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Assessment of multiple- and single-factor stress impacts on corn

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Abstract

Impacts of abiotic stress on growth and development of corn (*Zea mays* L.) plants have typically been investigated in isolation from influences of other stress factors. This approach usually results in both lost information and overly simplistic conclusions concerning the effects of single stress factors on the physiological response of corn. In reality, the impact of an individual stress on corn growth depends on the intensity and possible interactions among other stress factors. A superior approach for investigating the effect of a single stress would consider the effect of other stress factors using multivariable modeling. We used a mixed-model framework to estimate the relative impacts of soil physical stresses for a multiple-location-and-year experiment involving the response of corn to a range of tillage systems from no-till to conventional fall tillage. Covariates were treated as fixed effects in a multivariable mixed model; effects due to location, year, replication, and their interactions were treated as random effects. Relationships among soil physical factors that limited corn root and shoot growth in the field were identified. We present the underlying analysis in detail, along with several examples where interactions were identified among soil physical stress factors and early corn growth in specific tillage systems. In an example used to illustrate the model, high soil water contents (>0.30 $m^3\ m^{-3}$) during the first 4 weeks after seeding were more detrimental to no-till corn root and shoot development at low (19 °C) mean soil temperatures. In contrast, high soil water contents benefited no-till corn root and shoot growth at warm (23 °C) soil temperatures. Therefore, when corn seedlings encountered multiple stresses of low soil temperatures and high water contents, reductions in corn root and shoot growth were more likely to occur in no-till relative to conventional tillage. Future assessment of multiple stress impacts on corn development should employ multivariable statistical models more routinely to improve our understanding of interactions among the various stresses and also to help guide efforts in stress amelioration.

Keywords: *Zea mays* L.; Corn; No-till; Soil physical stresses; Multiple stress analysis; Mixed model; SAS PROC MIXED

1. Introduction

The productivity of corn, as with other crops, is constrained by the relative plasticity of the response to environmental stresses. The response of plant plasticity to abiotic stresses has recently been described as being either morphological or phenotypic in character (Duncan, 2000). Scientific understanding of the response of corn to particular short- and long-term stresses is limited even further by interactions with other stresses in the crop. Without considering biotic stress factors (such as insects and diseases), corn

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plants might simultaneously be exposed to combinations of abiotic stresses such as low air temperatures and saturated soils, high bulk density soils and high temperatures, nutrient deficiencies and droughty periods, or plant phytotoxins and low temperatures.

Although it is important to understand the genetic mechanisms involved in response to a specific stress, such understanding about individual stress factors will be limited unless crop physiologists can better understand the response of corn plants to multiple environmental stresses. The response of corn to multiple environmental stress factors may interact with genetics, the stage of development when the multiple stresses exert their impact, and the relative intensities (length and duration) of the multiple stresses.

To understand the response of corn to stress, crop physiologists usually expose plants to a single stress factor. The response of corn to a single stress can be complex depending on the intensity of stress, the duration of stress, and the stage of corn development when the stress is imposed. Impact of any single stress on the yield of grain corn depends on other stress factors encountered during corn development. The complexity in corn response to a single stress factor is described by Benoit et al. (1990) and Lizaso and Ritchie (1997). Benoit et al. (1990) observed in a field environment that the response of corn leaf area to variations in day/night temperatures depended on the initial size of the corn plants. They also observed that the optimum temperatures at night for corn growth depended on the maximum temperature during the previous day. Lizaso and Ritchie (1997) demonstrated that corn leaf area expansion was reduced more when the soil was saturated with water early (V3) versus late (V6), although the effect of saturation on corn growth at the later stage was more likely to be associated with premature leaf senescence.

Although stress factors are usually investigated with assumptions of independence from one another, some studies provide strong evidence that much information is lost when interactions among stress factors are ignored. Multiple stress factors have been investigated in corn (Dwyer et al., 2000; Schneider and Gupta, 1985; Tollenaar et al., 1997; Veen and Boone, 1990). These studies have been helpful in improving understanding of the occasionally additive, but frequently synergistic, impact of multiple stresses. For instance, Dwyer et al. (2000) observed poor stand establishment of corn with the combination of cool soil temperatures (<12.5 °C) and high soil water content. Schneider and Gupta (1985) reported that corn emerged slower in low soil temperature conditions when soil water content in the seed zone was below field capacity. Tollenaar et al. (1997) reported that individual stresses of low soil N and weed competition reduced aboveground dry matter at silking and maturity by 20%, compared to a 55% reduction with the combination of both stresses. Veen and Boone (1990) observed in a laboratory experiment that the combined stresses of high mechanical resistance and low soil water potentials had an additive effect on the growth rate of seminal corn roots. Corn response to treatment variables involving a range of soil or plant stress incidence in field experiments may be better understood if interactions among specific stress factors are anticipated during both the design of the experiment and the subsequent analysis.

2. Multiple stress analysis

Establishing field experiments to determine the response of corn to a single stress, or multiple stresses, are both costly and complex. Most physiologists defer to controlled-environment indoor experiments when they investigate the response of crops to multiple stresses. Even when the intensity of multiple stresses is adequately documented in experiments involving crop response to stresses, interpretation of cause and effect relationships are often too limited by the use of single variable models.

