# Analysis of Radiograms of Wheat Kernels for Quality Control

T. F. SCHATZKI and T. A. (BRYANT) FINE1

#### ABSTRACT

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Daily film radiograms were obtained of hard winter wheat kernels following one day exposure to each of the four major hidden insect pests of North American wheat (Sitophilus zeamais, Sitophilus oryzae, Ryzopertha dominica, and Sitotroga cerealella). Resulting films were viewed in transmitted light using microscopic lenses and image enhancement. When presented with the image of a single kernel for 1 sec, a trained operator

could detect the presence of a hidden insect of these four species, respectively, with 80% accuracy 8, 7, 27, and 15 days after oviposition. False negatives generally decreased exponentially with insect maturity. False positives amounted to 0.08%. The implications for wheat quality control are discussed.

Wheat quality standards address, among others, insect content of the wheat. Department of Agriculture standards (USDA/FGIS 1980, 1987) are based solely on the presence of visible insects (two live insects injurious to grain/1,000 g); these standards take no account of the immature insects that may inhabit grain kernels (hidden insects). Food and Drug Administration standards (USFDA 1980) are based on insect fragments found after milling and thus include effects of at least the more mature hidden insects. This inconsistency causes problems in classification of wheat quality. Further, neglect of hidden insects gives no warning of possible problems that may develop in stored wheat. Storey et al (1982) studied samples of wheat from U.S. commerce. Based on 1,000-g samples, 4% of samples contained at least one insect. Removing the visible insects and incubating the cleaned samples for a generation resulted in increasing contamination to 16%. This fourfold increase of infestation must result from hidden insects. The establishment of a quality control standard for hidden insects is therefore of considerable interest.

Among the methods that have been proposed for detecting hidden insects in grain are radiography of wheat kernels (Milner et al 1950b, 1952), staining of the egg plug deposited by the female on egg laying (Frankenfeld 1950, Goossens 1949, Milner et al 1950a), visual inspection for exit holes left by the emerging insects (Nicholson et al 1953a), flotation of kernels to detect internal voids left by the feeding insect (Apt 1952), cracking kernels and concentrating the removed insect parts (Harris et al 1952), crushing kernels and staining with ninhydrin to detect the amino acids representative of the living insect (Dennis and Decker 1962), detection of uric acid present in the excreta of insects (Galacci 1983, Roy and Kvenberg 1981, Wehling and Wetzel 1983), IR/CO2 gas analysis to determine the respiration of insects (Bruce and Street 1974, Bruce et al 1982, Street and Bruce 1976a,b), and sonic detection of chewing insects (Adams et al 1953, 1954). Not all these methods are compatible, in that some determine live insects only, some determine internal as well as external insects, some are

With respect to X-ray imaging in particular, Nicholson et al (1953c) investigated the pertinent exposure parameters, and the method was adopted as an official method (AACC 1969). Dry paper methods are commonly used for speed by millers. Exposure of film is used when the development of the immature insect is to be followed in detail (Kirkpatrick and Wilbur 1965, Mills and Wilbur 1967, Sharifi and Mills 1971a,b). Martel and Belanger (1977), using high voltage X-ray (295 KV), obtained good contrast for whole corn kernels using xerography. Pedersen and Brown (1960) noted that better resolution could be obtained by using a collimated X-ray beam (X-ray microscope), but, possibly because

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of cost and time required, this method has not been adopted. Stermer (1973) singulated the kernels to an automated, nonimaging X-ray system, similar in concept to a method for X-raying standing lettuce developed by Lenker and co-workers (Lenker and Adrian 1971, Schatzki et al 1981). However, to get good response Stermer had to vacuum inject the kernels with  $K_2CO_3$  solution, presumably to take advantage of the X-ray absorption of potassium.

While some of the methods are still under active development, the current method of choice by the industry is clearly X-ray radiography; approximately 40% of U.S. millers and processors use this method as quality control for accepting shipments (Arteman 1981). Nevertheless, the method is currently subjective; no quality standards exist for any method to determine hidden insects. Nor is it known how reliable the X-ray method is, particularly in detecting young insects. The current study was undertaken to determine what can be detected under idealized conditions. Single-kernel images of noninfested kernels or kernels containing insects of known maturity were presented to a trained operator for fixed time periods, and recognition rates were recorded. While these tests are clearly not representative of commercial conditions, they do yield limits on any method using recognition of X-ray images.

