Ergosterol Content in Relation to Grain Kernel Weight

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ABSTRACT

The relation between fungal biomass content and grain kernel weight was investigated in five lots of grain: three wheat, one rye, and one triticale. Fungal biomass was quantified as ergosterol content. Samples were fractionated on the basis of grain kernel weight using an automatic sorter. In all lots, ergosterol concentrations increased as kernel weight decreased. In four of the five lots, the absolute amount of ergosterol per kernel also increased with decreasing kernel weight. Within-cultivar variation in ergosterol levels could not be explained solely by differences in surface-to-volume ratio. Ergosterol concentrations in the impurities (material not classified as whole, sound kernels) were generally several times higher than those in the original lots. Although concentrations of ergosterol were relatively high in small kernels and impurities, removal of these fractions did not significantly influence the average ergosterol levels of the lots, due to the low relative weight of these fractions. Separation during handling of the grain might, however, lead to extreme ergosterol concentrations in certain locations of the storage bins. These locations can serve as potential starting points for mold growth.

Fungi are the most important spoilage organisms in cereal grains. They can severely reduce the value of the grain, both in the field and in storage. Many studies have focused on the effect of storage conditions on mold growth, especially moisture (water activity), temperature, and the gaseous atmosphere (Lacey and Magan 1991). Plant pathologists have often investigated the effects of specific fungal diseases, such as *Fusarium culmorum*, on yield and average kernel weight (Snijders and Perkowski 1990). Yet, little is known about the relationship between grain kernel-size-distribution and the degree of fungal invasion and growth.

In wheat, kernel size varies within spikelets, between spikelets on the same head (Rawson and Evans 1970, Grieve et al 1992), and between heads in the field (Dahlstedt 1985, 1991; Lesch et al 1992). Differences in final grain weight have been attributed more to the rate of dry matter accumulation in the grain than to the duration of fill (Housley et al 1982). At maturity, kernels in the central spikelets generally are larger than the corresponding kernels in the basal and upper spikelets. Furthermore, the central spikelets often contain four or five kernels of different size, whereas only one or two kernels develop in the distal spikelets. Kernels in the upper florets of the central spikelets can, however, be as small as those in the basal florets of distal spikelets. Dahlstedt (1985, 1991) reported that the average kernel weight in heads increased as tiller order decreased, and that primary heads on plants with many tillers contained the largest kernels in the field.

Sieving is the most common way to divide a grain sample into classes of different size kernels. However, with the normal set of screens, sieving gives only a few classes of grain and the result varies, depending on the sorting procedure and the precision of the device. In this study, we used an apparatus that automatically sorts kernels by weight. The apparatus (Regnér 1993) can provide narrow weight classes of kernels suitable for quantitative and regression analyses.

In 63 winter wheat samples from 1989 field trials (seven cultivars, three locations, and three N levels), the standard deviation of the kernel weights ranged from 8.0 to 13.1 mg, with an average of 10.1 mg. The average kernel weight ranged from 32.5 to 47.0 mg, with an average of 41.6 mg (S. Regnér, *unpublished*). The samples were cleaned only slightly before analysis.

Ergosterol appears exclusively in fungal membranes (Weete 1980) and is commonly used as a marker of fungal growth in grains (Seitz et al 1977, Young et al 1984, Müller and Lehn 1988, Schnürer and Jonsson 1992). The highest ergosterol concentrations are found in the outer layers of the kernels. Young et al (1984) found 85-90% of the total ergosterol content in the bran fraction after milling soft winter wheat. Schnürer (1991) reported that, in winter wheat, the flour contained only 3% of the total ergosterol content, whereas the fine and coarse bran fractions accounted for 43 and 54%, respectively.

Even if kernel density were independent of kernel size, as in wheat (Millet and Pinthus 1984), and all kernels had the same ergosterol content per unit surface area, the ergosterol concentrations of the single kernels would still differ in accordance to their shape and size. Thus, small kernels should have higher ergosterol concentrations than do large kernels because their surface-to-volume ratios are higher.

