# CHARACTERIZATION OF GRAIN MOLD RESISTANCE IN SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH]

A Dissertation

by

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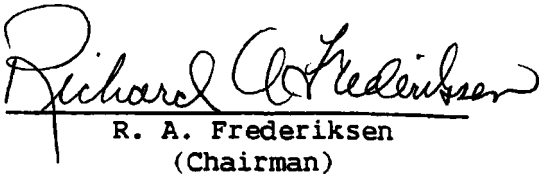

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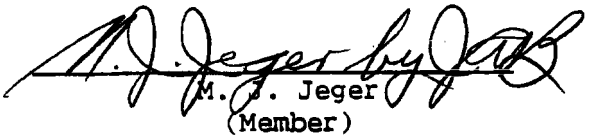

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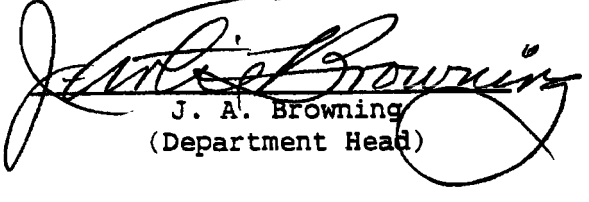

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## ABSTRACT

Characterization of Grain Mold Resistance in Sorghum [*Sorghum bicolor* (L.) Moench]. (December 1986)

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Chairman of Advisory Committee: Dr. R. A. Frederiksen

Sorghum grain lots with various levels of resistance to sorghum grain mold (SGM) were evaluated for several loss parameters: kernel weight, kernel density, electrolyte leachate level, percentage germination, vigor, and visual quality appraisal; and two indirect measures of disease intensity, ergosterol level and colony-forming units (CFU) of *Fusarium moniliforme*. Ergosterol level was significantly correlated with CFU (r= +0.94), visual rating (r= +0.92), leachate level (r= +0.90), and percentage germination (r= -0.86). Leachate level was highly correlated with germination (r= -0.98). In all cases, correlation coefficients for the standard deviation of kernel weight were higher than for the mean. Fitting variables to a theoretical linear model resulted in a highly significant cultivar x treatment (treatment = inoculated vs. noninoculated) interaction for kernel weight.

Two greenhouse inoculation procedures, vacuum infiltration and panicle spraying differented among resistant and susceptible cultivars and caused symptoms similar to those resulting from

natural infection or inoculation in the field. On susceptible cultivar TX2536, reduction in kernel weight due to inoculation by the vacuum and panicle spray methods were 31 and 41%, respectively, and reductions in percentage germination were 93 and 94%, respectively. On resistant cultivar SC0170-6, kernel weight reductions for vacuum and spray inoculations wer 10 and 22%, respectively

Paraffin- and resin-embedded spikelets of a susceptible and resistant cultivar were inoculated with *Fusarium moniliforme* and examined for differences in the invasive processes that may be related to resistance. Glumes, lemma, palea, and stamens were intra- and intercellularly colonized in TX2536, but not in SC0170-6. Necrosis and extracellular epidermal deposition characterized host response to the pathogen in SC0170-6. Necrosis appeared subsequent to colonization in TX2536.

SGM had a greater effect on phenolic compounds than on total phenolic acids (PA). Disease caused some PA (e.g., p-coumaric and gallic) to increase more in resistant cultivars than in suscepts. One unidentified compound increased as a result of disease in spikelets of suscept TX2536 and decreased in tissues of resistant cultivar SC0103-12.

ACKNOWLEDGMENT

I would especially like to thank Dr. R. A. Frederiksen, my committee chairman and project leader, for making this research opportunity possible, and for providing suggestions and resources whenever needed.

I also wish to thank my committee members, Drs. Craig, Jeger, and Waniska, for their constructive and encouraging comments throughout.

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# CHAPTER I

## INTRODUCTION

Grain sorghum is a major food source in many parts of the developing world, though in these areas yields are generally very low. The average yield in India in 1980 was 645 kg/ha. In contrast, yields are much higher in developed countries (3947 kg/ha in the US in 1980) where sorghum is grown for animal feed.

In an effort to combat low yields in developing countries, traditional sorghum cultivars are currently being bred to be photo-insensitive. As a result, flowering and grain fill can be made to coincide with the high soil moisture availability of the rainy season. Grain production during the rainy season can greatly increase yields, but also creates favorable conditions for parasitic and saprophytic colonization of the kernel by fungi.

Coupled with the change in flowering period, there has been an increase of international concern for grain quality. Just two decades ago, Tarr (45) considered grain mold to be of minor importance. Today, this disease constitutes one of the major problems facing sorghum workers around the world (6,13,52).

### What is Grain Mold?

Among researchers, the names of diseases are usually sufficient

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The journal used as a style pattern was *Phytopathology*.

to communicate a relatively precise idea of the organisms involved and the symptoms they cause. Unfortunately, the term "grain mold(s)" of sorghum has been applied in both narrow and broad sense to many different types of grain degradation.

In spite of global disaccord, a consensus regarding a functional definition of sorghum grain mold (SGM) has developed among some workers in the US (7), India (52), and Senegal (13). They agree that grain mold is the result of an infection of the sorghum spikelet at or soon after anthesis by parasitic fungi. These conclusions developed from isolations and inoculations (7,52), and histopathological observations (7).

The fungal pathogens most commonly associated with grain mold are: *Curvularia* spp. and *Fusarium* spp., with *Fusarium moniliforme* Sheld. being the most common single species. Grain mold pathogens also have saprophytic capabilities and are ubiquitous in nature, especially *Fusarium* spp. It can be safely assumed that some or all of these pathogens occur wherever sorghum is grown, though the epidemiological importance of inoculum density has never been reported.

Late season weathering can also diminish the quality of sorghum grain and complicate the study of grain mold. Weathering is a result of biological and physical degradation of mature grain (6,19,20), involving many different genera of fungi acting saprophytically. These fungi are easily isolated from surface sterilized grain along with grain molding pathogens. Since most grain mold assessment methods occur after grain maturity, the effects of weathering and

grain mold can be confounded. The division between grain mold and weathering is theoretically clear, but often becomes blurred in the field.

Another impediment to the development of a precise description of grain mold is the uncertainty about the period when most infections occur. Castor (7) showed that severe damage, in terms of yield and quality, can occur when heads are inoculated at flowering with a suspension of spores of either *Fusarium moniliforme* or *Curvularia lunata*. Histologically, he found colonization of floral parts by both fungi as early as 5 days after inoculation at anthesis. In addition to Castor's demonstration of the relationship between inoculation at anthesis and severe grain mold symptoms, little can be said about the type of damage which occurs if infection by one of these fungi takes place after flowering, when the kernel is maturing.

## Consequences of Grain Mold

Grain mold can cause losses in sorghum in several ways. Severe yield losses caused by incompletely filled grain have been associated with mold infection (22,28). Comparisons of yields and mold infection for several sites have also indicated that mold can be an important factor in yield reduction (13). Castor (7) reported reduced yield, test weight and 1000 kernel weight in sorghums due to molds caused by *Fusarium* spp. Castor and Frederiksen (8) noted substantial losses from grain mold on the Texas High Plans in 1974.

In addition to yield losses, sorghum grain can be affected by reductions in food or processing quality, as well as market value.

In India, moldy grain sold for about 20% less than clean grain (50,52). Glueck and Rooney (20) reported that highly molded grain are altered both physically and chemically. Other studies suggest that digestability may be enhanced by molding (2). *Fusarium* spp. are also known to produce potent toxins under laboratory conditions (14). The presence of toxins in moldy grain is a major concern of sorghum researchers (52).

Mold may also greatly reduce germination potential (7,17). Germinability is one of the primary interests of hybrid grain producers. The location of seed production centers in dry areas is principally a mechanism designed to avoid problems of low seed quality.

## Control of Grain Mold

Some researchers have controlled grain mold in the field with applications of benomyl (28). This approach might provide a good means of control in high-income systems, such as hybrid seed production, but will be economically impractical for other commercial and subsistance purposes. Most researchers agree that the only viable means of control (other than avoidance or escape) is host resistance (6,13,52).

Host plant resistance to grain mold has been identified by several workers (7,13,34). At ICRISAT (International Center for Research in the Semi Arid Tropics), major efforts are under way to breed grain mold resistant cultivars (34). Several characteristics have been associated with resistance, such as open heads, high tannin

content in the testa, vitreous endosperm, and enclosing glumes. These characteristics may be undesirable for many consumer and production requirements. Williams and Rao (51) have shown that head and grain characteristics are not always associated with mold resistance. Some non tannin lines may be as resistant as high tannin cultivars. Glueck and Rooney (20) determined that water uptake and retention were important factors associated with resistance to weathering. The longer the moisture content was at 18-20% or more, the greater the incidence and severity of weathering. Castor (7) compared susceptible and moderately resistant non tannin cultivars histologically. Sections at 5 and 11 days showed that decreased fungal colonization in the moderately resistant line may have been associated with pigmentation in the lodicule. He theorized that resistance may be manifested in floral tissues near the basal end of the kernel.

Of the many grain mold-related problems which remain unsolved, one which begs greater attention and lends itself to practical experimentation is the further characterization of resistance. A greater understanding of resistance mechanisms should facilitate efforts to breed cultivars which have both resistance to grain mold and high food and feed quality. The following research was designed to address these questions.

# CHAPTER II

## ASSESSMENT OF GRAIN MOLD

Resistance to SGM is routinely assessed in the field on mature plants by visual estimation of severity based on a qualitative scale (13,34). In addition to problems associated with visual estimation, evaluation of SGM is further complicated by the masking effects of postmaturity weathering. SGM has been quantitatively assessed under research conditions using two types of measure; 1) kernel loss parameters, and 2) indirect measures of disease intensity. Examples of kernel loss parameters are kernel weight (6,35,43), kernel hardness or density (43), leachate level (27), and percentage germination (6,17).

Disease intensity was defined by Chester (10) as "the amount of disease present on a plant, in a field, or in a geographic region, without reference to the damage caused". Since the actual measurement of the amount of disease on individual kernels or in lots is difficult, we propose the use of an indirect measure such as fungal colonization of host tissue. Quantification of the pathogen in the host tissue satisfies the definition given above by making no "reference to damage caused". Tissue colonization has been measured indirectly for SGM by the quantification of fungal metabolytes, principally ergosterol, in seed samples (41). Fungal colonization of root, stem and kernel tissue has been quantified directly with serial dilutions of ground tissue on selective media (31).

The objective of this study was to evaluate the relationship between kernel loss parameters and disease intensity, and to determine the effects of grain mold infection on several cultivars differing in grain characteristics.

## Materials & Methods

### Seed lots

Twelve grain lots comprising six cultivars and two treatments (inoculated vs. noninoculated) were evaluated. Cultivars were selected to provide a range in SGM susceptibility and grain types (Table 1), and consisted of: SC0630-11, SC0103-12, SC0599-6, TX412, CS3541, and TX2536. Leaf wetness was maintained throughout kernel development on inoculated plants for a minumum of 12 hr/day (8PM-8AM) using misters (Burgess vibrocrafters, Graysville, IL) with 4.14 $kg/cm^2$ of pressure. Plants were inoculated with a conidial suspension ($10^6$ spores/ml) of a *F. moniliforme* isolate derived from naturally infected grain. Inoculum was applied until runoff on three consecutive days during flowering with a hand-held spray bottle (model S-67, Delta Indust., Philadelphia, PA). Fifteen heads were harvested from each cultivar 50 days after anthesis. Samples were threshed and dried in a greenhouse for 3 wks before evaluation.

Loss parameters

Mean values were obtained on four 100-kernel samples for the following parameters: kernel weight, kernel density, electrolyte leachate level, and percentage germination. Individual kernel values were obtained on four 100-kernel samples for weight and leachate level. Volumes of 100-kernel samples used in density calculations were measured with an air compression pycnometer (model 930, Beckman Inst., Inc., P.O. Box C-19600, Irvine, CA 92713). Electrolyte leachate levels were measured on an automatic seed analyzer (ASAC-1000 Neogen Food Tech. Corp., Okemos, Mich. 48864) after 5 hr of imbibation. Two 300-kernel samples of each cultivar was assessed visually for the percentage of damaged kernels (i.e, small or discolored).

After removal from the analyzer, kernels were placed between moist paper towels, rolled, and incubated at 25 C for 72 hr. Percentage germination was recorded on all four samples for each cultivar. Radicle and shoot length were recorded for individual seedlings of the first 100-kernel sample.

Disease intensity parameters

Two techniques were employed to quantify colonization of kernel tissue by fungi in general and by *F. moniliforme*, specifically. General fungal colonization was determined by measuring ergosterol levels in 25 g samples according to previously published methods (40). *F. moniliforme* colonization was quantified by using Nash Snyder Agar (NSA) (31), a genus-specific, selective medium. Four

Table 1. Kernel characteristics of sorghum cultivars used to assess resistance to grain mold.

| Cultivar | Mesocarp thickness | Pigmented testa | Endosperm texture | Appearance | SGM Resistance[1] |
|---|---|---|---|---|---|
| SC0103-12 | thick | P | FL | reddish | R |
| SC0599 | intermediate | A | I | white | R |
| SC0630 | thin | A | I | red | R |
| CS3541 | thin | A | VT | white | MR |
| TX2536 | thin pearly | A | I | yellow | S |
| TX412 | thin | A | I | yellow | S |

[1] Based on several years of field observation at numerous national and international locations (unpublished data). R = resistant; MR = moderately resistance; S = susceptible.

100-kernel samples were ground for 5 s in a UDY mill fitted with a 40-mesh screen. The ground tissue was dispersed in 0.01% water agar at a density of 0.01 gm/ml. After 15 min on a rotary shaker, 0.5 ml of suspension and serial dilutions (1:10, 1:100, and 1:1000, v:v) were added to petri dishes with molten NSA. Colony-forming units (CFU) were counted after 7 days of incubation at 25 C.

## Results

The two disease intensity variables, CFU on NSA and ergosterol level were highly correlated (r= +0.94) (Table 2).

Correlations between loss variables and either intensity variable were similar, though generally slightly higher for ergosterol. The variable most highly correlated with ergosterol was leachate level (r= -0.90). Other variables significantly correlated with ergosterol were percentage germination (r= -0.85), leachate level standard deviation (r= +0.65), and mean shoot length (r= -0.64). There was no significant correlation between ergosterol level and either mean kernel weight (r= -0.28) or the standard deviation (r= +0.42). Kernel weight was not significantly correlated with any of the variables measured, but the standard deviation of kernel weight was significantly correlated with mean shoot length (r= -0.61). In all cases, correlation coefficients for the kernel weight standard deviation were higher than for the mean. Among quality variables, the highest correlation occurred between percentage germination and leachate level (r= -0.98). Leachate level and percentage germination were also highly correlated with shoot length

Table 2. Correlations among loss and disease intensity parameters[1] measured on 12 sorghum grain lots comprising both inoculated and noninoculated samples of six cultivars differing in resistance to grain mold

| | Leachate | $S_L$ | Germination | Kernel Wt. | $S_{sw}$ | Shoot $L_n$ | $S_{SI}$ | Ergosterol | BioAssay | Visual | Density |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Leachate | 1,000 | 0.93**** | -0.98**** | -0.13 | 0.49 | -0.75*** | -0.33 | 0.90**** | 0.86*** | 0.94**** | -0.08 |
| $S_L$ | | 1.00 | -0.88**** | -0.13 | 0.56 | - .076*** | -0.20 | 0.65* | 0.61* | 0.77*** | -0.27 |
| Germination | | | 1.00 | 0.09 | -0.47 | 0.72** | 0.22 | -0.85*** | -0.76** | -0.89*** | 0.16 |
| Kernel Wt. | | | | 1.00 | 0.03 | -0.19 | 0.07 | -0.28 | -0.22 | -0.21 | 0.03 |
| $S_{sw}$ | | | | | 1.00 | -0.61* | 0.13 | 0.42 | 0.36 | 0.38 | -0.33 |
| Shoot $L_n$ | | | | | | 1.00 | 0.46 | -0.64* | -0.63* | -0.67* | -0.14 |
| $S_{SI}$ | | | | | | | 1.00 | -0.46 | -0.51 | -0.46 | -0.25 |
| Ergosterol | | | | | | | | 1.00 | 0.94**** | 0.92**** | 0.01 |
| BioAssay | | | | | | | | | 1.00 | 0.85*** | 0.22 |
| Visual | | | | | | | | | | 1.00 | -0.03 |
| Density | | | | | | | | | | | 1.00 |

[1] Leachate = electrolyte leachate level mean; $S_L$ = electrolyte leachate level standard deviation; Germination = mean percentage germination of four 100-kernel samples; Kernel wt. = mean kernel weight of four 100-kernel samples; Shoot $L_n$ = mean individual seedling shoot length for one 100-kernel sample; standard deviation of shoot length among individual seedlings; Ergosterol levels of 1 sample (20-30 gm); Bioassay = CFV of four 100-kernel samples; Visual estimation of one sample containing approximately 500 kernels; and Density = mean density of four 100-kernel samples.

(r= -0.75 and +0.75, respectively), and with visual estimation (r= +0.94 and -0.84, respectively).

A linear model was used to explore the resistance discrimination power of several variables. The model was $M = a + b_1x + b_2y + b_3xy$ where M is the measurement variable, x is cultivar effect, and y is the effect of inoculation; a is the intercept, and $b_1$, $b_2$, and $b_3$ are multiple regression coefficients. Of the various model components, the one of interest in this study was the cultivar x inoculation interaction. As noted earlier, prior experience supported the existence of this interaction, because some cultivars were known to be resistant to SGM, relative to the susceptible check TX2536. Therefore, the mean square of this interaction should be high for variables which can be fitted to a theoretical resistance model and which are not characterized by excessive unexplained variation. F values representing the inoculation x cultivar variance to the model variance were examined.