# MATERIALS AND METHODS

## Incubation and X-Ray

Insect colonies of four species (Sitophilus zeamais [maize weevil], S. oryzae [rice weevil], Ryzopertha dominica [lesser grain borer], and Sitotroga cerealella [angoumois grain moth]) were obtained from USDA laboratories at Manhattan, KS, and Fresno, CA. Hard winter wheat (obtained from General Mills, Vallejo, CA) was brought to 13% moisture. Fifty (for S. oryzae) or 100 g of wheat (plus 1 teaspoon of flour for R. dominica) was exposed to unsexed parent insects of a single species (100, 92, 100, and 10 insects, respectively) in an incubator (25° C, 70% rh) for 24 hr, after which the parents were removed. Images were prepared using the method of Milner (1950b), as adapted by Mills and Wilbur (1967), with only slight modifications. Using a minimum amount of rubber cement, 650 kernels were mounted on a 1-mil polyethylene sheet overlaid with 6-mm square mesh chicken wire, one kernel per square. At least one such kernel array was prepared for each of the four species. Kernels exposed to S. zeamais were mounted crease down. For the remaining three species half the kernels were glued crease down, half crease up, in approximately the position they would lie when freely spread one layer thick. Plastic sheet and chicken wire were stapled to a wooden frame, which was gasketted and clamped between a framed copper screen (for ventilation) and plywood to prevent any emerging insects from escaping. Frames were kept in the incubator (except to X-ray) until all insects emerged, as were ventilated jars containing the remaining exposed wheat. Frames were removed from incubator and X-rayed daily. All emerged insects were counted and removed on the day of emergence, as were those that emerged in the jars. Radiography was done using a Faxitron 43804N (Hewlett-Packard,

<sup>&</sup>lt;sup>1</sup>Food Quality Research Unit, Western Regional Research Center, Agricultural Research Service, Albany, CA 94710

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McMinnville, OR), using 3 mA, 15 kV, 5 min, and Industrex M film (Kodak Co., Rochester, NY). Film was developed for 5 min, washed 1 min, fixed 5 min, washed, and dried. Care was taken to avoid damaging the emulsions by keeping the developed films in plastic envelopes, except when viewing.

Film was viewed using transmitted light (supplied by a bank of fluorescent lamps covered with a frosted glass plate) through a stereomicroscope (Nikon SMZ-10), equipped with iris stopped down to 6 mm and trinocular head. Film was aligned using ocular objectives, while image inspection was done using a videocamera with an ER Newvicon tube (Panasonic WV-1850) with black clamp and automatic gain control. The videocam-captured image, expressed as an RS-170 TV monochrome signal (as in Fink 1982), was passed to an image processing computer, where, after scaling and digitization, the image was stored in a frame buffer as a set of integers between 0 and 255. Scaling was adjusted to give a grey scale of approximately 60 for a fully exposed film and approximately 250 at the center of a solid kernel. The data, g, in the frame buffer was optionally transformed (enhanced) according to the expression

$$h = 256^{(g/255)} - 1,$$

and either g or h was displayed as a single kernel on a 13-in. CRT with resulting magnification of  $35 \times$  (Fig. 1).

## **Recognition Tests**

For each insect, a day after exposure was selected when hidden insects were mature enough to be easily seen on the radiogram (using the unenhanced g-display) but had not yet exited from the kernels (column 1 of Table I). The radiogram corresponding to that day was then inspected, and each of the 650 kernels was

TABLE I Infestation and Emergence

Insect	Day of Classification for Infestation	Kernels Infested/ 650	Emergence			
			Screen			
			No. Insects	Day	Bottle	
Sitophilus zeamais	28	28	26	36 ± 2	37 ± 2	
S. oryzae	28	110	106	$36 \pm 2$	$35 \pm 1$	
Ryzopertha dominica	40	89	90	$63 \pm 6$	59 ± 8	
Sitotroga cerealella	32	27	23	41 ± 4	$43 \pm 4$	