Statistical tools are readily available to help investigate interactions among stress factors impacting corn growth in natural environments. With sufficient data on possible individual stress factors impacting outdoor corn growth, these tools may enable more understanding of multiple stress impacts than that gained from experiments in controlled conditions or from existing crop growth models. Generalized linear models can be used to model multiple fixed (i.e., stress factors) and random (i.e., location, year, replication) effects (Neter et al., 1989; McCullagh and Nelder, 1989). When interactions are present, this multivariate approach can identify and quantify their existence (Hooker, 2000).

3. Stress factors affecting no-till corn

Variable seedbed conditions in no-till have been reported to be a major cause of inconsistent corn yields; no-till seedbeds have been often characterized by soil physical conditions which are unfavorable for early corn growth (e.g., Griffith et al., 1988; Kaspar et al., 1987; Vyn et al., 1994). These problems are exacerbated by cool and wet weather conditions during the seeding season, and especially on poorly drained soils. The cause and effect relationship for yield differences among tillage systems is not always apparent. In addition, relatively poor early growth does not necessarily result in lower yields at harvest. Early corn growth, however, is dependent on interactions among soil temperature, soil water, oxygen status and mechanical impedance (Letey, 1985). Tillage affects these four physical factors via the mechanical effects on soil structure (i.e., aggregate size distribution, density, and porosity) and surface residue cover (Fig. 1). The short-term impacts of tillage operations on these soil properties can exert major impacts on the early growth response of corn in the first weeks and months following seeding (Kaspar et al., 1987; Opoku et al., 1997; Vyn and Raimbault, 1993).

Because many soil physical factors differ across tillage treatments, drawing conclusions based on simple effects of crop response to one factor across different tillage systems is not plausible (Cruse et al., 1982). In a recent series of tillage experiments for corn following soybeans (*Glycine max* L.), we had the combined objectives of: (1) identifying soil factors responsible for reduced early corn performance and grain yield in alternative tillage systems; and (2) identification and quantification of soil physical factors (and their interactions) affecting the potential of grain corn yields through early development, root growth, and shoot dry matter accumulation. Once relationships among soil physical factors become more apparent in alternative tillage systems, the potential to modify those systems to produce corn yields consistently equal (if possible) to those after conventional tillage (i.e., fall moldboard plowing) should be improved. Although experimental details are outlined in Vyn and Swanton (1998) and Hooker and Vyn (2000), the main intent here is to demonstrate the utility of multivariate analyses on a complex data set involving measurements of several soil physical stress factors and associated corn response, and to give guidance on how to implement a mixed model analysis in a commonly used software package (SAS, 1995).

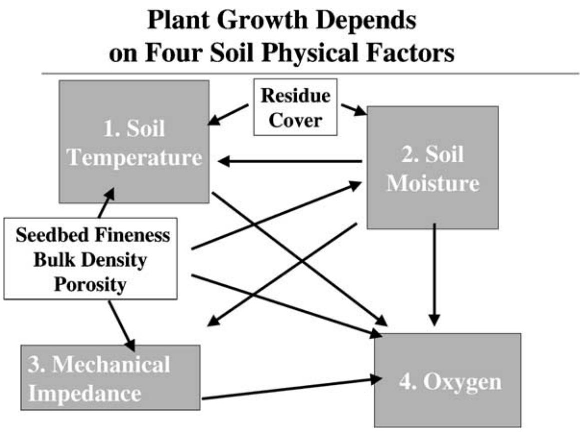

Fig. 1. An illustration of tillage system impacts on four soil physical factors affecting corn growth, and the consequent interrelationships.

4. Materials and methods

The experiments were conducted from 1994 to 1996 on two private farms in southern Ontario, Canada. One farm was near Alvinston (42°52′N, 81°53′W) on a Clyde Clay soil type (14% sand, 42% silt, and 44% clay) with 3.9% organic matter and a pH of 7.3. The other farm was near Fingal (42°40′N, 81°18′W) on a Toledo Silty Clay Loam soil type (18% sand, 52% silt, and 30% clay) with 3.8% organic matter and a pH of 7.1. In 1993, before the experiments were established, each site was split with one half cropped to soybean and the other to corn. In the autumn of 1993, eight tillage systems were replicated four times in a randomized complete block design after each crop. In each of the 3 years, tillage treatments for corn were imposed following soybeans for fields in a corn–soybean rotation, and each corn experiment within a location was conducted in adjacent areas in the same field. Furthermore, the tillage treatments imposed on plots in 1994 were repeated on the same plots in 1996.

The varying degrees of tillage intensity among tillage systems created a diversity of seedbed environments; however, only three of those tillage systems will be outlined in this paper: (1) fall moldboard plow (15 cm deep), plus two passes of spring secondary tillage with a heavy-duty field cultivator which operated at 5–7 cm depth (MB); (2) fall zone-tillage using a Trans-Till (Row-tech, Snover, MI) (17 cm deep) in the fall plus no-till (coulter) in the spring (FZT); and (3) no-till (coulter) seeding only with tillage in the immediate row area by using one planter-mounted 5 cm wide fluted coulter (NT).

