set 1	0	0.0	31	15.5	169	**84.5**
set 2	1	0.5	24	12.0	175	**87.5**
set 3	6	3.0	42	21.0	152	**76.0**
mean		1.2 ± 1.6		16.2 ± 4.5		**82.7 ± 6.0**

The classification accuracies by the trained BPNN for independent test sets were 75.7% (uninfested), 64.3% (larvae), 81.3% (pupae-adults). The false negatives were 10.4 and 1.2% for kernels infested by larvae and pupae-adults and false positives were 22.3 and 3.0% as infested by larvae and pupae-adults.

The classification percentages of true positives and true negatives determined by the parametric classifier, non-parametric classifier, and trained BPNN for the independent test sets were compared. The parametric classifier, non-parametric classifier, and BPNN identified the same percentages of uninfested kernels. The parametric classifier and BPNN identified significantly higher percentages of kernels infested by *C. ferrugineus* larvae and pupae-adults than the non-parametric classifier.

Canonical analysis

The categorization of sound and infested kernel classes using canonical functions is shown in Fig. 6. The R^2 values of the two canonical functions determined were: CAN1 = 0.58 and CAN2 = 0.34. Better separation of uninfested and infested kernels can be achieved using CAN1 discriminant function as shown in the plot. The overlap of kernels uninfested and infested by *C. ferrugineus* larvae resulted in a high percentage of false positives classified as infested by larvae. There was a distinct difference between the features derived from the uninfested and kernels infested by *C. ferrugineus* pupae-adults as evident from the canonical plot. Therefore, kernels infested by *C. ferrugineus* pupae-adults were identified with more than 98% accuracy by the BPNN. The overlap between 'Larvae' and 'Pupae-Adults' classes caused misclassifications between these two classes of infested kernels.

When kernels were X-rayed for infestations due to first instar, the larvae might not have consumed enough germ to create a difference compared to germ of sound kernels. This might have resulted in the high percentage of false positives classified as infested by larvae and false negatives in the 'Larvae' class. Hence, it was hypothesized that the removal of kernels infested by first instar larvae from the training and independent test sets would improve classification accuracies. Table 2 lists the classification percentages of different classes determined by the trained BPNN. There were no significant differences in the classification accuracies of sound and infested kernels determined with the first instar larva removed or included in the classification analysis.

The parametric classifier and BPNN identified a significantly higher percentage of infested kernels using all feature models. The non-parametric classifier requires the training sets to determine the class probability and to develop the discrimination functions (SAS 1990; Luo et al. 1999). The coefficients of the discriminant functions determined by the parametric classifier from the training sets and trained BPNN can be saved and hence the parametric classifier and BPNN can be applied independently of