The primary objective of this study was to ascertain whether the ergosterol content varies with grain kernel weight. This was indeed found to be the case. Thus, our second objective was to determine whether the variation could be explained by differences in kernel surface-to-volume ratio.

MATERIAL AND METHODS

Grain

Five lots (2 kg each) of different winter grain cultivars: wheat (Kosack, Pr 5559, Sleipner), rye (Danko), and triticale (Lad 285) were supplied by the Swedish Farmers Supply and Marketing Association. The grain was harvested in 1990 field-trial plots in Vreta Kloster (58°29'N, 15°30'E). Nitrogen was applied to the plots at a rate of 150 kg/ha. Fungicides were not used. Plants showed low levels of pathogen infection at heading; the two upper leaves were free of symptoms, and only Pr 5559 and Danko had more than 10% of the leaf area on the third upper leaf covered with fungal spots (*Septoria* spp and *Drechslera* spp). Low infection levels and no cultivar-related differences in the level of rust (*Puccinia recondita* f.sp *tritici* and *Puccinia striiformis*) were observed. Pr 5559 wheat and Lad 285 triticale, and to some extent, Danko rye, were attacked by powdery mildew (*Erysiphe graminis*) at heading. Pr 5559 normally is more severely attacked by powdery mildew than are the others; unfortunately, however, no observations were made at later stages of maturity. No *Fusarium* head blight was observed in the field for any of the cultivars. After harvest, the grain was dried in slightly heated air to a moisture content of approximately 14% and stored at about 10°C before further treatment.

Two samples (20 g) of each uncleaned original lot were taken for ergosterol determinations. Samples (500 g) of the different cultivars were manually cleaned; parts of straw and heads were removed. The remainder was sifted on a fine sieve to remove most of the dust and small pieces of kernels in the samples. The removed particles were combined into a single sample and stored. Kernels were not sorted until their moisture content had equilibrated with the air in the laboratory. After the samples were sorted, damaged (broken and insect-eaten) kernels were collected and added to the particles removed earlier. These are collectively referred to as *impurities*.

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The moisture content of the sorted kernels was determined by drying overnight at 105°C.

Sorting of Kernels

The grain samples were automatically sorted by weight. In the apparatus used, every kernel is weighed individually on a scale and transported by pressurized air to a desired container (Regnér 1993). Kernel weight data are stored in the computer controlling the system.

The weight ranges of the sorted grain classes were between 1 and 2.5 mg, except for those with the lowest kernel weights. These classes ranged from 0 mg to a maximum weight between 20.5 and 27.5 mg, depending on the cultivar. Danko rye contained so many kernels in this interval that it was divided into two subintervals.

Ergosterol Determination

Ergosterol was extracted using a modified version of Seitz et al (1977), as described by Schnürer (1991). A high-pressure liquid chromatography system (Waters Associates, Milford, MA) with a Novapak C18 column and methanol (2 ml/min) as the mobile phase, was used for the determination. Ergosterol was detected at 280 nm about 8 min after injection. The levels were converted to dry weight values.

Single determinations were made on each of the sorted fractions and each of the samples representing the original lots.

The amount of grain analyzed varied between fractions. Determinations normally are made on 20-g samples. However, to reduce the time needed for sorting, without having to widen the weight ranges of the classes too much, fraction weights as low as 5 g were accepted. Nevertheless, most of the analyzed samples contained 20 g of grain, and only the classes far out in the tails of the distributions contained the minimum of 5 g.

Model, Regression Analysis, and Calculations

A physically based model for the ergosterol concentration as a function of kernel weight was developed. It is based on the assumptions that all kernels have the same density, and that the ergosterol content is proportional to the kernel surface area (constant degree of infection per unit surface area):

$$A' = c_1 \times e^{-(1/c_2) \times \ln(m)} \quad (1)$$

where: A' = ergosterol concentration (ppm, dwb); m = mass of the kernel (mg); c_1 and c_2 = arbitrary coefficients.