Corn was seeded at a rate of 74 000 seeds ha^{-1} with a John Deere 7000 Conservation planter (John Deere, Moline, IL). Pioneer 3960 (Pioneer Hi-Bred, Chatham, Ont., Canada) was planted at both sites during 1994 and 1995, and Pioneer 3769 was used as a comparable replacement in 1996. Starter fertilizer (11-52-0) was applied at a rate of 125 kg ha^{-1}, and placed 5 cm below and away from the seed. Nitrogen was applied 8 cm deep between crop rows in the form of urea–NH_4NO_3 at 150 kg N ha^{-1} after seeding. Weeds were controlled throughout the study.

4.1. Soil measurements

Both soil temperature and soil water content were measured across three or four replications of the plots at each site. Soil temperature was recorded hourly with an automatic digital recorder. Two copper–constantan thermocouples were installed 5 cm beneath the row in each plot. Mean daily soil temperature was calculated by averaging the daily minimum and maximum temperatures. In-row soil water contents (volumetric) were measured in the surface 10 cm in each tillage system following seeding using time domain reflectometry (TDR) (Topp et al., 1980). At least eight measurements of soil water content were conducted per plot in the first 4 weeks after seeding using TDR. Mean soil water content and temperature values for growth analysis were calculated by averaging the measurements from seeding to 4 weeks after seeding. Mean air-filled porosity was estimated using these soil water content values, together with bulk density data from the surface 10 cm and with the assumption of a particle density of 2.65 Mg m^{-3}.

4.2. Corn measurements

In two-row segments in the middle of each plot, 10 consecutive plants were selected for development and growth monitoring. In these same rows, entire root systems of another 10 corn seedlings per plot were excavated when 5–7 leaf tips per plant were visible. The roots were washed, and analyzed for root length and width at the Root Image Processing Laboratory, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI. About 6 weeks after seeding, or when approximately 12 leaf tips were visible (approximately V6 stage), two sampling areas of approximately 1.5 m^2 were selected to represent each plot for shoot dry matter. Samples were oven-dried for at least 3 days at 80 °C and weighed. Corn grain yield was also determined, but was not the variable of interest presented in this paper; however, the persistence of one or more stress effects through harvest were evident in no-till because corn yields were approximately 10% lower than those after conventional tillage (Hooker, 2000).

4.3. Multivariable mixed models

One approach to modeling relationships between a soil physical variable and a crop response variable among tillage system treatments is to express the dependent variable as a simple linear function of each soil physical variable (i.e., covariate). Such an approach, however, assumes that the response curve of each covariate is similar across treatments. This is one assumption in conventional analysis of covariance (Lindman, 1992). We tested the assumption by investigating the crop response relationship with a covariate (i.e., soil physical factor) across tillage systems. Moreover, when two soil physical variables were investigated, interactions between the soil physical variables were examined across tillage system treatments.

Relationships among soil physical variables, tillage system, and corn response variables were modeled by choosing two soil physical variables (e.g., mean soil water content and mean soil temperature) for each crop response variable (e.g., shoot dry matter). The soil physical variables and tillage treatments and their interactions were treated as fixed variables. The effects of location, year, replication, and their interactions with other random effects or fixed effects were treated as random because inferences were not made on specific locations, years, and replications but from a "population" of locations and years. Although imposing the same tillage treatments on the same plots for corn in both 1994 and 1996 as part of the corn–soybean rotation may have the potential to account for some of the within-plot variability, we assumed that the performance of a tillage system within a plot in 1996, for example, was not affected by the tillage treatment imposed in 1994. Tillage history within a 3-year time frame has had no impact on corn response to no-till versus other tillage systems on clay loam soils with similar levels of organic matter in Ontario (T.J. Vyn, unpublished data).

The full model has the following initial form before non-significant effects are removed and pooled into other effects of the model (quadratic effects of the fixed terms were excluded for clarity):

$$\begin{aligned}Y_{ijkm} = {} & l_i + y_j + (ly)_{ij} + r_{(ij)k} + \tau_m + (l\tau)_{im} + (y\tau)_{jm} \\ & + (r\tau)_{(ij)km} + (ly\tau)_{ijm} + \alpha + \beta_1 \cdot X_{1ijkm} \\ & + \beta_2 \cdot X_{2ijkm} + \beta_{12} \cdot (X_{1ijkm} X_{2ijkm}) + \alpha_m \\ & + (\beta_{1m} \cdot X_{1m}) + (\beta_{2m} \cdot X_{2m}) \\ & + \beta_{12m} \cdot (X_{1m} \cdot X_{2m}) + \varepsilon_{ijkm}\end{aligned}$$

where Y_{ijkm} represents a specific crop response as observed at the ith location, in the jth year, in the kth replication in tillage system m. The random effects terms are as follows: let l_i represent location i, y_j represent year j, $r_{k(i,j)}$ represent replication k within location i and year j, $(ly)_{ij}$ represent the interaction between location i and year j, $(r\tau)_{(ij)km}$ represent the interaction between replication k and tillage m within location i in year j, $(l\tau)_{im}$ represent the interaction between location i and tillage m, $(y\tau)_{jm}$ represent the interaction between year j and tillage m, and finally, $(ly\tau)_{ijm}$ represent a 3-way interaction among location i, year j, and tillage m. Let the fixed-effects terms α, τ, $\beta_1 \cdot X_{1ijk}$, $\beta_2 \cdot X_{2ijk}$, and $\beta_{12} \cdot (X_{1ijk} X_{2ijk})$ represent the average dependence of two covariates, X_1 and X_2, across tillage systems τ. This includes the intercept α, the main effect or tillage system τ, the slope β_1 for covariate X_1, the β_2 slope for covariate X_2, and the coefficient β_{12} for the interaction between covariates X_1 and X_2. The last terms in the full model α_m, $\beta_{1m} \cdot X_{1ijkm}$, $\beta_{2m} \cdot X_{2ijkm}$, and $\beta_{12m} \cdot X_{1ijkm} \cdot X_{2ijkm}$ represents the average dependence and unique characteristics of tillage system m. The ε_{ijkm} term represents the random variation in crop response that is not accounted for in the model. It is assumed that location l_i, year y_j, replication $r_{k(ij)}$, and ε_{ijklm} are all random quantities, each from a normal distribution with a mean deviation from the population equal to zero.