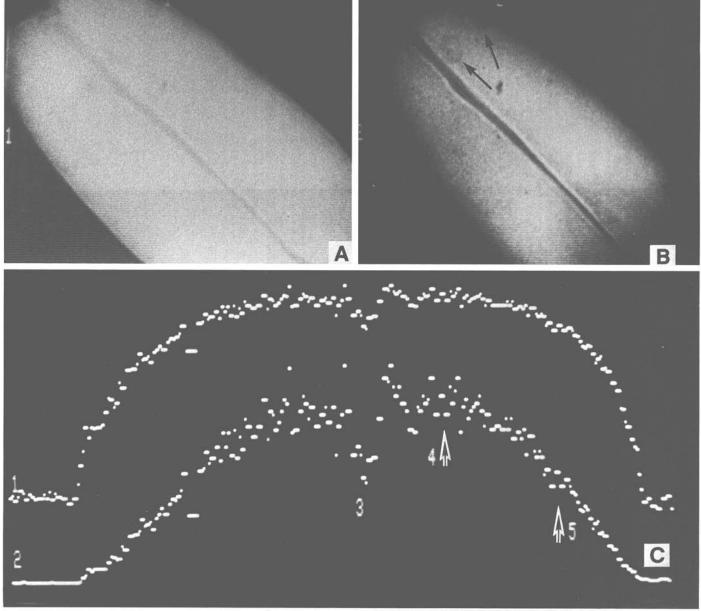


Fig. 1. A, X-ray image of wheat kernel three days after oviposition by Sitophilus zeamais. B, Same kernel after grey-scale enhancement, egg holes at arrows. C, Density contours along lines from 1 to 1 in A and from 2 to 2 in B; 3 marks crease, 4 and 5 mark holes.

classified as infested or not according to whether an insect of proper maturity was visible. This classification was then used for all radiograms.

To test recognition of infested kernels, a sequence of approximately 2,000 single kernel images was prepared using the X-ray films produced as described above. By use of a random number table the sequence was randomized as to insect exposure, maturity, infestation, and kernel orientation. One-half of the images presented were of kernels exposed to S. oryzae, one sixth each to the other three insects. Six days were chosen for each insect, the time span covered the range from oviposition (for S. zeamais and S. orvzae) or entrance (for R. dominica and Sitotroga cerealella) until approximately the second instar (Fig. 1). Half the images were of infested kernels. Also, half were of crease up kernels (except S. zeamais where all were crease down). No image was presented more than once in the sequence, although a given kernel could appear at several maturities.

Using one operator, the "presentor," the X-ray film was placed and positioned as required in the optical path and in the sequence specified. The presentor was aware of maturity and insect type, by selection of the actual film sheet used, but not of infestation; that information was kept in a closed file. The enhanced (h-display) image was flashed for precisely 1 sec on a screen that was observed by a second trained "observer." The decision of the observer as to infestation was recorded. Because in a field situation an inspector would presumably take a thorough second look at any suspected positives, the observer similarly was permitted to review an image recognized as positive for several seconds and change the decision; this did not apply to images recognized as negative. The observer at no time viewed the unenhanced image. A new image was presented approximately every 28 sec, the remaining time being required for film handling, positioning, and recording. Operator exhaustion limited viewing to about 400 images/day.

At the completion of the test, (revised) observer decisions were compared to actual classification of each image and correct recognition computed as a function of species, maturity, and kernel orientation. These results are expressed as p = fraction of correctly recognized infested kernels and q = fraction of correctly recognized noninfested kernels. All images that had resulted in false positives were reinspected to establish the cause of the misclassification and, in particular, to segregate the false positives arising from insect attack from those due to inherent wheat defects. Images and the numbers of each type of false positive error were recorded. This reinspection was done in the "sighted" mode, i.e., with knowledge that the kernels had been exposed to egg-laying adults but were, in fact, noninfested.