Kernel weight had the highest F value (70.94), and an R-square value for the complete model of 0.96 (Table 3).
The R-square is a measure of the proportion of variation in the data which is explained by the model. Possible sources of unexplained variability are measurement error and sampling error. Percentage germination had the second highest F value (21.00) and an R-square of 0.96. Leachate level had an F value of 14.33 (R-square = 0.94), and CFU had an F value of 12.36 (R-square = 0.82). The only variable which could not be fitted to the model with

Table 3. F and R-square values for several loss and disease intensity parameters measured on 12 sorghum grain lots comprising both inoculated and noninoculated samples of six cultivars differing in resistance to grain mold

| Parameters | F value for interaction Cultivar x Inoculation | R-square |
|---|---|---|
| Density | 0.83 | 0.31 |
| Leachate level | 14.33**** | 0.94 |
| Leachate standard deviation | 5.48*** | 0.92 |
| Percentage germination | 21.00**** | 0.96 |
| Kernel weight | 70.94**** | 0.96 |
| Kernel weight standard deviation | 3.90** | 0.89 |
| Bioassay | 12.36**** | 0.82 |

**, *** = 0.01 and 0.001 level of significance.

statistical significance was kernel density (F = 0.83, R-square = 0.31).

There was little difference in kernel density between inoculated and noninoculated lots, except for cultivar TX412, where inoculation resulted in a noticeable reduction (Fig. 1). All other loss variables appeared to be greatly affected by inoculation. Cultivars SC0630-11, SC0103-12, and SC0599-6 were similar in response to inoculation, and in general performed much better than the other three cultivars. Exceptions were the resistant-like reaction of CS3541 for mean kernel weight and the susceptible-like reactions of SC0630-11 and SC0103-12 for the kernel weight standard deviation.

For all cultivars, inoculation caused a reduction in mean kernel weight from 26.59 gm/1000 to 22.33, and an increase in the standard deviation from 7.18 gm/1000 to 7.25 (Fig. 2).

The effects of inoculation on weight frequency distributions were more noticeable at the cultivar level (Fig. 2). For resistant cultivar SC630-11 there was an increase in the kernel weight standard deviation (5.91, noninoculated, 6.15, inoculated), and a decrease in the mean (27.67, noninoculated, 23.87, inoculated). For susceptible cultivar TX2536, the effect of inoculation was greater. The standard deviation values for inoculated and noninoculated were 32.21 and 21.29, respectively. Mean values for inoculated and noninoculated were 7.19 and 5.36, respectively.

Weight distributions for noninoculated lots were skewed toward higher weights, reflecting the large proportion of normal,

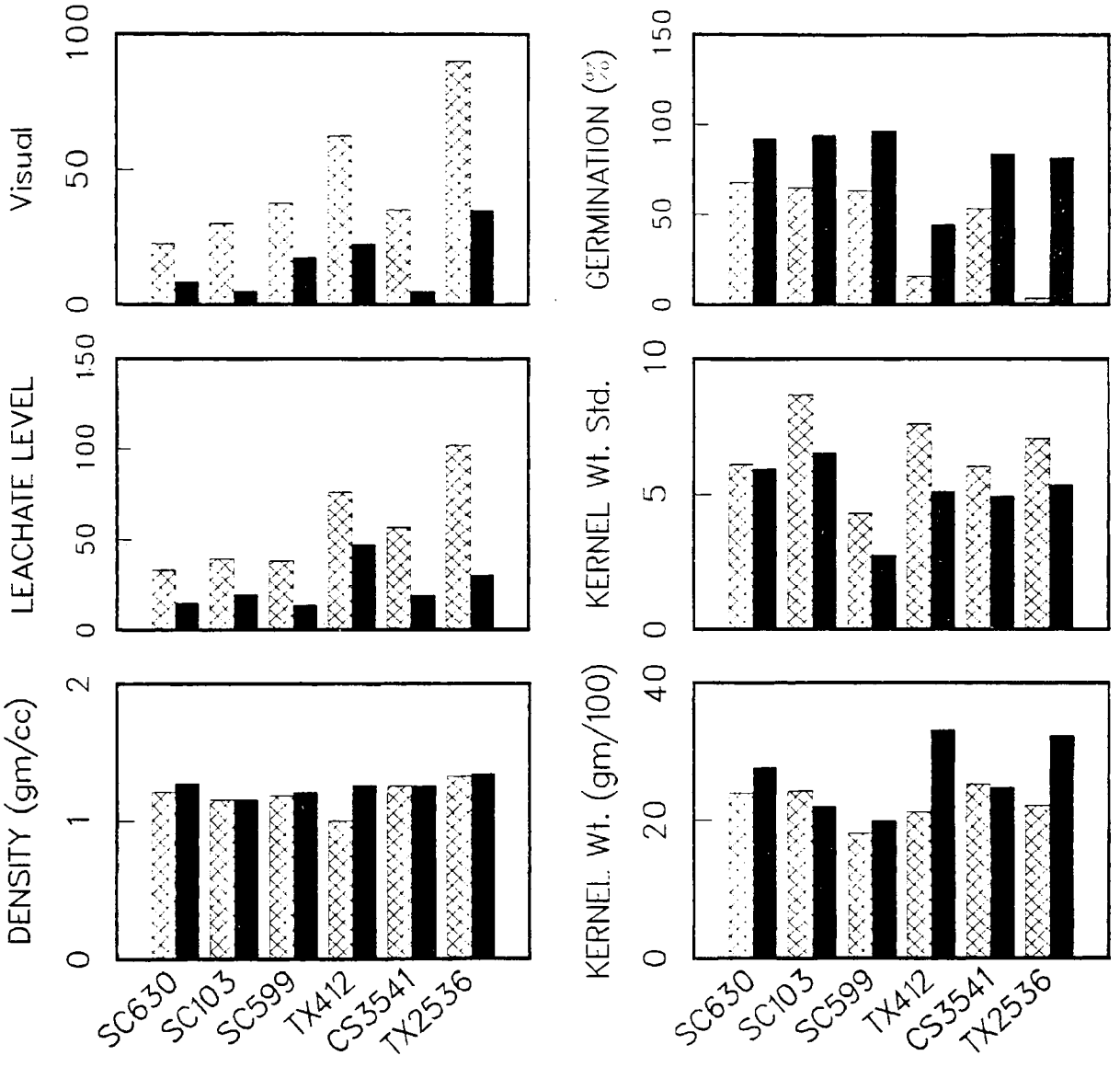


Figure 1. Comparison of six sorghum cultivars (inoculated and noninoculated) for several kernel quality variables. Hatched = inoculated, solid = noninoculated.

well-developed kernels. Weight distributions of the inoculated lots were less noticeably skewed, reflecting heterogeneity in disease intensity among kernels.

## Discussion

As noted, CFU of *F. moniliforme* and ergosterol levels were highly correlated (r= +0.94). Much of the correlation, however, reflected the effects of inoculation in general. When comparing the two variables, grain lots tended to clump into inoculated and noninoculated groups (Fig. 3).

If only values for inoculated lots are considered, there is a positive, significant correlation (r= +0.93). The pattern of noninoculated cultivars, however, suggests a very different relationship. The correlation is negative (r= -0.73), though only significant at the 10% probability level. The reasons for this trend are not clear but may be related to a competitive interaction between *F. moniliforme* and other species which produce less ergosterol.

The lack of significant correlation between the two intensity variables for noninoculated lots does not preclude the use of either assay under natural conditions. Noninoculated lots in our experiment were intended as controls so that assessment measures could be compared for lots of high and low disease intensity. Further studies should be instigated to determine the relationship between *F. moniliforme* colonization and ergosterol level where natural disease intensity is high.

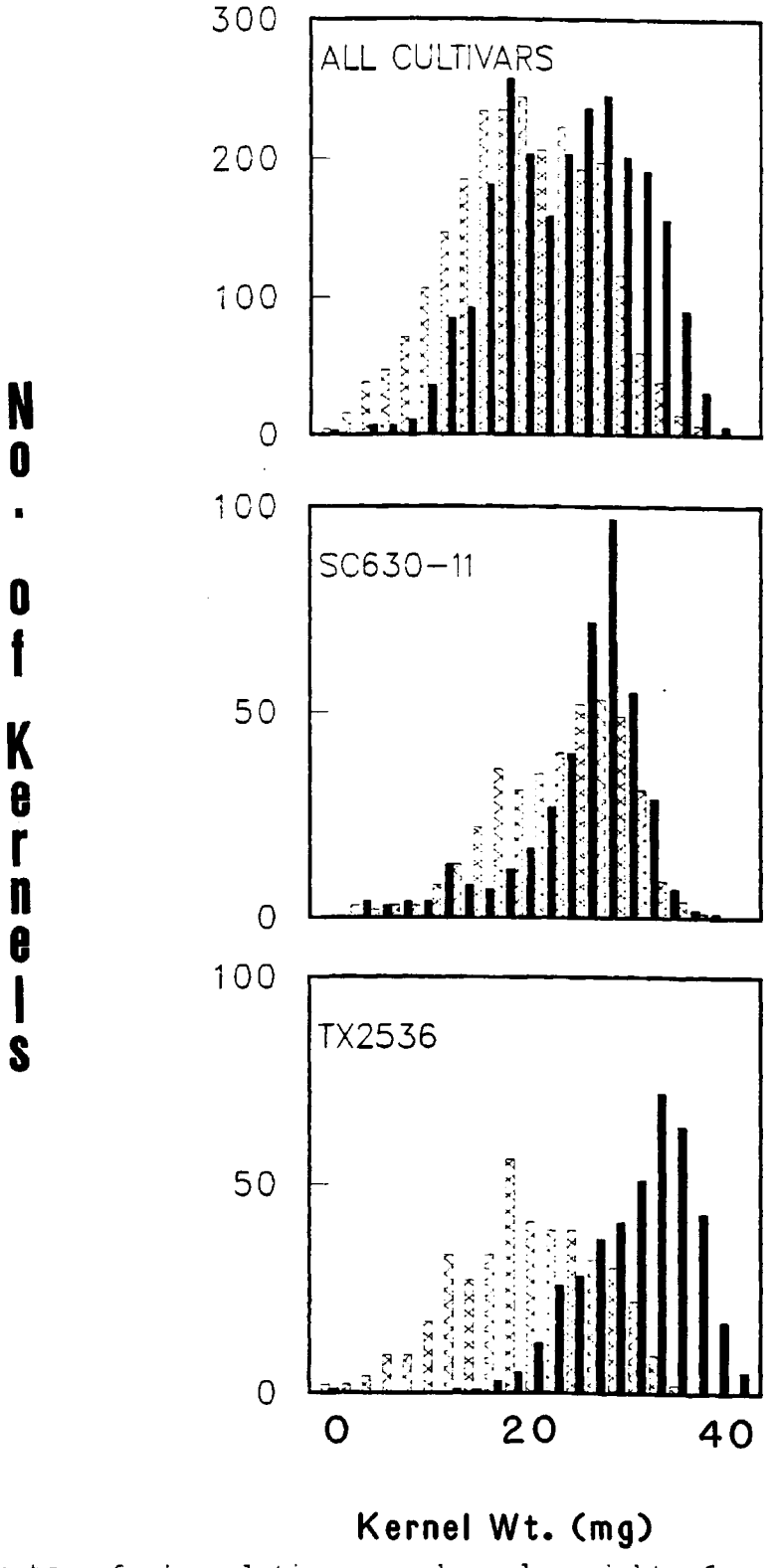


Figure 2. Effects of inoculation on kernel weight frequency distributions for all cultivars, SC0630-11 (resistant), and TX2536 (susceptible). Hatched = inoculated, solid = noninoculated.

Measurement of CFU or pathogen propagules in seed have generally been made to determine incidence of seed-borne pathogens (43,46), but seldom to quantify colonization of seed tissues. Cantone et al (5) found the number of fungal propagules in maize infested with *Aspergillus* and *Penicillium* spp. to be highly correlated with ergosterol and visual mold contamination. It appears that simple bioassays could be a useful substitute or complement to the more demanding ergosterol assessments. Success of a bioassay, however, is often dependent upon the existence of a selective medium. For *F. moniliforme*, NSA gave excellent results, and contamination was seldom a problem. Serial dilutions are laborious for numerous samples, but the procedure could probably be modified to use an Anderson sampler and dried, ground tissue, as has been done for the assessment of *Verticillium* wilt (13).

Our results indicate that SGM does not affect all loss parameters in the same way. Leachate level and percentage germination were highly correlated with CFU of *F. moniliforme* and ergosterol level. Kernel weight was not significantly correlated with any variables measured, yet had the highest F value for the cultivar x inoculation interaction

The high R-square for kernel weight can be attributed to the precision with which this variable can be measured. The unexplained variability that did occur was probably a result of sampling error, since weighing per se should involve little or no measurement error. Ross and Kofoid (35) showed that 125-kernel samples were optimum for estimating 1000-kernel weight.

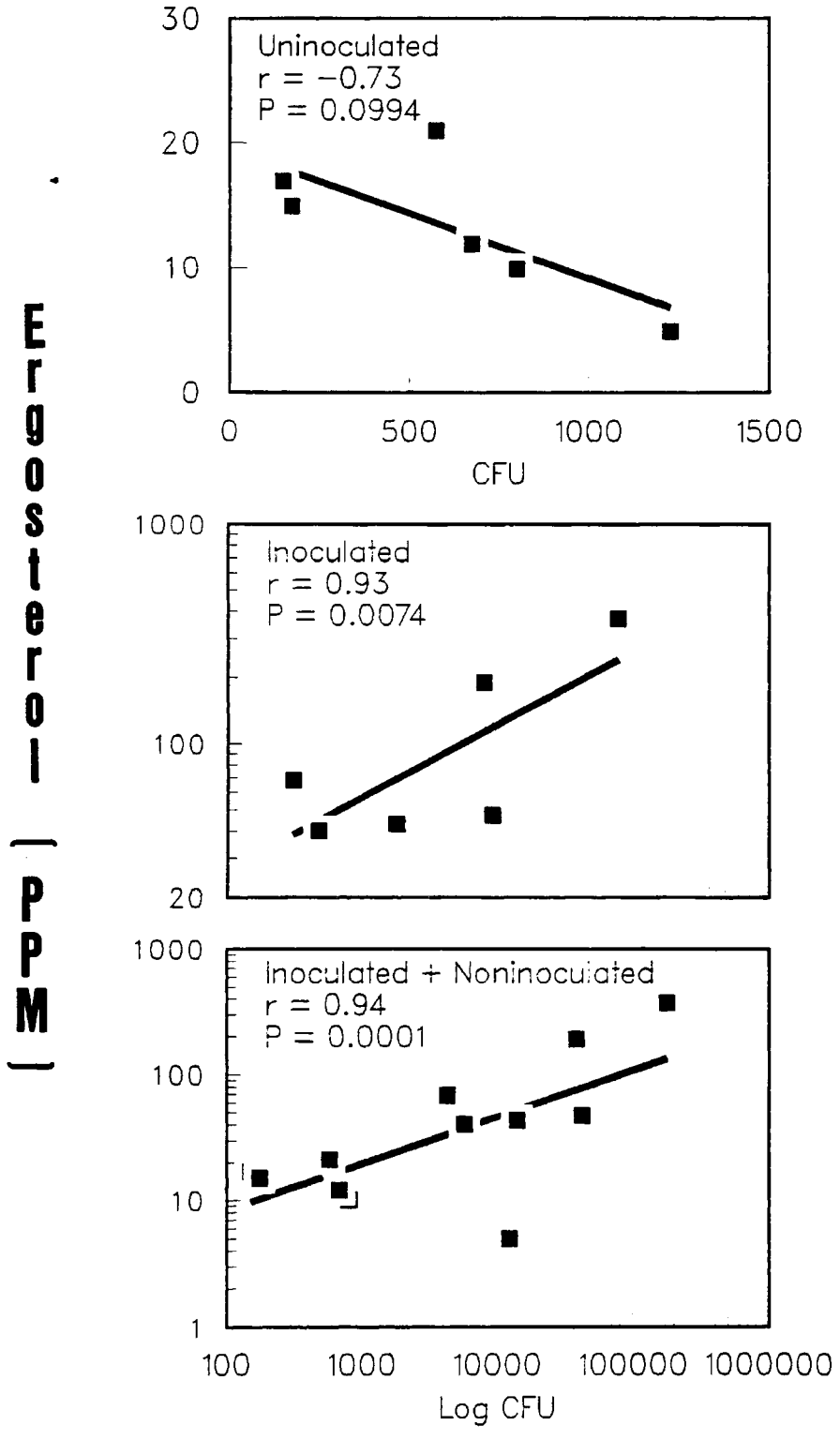


Figure 3. Relation between ergosterol level and colony-forming units (CFU) of *F. moniliforme* for noninoculated, inoculated, and combined lots.

The highly significant cultivar X inoculation interaction for kernel weight was due to reductions in inoculated lots of two susceptible cultivars, TX2536 and TX412. Little or no effect on mean weight was evident among resistant cultivars, even though standard deviations indicated greater heterogeneity among individual kernels. Apparently, precision of mean kernel weight for measurement error is somewhat compensated by its lack of sensitivity in differentiating among resistant and moderately resistant cultivars.