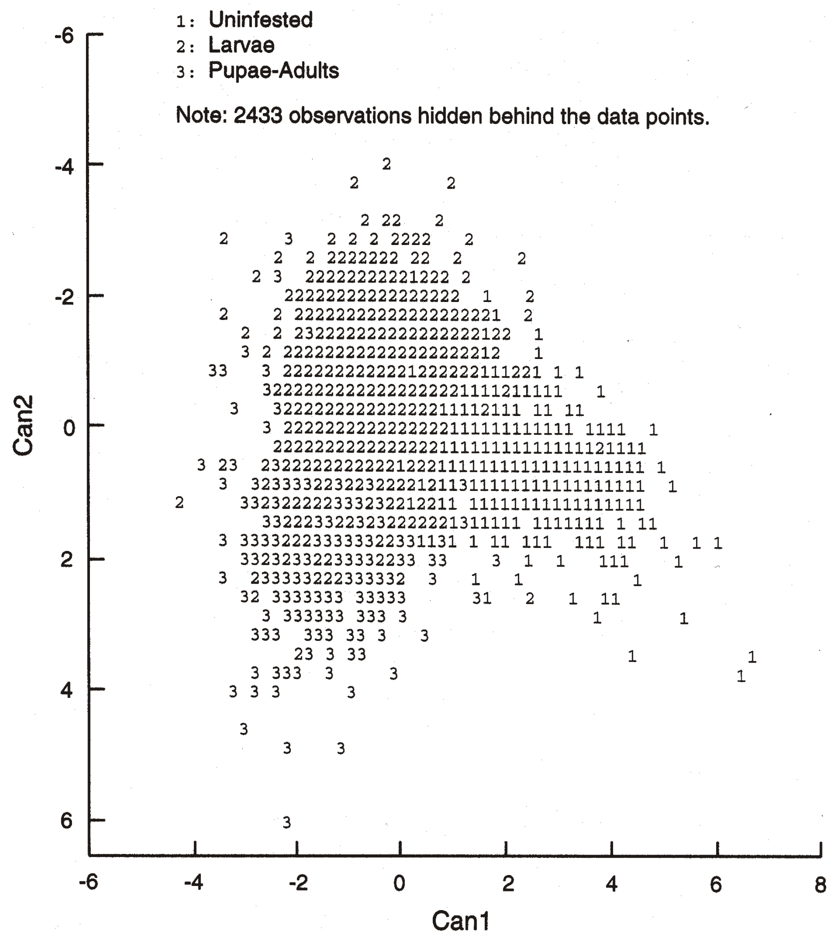

Fig. 6. Canonical plot for wheat kernels uninfested and infested by *Cryptolestes ferrugineus*.

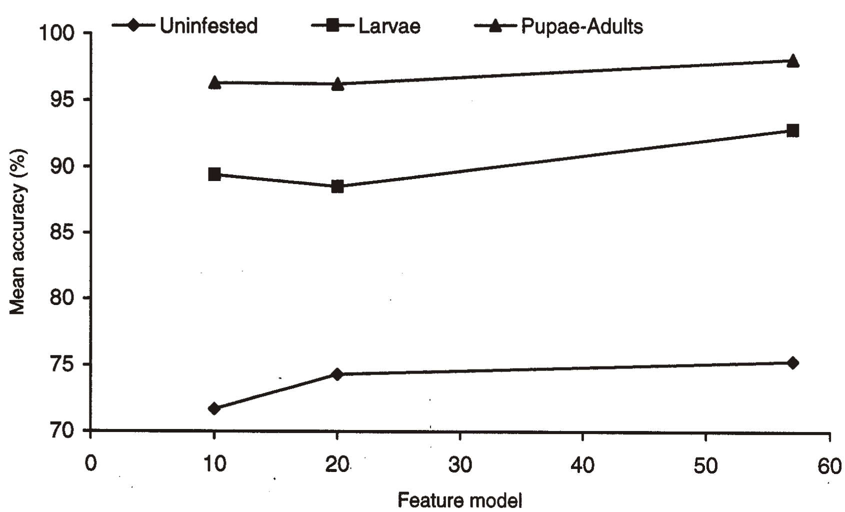

Fig. 7. Evaluation of feature models by the 4-layer BPNN for the classification of wheat kernels uninfested and infested by *Cryptolestes ferrugineus*.

the training set. However, there is no theoretical method available to design an optimal neural network classifier. If a neural network is to be used for classification, experience and extensive experimentation with different architectures are required to find the best neural network classifier for the particular application. From this study, the linear-function parametric classifier and BPNN were selected as the best classifiers for the detection of infestations caused by *C. ferrugineus* in wheat kernels.

There were no significant differences among the statistical classifiers and BPNN for the identification of sound kernels determined using the histogram, texture, and combined feature models. Kernels infested by *C. ferrugineus* larvae were identified significantly more accurately using the texture and combined feature models than the histogram feature model by the parametric classifier. Smaller density differences in the germ area due to feeding by first and second instars might not contribute significantly to the histogram features but can be detected by the textural features using gray value distributions.