The recognition rate, p, of infested kernels is expected to depend on species and to increase asymptotically to 1 as the insects mature. The rate might also depend on kernel orientation since a kernel lying crease up on an X-ray film will give an appearance quite different from a kernel lying crease down. This relationship may be approximated by

$$\hat{p}(t) = 1 - b \exp(-ct),\tag{1}$$

where  $\hat{p}(t)$  is the predicted recognition rate of infested kernels t days after oviposition, and the parameters b and c will depend on species and orientation. It is more important to express  $\hat{p}(t)$  in terms of the day  $t_0$  when  $\hat{p}(t) = 0$ , and the day  $t_{80}$  when 80% recognition is predicted, rather than b and c. Rewriting (1) one obtains

$$\hat{p}(t) = 1 - \exp[-(\ln 5)(t - t_0)/(t_{80} - t_0)] \tag{2}$$

Again,  $t_0$  and  $t_{80}$  may depend on species and orientation. For R. dominica and Sitotroga cerealella no infestation can be seen before the larvae enter the kernels, so that  $t_0$  may be taken as an estimate of day of initial larval entry. In this case  $\hat{p}(t)$  is set to 0 for  $t < t_0$ . On the other hand, Sitophilus eggs are inserted into the kernel on day t = 0, and here p(t) > 0 for all t > 0. In this case  $t_0$  is simply a parameter which will be negative.

The parameters  $t_0$  and  $t_{80}$  are obtained by fitting the observed p

to the predicted  $\hat{p}$  by weighted least squares, separately for each species and orientation. The weighting function to be used is the standard deviation of p(t), s(t). Since p is a fraction, this is given by

$$s = \sqrt{p(1-p)/n}$$

where n(t) is the number of kernels inspected corresponding to day t. We thus minimize the sum of squares

$$SS = \sum (p - \hat{p})^2 / s^2 = \sum n(p - \hat{p})^2 / [p(1 - p)], \tag{3}$$

by (nonlinearly) adjusting the two variables  $t_0$  and  $t_{80}$ . The sum goes over six days. The significance of the orientation variable was tested for by an F test and was found nonsignificant at the 0.05 level in the three species tested. This result can presumably be assigned to the exponential nature of the enhanced grey scale, h. Accordingly, both orientations for a given day were lumped in further calculations of  $t_0$  and  $t_{80}$ .

Because the sample of noninfested wheat was small, and to assure that the segregation between insect-attack and clean-wheat defects was valid, a large sample (500 g or approximately 15,000 kernels) of wheat never exposed to insects was subsequently Xrayed and inspected. A kernel-by-kernel search for images that could be mistaken for insects was carried out knowing that the wheat was clean, using the same enhancement and enlargement as above. Images found in this way were recorded and again classified as to type of defect.

## RESULTS AND DISCUSSION

#### **Insect Behavior and Appearance**

Observations on insect behavior are in agreement, by and large, with those of previous investigators. Some additional observations in X-ray images are noted here. A total of 94 images that appeared to have egg holes were noted in exposed kernels that did not develop larvae. It is not clear whether these holes were a consequence of feeding by adults, empty holes, or egg mortality. We assume the latter two causes predominate, because all but one hole occurred in kernels exposed to Sitophilus. Insect morbidity could not be expected to be affected by irradiation that amounted to less than 20 roentgens/day (whole kernel basis). Dennis (1961) noted that LD<sub>50</sub> for adult S. oryzae amounted to about 10<sup>3</sup> roentgens. Nondeveloped holes were noted in 59 separate kernels. This should be compared to the 138 adult Sitophilus that were bred in this sample. Very little morbidity for partly grown larvae was noted; the number of insects seen on the day when images were classified as to infestation (column 2 of Table I) was essentially identical to the number of insects that emerged (column 3). X-ray images cannot distinguish live from dead insects unless desiccation takes place. In the present experiment, the time was apparently not long enough or the humidity low enough for such desiccation to occur. One image showed larvae that stopped developing, but the X-ray density observed was essentially that of live insects. No change in density was observed during the course of observations that extended 30 days past the estimated date of

Development time was quite uniform in populations, as noted by the standard deviations of emergence dates. Adult insects emerged from irradiated, mounted kernels in the same time from ovipositions as from nonirradiated kernels that had been kept in the same environment (Table I). The rather large deviation noted for R. dominica is not an indicator for nonuniformity. Rather, these adults use the kernels as nests even after maturation. Instances were noted where adults would be positioned in one direction in a tunnel one day, the reverse the next day, which indicates emergence and reentrance. The maturation times may also be too long for the species; it was difficult to establish maturation because mature insects had to be pried loose from the kernels to remove them. For this reason counting live adults by sifting and observing the dockage, as done in standard quality tests, will almost certainly yield a low value for R. dominica infestation. Multiple occupation of kernels by this insect was frequently seen. Up to three viable insects would occupy a single tunnel in tandem. Double occupation to maturity was seen in two kernels occupied by *S. oryzae*, but in no other cases.