The model accounts for realistic situations where the covariates describe the average crop response across tillage systems. This is the standard analysis of covariance, which assumes that the slopes or curves of the covariates, relative to the dependent variable, are homogeneous across the categorical variable (i.e., tillage systems). However, the model also accounts for the fact that the crop response relationship between the covariates may depend on the tillage system. In other words, the slopes or curves for each covariate may be different among tillage systems. Therefore, the model accounts for interactions between specific soil factors (i.e., the covariates).

Relationships among soil factors (i.e., covariates), tillage system, and a crop response variable were identified by fitting the full model using the PROC MIXED procedure in SAS, version 6.12 (Littell et al., 1996; SAS, 1995). A modified Shapiro–Wilk W-test using Royston's algorithm tested for normality, and also determined if transformations were necessary. Appropriate transformations were performed on the crop response variable to conform to the assumptions of ANOVA. A listing of the SAS code for the analysis of root surface area (ROOT) as affected by mean soil temperature (TEMP) and mean soil water content (WATER), for example, is provided in Fig. 2. For illustration purposes, quadratic effects of the covariates were tested but omitted from the example because they were deemed insignificant to the relationship ($P > 0.10$). The RANDOM statement specified the random effects of location, year, and replication.

Before testing the fixed effects, individual random effects in the model (i.e., location, year, replication, and their associated interactions) were tested to determine their effect on reducing the error variance. Apparently, the test of significance for random effects is rather conservative in PROC MIXED; a $P > 0.35$ was considered sufficient evidence to exclude the effect from the model (personal communication, W. Sears, Ashton Statistical Laboratory, Univ. of Guelph, Guelph, Ont., Canada). Furthermore, random effects that are poorly estimated have 95% confidence limits that may range from several orders of magnitude. An example of an SAS Output is presented in Fig. 3, showing the SITE × YEAR effect as the only significant random effect in the model; the other random

```
DATA ALL; * entire dataset across year, location, replication, and tillage;
* year = 3 levels;
* location or site = 2 levels;
rep or replication = 4 levels;
* tillage = 8 levels (3 illustrated here);
       MB = Moldboard plow, FZT = Fall zone-till, NT = No-till


PROC MIXED NOCLPRINT NOITPRINT CL COVTEST DATA=ALL;
CLASS YEAR SITE REP TILLAGE;
```

Initial "testing model" to produce ANOVA; includes fixed effects and their interactions. Delete this model when determining parameter estimates with the "estimation model" (below).

```
MODEL ROOT=TILLAGE TEMP WATER TEMP*WATER TEMP*TILLAGE WATER*TILLAGE TEMP*WATER*TILLAGE/P;
```

Subsequent "estimation model" to determine parameter estimates and their significance in tillage system treatments; this model presumes the same fixed effects and significant random effects as determined in the above "testing model", but main covariate effects and their interaction are not included. Delete this model when using "testing model".

```
MODEL ROOT=TILLAGE TEMP*TILLAGE WATER*TILLAGE TEMP*WATER*TILLAGE/NOINT SOLUTION;
```

NOINT suppresses the constant term in model for direct output of parameter estimates

RANDOM statement initially includes all random effects. Important ones are determined in the testing model; these are retained in the "estimation model".

```
RANDOM SITE YEAR SITE*YEAR REP(SITE YEAR) REP*TILLAGE SITE*TILLAGE YEAR*TILLAGE
       SITE*YEAR*TILLAGE;
```

Estimate Statements for comparing parameter estimates among tillage system treatments

```
ESTIMATE 'MB,FZT VS NT INTERCEPT'         TILLAGE  1  1 -2/DIVISOR=2;
ESTIMATE 'MB VS FZT INTERCEPT'            TILLAGE  1 -1;

ESTIMATE 'MB,FZT VS NT SLOPE TEMP'        TILLAGE*TEMP   1  1 -2/DIVISOR=2;
ESTIMATE 'MB VS FZT SLOPE TEMP '          TILLAGE*TEMP   1 -1;

ESTIMATE 'MB,FZT VS NT SLOPE WATER'       TILLAGE*WATER   1  1 -2/DIVISOR=2;
ESTIMATE 'MB VS FZT SLOPE WATER '         TILLAGE*WATER   1 -1;

ESTIMATE 'MB,FZT VS NT TEMP*WATER'        TILLAGE*TEMP*WATER   1  1 -2/DIVISOR=2;
ESTIMATE 'MB VS FZT TEMP*WATER '          TILLAGE*TEMP*WATER   1 -1;

RUN;
```

Fig. 2. SAS code for the analysis of corn root growth as affected by the mean soil temperature (TEMP) and soil water content (WATER) covariates in the MB, FZT and NT tillage systems.

terms could either not be estimated, or were deemed non-significant at $P > 0.35$. Once a random effect is removed from the model, the degrees of freedom associated with the effect are partitioned to the residual error variance.