There are at least three plausible explanations for this phenomenon. First, reduction in kernel weight may reflect colonization of spikelet tissues extraneous to the kernel. This could explain a susceptible reaction for kernel weight from a cultivar with low disease intensity in the kernel. The second explanation is that cultivars may differ not only in resistance but also in tolerance to *F. moniliforme*. Infection of sorghum spikelets with *F. moniliforme* has been shown to reduce kernel development by inducing premature black layer formation (15). It is not known if the level of disease intensity required to induce black layer is the same for all cultivars. Finally, mean kernel weight may be unaffected by low levels of infection because of the compensatory growth of healthy kernels.

We found visual appraisal to be highly correlated with quality and intensity measures. Our visual estimations were made on threshed grain in the laboratory, and were based on an estimate of the percentage of kernels damaged (i.e., small or discolored). A percentage is a quantitative measure easily compared with other

variables and allows for statistical inferences. Field assessments based on qualitative scales may be less useful for comparison with other variables, but have been widely used for resistance screening.

# CHAPTER III

# COMPARISON OF INOCULATION TECHNIQUES

## Introduction

Many biotic and abiotic factors influence grain quality and subsequent seedling vigor of grain sorghum. One of these, sorghum grain mold (SGM) caused by *Fusarium moniliforme* Sheld and *Curvularia lunata* (Wakkes) Boed. is receiving increasing attention as a potentially damaging disease with world-wide significance (52). Inoculation in the field with suspensions of *F. moniliforme* conidia are routinely used in sorghum improvement programs (4). Plants are generally inoculated soon after anthesis and exposed to natural or artificially-produced high relative humidity (RH).

Greenhouse inoculations of sorghum with *F. moniliforme* have resulted in symptoms similar to those found in the field (L. L. Castor, personal communication). These inoculations involved spraying sorghum panicles at anthesis with an aqueous conidial suspension, followed by 5- 8 days of high RH.

Greenhouse and field inoculation hypothetically allow for different modes of infection. Some conidia could land between partially opened glumes, placing the inoculum in the infection court at the moment of inoculation. Alternatively, conidia may adhere to the exterior of the spikelet, germinate, and subsequently penetrate the glume junction by mycelial growth. Histological studies suggest that infection of the glume does not occur

(L. L. Castor, personal communication). Long incubation favors mycelial growth and delays drying of spent anthers, which may adhere to the spikelet apex, providing a rich energy source for the fungus. Mycelial tufts can often be seen on spikelet apexes of plants inoculated and bagged (i.e., exposed to high RH) for several days. While long incubation is useful for resistance screening, certain research objectives would be facilitated by greater control over infection timing. Two of these objectives are: 1) study the invasion process histologically; and 2) examine of the relation between host maturity and resistance. Neergaard (32) points out that resistance to several types of floral infection can change over periods of one to several days. Resistance is often closely linked with pollination.

The following study was designed to compare two inoculation techniques and to test the hypothesis that a short incubation period is sufficient for infection, provided the inoculation procedure delivers inoculum to the infection court.

## Materials & Methods

For vacuum infiltration, sorghum panicles were inserted horizontally into a 500-ml flask with side tubulation which was partially filled with inoculum. The flask was inverted so that all spikelets were submerged. Two halves of a rubber stopper with a 0.55-cm hole in the center were fitted on the peduncle and into the flask, sealing the juncture of the flask and plant. A negative pressure of 400 mm was exerted through the side tubulation for 10

s. Plants were incubated for 24 hr at 25 C in an unlighted dew chamber (model 1-35-D, Percival Mfg. Co., Boone, IO 50036).

For the second inoculation treatment, panicles were sprayed at anthesis until run off and immediately transferred to a plastic tent in the greenhouse, where they were incubated for 6 days. Leaf wetness was maintained with conventional room humidifiers. Panicles were sprayed with inoculum on three consecutive days.

Inoculum for both treatments was produced by culturing two isolates of *F. moniliforme* on autoclaved sorghum grain for 14 days at 25 C, comminuting the infested grain with distilled water in a blender and filtering the suspension through cheese cloth. Inoculum density was adjusted to $10^6$ spores/ml.

Inoculation techniques were compared on susceptible cultivar TX2536 and resistant cultivars SC0170-6 and SC0103-12. Cultivars differ in grain structure characteristics (Table 4). Prior knowledge of resistance levels is based on numerous, multi-locational field trials (unpublished data).

Resistance was evaluated by measuring mean kernel weight and percentage germination per panicle. Mean kernel weight was based on full panicle yield, and percentage germination was assessed on one 100- kernel sample.

## Results

When both inoculation techniques were compared with controls the treatment effect (i.e., vacuum vs spray vs control) was highly significant for both kernel weight ($P = 0.0001$) and

Table 4. Grain characteristics and resistance ratings of cultivars used to compare vacuum and spray inoculation procedures

| Cultivar | Mesocarp Thickness | Pigmented[1] testa | Endosperm[2] texture | Appearance | SGM resistance |
|---|---|---|---|---|---|
| SC0103 | thick | P | Fl | brown | R |
| SC0170-6 | thin | A | Vt | white | R |
| TX2536 | thin pearly | A | I | yellow | S |

[1]P = present, A = absent.

[2]Fl = floury, Vt = vitrious, I = intermediate.

[3]Based on several years of field observation at numerous national and international locations (unpublished data).

percentage germination (P = 0.0001) (Table 5). The cultivar and treatment x cultivar interaction effects, however, were only significant for percentage emergence (P = 0.0001 and 0.0006, respectively). When only vacuum inoculation was compared with controls, all factors were significant for percentage germination. The treatment (i.e., vacuum vs control) x cultivar interaction was significant for kernel weight (P = 0.0081). The treatment main effect was also significant for kernel weight. In these analysis of variance models, the interaction effect is important because it indicates a differential response among the cultivars to infection by *F. moniliforme*.

All factors were significant for percentage emergence when only spray inoculation was compared with controls (Table 5). For kernel weight neither the cultivar nor cultivar x treatment interaction effects were significant at the 5% level.

Based on R-square values, the model much better explained variation in percentage germination than in kernel weight. R-square values for kernel weight and percentage germination were higher for vacuum inoculation (0.75 and 0.95, respectively) than for spray inoculation (0.69 and 0.87, respectively).

Vacuum and spray inoculation caused reductions in percentage germination over control of 93 and 94%, respectively, for TX2536, 41 and 39%, respectively, for SC0103-12, and 35 and 24%, respectively, for SC0170-6 (Table 6). Reductions in kernel weight resulting from vacuum and spray inoculation were 42 and 41%, respectively, for TX2536, 33 and 20%, respectively, for SC0103-12

Table 5. Analysis of variance for kernel weight and percentage emergence of grain samples harvested from panicles inoculated with *F. moniliforme*

| Source of variance | Kernel weight | | | | Percentage emergence | | |
|---|---|---|---|---|---|---|---|
| | df | MS | P | $R^2$ | MS | P | $R^2$ |
| $Treatment_{com}$ (T)[1] | 2 | 3.66 | 0.0001 | | 10272.03 | 0.0001 | |
| Cultivar (C) | 2 | 0.03 | 0.1677 | | 7263.03 | 0.0001 | |
| TXC | 4 | 0.69 | 0.0985 | | 2374.89 | 0.0006 | |
| Error | 27 | 0.16 | | | 174.07 | | |
| Model | 8 | 1.16 | 0.0001 | 0.68 | 4977.48 | 0.0001 | 0.89 |
| $Treatment_{vac}$ | 1 | 3.73 | 0.0001 | | 14065.04 | 0.0001 | |
| Cultivar | 2 | 0.26 | 0.0991 | | 3531.79 | 0.0001 | |
| TXC | 2 | 0.64 | 0.0081 | | 4077.08 | 0.0001 | |
| Error | 18 | 0.09 | | | 68.45 | | |
| Model | 5 | 1.10 | 0.0001 | 0.75 | 5041.14 | 0.0001 | 0.95 |
| $Treatment_{spr}$ | 1 | 6.79 | 0.0001 | | 16642.67 | 0.0001 | |
| Cultivar | 2 | 0.13 | 0.5111 | | 2728.67 | 0.0002 | |
| TXC | 2 | 0.32 | 0.2141 | | 1441.17 | 0.0047 | |
| Error | 18 | 0.18 | | | 196.75 | | |
| Model | 5 | 1.54 | 0.0004 | 0.69 | 4996.46 | 0.0001 | 0.87 |

[1] $Treatment_{com}$ = combined model comparing vacuum infiltration, panicle spray and uninoculated, panicle spray and uninoculated control. $Treatment_{vac}$ compares vacuum inoculation with controls. $Treatment_{spr}$ compares panicle spray with controls.

and 22 and 10%, respectively, for SC0170-6.

Infected kernels of TX2536 from all three inoculation procedures were reduced in size, discolored, and often showed signs of *F. moniliforme*. Discoloration and signs originated at the hilar pole and progressed acropetally. Minor infection was less obvious prior to threshing because symptoms and signs were covered by the glumes.

Symptoms on kernels of SC0170-6 resembled those of TX2536, but incidence of severe infection was lower. Discoloration was not readily evident on SC0103-12 due to the brown pigmentation of the pericarp. Severely infected kernels of both resistant cultivars showed signs of *F. moniliforme* at the hilar end.

## Discussion

Symptoms observed in these experiments support the distinction between SGM and weathering put forth by Castor (6). SGM involves infection at the hylar pole that subsequently radiates acropetally. The most severely affected part of the kernel is covered by the glumes. Weathering results from exposure to biotic and abiotic elements and is generally limited to the area not covered by the glumes. Threshing aids in distinguishing between the two phenomena, and has been advised as a preliminary step in kernel quality assessment.

In our experiments 24 hr of incubation was sufficient for infection; once inoculum had been delivered to the infection court via vacuum infiltration. On TX2536, symptoms were evident

Table 6. Kernel weights and germination percentages of grain samples harvested from panicles inoculated with F. moniliforme using vacuum infiltration and panicle spraying.

| Cultivar | Treatment | Percentage germination | Kernel weight |
|---|---|---|---|
| TX2536 | Control | 90.00 | 3.44 |
| | Spray | 6.50/93[1] | 2.01/42 |
| | Vacuum | 5.75/94 | 2.04/41 |
| SC0103 | Control | 97.75 | 3.42 |
| | Spray | 57.75/41 | 2.29/33 |
| | Vacuum | 59.75/39 | 2.72/20 |
| SC0170-6 | Control | 99.50 | 2.92 |
| | Spray | 65.00/35 | 2.28/22 |
| | Vacuum | 76.00/24 | 2.64/10 |

Standard error of difference between two means = 9.33 for percentage germination and 0.28 for mmean kernel weight, where n = 4.

[1] Numbers on the left of the slash represent actual germination percentage; numbers on the right represent percentage reduction over controls.

4-5 days after inoculation and progressed though maturation under environmental conditions (greenhouse) which are not conducive to extra-spikelet fungal growth or new infections. Mycelium was never visible on the surface of inoculated spikelets nor on the portion of the kernel not covered by the glumes. Based on these observations, a logical question appears to be whether incubation is necessary at all, once inoculum is introduced into the spikelet

The role of moisture in the overall (field-wide) intensity of SGM may be principally epidemiological, increasing incidence of infected kernels rather than the severity of SGM on individual kernels. High moisture would permit spore germination and mycelial growth on the exterior of the spikelet and subsequent penetration between the glumes into the infection court. High moisture may also increase available inoculum by promoting sporulation on plant debris. Conidia may also be carried into the spikelet in drops of dew or rain.

Both inoculation procedures differentiated between resistant and susceptible cultivars. Vacuum infiltration however, affords the advantage of greater control over infection timing. This can greatly facilitate efforts to histologically trace patterns of spikelet invasion and tissue colonization. Spray inoculation resulted in generally greater reductions in kernel weight and germination percentage (Table 6).

R-square values, however, indicate that infection was more uniform among panicles with vacuum infiltration.

# CHAPTER IV

# HISTOLOGICAL STUDY

## Introduction

Because of consensus among sorghum workers that SGM constitutes one of the major biotic constraints to grain sorghum production, the disease has received considerable attention in recent years (34,52). Unfortunately, little is known about the location or timing of initial infection. Some of the mechanisms of frequently-observed field resistance, such as presence of a pigmented testa, or drooping panicles are well documented (15,52); other mechanisms are poorly understood (52). Histological studies indicate that one type of resistance may involve impedance of fungal colonization of basal spikelet tissue. In both a susceptible and resistant cultivar, the lemma, palea, and stamens were infected early. The pathogen then colonized basipetally toward the pedicel. Disrupted lodicule tissue at the ovary base appeared to be a source of energy for fungal growth in the susceptible cultivar, and a site of impedance in the resistant cultivar (unpublished data).

The objective of this study was to further clarify differences in invasive patterns of *F. moniliforme* in resistant and susceptible spikelets of grain sorghum.

## Materials & Methods

Two sorghum cultivars, SC0170-6 (resistant) and TX2536 (susceptible), were inoculated with a suspension of *F. moniliforme* conidia using partial vacuum infiltration of spikelets in a manner previously described.

### Paraffin embedding

After inoculation at anthesis, spikelets were sampled 5, 7, 12, 15 and 20 days after anthesis. Samples were fixed in formalin-acetic-alcohol (FAA), dehydrated in an n-butanol series and embedded in Paraplast + (Lancer Division of Sherwood Medical, A Brunswick Co. St. Louis, Mo.). Sections (10-20 μm) were stained in Sass's Hemalum (38) for 15 - 30 min. Selected sections were deparaffinized and prepared for scanning electron microscopy (SEM) as previously described by Gaudet and Kokko (18). As a slight modification of their technique sections were critical-point dried after rinsing in amyl acetate. Air drying from ethanol distorted fungal tissue.

### Resin embedding

After inoculation, spikelets were sampled on days 1 and 3 after anthesis, in addition to periods mentioned above. Samples were fixed in 3% gluteraldehyde (v/v) in 0.1 M tris-phosphate buffer. Samples were dehydrated in ethanol and embedded in Historesin (LKB Produkter AB, S16126 Bromma, Sweden). Sections (1-2 um thick) were cut with glass knives, stained with 0.5% aqueous methyl green, and

counter stained with 0.001% acridine orange in a boric acid - borax buffer, pH 8.6 (39). Tissue was examined and photographed on a Olympus microscope equipped with an BH-RFL vertical fluorescence illuminator, with a mercury lamp. Fluorescence accessories included two B6-12 exciter filters, a B(PM-500+0-515) dichroic mirror and a 0-530 barrier filter.

## Results

The invasive process resistant and susceptible spikelets was studied using both scanning electron microscopy (SEM) and light microscopy (Fig. 4-12).

There were no signs of the pathogen in the lower interstitial voids of the spikelet one day after inoculation. Apparently conidia did not enter deep into most spikelets during inoculation, in spite of vacuum infiltration.

Basipetal invasion of interstitial voids was rapid for both TX2536 and SC0170-6. By day 5 after inoculation mycelium could be seen throughout the spikelet (Fig. 4A). SEM showed that mycelium grew in close association with plant tissues, sometimes forming a dense sheath-like matrix (Fig. 4B). The hyphal matrix enveloped the lodicules which apparently serve as a source of nutrients, but was not profusely colonized.

In TX2536, the early invasive process also involved inter- and intracellular colonization of glumes (Fig. 7), lemma, palea, and stamens. Initial colonization of glume tissue was generally not associated with loss of cellular integrity of the

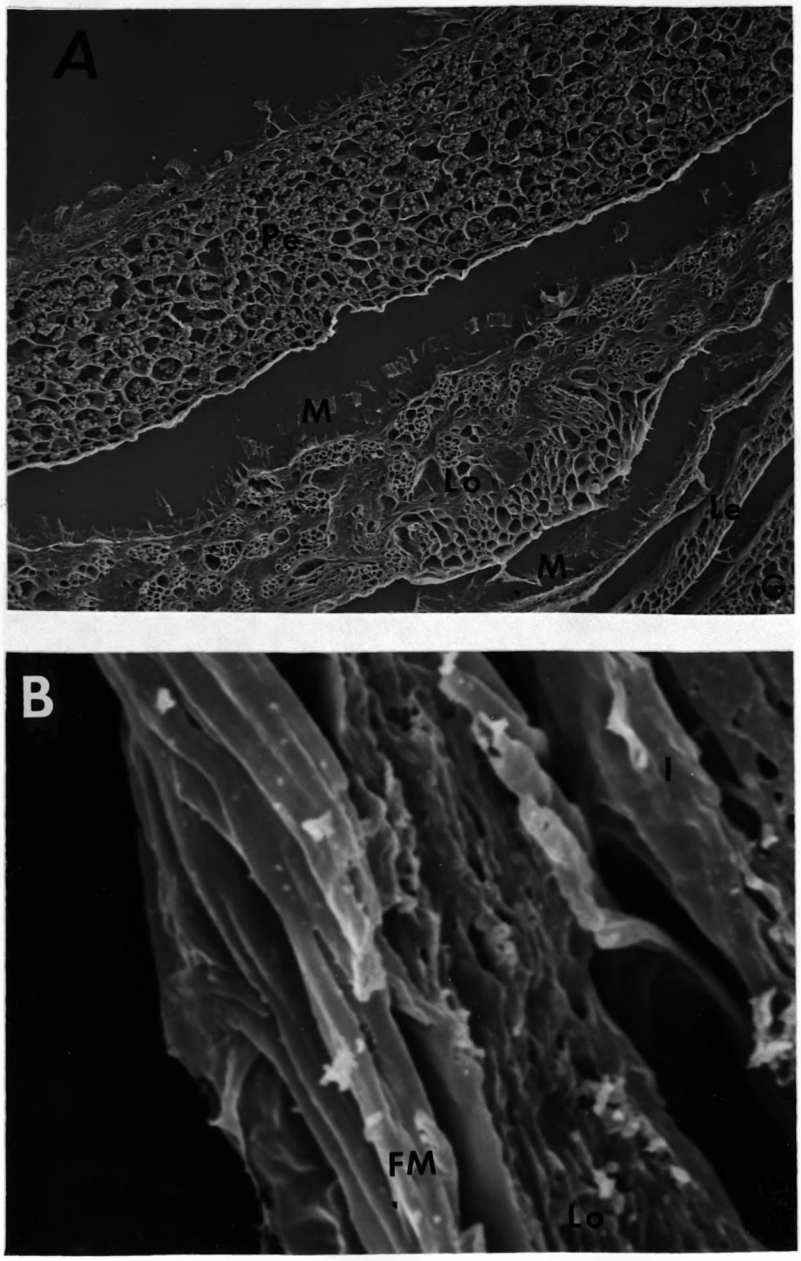


Figure 4. Scanning electron micrographs of deparaffinized tissue of SC0170-6. A, Basal portion of spikelet. Mycelium (M) growing in voids between pericarp (Pe), lodicule (Lo), lemma (Le), and glume (G). B, fungal matrix (FM) developing lodicule (Lo).