We noted a strong propensity for R. dominica and Sitotroga cerealella to enter in the germ region of the kernel. Whereas 14% of Sitophilus began their development in or near the germ portion (approximately one-seventh of the total kernel surface corresponds to germ surface) fully 98% of R. dominica and 89% of Sitotroga cerealella were first seen in the germ region. We did not see (and did not look for) larvae of R. dominica and Sitotroga cerealella before entrance. We do note that such larvae from the moment of entrance show considerably more detailed structure than those of young Sitophilus. The latter, both in shape and lack of structure, are visually difficult to distinguish from the germ portion of the kernel. All insects are easily recognized from the third instar on. We also note that Sitophilus produces a frass that is very fine and tightly packed into the kernel and thus difficult to distinguish from undamaged endosperm. The other two species, on the other hand, leave a marblelike appearing frass that is easily recognized.

## **Optics**

Overall image quality depends on the optical system: backlight, film, microscope optics, videocam, image processing computer, monitor, and viewing conditions. Recognition of inclusions in a kernel is only affected by local aspects of the image (Marr 1982). Thus spatial resolution (sharpness) and grey scale (contrast) are important, whereas slow variations caused by nonconstant lighting or distortions are not significant. Loss of spatial resolution can arise from three sources: film production, analogue optics, and digitization. Visual inspection of the exposed X-ray film through the microscope suggests that the finest object that can clearly be seen is approximately 35  $\mu$ m wide, whereas film grain size is of the

order of 1  $\mu$ m. Most of this loss of film resolution arises presumably from X-ray scattering, which is difficult to reduce with the Faxitron instrument used. Next, the lens optics could blur the image seen by the video camera. Blurring, if present, can be corrected for by applying a Laplace transform to the digital image (Marr 1982). Application of this transform did not sharpen the images used here, which indicates that analogue blurring does not occur at the present magnification. Finally, the pixel spacing, introduced by the videocam as well as the 480 × 512 resolution of the image processing computer, must be fine enough to reproduce the spatial detail in the image. Strictly, it must be less than one-half of the shortest wavelength seen (Nyquist 1928). At the magnification used, one pixel corresponds to 11.5 µm. This is well below the Nyquist limit if 35  $\mu$ m is used for the shortest wavelength present. One therefore concludes that the X-ray film spatial resolution is fully preserved in the present system.

Grey scale, g, varied from approximately 60 to 250 when no enhancement was used. This light intensity is subject to a standard deviation of 6 units, due to shot noise (dark current) in the videocam, as measured in regions between the kernels (fully

TABLE II Recognition of Infested Kernels in Blind Tests

Insect	Total Images	Parameters of Expression (2)			
		$t_0$	t <sub>80</sub>	Residual Sum of Squares	
Sitophilus zeamais	161	-5.6	8.4	6.6	
S. oryzae	495	-5.8	9.3	97.5	
S. oryzae					
t = 8 excluded	413	-3.4	6.0	4.8	
Ryzopertha dominica	177	9.0	27.2	1.8	
Sitotroga cerealella	143	8.1	15.7	12.6	

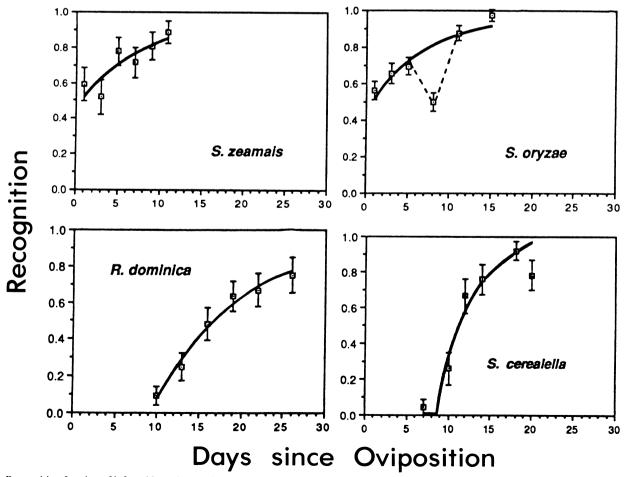


Fig. 2. Recognition fraction of infested kernels. 