Historically, the random effect of the interaction term between replication and tillage ($r\tau$) has been pooled with the overall error term; this pooling reduces complications for constructing ANOVA tables and for using the correct error terms and standard errors for testing other effects. PROC MIXED, however, obviates both of these complications. Although the interaction between replication and tillage should, on average, be unimportant or non-significant with proper randomization, the effect should be explicitly tested for significance. If indeed the effect is significant or important in the development of the potential error structure in PROC MIXED, then retaining the term would improve the testing of fixed effects (personal communication, William C. Sears, Ashton Statistical Laboratory, Univ. of Guelph, Guelph, Ontario, Canada).

From Testing Model

```
                    Tests of Fixed Effects (ANOVA)

Source               NDF   DDF   Type III F   Pr > F

TILLAGE                3     3        2.92    0.2013
TEMP                   1    26       10.36    0.0034
WATER                  1    26        8.97    0.0060
TEMP*WATER             1    26        8.37    0.0076
TEMP*TILLAGE           3    26        2.87    0.0559
WATER*TILLAGE          3    26        3.28    0.0369
TEMP*WATER*TILLAGE     3    26        3.19    0.0401
```

Interaction between soil temperature and water content; different across tillage systems

From Testing Model

```
                    Covariance Parameter Estimates (REML)

Cov Parm            Estimate     Std Error      Z   Pr > |Z| Alpha    Lower    Upper

SITE*YEAR           0.35394786   0.22950883  1.54    0.1230   0.05   0.1355   2.2728
REP(SITE YEAR)      0.00000000
REP*TILLAGE         0.00000000            .     .         .      .        .        .
YEAR*TILLAGE        0.00000000
SITE*TILLAGE        0.00000000            .     .         .      .        .        .
SITE*YEAR*TILLAGE   0.00000000            .     .         .      .        .        .
Residual            0.04573000   0.00888195  5.15  2.62E-07   0.05   0.0323   0.0697
```

Estimation of Random Effects; Year*Site Significant

From Estimation Model

```
                    Solution for Fixed Effects

Effect              TILLAGE     Estimate      Std Error      DF      t      Pr > |t|

TILLAGE                MB      4.56569638    5.12279866       3    0.89     0.4385
TILLAGE               FZT     10.64504221    6.46664933       3    1.65     0.1983
TILLAGE                NT     29.16723949    7.37321894       3    3.96     0.0288
```

Intercept in Moldboard Plow (MB), Fall zone-till (FZT), and No-till (NT)

```
TEMP*TILLAGE           MB     -0.11591571    0.23380948      26   -0.50     0.6242
TEMP*TILLAGE          FZT     -0.40895938    0.30062186      26   -1.36     0.1854
TEMP*TILLAGE           NT     -1.24085073    0.33843662      26   -3.67     0.0011
```

Soil temperature slope in MB, FZT, and NT

```
WATER*TILLAGE          MB     -2.45090631   15.89185748      26   -0.15     0.8786
WATER*TILLAGE         FZT    -27.59769428   21.74518362      26   -1.27     0.2156
WATER*TILLAGE          NT    -86.78112827   23.75498207      26   -3.65     0.0011
```

Water content slope in MB, FZT, and NT

```
TEMP*WATER*TILLAGE     MB      0.04509769    0.72435851      26    0.06     0.9508
TEMP*WATER*TILLAGE    FZT      1.23457546    1.01174038      26    1.22     0.2333
TEMP*WATER*TILLAGE     NT      3.87719678    1.09408272      26    3.54     0.0015
```

Soil temperature * Water content interaction in MB, FZT, and NT

```
                    ESTIMATE Statement Results

Parameter                  Estimate      Std Error    DF       t    Pr > |t|

MB+FZT vs NT intercept   -21.56187019    7.97510355    3    -2.70    0.0736
MB vs FZT    intercept    -6.07934583    7.56293153    3    -0.80    0.4803
MB+FZT vs NT slope TEMP    0.97841318    0.36812134   26     2.66    0.0133
MB vs FZT    slope TEMP    0.29304367    0.35214458   26     0.83    0.4129
MB+FZT vs NT  slope WATER 71.75682798   26.02762807   26     2.76    0.0105
MB vs FZT    slope WATER  25.14678797   24.63384837   26     1.02    0.3167
MB+FZT vs NT  TEMP*WATER  -3.23736021    1.20221397   26    -2.69    0.0122
MB vs FZT    TEMP*WATER   -1.18947777    1.14549497   26    -1.04    0.3086
```

Contrasts show MB and FZT similar; but NT different slopes and interaction

Fig. 3. An abbreviated SAS Output from the SAS code in Fig. 2; the ANOVA, parameter estimates and contrast statements from the analysis of corn root growth as affected by mean soil temperature (TEMP) and soil water content (WATER) across three tillage system treatments.