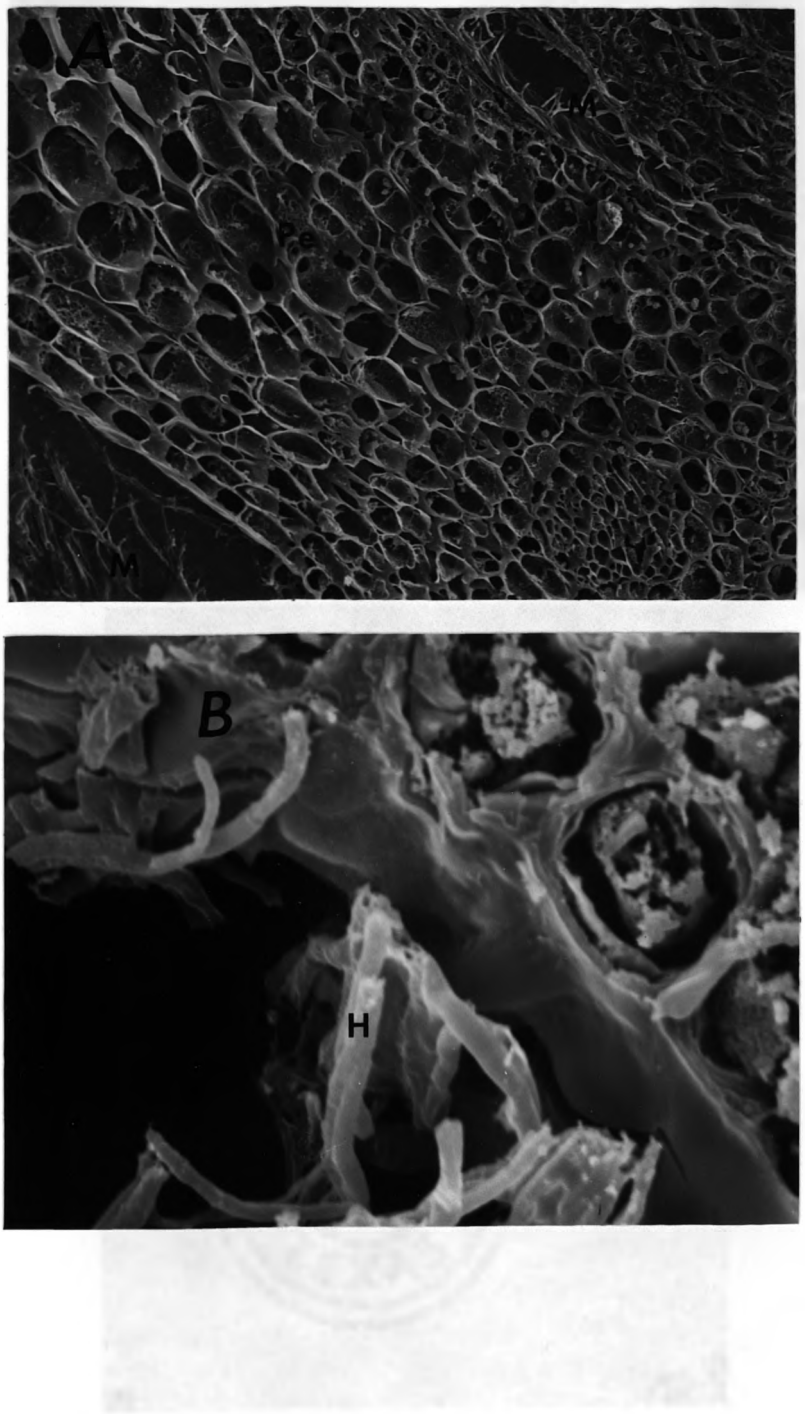


Figure 5. Scanning electron micrographs of deparaffinized tissue of TX2536. A, Pericarp (Pe) wall of TX2536 12 days after anthesis. Mycelium (M) can be seen outside and inside the pericarp, though cellular structure of plant tissue remains intact, and fungal colonization of pericarp cells is not visible. B, hyphae (H) in close association with pedicel-pericarp juncture, 12 days after anthesis.

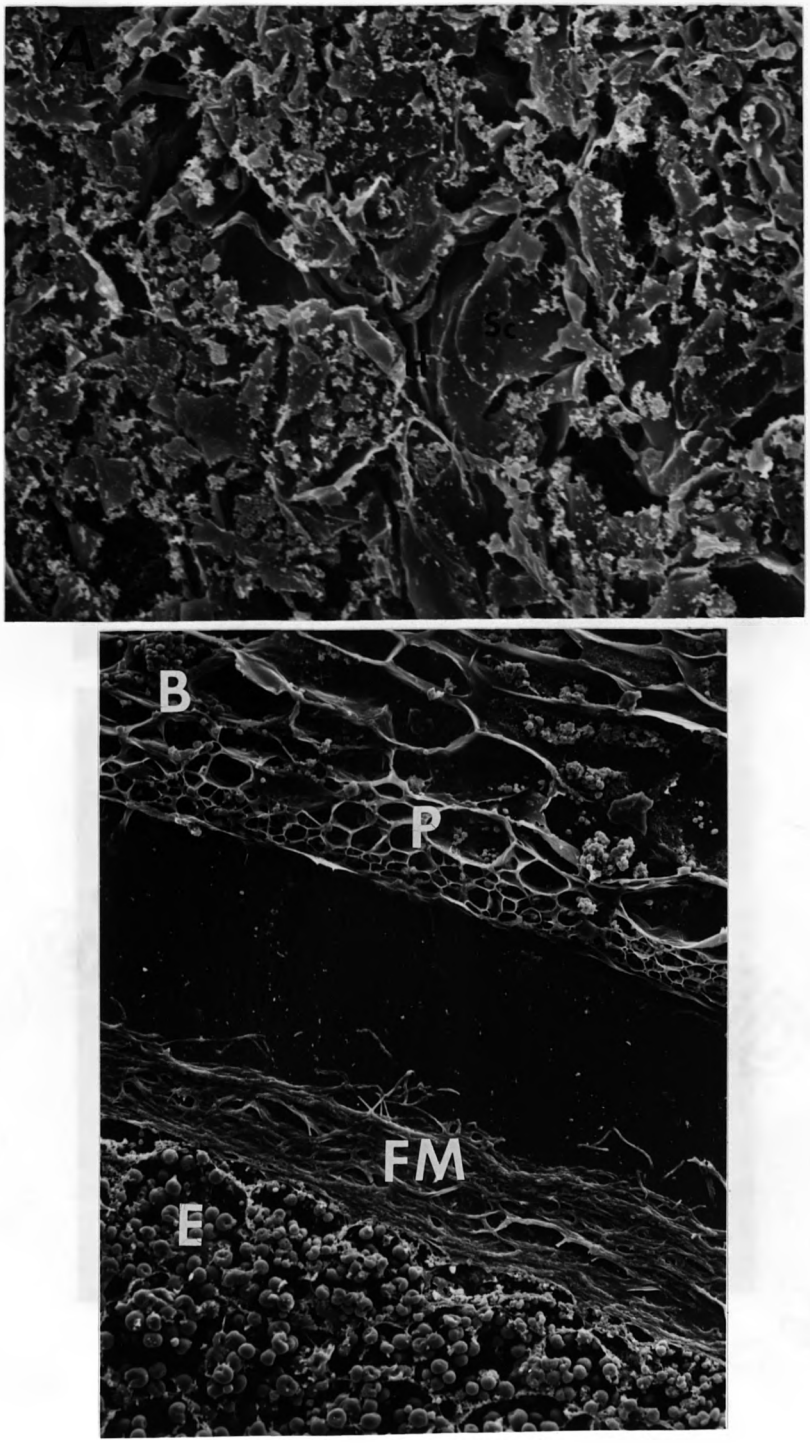


Figure 6. Scanning electron micrographs of deparaffinized tissue of TX2536 - advanced colonization. A, Scutellum tissue (Sc) and intercellular hyphae (H) of TX2536, 12 days after anthesis. B, Fungal matrix (FM) between endosperm (E) and Pericarp (P) of TX2536, 12 days after anthesis.

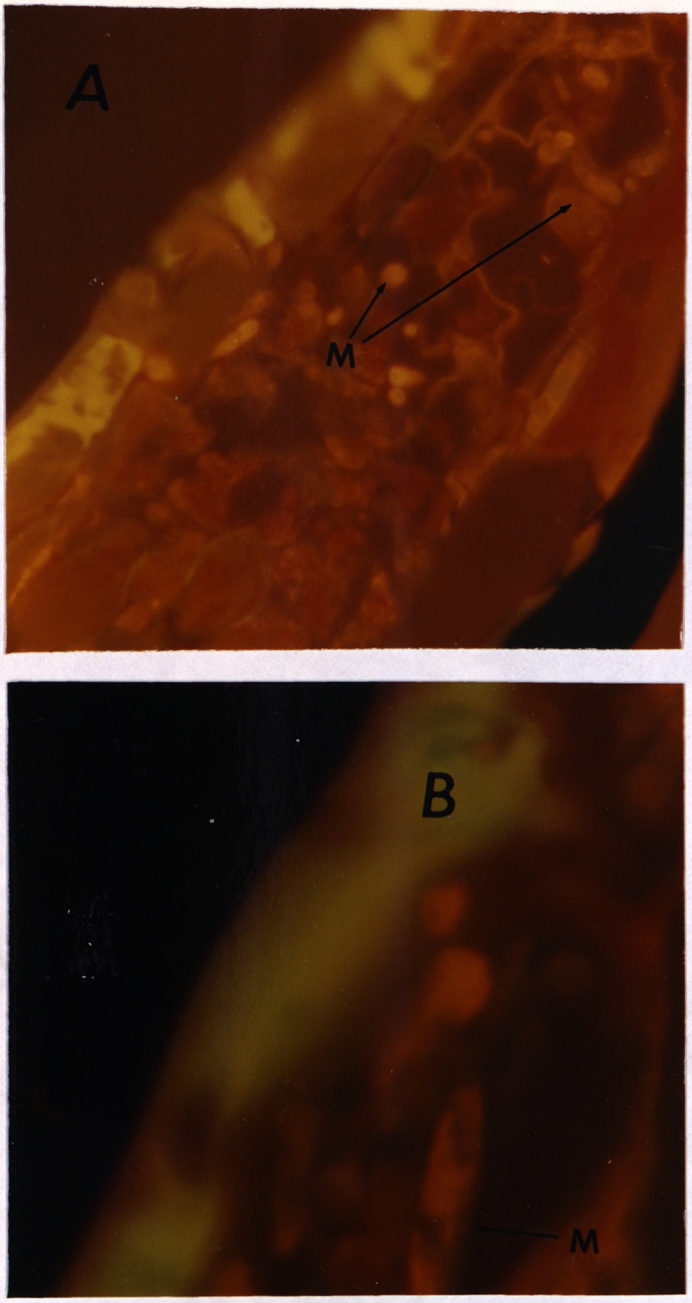


Figure 7. Light micrographs of colonization of suscept TX2536 tissue. A, colonization of glume of TX2536, 5 days after anthesis. Mycelium (M) present in both internal and external sub-epidermal tissue. B, Intra-cellular colonization of TX2536 glume.

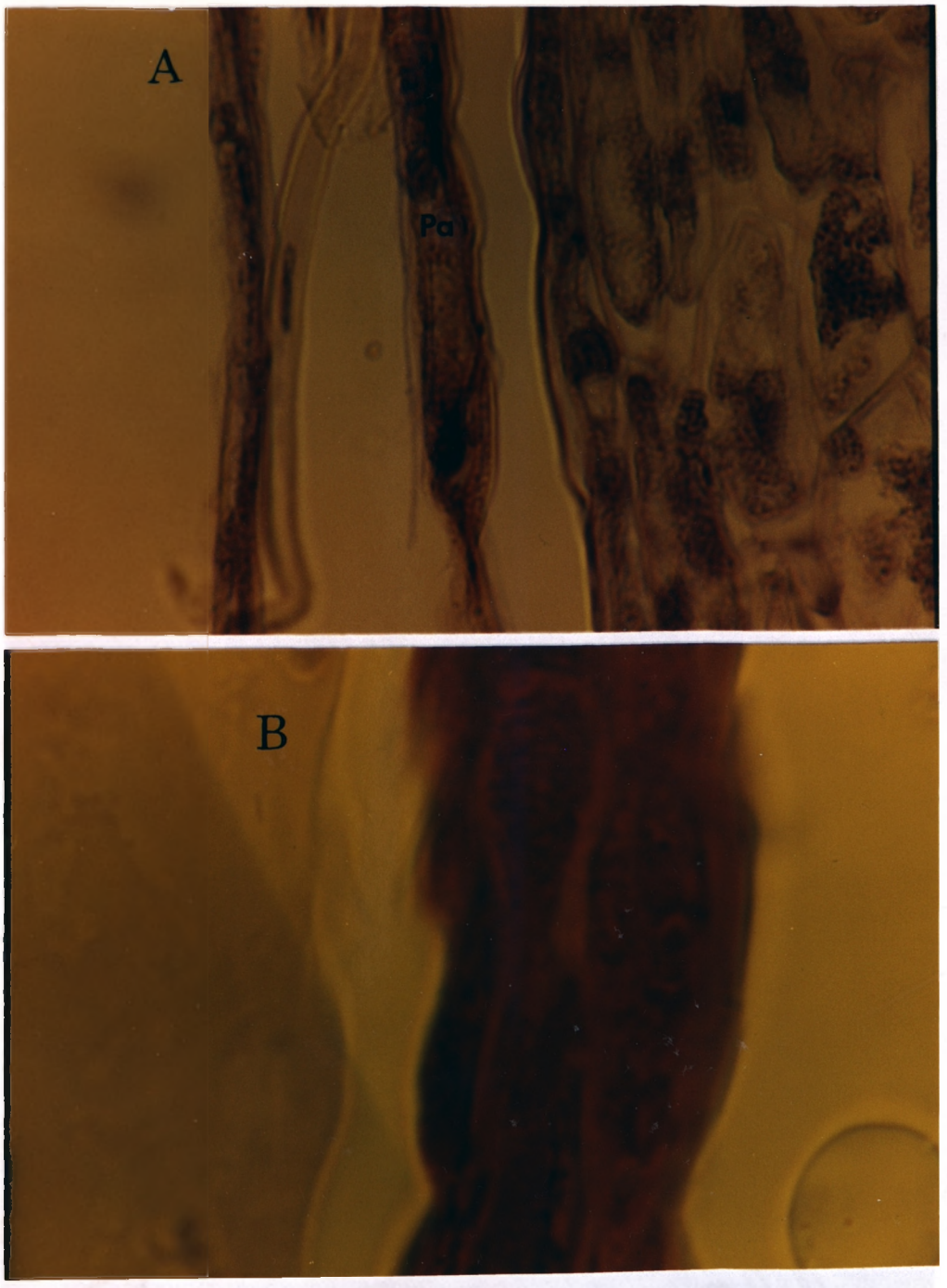


Figure 8. Light micrographs of disease reaction of resistant cultivar SC0170-6. A, Pigmented palea (Pa) of SC0170-6. Lesion developed in response to fungal tissue. B, Pigmented, condensed cells of SC0170-6, 12 days after anthesis.