The value of the coefficient c_2 is determined by the shape of the studied particles. For different-size, three-dimensional particles of the same shape, such as spheres or cubes, the value of c_2 is 3. Corresponding c_2 values for infinitely long cylinders and infinite plates are 2 and 1, respectively. Grain kernel shape is something between a sphere and a long cylinder; therefore, they should have a c_2 value between 2 and 3. Toftdahl Olesen (1987) suggested that the c_2 value should vary from 2.6 to 2.8 for kernels of common grain species.

Two spheres with a weight ratio of 1:2 have a surface ratio of 1:1.59; thus, the surface-to-weight ratio is 1:0.79 (1.26). Calculations based on the assumptions above show that the ergosterol concentration should be 26% higher in the small spherical kernel than it is in the larger one. Corresponding percentages for cylinders, plates, and grain kernels (c_2 = 2.7) are 41, 100, and 29% respectively. Note that the surface areas of the two plates are the same, and that at c_2 values less than unity even the absolute ergosterol content is higher in small kernels than it is in large kernels.

By fitting the coefficients in Equation 1 to the experimental data, the obtained c_2 values indicate whether the variation in ergosterol concentrations within the cultivars is due only to the surface-to-weight ratio.

The regression analysis was made with PROC GLM (SAS 1985). The median masses of the weight classes were used for the regression analysis. The median mass is the kernel weight dividing a sample into two parts with equivalent weights.

The ergosterol concentrations in the cleaned lots and within certain weight ranges were calculated based on the kernel-weight data and the results of the regression analysis. A modified version of Equation 1 with a constant (c_3) added to the right-hand side was used in these calculations to obtain a slightly better fit to the data.

RESULTS

For all varieties and kernel sizes, the sorted kernels appeared sound, well developed, and of good color.

The kernel-weight distributions varied considerably among the cultivars. The average kernel weight in the lots ranged from 35 to 49 mg, while the standard deviations of the kernel weights ranged from 10.4 to 14.1 mg (Fig. 1).

For all cultivars, the highest ergosterol concentration was found in the smallest kernels, and the concentration gradually decreased with increasing kernel weight (Fig. 1). There were, however, differences in the pattern of the decrease between cultivars.

The Equation 1 coefficients giving the best fit to the data are presented in Table I. For four of the five lots, the best-fit value of c_2 was less than unity, whereas it was 2.76 for Danko rye. The standard deviation of the residuals ranged from 0.2 ppm for Kosack wheat to 1.4 ppm for LAD 285 triticale. When the constant c_3 was added to the model, the corresponding values decreased to 0.1 and 0.9 ppm, respectively.

Mean measured ergosterol concentrations in the lots ranged from 1.8 ppm in Sleipner wheat to 4.9 ppm in Danko rye (Table II). For three of the five cultivars, the concentrations calculated from kernel-weight distributions, fitted equations, contents of