Once the insignificant random effects were removed from the model, relationships among soil physical factors, tillage system, and a corn response variable were determined using ANOVA. Significant linear and/or quadratic relations of two soil physical factors (i.e., covariates) to the dependent variable were investigated. Because quadratic effects are important in many biological processes, a P value of 0.10 was used as a level of significance for retaining quadratic effects in the model. Quadratic effects were always tested and prioritized before linear effects. After quadratic effects were investigated, the first test was to determine whether the crop response to either or both soil physical variables interacted with each other and whether this interaction depended on the tillage system (i.e., a 3-way interaction). If this 3-way interaction was significant ($P < 0.05$), then strong evidence exists that the crop response curves differ between the two covariates, and that this difference was affected by the tillage system. If the 3-way interaction among both covariates and tillage was not significant, then a second test was performed to examine 2-way interactions of whether the crop response to either covariate was different across tillage systems. If none of the covariates interacted with tillage, then little evidence exists that crop response curves were affected by tillage. A third test of an interaction between the covariates (i.e., averaged across tillage systems) should then be examined, followed by a fourth test for non-zero slopes for each covariate. Finally, if even the F-test for a non-zero slope of a covariate is not significant ($P > 0.05$), then little evidence exists of a relationship between the crop response variable and the covariate.

Parameter estimates for modeling response curves for individual tillage systems were determined using the same terms in the MODEL and RANDOM statements as in the model for testing the significance of relationships, but without the simple effects of each covariate and the interaction between each covariate in the MODEL statement (see second model statement in Fig. 2). However, if the testing model shows little evidence that the covariates interact with tillage, then the main effects of each covariate and their interaction should be retained, and the main tillage effect and the interactions with tillage removed from the MODEL statement. In either case, the NOINT option should be used to suppress the automatic inclusion of a constant term in the model, so redundancy is avoided with other predictors. The initial testing model could be used to determine parameter estimates, but then the intercept and slope for each covariate and tillage system would need to be adjusted relative to the mean of the covariate or the constant term in the model. The absence of these terms alters the sums of squares of the model; therefore, the ANOVA that is consequently produced should be ignored.

A three-dimensional response surface was plotted for each tillage system when significant differences among tillage systems were detected ($P < 0.05$). If a response was different across tillage systems, the parameter estimates were tested for differences among other tillage systems using estimate and contrast statements. Curves from tillage systems with a similar response ($P > 0.05$) were pooled into a dummy tillage system variable, and then re-analyzed to determine parameter estimates. Reducing the number of response surfaces across tillage systems simplified the interpretation of relationships.

For presentation purposes, each response surface was "sliced" into three two-dimensional sections across one independent axis for comparing tillage systems (Fig. 4). A range of values for each covariate was carefully chosen to ensure that the values in each tillage system were within the experimental region. A conservative range was defined as one standard deviation from the mean in each tillage system across the entire dataset of six location-years. Two of the slices were derived from the extremes of this independent axis, while one slice represented the middle value on the axis. The original three-dimensional surface could be visualized for each tillage system with visual integration of all three slices. The illustrations aided the interpretation, especially when there was a differential response among the tillage systems.

The multivariable analysis was compared to an analysis using multiple stepwise regression procedures. Relationships between the corn response variables and soil physical factors, plus interactions between those factors, were performed using PROC REG (SAS) using stepwise procedures. Dummy variables were used in PROC REG to test the effects of tillage on these relationships, as described by Bowerman and O'Connell (1990).