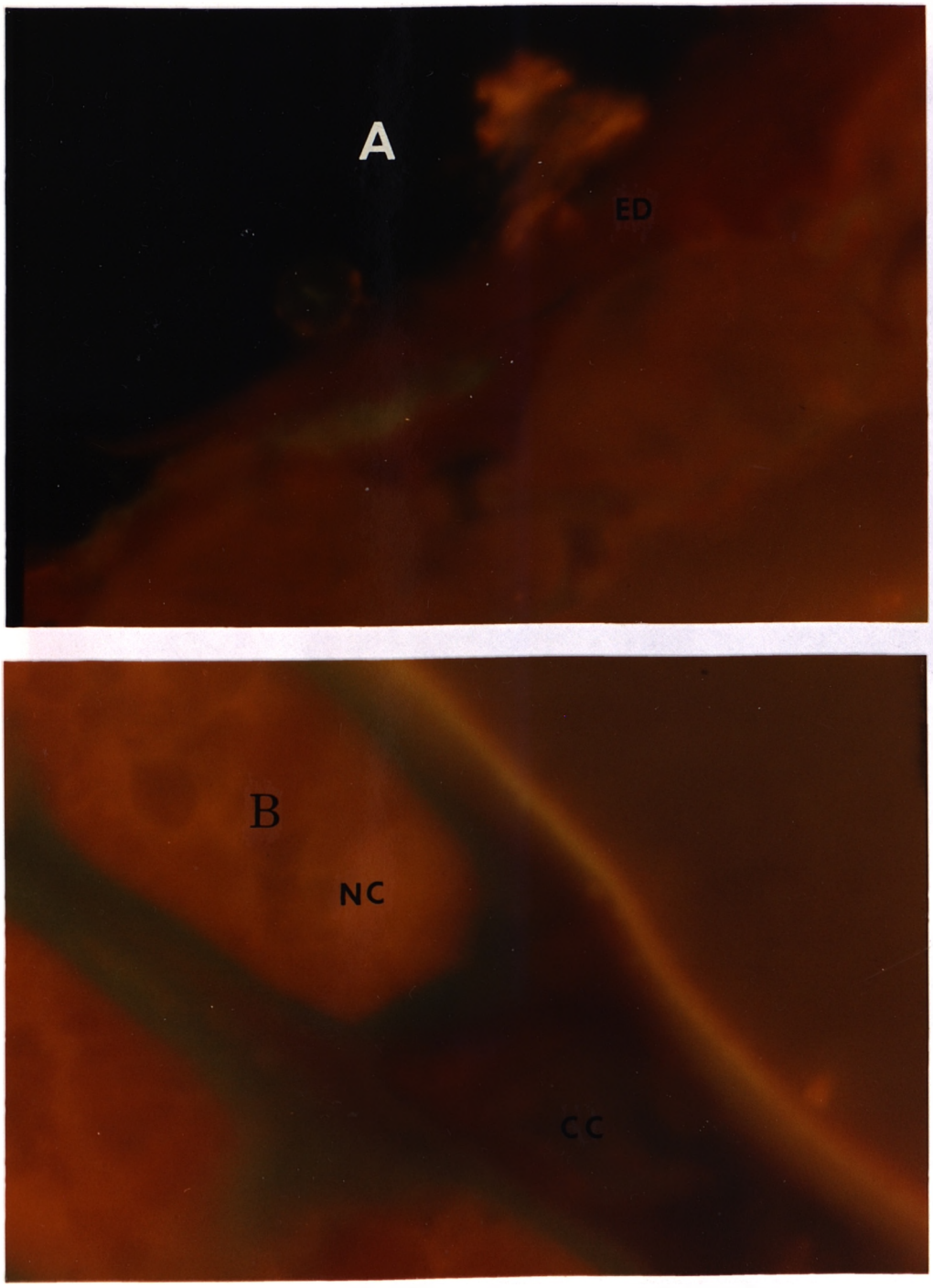


Figure 9. Light micrographs of epidermal deposition in resistant cultivar SCO170-6. A, Dense extra-cellular epidermal deposition (ED) on pericarp of SCO170-6, 12 days after anthesis. B, Collapse of pericarp epidermal cell of SCO170-6, 6 days after anthesis, NC= normal cell, CC= collapsing cell.

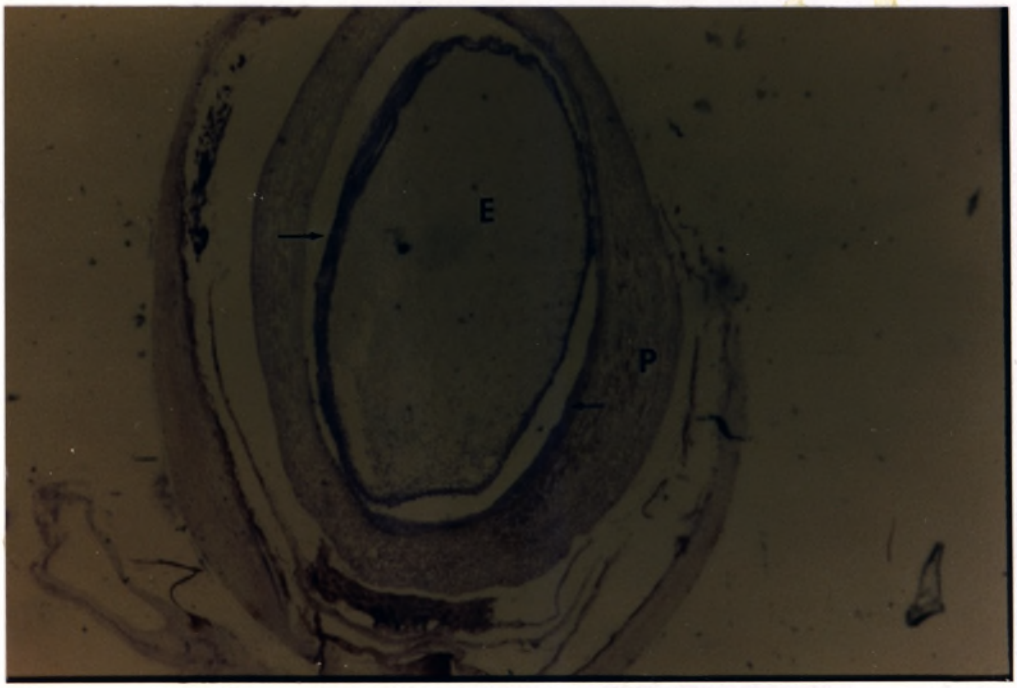


Figure 10. Light micrograph of longitudinal section of grain mold suscept, TX2536, kernel 12 days after anthesis. Arrows indicate hyphal mat between pericarp (P) and endosperm (E).'

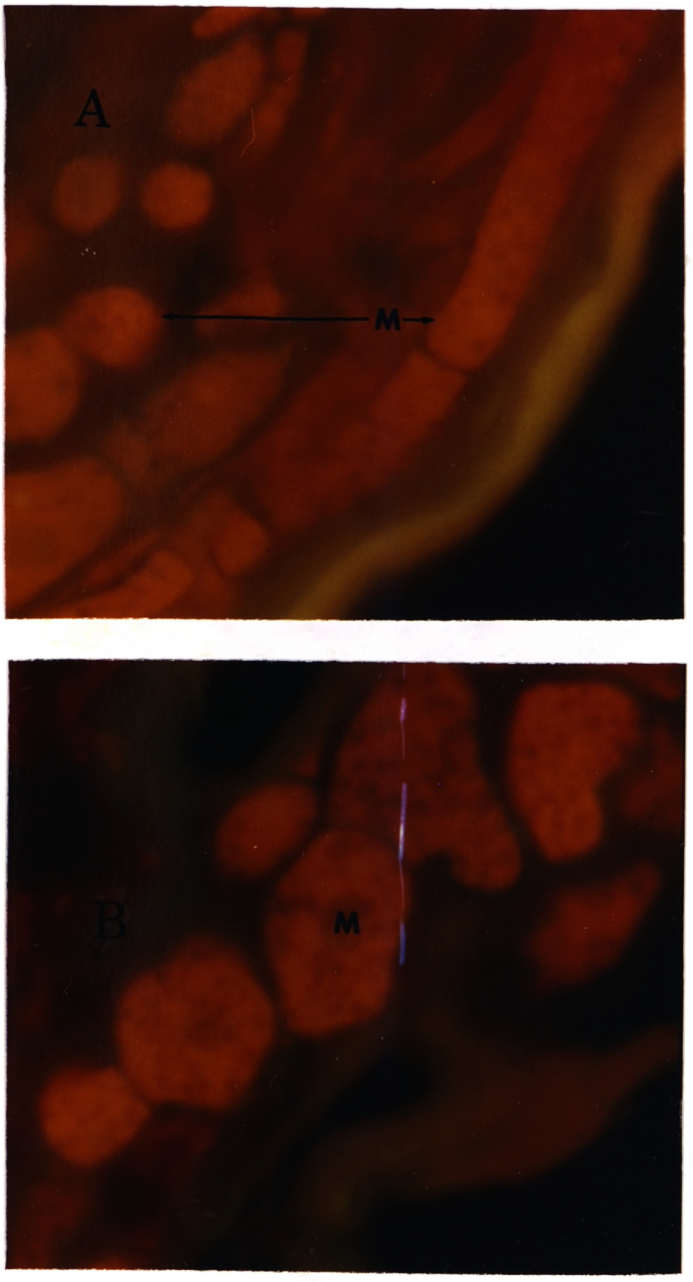


Figure 11. Light micrographs showing colonization of TX2536 kernel 12 days after anthesis. A, Fluorescence of *F. moniliforme* mycelium (M) in pericarp tissue. C, Amorphorus growth of mycelium (M) in pericarp tissue.

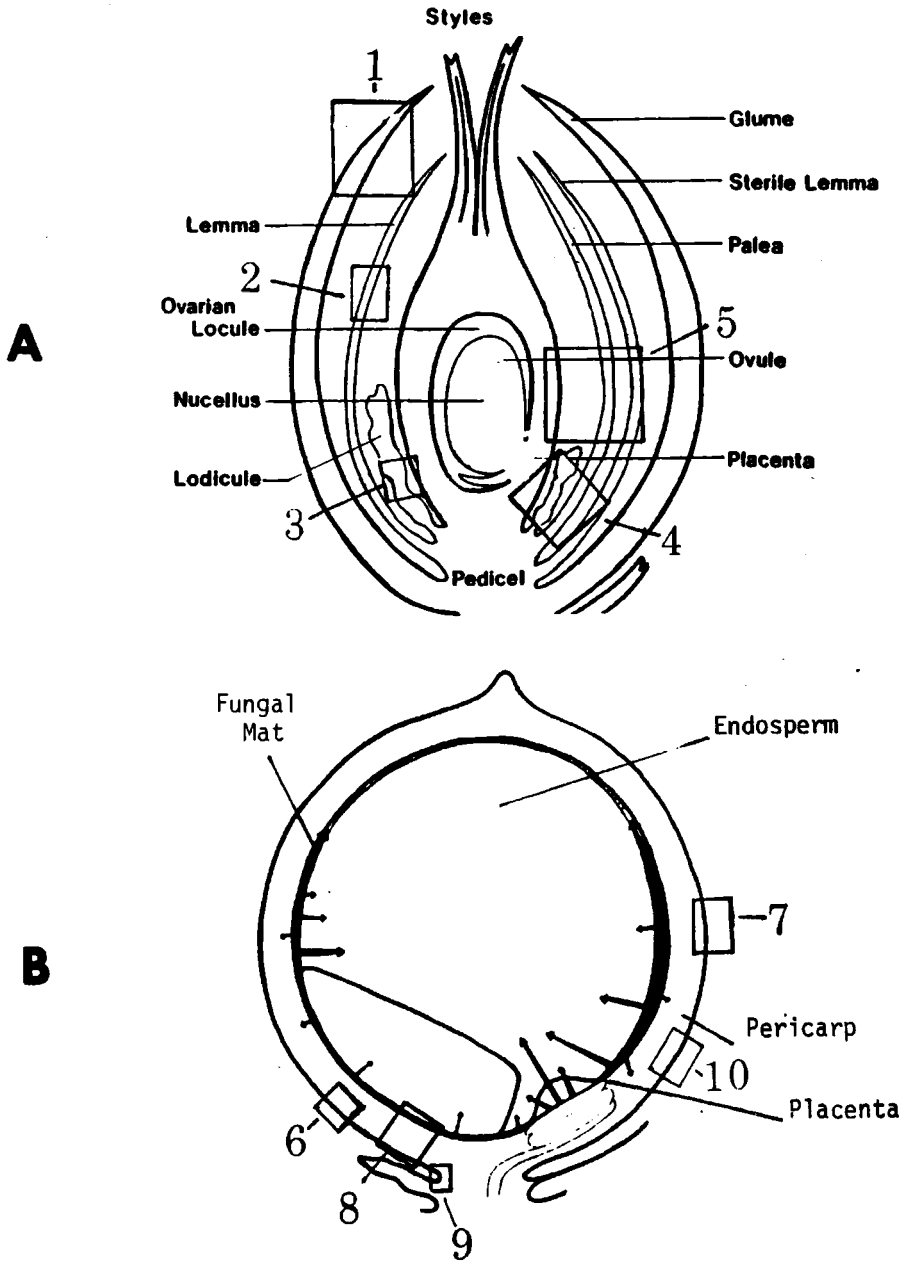


Figure 12. Schematic representation of sorghum spikelet (A) and kernel (B). Numbered rectangles represent photographic orientations to aid interpretation of photomicrographs (Fig. 1-8). Arrows (in B) represent colonization pattern of *F. moniliforme* in TX2536 at 12 days after anthesis. The relation between numbered rectangles and photomicrographs is: 1-4A,4B; 2-5B; 3-1B; 4-1A; 5-5A; 6-8A,8B; 7-6B; 8-2A; 9-2B; 10-6B.

host or pigment formation (Fig 7A). Dark pigmentation and disrupted cells were present in tissues which apparently had been colonized for several days.

Colonization of glumes of TX2536 often occurred near the epidermis (Fig. 7A). It was difficult to determine whether colonization began within the spikelet and progressed outward, or if initial infection had occurred on the outer epidermis of the glume.

In SC0170-6, early interstitial colonization was not accompanied by profuse colonization of lemma, palea, stamens or glumes. Instead, these tissues and pericarp developed brightly-pigmented "lesions" in association with mycelial growth (Fig. 8A, B). No signs of the fungus were found in lesions, although pigmented areas did not fluoresce, and pigments may have inhibited fungal fluorescence. Upon close examination, lesions on glumes and pericarp appeared to be covered with a thick, dense layer of unknown composition (Fig. 9). In some cases the layer originated from the collapse of the outermost epidermal cells (Fig. 9B). Thinner structures, such as lemma, palea and stamens collapsed entirely, forming dense, undifferentiated tissue (Fig. 8).

In TX2536, fungal invasion of the ovary wall, or pericarp, occurred between 5 and 12 days after inoculation. By day 12, mycelium often formed a mat surrounding the endosperm and the embryo (Fig. 10, 12) Fungal tissue was most abundant in the cross and tube cells (Fig. 6B), with some colonization of the endosperm, and little or no colonization of mesocarp tissue. The scutellum

and embryo were heavily colonized by day 12 after anthesis (Fig. 6A).

In some sections mycelium could be seen crossing the pericarp at the ovary base, usually at the point where the lodicule rests against the ovary wall (Fig. 11A). Mycelium could be seen growing on both inner and outer sides of the pericarp wall, but could not be detected within the wall with SEM using deparaffinized sections (Fig. 5A). With fluorescenc microscopy, mycelium was veiwed growing inter- and intracellulary in the pericarp (Fig. 11). Intracellular mycelium was often amorphous (Fig. 11B) in addition to typically filamentous.

Kernels of SC0170-6 were not colonized 12 days after inoculation. Penetration of the pericarp epidermis generally did not occur. Lemma, palea and glumes were necrotic, and did not fluoresce. Interstitial mycelial growth was reduced relative to colonization levels at 5-7 days after inoculation. By 12 days after inoculation some mycelia fluoresced weakly or not at all.

## Discussion

The presence of fungal tissue was associated with a necrotic reaction on SC0170-6, including the formation of a dense epidermal deposition on glumes and pericarp. Total cellular collapse of lemma, palea and stamens occurred as a result of association with the fungus. Necrosis did not appear to inhibit interstitial mycelial growth, but probably eventually reduced substrate availability. At day 12, mycelium was less abundant than at early stages, indicating a diminution of the invasion

process.

Shans et al. also detected deposition layers on the epidermis of rape seed (39). The depositions were determined to be mucilage based on an earlier study (47). In rape seed, mucilage was believed to delay penetration of all layers beneath the epidermis. Extracellular deposits have also been reported in tomato leaves infected with *Cladosporium fulvum* (29). Deposits were considered to be phenolic material lost from dead and dying host cells. Xylem vessels of wilt-infected plants often became lined with deposits (26).

Colonization of spikelet tissue in TX2536 did not cause immediate necrosis, collapse of cells or extracellular depositions. The occurrence of both intracellular mycelium with no apparent cell disruption and generalized necrotic areas suggests that necrosis results subsequent to inter and intracellular colonization.

Colonization of glumes of TX2536 occurred several days after inoculation and involved the inner and outer epidermal layers. The presence of mycelium in outer epidermal tissue suggests that the glumes may not be impenetrable to the fungus. If infection can occur directly through the glumes of TX2536, it would be an important aspect of the susceptibility of this cultivar.

Of the different microscopical techniques used, resin-embedded tissue viewed with a fluorescence microscope was superior for detecting early invasion phenomena. SEM using deparaffinized sections was excellent for the study of interstitial fungal

growth in association with host tissue, but was not sensitive for detection of incipient infection.

# CHAPTER V

# PHENOLIC ACID ANALYSIS

## Introduction

Resistance to SGM has been associated with the presence of phenolic compounds known as tannins, which are located in the subcoat, or testa, of the kernel. High-tannin sorghums, however, are lower in food and feed quality than those those not possessing a pigemented testa (23).

While only a small group of sorghums contain condensed tannins, virtually all cultivars contain a closely related group of compounds called phenolic acids (PA), which are derivatives of either benzoic or cinnamic acid. Free PA are water soluble and are found in the outer layers of the kernel (pericarp, testa and aleurone). Bound PA are usually found in association with cell walls (23). Levels of bound PA are thought to be relatively constant for all cultivars, but free PA levels are extremely variable (24).

Some low-tannin sorghums are resistant to SGM. Hahn et al. (24) suggested that PA may be involved in resistance mechanisms not associated with tannins. PA are known to reduce fungal spore germination and mycelial growth (48). Certain PA are thought to act as precursors in the formation of physiological barriers (26). Enzymes also may oxidize preexisting phenolic compounds which are involved in resistance (4).

The objective of this study was to assess the relation between SGM resistance and accumulation of phenolic compounds (PC) and PA in

tissues of the sorghum spikelet. PA levels were evaluated at different physiological periods and on both *F. moniliforme*-inoculated and noninoculated samples.