— Observed, with one standard deviation shown. Solid curve, predicted with parameters from Table II.

exposed film). Since the eye can distinguish about 64 intensity levels, the shot noise is barely visible as a fine graininess in the darker regions of the image. The contrast of Figure 1A is not adequate, however, to detect egg holes. This result follows from Beer's law. The film exposure and the grey level are proportional to  $I_{00} - (I_{00} - I_0)e^{-ux}$ , where x is the absorbing thickness, u is constant and  $I_{00}$  and  $I_{0}$  refer to fully unexposed and fully exposed film. An egg hole or small larva will have only a minor effect on g if it occurs in a region of large x. But this is where many of these holes are; weevil holes, for example, tend to be excavated towards the center

TABLE III
Classification of Noninfested Kernels in Blind Tests

Kernels Exposed	Incorrectly Recognized					
	Eggs	Larvae	Flour	Damage	Germ	
145	6	1	0	0	0	
507	87	0	0	4	1	
155	0	0	2	1	1	
151	1ª	0	0	2	0	
	145 507 155	Exposed         Eggs           145         6           507         87           155         0	Exposed         Eggs         Larvae           145         6         1           507         87         0           155         0         0	Exposed         Eggs         Larvae         Flour           145         6         1         0           507         87         0         0           155         0         0         2	Exposed         Eggs         Larvae         Flour         Damage           145         6         1         0         0           507         87         0         0         4           155         0         0         2         1	

<sup>&</sup>lt;sup>a</sup>Presumed damage.

of the kernel, 0.5 mm deep. Most of the kernel is imaged at high g; note the upper curve in Figure 1C. If the image were presented as in Figure 1A these holes would be missed. There are, in fact, two maize weevil egg holes, three days old, in the presented kernel. To compensate for this effect, a transform h is used that increases the contrast at high g, albeit at the cost of higher noise at high g and less contrast at low g. A convenient form is the exponential  $h = 256^{(g/255)}$ -1, which matches g at 0 and 255 but increases contrast for g > 176. Transformations like that of g to h, depending only on the value of the pixel in question, can be done in hardware at display speeds (30 msec) and at very low cost. Transforming Figure 1A from a g-to an h-grey scale results in Figure 1B. We now clearly recognize two holes. It is worth noting that this recognition still depends very much on the human eye. A grey-scale contour along a line that passes through the eggs is displayed in Figure 1C for both the gand h-display. The small dips in grey scale correspond to the dark rings seen in Figure 1B. They are barely visible on the contour, yet the rings are clear on the image. The recognition one notes on viewing Figure 1B arises from the integration the eye does when the entire surround of the egg is viewed. This type of global integration and averaging of noise, so common in human vision, is extremely