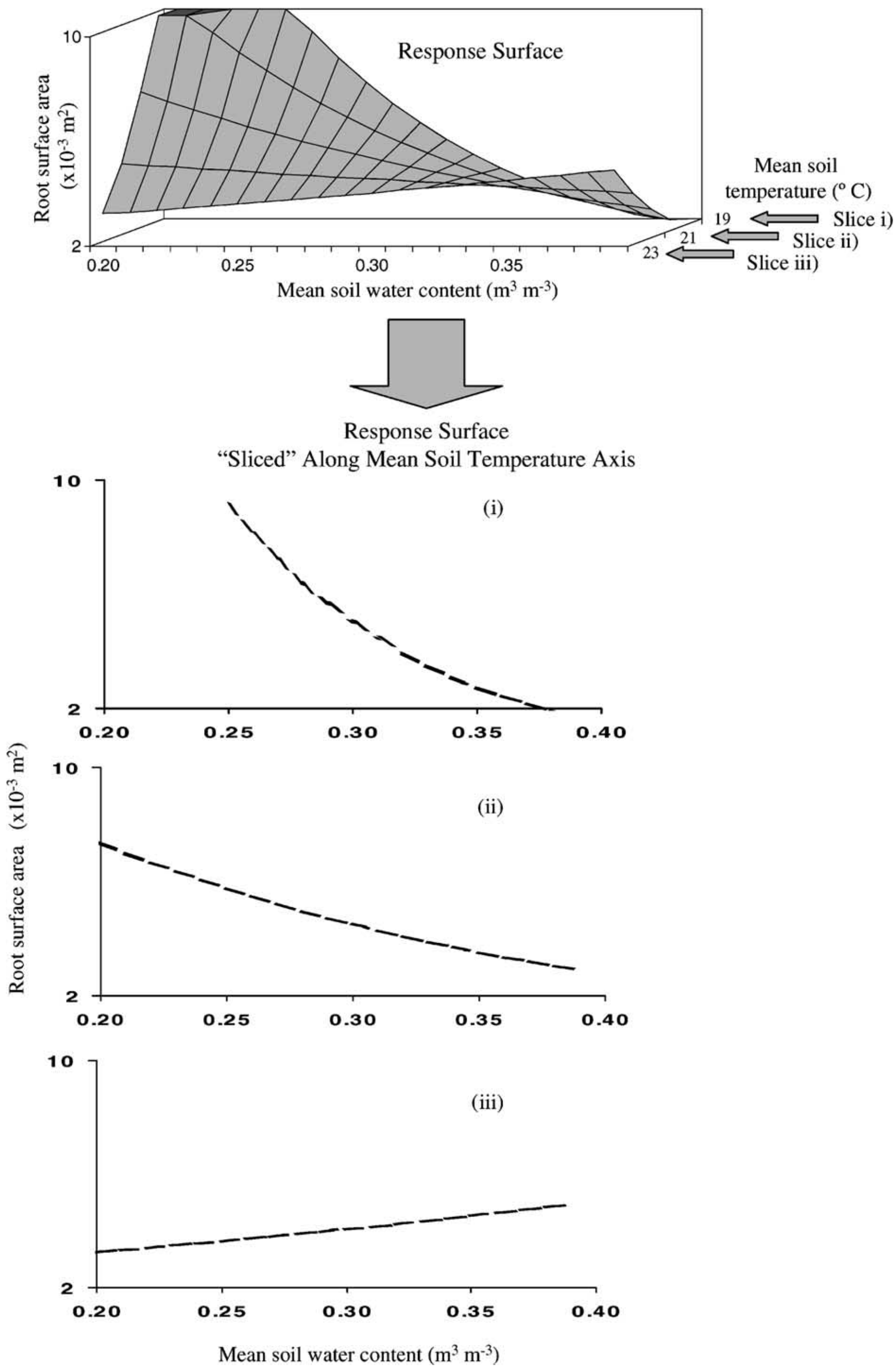

Fig. 4. Root surface area response of corn seedlings at the 6th leaf tip stage in NT to mean soil temperature and soil water content 4 weeks after seeding: conceptual three-dimensional response surface illustrated as three two-dimensional response surface ''slices'' along one independent axis (soil temperature). (i) Mean soil temperature = 19 °C, (ii) mean soil temperature = 21 °C, (iii) mean soil temperature = 23 °C.

5. Results of multivariable analysis using illustrations of multiple soil stresses

The root surface area of corn seedlings at the sixth leaf (visible tip method) in no-till responded to mean soil water content and soil temperature recorded during the first 4 weeks after seeding (Fig. 5). No relationship between soil water or soil temperature was detected in the moldboard plow system or the fall zone-till system. The results can be explained in more