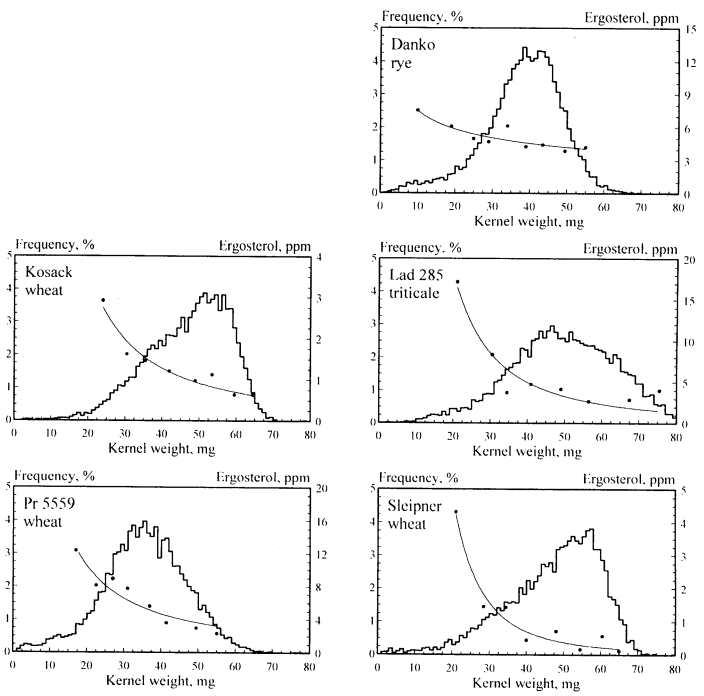

Fig. 1. Kernel-weight frequency distributions (thick line) and relationship between measured ergosterol concentration (●) and kernel weight together with the fitted curve (thin line). Note different scaling of axes for ergosterol concentration.

TABLE I
Values of the Coefficients (c_1 and c_2) Allowing Best Fit to Data

Cultivar	c_1	c_2
Danko	180	2.76
Kosack	340	0.67
Lad 285	4,900	0.54
Pr 5559	340	0.86
Sleipner	21,000	0.39

impurities, and ergosterol concentrations in the impurities were similar to those of the measured concentrations. For the Kosack and Pr 5559 wheat, the measured and calculated concentrations differed by about 1.5 ppm.

Amounts of impurities and ergosterol concentrations in the lots are presented in Table II. Ergosterol concentrations in the impurities were generally several times higher than those in the uncleaned lots.

DISCUSSION

Average ergosterol concentrations in the studied lots were similar to those of other European studies (Müller and Lehn 1988, Schnürer and Jonsson 1992).

The kernel-weight distributions for Kosack and Sleipner, which had the lowest average ergosterol concentrations, showed a higher degree of negative skewness than did those for the other cultivars. However, earlier we found that the standard deviation and skewness of the kernel-weight distribution were not affected by the use of fungicide, even when the average kernel weight differed by 10% (S. Regnér, *unpublished*). This suggests that the degree of fungal infection has little influence on the skewness of the distribution. The effect of fungal infections on the kernel properties might, however, depend on when the infection occurs and on the type of invading fungus.

In the four cultivars with c_2 coefficients less than unity, not only the ergosterol concentrations, but also the absolute ergosterol contents, were higher in small kernels than they were in large ones. Thus the model used, which is based on the assumption that there is a constant degree of infection per unit surface area, describes a kernel shape whose surface area increases with decreased kernel weight. Obviously this is not true. Instead, the result indicates that the degree of infection per unit surface area depends on kernel size, at least for these four cultivars.

Fungal diseases can affect the development of heads and kernels in different ways and to varying degrees. The relatively high ergosterol contents in the small kernels could be explained by lower kernel filling rates of the most severely infected kernels. However, the low degree of fungal infection in the field and the uniform kernel appearance suggest that there were no large differences in infection levels between kernels. Thus, we do not believe that the low c_2 values should be attributed to different kernel filling rates caused by fungal infections. Instead, the relationship could be a size-related difference in the average climatic conditions to which kernels were exposed. Another possible explanation for the relationship is that kernels of different size differ in their stages of their development—and thus, probably, in their susceptibility—when climatic conditions are favorable for fungal invasion and growth.

For Danko rye, the fitted value of the c_2 coefficient certainly is within the range of values predicted, based on the surface-to-volume approach. This indicates a constant degree of infection per unit surface area on the rye kernels. One possible explanation for this size independence could be that the average climatic conditions to which different-size kernels were exposed were more uniform for rye than they were for wheat and triticale. Only one lot each of rye and triticale were analyzed, so we cannot be sure that the differences between the rye and the others are species-specific. However, the configuration of the kernels in rye heads differs from that in wheat and triticale heads. Wheat and triticale spikelets often contain several kernels of different sizes, whereas the rye spikelet normally holds only two kernels. The largest kernels in the wheat and triticale spikelets are situated at both ends of the spikelets and might be better ventilated than the smaller kernel pressed between them. Another difference between the species is that at the end of the growing season, the wheat and triticale kernels are normally well hidden in the spikelets, whereas the rye kernels are more exposed.