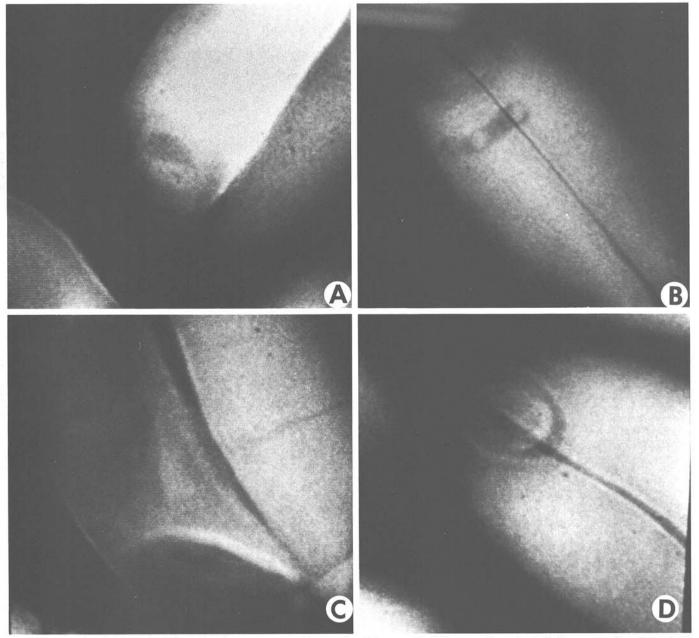


Fig. 3. False positive images. A, Egg hole like, clean wheat. B, Dead larvae, Sitophilus zeamais. C, Damage, clean wheat. D, Germ separation, clean wheat.

difficult to reproduce by computation. As a result, fully automatic recognition is extremely difficult to implement.

## Infestation Levels

An actual field sample would be expected to contain mostly clean wheat unattacked by insects. Assuming a quality control level of one hidden insect per 1,000 g, only one kernel out of approximately 30,000 would show infestation, requiring a false positive rate of less than 1/105 for adequate standards. Samples with realistic infestation levels would have to be very large to obtain adequate statistical information on infested kernels. To avoid excessive labor, recognition was tested instead by using samples that were highly enriched (50% infested kernels and 50% kernels that had been exposed to insects but were not infested). The results were then adjusted to account for true field conditions. The "observer" in the present test was permitted to scrutinize a kernel tentatively identified as positive for several seconds and change the recognition decision, if desired. Only the reconsidered decision was recorded. This did not apply to kernels recognized as negatives. It was important to establish whether false positives were due to clean wheat defects, and thus would scale up if the test sample were to be diluted with clean wheat, or whether they were due to insect damage and would, therefore, not increase on dilution. Hence, great care was taken to identify the cause of false positives after test completion, essentially from the appearance of the image.

The computation of the parameters  $t_0$  and  $t_{80}$  in expression (2), describing the expected recognition rate  $\hat{p}(t)$ , was given in the Methods section. The results for  $t_0$  and  $t_{80}$  are shown in Table II. The last column shows the total number of infested kernels inspected for each species, approximately one-sixth of these were viewed for each day of measurement. Figure 2 shows the fit graphically, the vertical bars corresponding to  $\pm s$ . The strong dip at t = 8 for S. oryzae is not a statistical aberration; it is noted in subsets of the data, and a somewhat smaller dip was noted at t = 7in a preliminary test. This recognition failure occurs upon molting (first to second instar), when the larva fully fills the available cavity, leaving little dark contrast region to recognize. The dip in p is as pronounced as it is because all the larvae are exactly the same age. A better representation would be the dashed curve (fitting  $\hat{p}$  to the remaining five data points,  $t_0 = -4.3$ ,  $t_{80} = 6.7$ , SS = 4.9). However, in a field sample, all ages would be present, and the solid curve adequately represents the data. The dips in p noted for S. zeamais may be of similar origin.

## Recognition of Noninfested Kernels

The recognition rate, q, of noninfested kernels might again depend on orientation. In a preliminary test (observation time 1.3) sec instead of 1 sec, all images from a single species, and no reconsideration of positives), results showed a marginal orientation effect only for R. dominica ( $q = 0.87\pm0.04$  and 0.95±0.03 for crease up and down, respectively). Hence, a pooled estimate of q was used, as with p. Results are shown in Table III, where the first column simply indicates the insect to which the kernel was exposed. All images recognized incorrectly as infested were reexamined following test completion and categorized as to source of the recognition error. The majority of false positives arose from egg holes that did not develop. Two misclassifications could be assigned to the presence of flour particles that would not be expected to be present in a vacuumed field sample. The remaining 10 false positives showed kernel damage that could be traced back to the images taken the first day. Of course, there was considerable additional kernel damage, but these 10 were confused with young insects, i.e., they appeared as larvae or eggs. In eight cases the damage appeared external, of the type caused by feeding insects. The remaining two images showed separation between germ and endosperm. The resulting germ region appeared so similar to a second instar Sitophilus larvae that it could not be clearly distinguished therefrom (Fig. 3D). Only the latter two false positive recognition cases were assigned to what might be expected in clean wheat. A similar inspection following the preliminary test found three such images in 940 noninfested kernels.