## Materials and Methods

### Samples

Six sorghum cultivars differing in kernel characteristics and resistance to SGM were grown in College Station in 1985. A minimum of 15 panicles of each cultivar were inoculated with a conidial suspension of *F. moniliforme* and exposed to artificially-maintained periods of leaf wetness, or were grown as controls using standard agronomic practices. Cultivar designations, kernel characteristics, resistance levels, and inoculation procedures were previously described (Table 1, Page 9). Rachis brances were tagged at anthesis and collected on the following days relative to anthesis: -2, 0, 1, 3, 5, 7, 10, 14, 20, and 27.

### Chemical analysis

Whole, fertile spikelets were assessed through 7 days after anthesis (DAA), after which kernels and glumes (including glumes, lemma and palea) were treated separately. Samples sizes ranged from 50 - 100 units, with two replications.

Initial extraction involved grinding samples in acidified methanol (1.0% hydrochloric acid in methanol) with a polytron homogenizer (Pt-10 probe, Brinkman Instr., Westbury, NY) for 15 s, and shaking for 1 hr at 20 C. Extracts were rotoevaporated and

subjected to alkaline hydrolysis (10 ml of 4.0 N NaOH) for 10 - 15 hr at 20 C *in vacco*. The hydrolysate was neutralized to pH 2 with 12 N hydrochloric acid and extracted 3 times with 20 ml of ethyl acetate. The ethyl acetate was separated and rotoevaporated; and the residue was dissolved in methanol.

Phenolic compounds (PC) were determined on three subsamples of the acidic methanol extract using the Folin-Ciocalteu procedure (23) based on a gallic acid standard. Phenolic acids (PA) were determined on two subsamples of the alkaline-hydrolyzed components with a high performance liquid chromatography (HPLC) system (24).

## Results

### Average of All Cultivars

Total phenolic compounds (PC), measured by the Folin assay, were compared with the sum of phenolic acids (PA) measured with HPLC (Fig. 13).

Over all dates, PC were generally higher and affected more by inoculation than were valuse for the sum of PA. There was no apparent effect of inoculation on either PC or PA through 7 DAA. Both PC and PA decreased from initial preanthesis levels before ascending at 7 DAA. Preanthesis levels may have been affected by the presence of pollen-filled anthers in the spikelets.

Total PA values rose steadily from 10 through 27 DAA in noninoculated kernels (Fig. 13). The rise in PC for inoculated samples was maximum at 21 DAA, then fell. For noninoculated samples, there was no fall in PC after 21 DAA, but the increase occurred at a

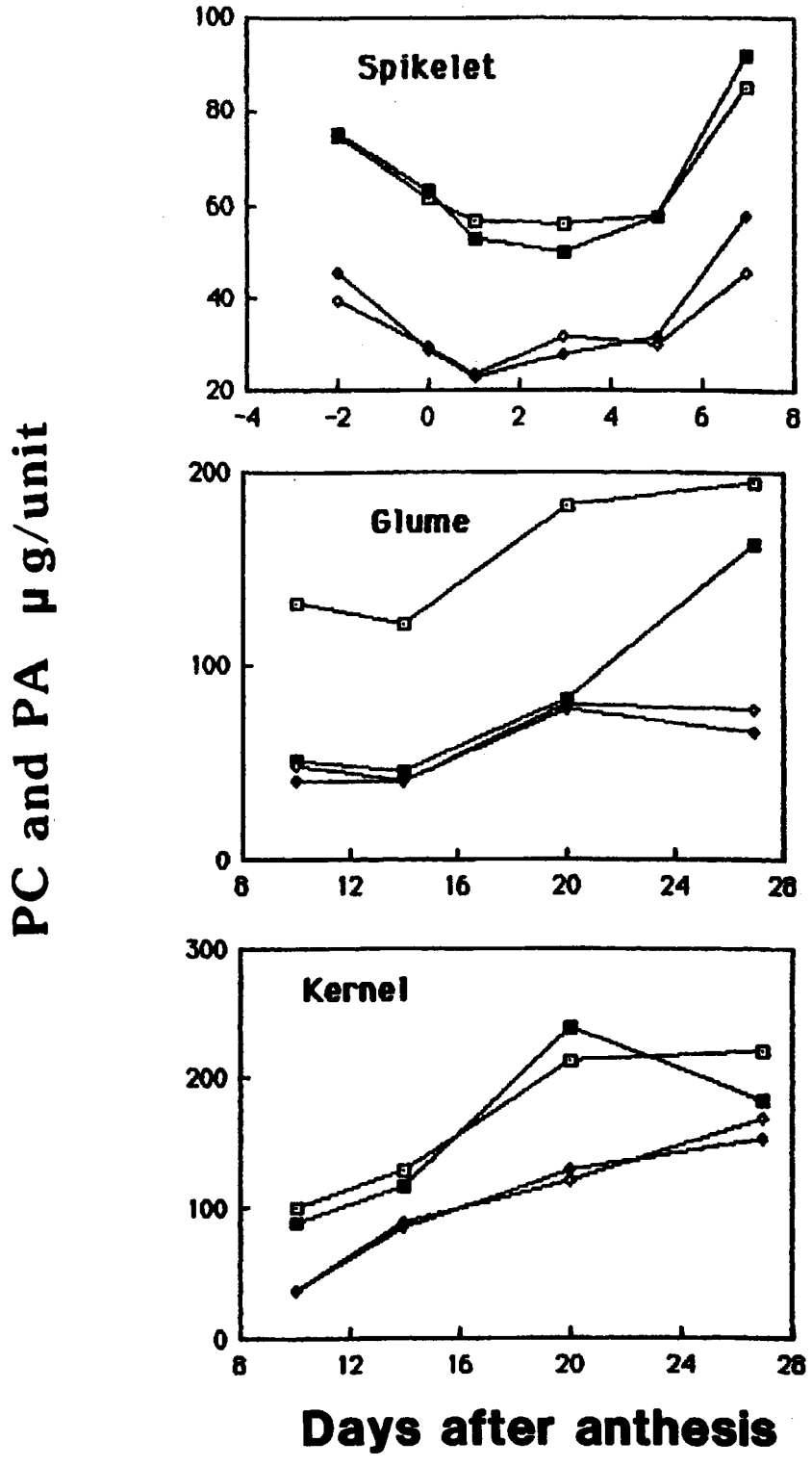


Figure 13. Phenolic compounds (PC) and phenolic acids (PA) for inoculated and noninoculated samples. Open and solid box = inoculated and noninoculated PC, respectively. Open and solid diamond = inoculated and noninoculated PA, respectively. Values represent means of six cultivars differing in resistance to sorghum grain mold.

slower rate.

The effects of inoculation on PC were most apparent in glumes (Fig. 13). Levels in inoculated glumes were approximately 100 µg/unit higher than in noninoculated from 7 through 21 DAA. This trend changed between 20 and 27 DAA when the rate of accumulation of PC was higher in noninoculated than inoculated.

Total PA in glumes were relatively stable from 8 through 27 DAA. Levels peaked at 21 DAA at approximately 70 µg/unit, changing little through 27 DAA.

Average According to Disease Resistance

In order to examine the relation between disease resistance and content of PC and PA, cultivars were grouped according to resistance to SGM as follows: highly resistant (H) SC0103-12, resistant (R) SC0630-11, moderately resistant (M) CS3541 and SC0599-6, and susceptible (S) TX2536 and TX412. Resistance ratings were based on years of field observation (R.A. Frederiksen, personal communication). For resistance groups comprising two cultivars, values represent the mean of both cultivars. PC in the H cultivar began to increase in the inoculated samples 3 DAA (Fig. 14).
In all other resistance groups, PC began to rise between 5 and 7 DAA. For noninoculated samples PC trends were similar, but there was a delay in the accumulation. By 7 DAA, only the H cultivar showed an accumulation of PC in the spikelet (Fig. 14).

The effect of inoculation on PC was greater for glumes than for kernels (Fig. 15).

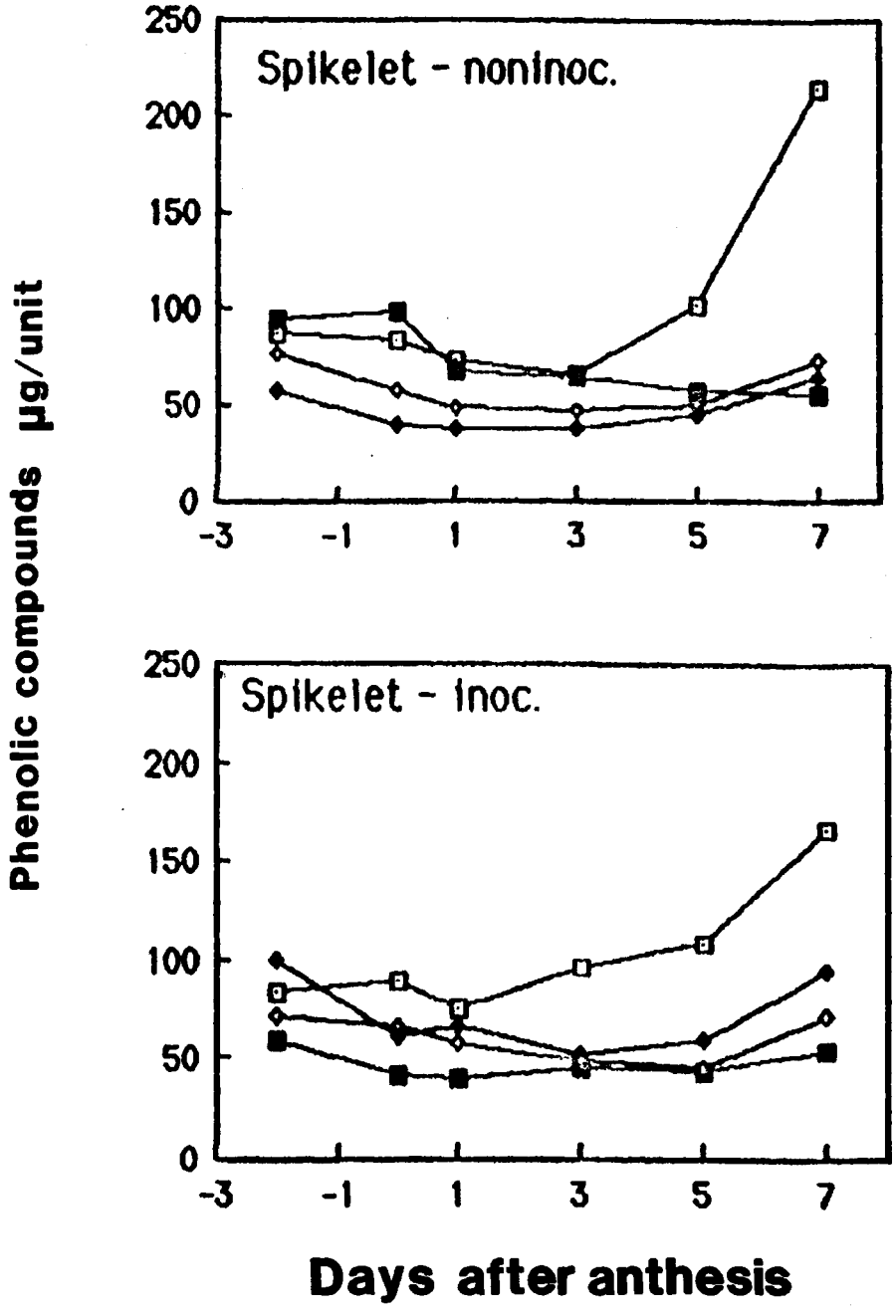


Figure 14. Phenolic compounds in noninoculated and inoculated spikelets of 4 cultivar groups differing in resistance to sorghum grain mold. Highly resistant (open box) = SC0103-12, resistant (solid diamond) = SC0630-11, moderately resistant (solid box) = CS3541 and SC0599-6, and susceptible (open diamond) = TX2536 and TX412.

By 20 DAA PC reached 400 μg/glume in the inoculated H cultivar, and increased slightly through 27 DAA. Accumulation of PC in noninoculated H cultivar was much later and reached only 300 μg/glume by 27 DAA. PC accumulated to between 80 and 150 μg/glume in the inoculated samples of the other resistance groups during 10 - 14 DAA. This level was approximately 50 μg/glume higher than that of noninoculated samples. Accumulation of PC in noninoculated glumes of R and M groups occurred during the period between 14 and 27 DAA.

In kernel tissues, PC rose sharply till 20 DAA (physiological maturity) in the H cultivar, then fell, in both inoculated and noninoculated samples (Fig. 15). The maximum for the inoculated H cultivar was 1400 μg/unit, about 500 μg/unit higher than the maximum for noninoculated. Between 20 and 27 DAA there was a slight increase in PC of the M cultivar. For other resistance groups, PC increased less than 50 μg/kernel throughout the period.

## Correlation of PA with Resistance Measures

To further examine the possible association between resistance and PA, individual phenolic acids were correlated with three measures of resistance to SGM: ergosterol level, percentage germination, and electrolyte leachate level. Resistance parameters were measured on grain harvested 50 DAA. A full description of assessment procedures is given in chapter II. Ergosterol and leachate level are negative measures of general kernel quality (i.e., high values for either measure indicate poor kernel quality). Germination is positively correlated with kernel quality.

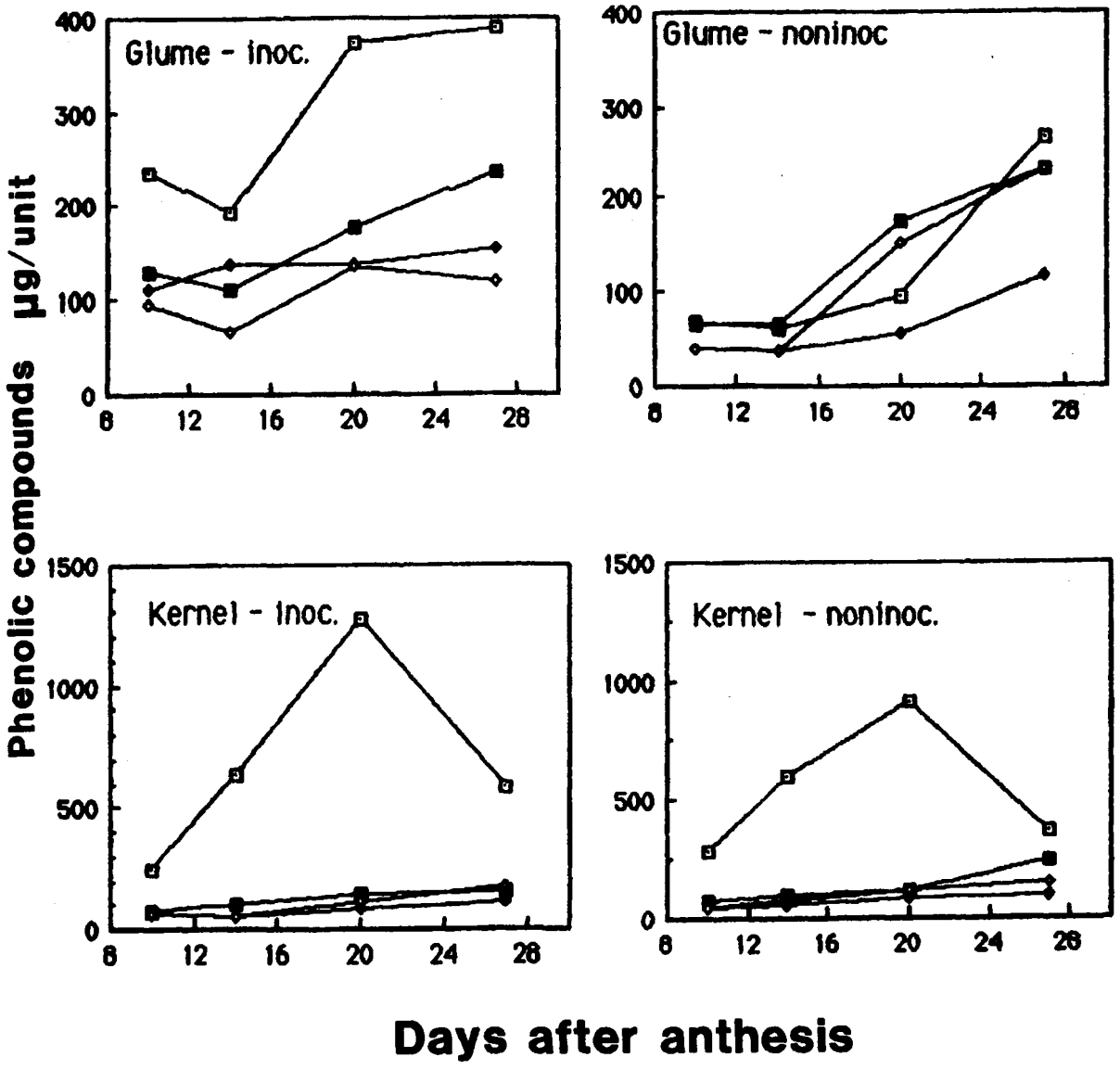


Figure 15. Phenolic compounds of resistance groups for inoculated and noninoculated glumes and kernels. Same code for resistance groups as in Fig 14.

Correlations were made separately for 10, 14, and 20 DAA, because this appeared to be the period of greatest PA accumulation. Correlations include all cultivars except SC0103-12. The high concentration of tannins in this cultivar could drastically affect results and mask smaller differences among other cultivars. The greatest number of significant correlations occurred at 20 DAA, though trends in coefficients of other DAA were similar. Only data for 20 DAA are presented (Table 7).