The difference in c_2 coefficient might also be due to variation in the degree of tillering between stands. Average kernel weight normally decreases with increased tiller order; the higher the order of the tiller, the deeper in the stand the head will be situated (Dahlstedt 1985). Large kernels in the primary tillers are, therefore, better ventilated than the smaller kernels in tillers of higher order.

Seitz et al (1986) evaluated different cleaning methods with regard to their effectiveness in decreasing the content of deoxynivalenol (DON), a metabolite of *Fusarium graminearum*, in samples of hard red winter wheat. The degree of DON removal varied considerably among the studied lots. This, like other results in their study, show that the distribution of DON within the grain varied between lots. The ratio between the concentration of DON in the removed material and that in the uncleaned grain ranged from 3 to 18 for the different lots and methods, which is similar to the corresponding range of the ergosterol ratios in the present study (Table II). However, the relative decreases in DON concentrations obtained by cleaning were generally larger than the corresponding decreases in ergosterol concentrations obtained when the impurities were removed.

Bechtel et al (1985) assigned winter wheat kernels infected with *Fusarium graminearum* to one of three categories, based on appearance: 1) normal kernels appearing sound and of good color and weight; 2) lightly infected kernels of normal size but of light weight and color; and 3) heavily infected kernels that were shriveled and light colored. The 1,000-kernel weights in these categories were 29.9, 25.6, and 13.1 g, respectively. The relationship between the concentrations of ergosterol (2.8, 29.0, and 103.0 ppm) and those of DON (0.43, 22.7, and 68.7 ppm) in the different categories support our conclusion that the variation in ergosterol concentration is not solely a function of surface area.

Although ergosterol concentrations were relatively high in the small kernels and in the impurities, the relative weight of these fractions was too low to significantly influence the average ergosterol concentration in the lots. This indicates that aspiration of grain will not markedly influence the ergosterol content of the grain unless its content of impurities and small and broken kernels is extremely high.

The ergosterol concentration found in the impurities was high, and because this kind of material often separates during transport and bin loading, the ergosterol concentration (fungal biomass) in grain parts can become very high. Grain deterioration and mold growth is probably more easily started in grain portions high in impurities and infected grain than they are in grain of better quality. Thus, there is good reason to clean the grain before storage. The differences between measured and calculated ergosterol concentrations in the lots might have been due to separation, even though a sample divider was used for the sampling. The average difference between the measured duplicates was 1.6 ppm. For Pr 5559 and Kosack wheats, which showed the largest difference between measured and calculated concentrations, the corresponding differences were 1.9 and 3.2 ppm, respectively.

More investigation is needed to validate the relationship between fungal infection and kernel weight that was found in this study. Preferably, such studies should be made on grain samples with a wide range of ergosterol contents, and they should include determinations on kernels from different kinds of heads and different parts of the heads. Furthermore, the effects of specific fungal diseases should be clarified.

TABLE II
Ergosterol Concentrations in Original Lots and Impurities and Relative Amounts of Impurities in Lots

	Ergosterol Concentration, ppm				
	Calculated[a]		Measured[b]		Amount of
Cultivar	Cleaned	Uncleaned	Uncleaned[c]	Impurities[d]	Impurities, %
Danko	4.6	4.7	4.9	12.8	0.9
Kosack	1.1	1.4	3.2	63.6	0.5
Lad 285	4.9	5.0	4.8	16.2	0.5
Pr 5559	5.3	5.7	4.2	20.6	2.5
Sleipner	2.0	2.1	1.8	23.9	0.9

[a] Based on fitted equation and sample data.
[b] Modified version of the method of Seitz et al 1977 (Schnürer 1991).
[c] $n = 2$.
[d] Material not classified as whole, sound kernels.

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