All the tested noninfested kernels had, in fact, been exposed to

egg-laying adults. Hence, one could not be sure that separation of the last 10 errors into insect-caused and inherent damage was valid. Furthermore, the sample size was too small to assure good statistics. Accordingly, X-ray radiograms of 500 g of hard winter wheat that was clean and had not been exposed to insects was inspected. Each of the approximately 15,000 kernel images was inspected separately using the magnification and enhancement described above. All images that would be classified as infested by a trained observer were identified, stored on disk, and categorized. Twelve images that could lead to misrecognition were found; eight involved the germ region but contained enough structure that made them difficult to distinguish from larvae (similar to the two noted above), two were of damaged kernels appearing similar to preadult moths (Sitotroga cerealella), and two showed holes similar to egg holes. Examples are given in Figure 3. These twelve amounted to a false positive rate of 0.08%. If this is the true false positive rate, a Poisson distribution test (e.g., Bulmer 1965) would predict two or more false positives in a sample of 955 kernels 18% of the time, three in 940 kernels 4% of the time. The results of the two small samples are therefore not inconsistent with a false positive rate of 0.08% obtained from virgin wheat. Kernel damage similar to that shown in Figure 3 has also been illustrated by Nicholson et al (1953b).

## CONCLUSIONS

The purpose of the present work was to establish whether inspection of X-ray radiograms of wheat could be developed into a method of quality control. Clearly, the technique presented here is too slow to be practical. To inspect 1,000 g of wheat one would need to prepare approximately 20 sheets of  $20 \times 25$  cm film. Inspection alone would require over 4 hr, not counting exhaustion; film exposure would require an additional 5 min per film. Here only 13 g of wheat was inspected per day. However, one can derive from these results hints as to where the method needs to be modified and, more important, what the inherent limits of the method are.

Film preparation time needs to be decreased by a factor of 10 or so. Use of higher voltage X-rays may possibly compensate for the expected loss of contrast that shorter exposure times cause. One cannot practically shorten the time taken to inspect an image. The field of view must be increased instead if one is to inspect more kernels in a given time. Because roughly 1,000 sec are available for field inspection of 1,000 g, at least 30 kernels must be contained within an image. This implies a resolution from one-fifth to onesixth of the current one, and one-eighth is probably more realistic, yielding a resolution of approximately 0.25 mm. At that resolution (4× magnification, or roughly what the naked eye can see) it will not be possible to recognize eggs or young larvae, and thus p is expected to drop to 0, at least until the third instar or so is seen. Nicholson et al (1953b) similarly noted that 1-2 sec/kernel was required to inspect for "minor" damage, although no quantitative results for such recognition were given.

Recognizing noninfested kernels, i.e., distinguishing whether a kernel is a true positive or not, does not suffer from this speed limitation. One expects very few possible positives; any suspects can always be inspected microscopically. Instead, the problem remains that of false positives. Several approaches might be used to obtain a level as low as 0.001%, which is required for a control level of 1 hidden insect/1,000 g. First, none of the false positives seen, either in exposed or clean wheat, resembled a third instar or older stage. If this remains true for larger samples and more varied wheat samples, restriction of the quality test to older hidden insects could resolve the problem. Second, one might improve the physical sharpness of the X-ray image, possibly along with optics used. Third, given enough false positive examples, one might be able to develop a pattern recognition approach to separate false and true positives better than is done here. Combining the last two approaches could push the date of earliest recognition back. Finally, even if one has an 0.08% false positive rate, one could combine X-ray with some older, uncorrelated, test with a false positive rate no higher than 1%, to reach the desired 0.001%. In

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summary, application of X-ray radiography to detect only third and later instar hidden insects appears to be feasible; detection of earlier instars seems problematic.

The method described here offers the possibility not only of establishing infestation levels, but also of measuring the age distribution of hidden insects. From the latter one can then predict this distribution and hence quality at a later date. This is particularly the case if one defines quality in terms of flour quality, as does the FDA. This forward projection will depend, in turn, on how early in the life cycle insects can be detected. This provides the motivation to push that date back as far as possible. Finally, it should be noted that no difference between live and dead insects was seen.

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