Ranking of features

Though all features were used to determine the classification accuracies, some features might not contribute significantly to the classification accuracies. The STEPDISC procedure selected the total gray value and BPNN selected the long run of the run length matrix as the most significant features. The classification accuracies were determined by the BPNN using the first 10 and 20 selected features for the independent test sets and were compared with the 57 feature model (Fig. 7). There were no significant differences in the identification percentages of uninfested and infested kernels by the 10, 20, and 57 feature models. The BPNN took 1.4, 2.0, and 13.4 min, respectively, for training using 10, 20, and 57 feature models. Considering the time and classification accuracies, the first 10 features selected BPNN can be used to identify kernels infested by *C. ferrugineus.*

Comparison with Berlese funnel method

The efficiency of the soft X-ray method to identify wheat kernels infested by *C. ferrugineus* was compared with the extraction efficiency of the Berlese funnels (Karunakaran et al. 2003b). The Berlese funnels extracted 67.2, 50.5, 81.0, and 11%, of second, third, fourth instar larvae, and adults, respectively, in 6 h whereas the soft X-ray method correctly identified all infested kernels. It was determined that the lowest recovery percentage for kernels infested by adults was due to uneven maturity so that some pupae and developed adults that did not exit from the kernels were killed by lamp heat and were subsequently identified from the dissected kernels.

Limitations of X-ray method

The Berlese funnel method currently used in grain elevators to detect insect infestations can detect only live insects in grain samples. However, pneumatic grain samplers commonly used in the terminal and transfer elevators to take samples from trucks, railcars, and ships can kill a considerable percentage of live insects in a sample (CIGI 1993). For example, the pneumatic sampler kills 73% of *C. ferrugineus,* 65% of *T. castaneum,* 22% of *S. granarius,* and 65% of *O. surinamensis* adults (Bryan and Elvidge 1977). In the pneumatic conveyance system that is used to handle bulk grain, mortality is 98% for the adults and 35-85% for the immature stages of *C. ferrugineus* (Paliwal et al. 1999). This may be due to less impact force at the low pressures used to handle small quantities of grain compared to pneumatic systems used to handle large bulks of grain. So there is a possibility of live insects being transferred with the sample and the X-ray method can be used to detect whether the infestation contains live or dead insects (Karunakaran et al. 2003a). If the larva or adult insect has been dead for a long time, it would be dry and shriveled. With experience, one can become familiar with the appearance of insects that have been dead for some time (Fenton and Waite 1932; Nicholson et al. 1953). However, machine recognition between the insects killed before or during sampling might be difficult. If the pneumatic sampling method kills 100% of insects, none of the infestation detection methods (Berlese funnel, X-rays, and other methods) can be used to detect the presence of live insects. In that case, considerations should be made to make use of other methods of taking grain samples.

The soft X-ray method may not be helpful to detect live insects outside the grain kernels as the X-rays will penetrate the insect completely and could not be distinguished from the background (Karunakaran et al. 2003b). However, if the infestation is extensive and adults are crawling throughout a grain sample, there should be enough larval and pupal stages of the insects that feed inside and on grain kernels that they can be easily detected by the X-ray method.

CONCLUSIONS

The soft X-ray method has the potential to identify uninfested and kernels infested by *C. ferrugineus*. Sound kernels were correctly identified with more than 55.0, 58.7, and 75.3% by the all classifiers using histogram, texture, and combined feature models.

True negatives infested by *C. ferrugineus* larvae were identified with more than 70.1, 89.7, and 86.5% accuracy, and kernels infested by pupae-adults were identified with more than 91.2, 96.2, and 95.7% accuracy by all classifiers using histogram, texture, and combined feature models, respectively. Kernels infested by *C. ferrugineus* larvae and pupae-adults identified by the parametric classifier and BPNN were significantly higher than the non-parametric classifier.

The successful commercial application of the soft X-ray technique to detect insect infestations in grain samples will depend on the development and testing of a line-scan X-ray imaging system. Further work must be done to determine the efficiency of the method to identify insect infested kernels in real time using a line-scan X-ray imaging system.

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