Analysis of inoculated samples indicated that several compounds were highly correlated with kernel quality as indicated by the three resistance measures: ergosterol and leachate levels, and percentage germination (Table 7). The presence of an unidentified compound (Unknown 3) in kernels of inoculated samples was negatively associated with kernel quality by the following significant correlaitons with the resistance measures: ergosterol (r= +0.90), percentage germination (r= -0.97), and leachate (r= +0.90). In contrast, gallic acid was positively associated with kernel quality: ergosterol (r= -0.90, kernel), and percentage germination (r= +0.81, glume). Salycylic acid in glumes was also positively associated with kernel quality: percentage germination (r= +0.81), and leachate (r= -0.79).

For noninoculated samples, the association between salyclic acid and kernel quality changed from that of inoculated samples. For noninoculated samples the correlation coefficient between salicylic acid and percentage germination was -0.81 (Table 7). Also, p-coumaric acid was positively associated with kernel qualtiy:

Table 7. Correlations among quality measures and selected phenolic acids for inoculated and noninoculated samples of six sorghum cultivars differing in kernel composition and resistance to grain mold.

| Phenolic acid | Quality measure | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated | | | Noninoculated | | |
| | Ergosterol | % Germ. | Leachate | Ergosterol | % Germ. | Leachate |
| Kernel | | | | | | |
| Naringen | -0.18 | 0.47 | -0.34 | 0.80* | -0.95** | 0.89* |
| Gallic | -0.90** | 0.75 | -0.72 | 0.14 | 0.27 | -0.32 |
| Cinnamic | 0.20 | 0.13 | 0.14 | 0.68 | -0.81* | 0.82* |
| Salicylic | -0.62 | 0.70 | 0.60 | 0.53 | -0.81* | 0.67 |
| Unknown 3 | 0.90** | -0.97** | 0.90** | 0.86* | -0.66 | 0.64 |
| Glume | | | | | | |
| Gallic | -0.61 | 0.81** | -0.66 | 0.73 | -0.32 | 0.37 |
| Salicylic | -0.59 | 0.81** | -0.79* | -0.56 | 0.54 | -0.57 |
| P-coumaric | -0.40 | 0.45 | -0.34 | -0.91** | 0.88** | -0.89** |

* = significant at 0.05% level of probability
** = significant at 0.01 level of probability

ergosterol (r= -0.91), percentage germination (r= +0.88), and leachate (r= -0.89). Analysis of noninoculated kernels also indicated that naringen was significantly correlated with all three variables: ergosterol (r= +0.80), percentage germination (r= -0.95), and leachate level (r= +0.89).

Principal Component Analysis of Individual Phenolic Acids

To further evaluate the relation between resistance and individual PA, PA were analyzed using principal component analysis (1). Principal component analysis reduces variation associated with many variables (in this case individual PA) into a few non correlated variables i.e., principal components. Analysis of principal componets (PCM) is easier, because of the reduction in variable number and the violation of fewer assumptions associated with standard statistical tests. As with correlations, analyses were made separately for days 10 - 27 after anthesis. Unlike correlation analysis, however, all lots (i.e., inoculated and uninoculated) were analyzed simultaneously to meet certain requirements of the statistical procedure.

The three quality measures described above were regressed on one or more of the first five principal components (PCM) using stepwise regression. Several statistical models were significant at 10 % for all days (Table 8). Results of day 20 are presented to facilitate comparison with the correlation analysis.

In the analysis of glume tissue, only two PCM were found through stepwise regression to be associated with kernel quality (Table 8).

Table 8. Regression analyses of selected resistance measures on principal components of phenolic acid values for glume and kernel tissues, 20 days after anthesis.

| Tissue | Quality measure | Principal component (B*) | P > F | R-square |
|---|---|---|---|---|
| Glume | | | | |
| | % Germination | PCM 2 (+2.3)<br>PCM 3 (+4.2) | 0.06 | 0.34 |
| | Leachate | PCM 2 (-2.0) | 0.06 | 0.37 |
| Kernel | | | | |
| | Ergosterol | PCM 5 (+24.2) | 0.06 | 0.37 |
| | Leachate | PCM 5 (+5.40) | 0.07 | 0.37 |

* Regression coefficient for corresponding principal component

PCM 2 and 3 (glume) accounted for 34 % of the variation in percentage germination (Table 8). PCM 2 (glume) accounted for 37 percent of the variation in leachate level. Signs of regression coefficients indicated that PCM 2 and 3 were positively correlated with kernel quality.

In the analysis of kernel tissue, two models involving PCM 5 were significant at 10 %: ergosterol (P = 0.06) and leachate level (P = 0.07) (Tables 8, 9). PCM 5 accounted for 37 % of the variation in ergosterol, and 34% for leachate level. Regression coeffecients indicated that PCM 5 was negatively correlated with kernel quality.

Based on regression analyses, three PCM were selected for further examination (Table 9). These were PCM 2 and 3 from glumes and PCM 5 from kernels. PCM 2 indicates a high positive association with p-coumaric and a negative association with gallic acid (Table 9). PCM 3 indicates a high positive association with salicylic, p-coumaric and gallic acids. All associations in PCM 5 (kernel) were positive and involved gentisic, p-coumaric, and salicylic acids.

One interesting aspect of the PCM analysis, as well as above-mentioned, correlation analysis, is the repeated appearance of certain PA, including gallic, gentisic, p-coumaric and salicylic acids. For further examination, gallic and salicylic acids, and unknown 3 were plotted over the period of 10 - 27 DAA according the resistance classification and inoculation treatment (Fig. 16-18).

There appeared to be little effect of inoculation on gallic acid levels in kernels (Fig. 16). Galic acid levels in H cultivar (SC0103-12) were approximately 3 µg/kernel higher for inoculated than

Table 9. Eigenvectors demonstrating association of principal components with individual phenolic acids for glume and kernel tissues, 20 days after anthesis

| | Eigenvectors | | |
|---|---|---|---|
| | Kernel | Glume | |
| Phenolic acid | PCM 5 | PCM 2 | PCM 3 |
| Gallic | 0.08 | -0.61 | 0.48 |
| Protochatechuic | 0.01 | 0.03 | -0.01 |
| Gentisic | 0.42 | -0.01 | 0.01 |
| Unknown 1 | -0.01 | -0.01 | 0.13 |
| P-Hydroxybenzoic | 0.08 | -0.02 | -0.08 |
| Unknown 2 | -0.01 | -0.01 | -0.13 |
| Vanillic | 0.06 | 0.21 | 0.18 |
| Caffeic | 0.02 | -0.03 | 0.33 |
| Syringic | 0.08 | 0.04 | 0.02 |
| P-Coumaric | 0.31 | 0.64 | 0.47 |
| Salicylic | 0.81 | 0.21 | 0.44 |
| Ferulic | 0.11 | 0.26 | 0.22 |
| Sinapic | 0.04 | 0.18 | 0.01 |
| Cinnamic | 0.13 | 0.05 | -0.21 |
| Unknown 3 | 0.01 | 0.02 | -0.01 |
| Unknown 4 | 0.01 | 0.01 | 0.21 |
| Unknown 5 | 0.01 | 0.06 | 0.00 |

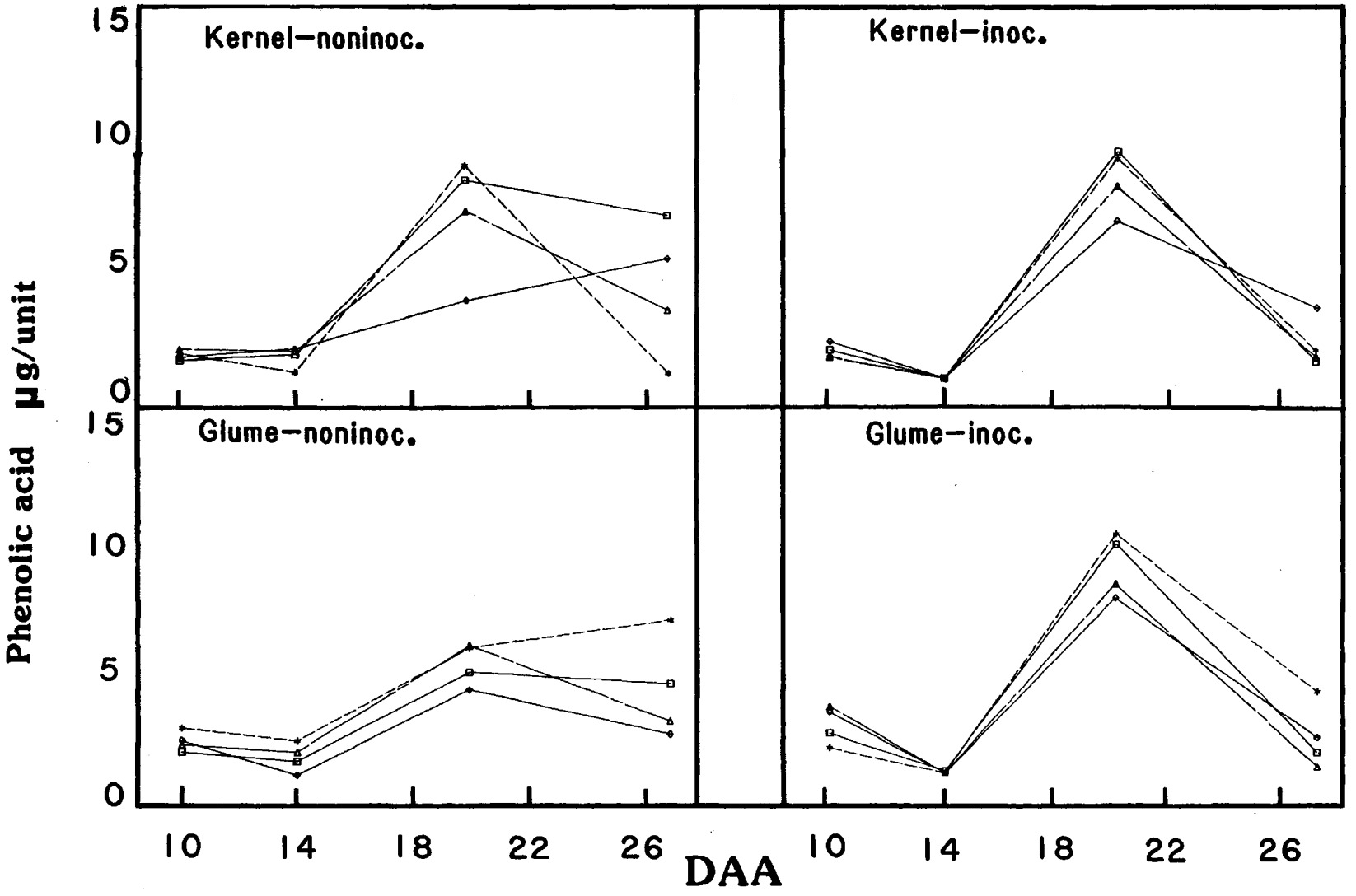


Figure 16. Galic acid levels in noninoculated and inoculated grain lots. Code: diamond = I (SC0103-12), square = M (CS3541 and SC0599-6), star = R (SC0630-11), and triangle = S (TX2536 and TX412). DAA = days after anthesis.

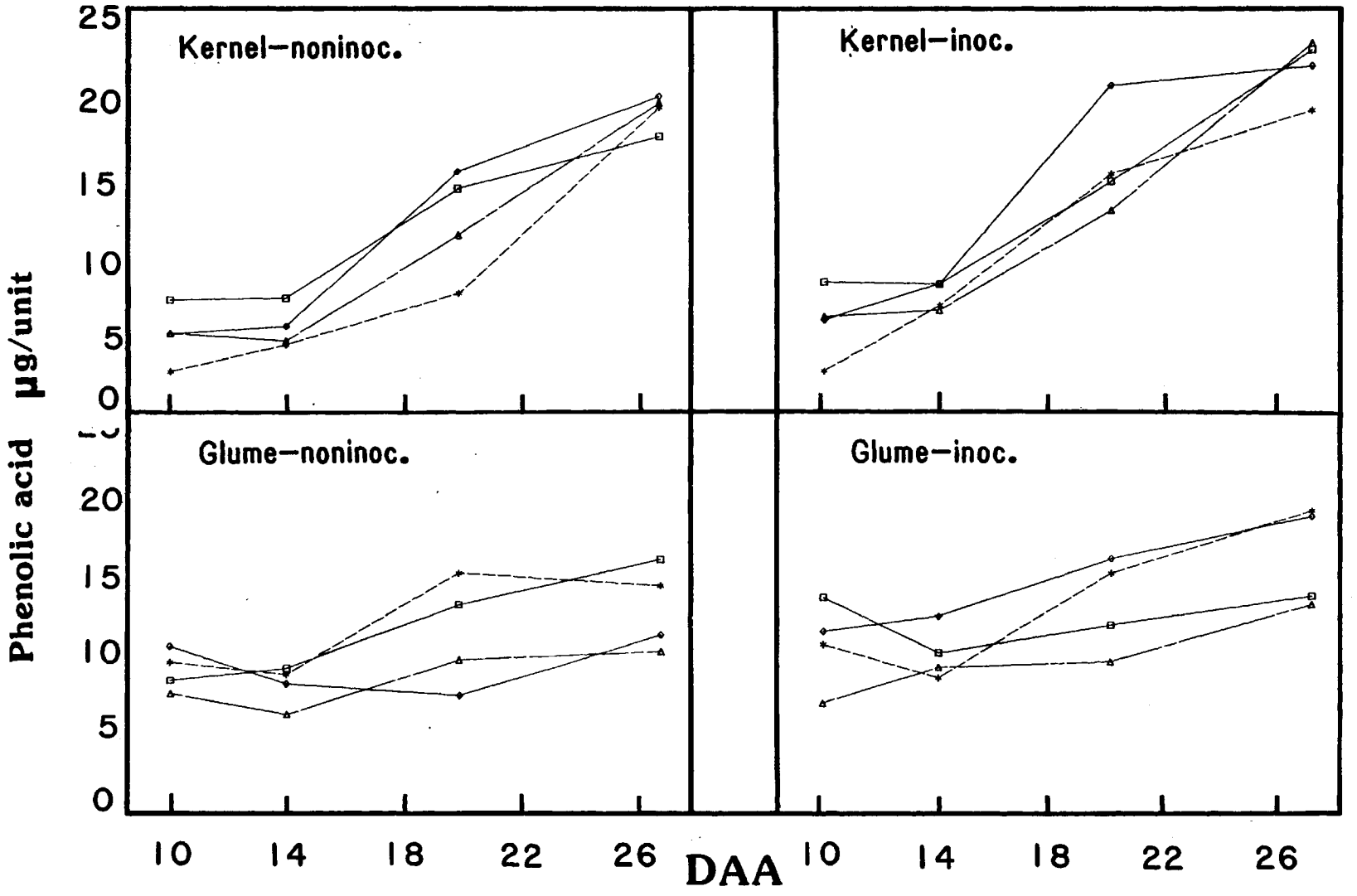


Figure 17. P-coumaric acid levels in noninoculated and inoculated grain lots. Code: diamond = I (SC0103-12), square = M (CS3541 and SC0599-6), star = R (SC0630-11), and triangle = (TX2536 and TX412). DAA = days after anthesis.

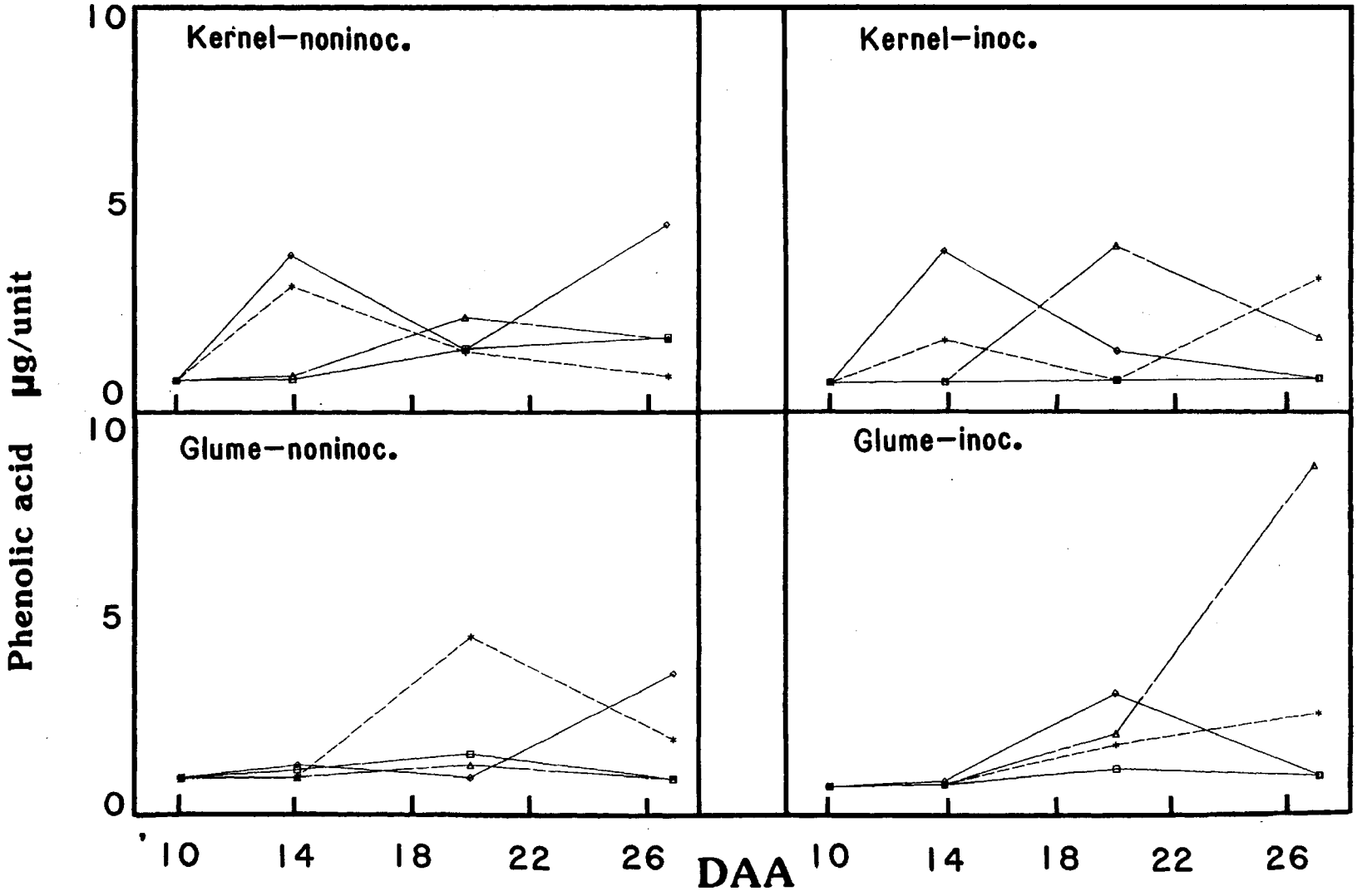


Figure 18. Unknown 3 levels in noninoculated and inoculated grain lots. Code: diamond = I (SC0103-12), square = M (CS3541 and SC0599-6) star = R (SC0630-11), and triangle = (TX2536 and TX412). DAA = days after anthesis.

for noninoculated at 20 DAA. Accumulation of gallic acid in glume tissues was higher in inoculated than in noninoculated cultivars at 20 DAA. Inoculated lots also generally exhibited a more precipitous decrease in gallic acid beginning at day 20 DAA in both glumes (resistance groups H,R,M, and S) and kernels (resistance groups H,M, and S).

Inoculation had a greater affect on p-coumaric acid (Fig. 17) than on gallic acid. In kernels, inoculated H and R groups contained higher p-coumaric acid levels 20 DAA than noninoculated. P-coumaric acid did not change with resistance for the other groups. In glumes, at 20 DAA H cultivar had the lowest content for noninoculated and the highest with inoculation. The S group had the lowest p-coumaric acid content for both treatments.

Inoculation resulted in a diminution of Unknown 3 content in both glumes and kernels of cultivar H at 27 DAA (Fig. 18). The effect of inoculation on Unknown 3 content in the S group was basically opposite. With inoculation, Unknown 3 content in S cultivar markedly increased at 20 DAA in kernels and at 27 DAA in glumes. Unknown 3 content in glumes and kernels of R cultivar increased after 20 DAA with inoculation.

## Discussion

These results suggest that PC and PA accumulation in glumes and kernels of sorghum changes through maturation. These changes were affected by colonization with *F. moniliforme* and by cultivar biochemistry. Certain field observations were interesting in light

of these findings. Inoculation with *F. moniliforme* caused a rapid accumulation of pigments (phenolic compounds) in the glumes of many cultivars, suggesting that PC accumulation could be grossly quantified in the field. Two cultivars which produce the brightest pigments in the glumes with field inoculation are SC0103-12 and SC0630-11, the two most resistant cultivars. Coloration of glumes, however, can be misleading in tan-pigmented plants such as CS3541. Inoculation produces little visible coloration in glumes of this cultivar, which is moderately resistant to SGM.

The increase in gallic acid at 20 DAA (Fig. 16) and the subsequent decline could be explained by two hypotheses. 1) gallic acid may be involved in active defense of the host, decreasing as substrate availability and fungal colonization subside, or 2) gallic acid may also be produced by the pathogen as a product of pathgenises. Whether associated with host defense or fungal pathogensis, compounds like gallic acid which decrease after or near physiological maturity probably reflect a general diminution of biotic activity associated with formation of black layer and the cessation of the influx of nutrients and water into the kernel.

The effects of inoculation on glume biochemistry were very different for PC measured by the Folin test and the total of PA measured by HPLC. The latter is a general assessment of many types of phenolic compounds. With inoculation there appeared to be a greater increase in PC than in PA. This could be interpreted as an indication that compounds other than PA are involved in disease defense or symptoms (Fig. 18).

Compensatory effects among individual compounds probably acted to reduce the effect of inoculation on total PA. Correlation analysis of inoculated lots indicated that Unknown 3 was negatively correlated with percentage germination and positively correlated with ergosterol and leachate levels (Table 7). Diseased kernels of S (susceptible) group cultivars had a noticeable increase in Unknown 3 after day 20 (Fig. 17).

In cortrast, gallic acid content was positively correlated with kernel quality (i.e., negatively correlated with ergosterol level) (Table 7), and p-coumaric and salicylic acids correlated with quality measures in noninoculated grain. In these cases, quality was associated with accumulation of PA. Therefore, these data indicate that infection with *F. moniliforme* causes some PA to decrease, and others to increase. Future investigations of the relation between disease development and PA must anticipate the possibility of compensatory interactions among PA. Future research must involve an assessment of the role of individual PA in disease development.

# CHAPTER VI

## SUMMARY AND DISCUSSION

Both histological and biochemical studies indicate an important role for glumes in the development of SGM. Sections of spikelet tissue showed early colonization of glumes of TX2536, but not of resistant cultivar SC0170-6. Resistance, therefore, was associated with extracellular deposition of pigmented compounds, which could be a product of phenolic compound metabolism (26).

Analysis of phenolic compounds (PC) and phenolic acids (PA) demonstrated that phenolic compound metabolism in glumes changes through maturation, and that changes are affected by disease. Apparently, however, accumulation of particular PA differs for glumes and kernels, and does not occur only in resistant cultivars.

The exact role that PA may play in disease development still remains unclear. Changes in phenol metabolism may be part of active or passive defense mechanisms in resistant cultivars, or a symptom of disease in susceptible cultivars. The increase in Unknown 3 in disease tissue of TX2536 seems to indicate that some PA are elaborated in the last periods of disease expression. The possibility of phenolic compounds being associated not only with resistance, but also with pathogen development, serves to make the effect of disease on any given PA unpredictable. The effects may change with time, cultivar, or pathogen isolate.

The directions that future SGM research should take seem to diverge into either more practical or more basic studies. The

results of this study are based on comparisons of healthy kernels (or spikelets) with field or greenhouse-inoculated kernels. Similar studies on grain produced where natural infection is high could lend practical verification.

For more basic research, a highly coordinated histological and chemical analysis seems to be the logical next step. Histological analysis demonstrated that invasive processes are different between a resistant and susceptible cultivar, and that resistance was associated with deposition of pigmented extracellur material. With controlled inoculation, extracellular deposition could be studied in close conjunction with biochemical analyses. All studies should be done on the same sample (i.e., the same cultivar or set of a few cultivars) to facilitate comparison of results from different laboratories. TX2536 appears to be a good candidate for a standard susceptible check. SC0170-6, as a standard resistant cultivar, would enable comparison with histological results presented in chapter IV. Ideally, the susceptible check should be disease free. This would be achieved most easily in the greenhouse or in growth chambers. The possible effects of artificial (i.e., greenhouse or growth chamber) enviornment on phenolic compound metabolism should affect both resistant and susceptible cultivars alike. The advantage of greater control over disease development, afforded by greenhouse experiments, should outweigh possible disadvantages.

With increased control over inoculation and the world-wide importance of sorghum grain quality, SGM caused by *F. moniliforme* seems to be a good model for basic research on the relation between

phenolic compound metabolism and disease development.

# LITERATURE CITED


1. Afifi, A.A., and Clark, V. Computer-aided Multivariate Analysis. LLP Pub. Belmont, CA 94002. 458 pp.

2. Breuer, L.H. Jr., Dohm, C.K. 1972 Comparative nutritive value of several sorghum grain varieties and hybrids. J. Agr. Food Chem. 20:83-86.

3. Burroughs, R., and Sauer, D.B. 1971. Growth of fungi in sorghum grain stored at high moisture contents. Phytopathology 61:767-772.

4. Byrde, R.J. Fielding, A.H., and Williams, A.H. 1960. The role of oxidized polyphenols in varietial resistance of to brown rot. Page 95 In: Phenolics in Plants and Health and Disease. eds. Pridham, J.B. Pergamon Press. New York.

5. Cantone, F. A., Tuite, T., Bauman, L. F., and Stroshine, R. 1983. Genotypic differences of stored corn kernels in reaction to attack by selected *Aspergillus* and *Pencillium* spp. Phytopathology 73:1250-1255.

6. Castor, L.L. 1977. Seed molding of grain sorghum. Development of high yielding disease and insect resistant sorghum cultivars. Third Ann. Prog. Rept. TAES-US/AID Contract ta-c-1092. Tx. Agric. Exp. St., College Station, Tx.

7. Castor, L.L. 1981. Grain mold histopathology, damage assessment, and resistance screening within *Sorghum bicolor* (L.) Moench lines. Ph.D. Dissertation Texas A&M Univ. College Station 177 pp.

8. Castor, L.L., and Frederiksen, R.A. 1980. Fusarium head blight, occurrence and effects on sorghum yields and grain characteristics in Texas. Plant Dis. 64: 1017-1019.

9. Castor, L.L., and Frederiksen, R.A. 1980. *Fusarium* and *Curvularia* grain mould in Texas. In: Sorghum Diseases, A World Review, Proc. Inter. Workshop Sorghum Diseases, India. Eds. Williams, R.J., Frederiksen, R.A., Mughogho, L.K., and Bengston, G.D. ICRISAT, Patancheru, India.

10. Chester, K. S. 1950. Plant diseases losses: their appraisal and interpretation. Plant Dis. Supl. 193:190-362.

11. Colhoun, J. 1983. Measurement of inoculum per seed and its relation to disease expression. Seed Sci. & Technol. 11:665-671.

12. Davis, J. R., Pavek, J. J., and Corsini, D. L. 1983. A sensitive method for quantifying *Verticillium dalhliae* colonization in plant tissue and evaluating resistance among potato genotypes. Phytopathology 73:1009-1004.

13. Denis, J.C. and Girard, J.C. 1980. Factors affecting the development of sorghum grain moulds in Senegal. In: Sorghum Diseases, A World Review, Proc. Inter. Workshop Sorghum Diseases, India. Eds. Williams, R.J., Frederiksen, R.A., Mughogho, L.K., and Bengston, G.D. ICRISAT, Patanchderu, India.

14. Diener, U.L., Davis, N.D., Morgan-Jones, G., and Wagener, R.E. 1979. Toxigenicity of fungi isolated from grain sorghum. Phytopathology 69:1026 (Abstract).

15. Frederiksen, R. A., Castor, L. L., and Rosenow, D. T. 1982. Grain mold, small seed and head blight: the *Fusarium* connection in sorghum. Proc. Thirty-seventh Ann. Corn Sorghum Ind. Res. Conf. 37:26-36.

16. Foudin, A.S., and Calvert, O.H. 1982. *Schizophyllum commune* as a possible mycotoxin producer in association with sorghum. Mycologia 74:1041-1043.

17. Frezzi, M.J., Parodi, R.A. and Scantamburlo, J.L. 1970. Fallas de germinacion en sorgo y sus causes. IDIA 272:45-57.

18. Gaudet, D. A., and Kokko, E. G. 1984. Application of scanning electron microscopy to paraffin-embedded plant tissues to study invasive processes of plant pathogenic fungi. Phytopathology 74:1078-1080.

19. Glueck, J.A. 1979. Identification and characterization of *Sorghum bicolor* lines with resistance to preharvest grain deterioration. Ph.D. Dissertation. Texas A&M Univ. 147 pp.

20. Glueck, J.A., and Rooney, L.W. 1978. Chemistry and structure of grain in relation to mold resistance. In: Sorghum Diseases, A Would Review, Proc. Inter. Workshop Sorghum Diseases, Eds. Williams, R.J., Frederiksen, R.A., Mughogho, L.K., and Bengston, G.D. ICRISAT, Patanchderu, India.

21. Gopinath, A. and Shetty, H. S. 1985. Occurrence and location of *Fusarium* species in Indian sorghum seed. Seed Sci. & Technol. 13:521-528.

22. Gray, E., Lacefield, G.D. and Lowe, J.A. 1971. Head mold on grain sorghum. Plant Dis. Rep. 55:337-339.

24. Hahn. D.H., Faubion, J.M., and Rooney, L.W. 1983. Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. Cereal Chem. 60:255-259.

23. Hahn, D.H, Rooney, L.w., and Earp, C.F. 1984. Tannins and phenols of sorghum. Cereal Foods World 29: 776-779.

25. Harris, H.B., and Burns, R.E. 1973. Relationship between tannin content of sorghum grain and preharvest seed molding. Agron. J. 65:902-904.

26. Heath, M. C. 1980. Reactions of nonsuscepts to fungal pathogens. Ann. Rev. Phytopathol. 1980. 18:211-236.

27. Hepburn, H. A., Powell, A. A., and Mathews, S. 1984. Problems associated with the routine application of electrical conductivity measurements of individual seeds in the germination testing of peas. Seed Sci. & Technol. 12:403-413.

28. Hepperly, P.R. 1983. Chemical control of seedborne fungi of sorghum: seed quality and germination in Puerto Rico. Plant Dis. 66:902-904.

29. Lazarovits, G., Higgins, V. J. 1976. Ultrastructure of susceptible, resistant, and immune reactions of tomato to races of *Cladosporium falvum*. Can. J. Bot. 54:235-249.

30. Lichtenwalner, R.E., Rooney, L.W., Glueck, J.A., and Reyes, L. 1979. Nutritive value of "weathered" sorghum grain for ruminants. J. Animal Sci. 49:183-180.

31. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.

32. Neergaard, P. 1977. pp. 362-365. In: Seed Pathology. The Macmillan Press, Ltd. London. 1187 pp.

33. Raghunathan, A.N. 1969. Control of internal fungi of sorghum by fumigation. Chem. J. Stored Prod. Res. 5(4):389-392.

34. Rao, K.N., and Williams, R.J. 1980. Screening for sorghum grain mold resistance at ICRISAT. In: Sorghum Diseases, A World Review, Proc. Inter. Workshop Sorghum Diseases, India. Eds. Williams, R.J., Frederiksen, R.A., Mughogho, L.K., and Bengston, G.D. ICRISAT, Patanchderu, India.

35. Ross, W. M., and Kofoid, K. D. 1978. Determining 1000-seed weight in grain sorghum. Crop Sci. 78:507-508.

36. Rukmini and Bhat, Ramesh, V. 1978. Occurrence of T-2 toxin in *Fusarium*-infested sorghum from India. J. Agric. and Food Chem. 27:647-649.

37. Sanders, E.H. 1955. Developmental morphology of the kernel in grain sorghum. Cereal Chem. 32:12-25.

38. Sass, J.E. 1940. Elements of Botanical Microtechnique. McGraw-Hill, Inc. New York. 320 pp.

39. Schans, J., Mills, J. T., and Van Caeseele, L. 1982. Fluorescence microscopy of rapeseeds invaded by fungi. Phytopathology 72:1528-1586.

40. Seitz, L.M., Mohr, H.E., Burroughs, R., and Glueck, J.A. 1983. Preharvest fungal invasion of sorghum grain. Cereal Chem. 60:127-130.

41. Seitz, L. M., Sauer, D. B., Burroughs, R., Mohr, H. E., and Hubbard, J. D. 1979. Ergosterol as a measure of fungal growth. Phytopathology 69:1202.

42. Seitz, L.M., Sauer, D.B., Mohr, H.E., and Burroughs, R. 1975. Weathered grain sorghum: natural occurrence of alternariols and storability of the grain. Phytopathology 65:1259-1263.

43. Singh, D. 1983. Histopathology of some seed-borne infections: A review of recent investigations. Seed Sci. & Technol. 11:651-663.

44. Sullins, R.D., and Rooney, L.W. 1975. Light and scanning electron microscopy studies of waxy and nonwaxy endosperm sorghum. Cereal Chem. 52:361-366.

45. Tarr, S. A. J. 1962. Diseases of Sorghum, Sudan Grass and Broomcorn. Comm. Myc. Inst., Kew, Surrey. 380 pp.

46. Taylor, J. D. 1970. A quantitative estimation of the infection of bean seed with *Pseudomonas phaseolicola* (Burkh.) Dowson. Ann. Appl. Biol. 66:29-36.

47. Van Caeseele, L., Mills, J. T., Summer, M., and Gillespie, R. 1981. Cytology of mucilage production in the seed coat of Candlecanola *Brassica campestris*. Can. J. Bot. 59:292-300.

48. Verdcourd, B. 1952. The effect of certain phenolic compounds on the germination and growth of microfungi. Mycologia 44: 377-386.

49. Vidhyasekaran, P. 1983. Control of *Fusarium moniliforme* infection in sorghum seed. Seed Sci. and Technol. 11:435-439.

50. Von Oppem, M., and Jambunathan, R. 1978. Consumer preferences for cryptic and evident quality characters of sorghum and millet. Diamond Jubilee Sci. Session Nat. Inst. Nutri., Hyderabad, India.

51. Williams, R.J., and Rao, K.N. 1980. The international sorghum grain mold nursery, moulds in Senegal. In: Sorghum Diseases, A World Review, Proc. Inter. Workshop Sorghum Diseases, India. Eds. Williams, R.J., Frederiksen, R.A., Mughogho, L.K., and Bengston, G.D. ICRISAT, Patanchderu, India.

52. Williams, R.J., and Rao, K.N. 1981. A review of sorghum grain moulds. Trop. Pest Manag. 27:200-211.