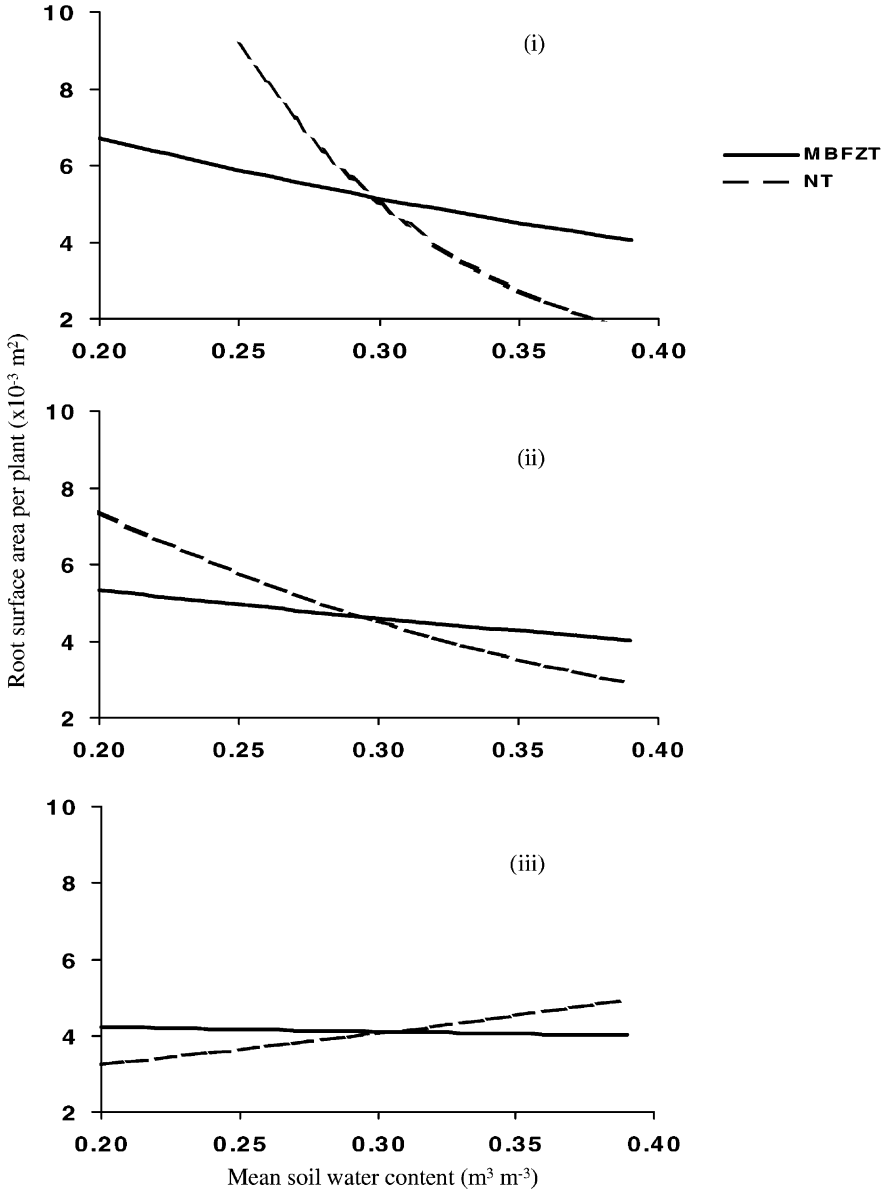

Fig. 5. Response surface slices of corn seedling root surface area as affected by mean in-row soil temperature at 5 cm depth (A), and soil water content (B) in the upper 10 cm layer. MBFZT = moldboard plow and FZT data combined: $Y = \exp(7.61 - 0.26A - 15.03B + 0.64AB)$; NT = no-till: $Y = \exp(29.17 - 1.24A - 86.78B + 3.88AB)$. $R^2 = 0.90$, $n = 75$ (includes all tillage systems).(i) Mean soil temperature = 19 °C, (ii) mean soil temperature = 21 °C, (iii) mean soil temperature = 23 °C.

detail for this illustration, with an abbreviated SAS Output in Fig. 3.

Mean soil temperature interacted with mean soil water content on the response of root surface area in corn seedlings. More importantly, however, the response depended on (i.e., interacted with) the tillage system ($0.037 < P < 0.056$ in the ANOVA table). The intercepts and slopes for each tillage system describe the relationship between soil temperature and soil water content on root surface area. In both the moldboard plow (MB) and fall zone-till (FZT) systems, the evidence is weak for rejecting the null hypothesis that the slopes are zero ($P > 0.19$); this suggests that root surface area of corn seedlings in the MB and FZT systems did not respond to the range of mean soil temperatures or soil water contents inherent in those tillage systems across locations, years, and replications. Furthermore, because there was no evidence that the response curves were different between fall MB and FZT in the "Estimate Statement Results", parameter estimates (i.e., curves) were combined for presentation purposes (Fig. 5). In the no-till (NT) system, however, there was strong evidence that root surface area responded to soil temperature and soil water ($0.001 < P < 0.029$). There was also strong evidence that the response in NT was different from either MB or FZT ($0.012 < P < 0.074$), as shown in the results of the "Estimate Statement"; therefore, a separate response surface was plotted for the NT system (Fig. 5).

Overall, there was a trend of reduced root surface area with increasing soil water content, especially in cool soil (Fig. 5). NT corn roots were more responsive to soil water than roots in other tillage systems at cool soil temperatures. Root surface area in relatively wet soil at 0.35 $m^3 m^{-3}$ was approximately one-third of that in relatively dry soil at 0.25 $m^3 m^{-3}$ in the NT system (Fig. 5). Root growth was enhanced when favorable conditions existed; however, compared to other tillage systems, the growth of roots was further reduced in NT because of stresses caused by cool temperatures and excessive soil water.

None of the relationships found with multivariable analysis were identified using conventional multiple regression techniques. In the response of root surface area to soil water content and soil temperature example, the simple effects of these soil physical factors were investigated, along with their interaction, using multiple regression techniques. The regression was performed for each of the six location-years because of a significant location by year interaction ($P < 0.05$). In brief, only 38% of the variability in root surface area was explained in any location-year when both soil water content and soil temperatures variables were included in the conventional multiple regression model. No differential response was detected among tillage systems using tillage as a dummy variable in the regression.

In another example of multivariate mixed model analysis, the response of shoot dry matter to both mean soil water content (Fig. 6) and air-filled porosity (Fig. 7) depended on mean soil temperature during the 4-week period; however, the response to soil temperature and soil water content depended on the tillage system (Fig. 6; $P = 0.009$), while the response to air-filled porosity was the same regardless of tillage. Curves from tillage systems with the same overall response (i.e., evidence against the null hypothesis of similar intercepts and slopes among those tillage models was very weak) were combined for illustration purposes (Fig. 6). Shoot growth was reduced in NT as soil water content increased when soil temperatures were between 19 and 21 °C (Fig. 6). There was also a trend for lower dry matter accumulation in the MB and FZT system with increasing soil water content, but this trend was significant only in low (19 °C) temperature conditions.

The magnitude of the interaction between temperature and soil water content on corn shoot growth (Fig. 6) was greater than the interaction between temperature and air-filled porosity (Fig. 7), even though soil water content and air-filled porosity are inversely related by definition. The influence of other soil physical factors—in addition to soil water and air-filled porosity in this illustration—contributes to the differential response among soil water content, air-filled porosity, and tillage systems (Hooker, 2000). Air-filled porosity, for instance, is dependent on both the water content and bulk density of the soil. If other soil physical factors or stresses are constant across tillage systems, then corn should respond the same in one tillage system as in another. Although the analysis and interpretation of a 3-way interaction among soil physical factors is complex, the differential response among tillage systems is evidence that any one soil physical factor may explain the variation in corn

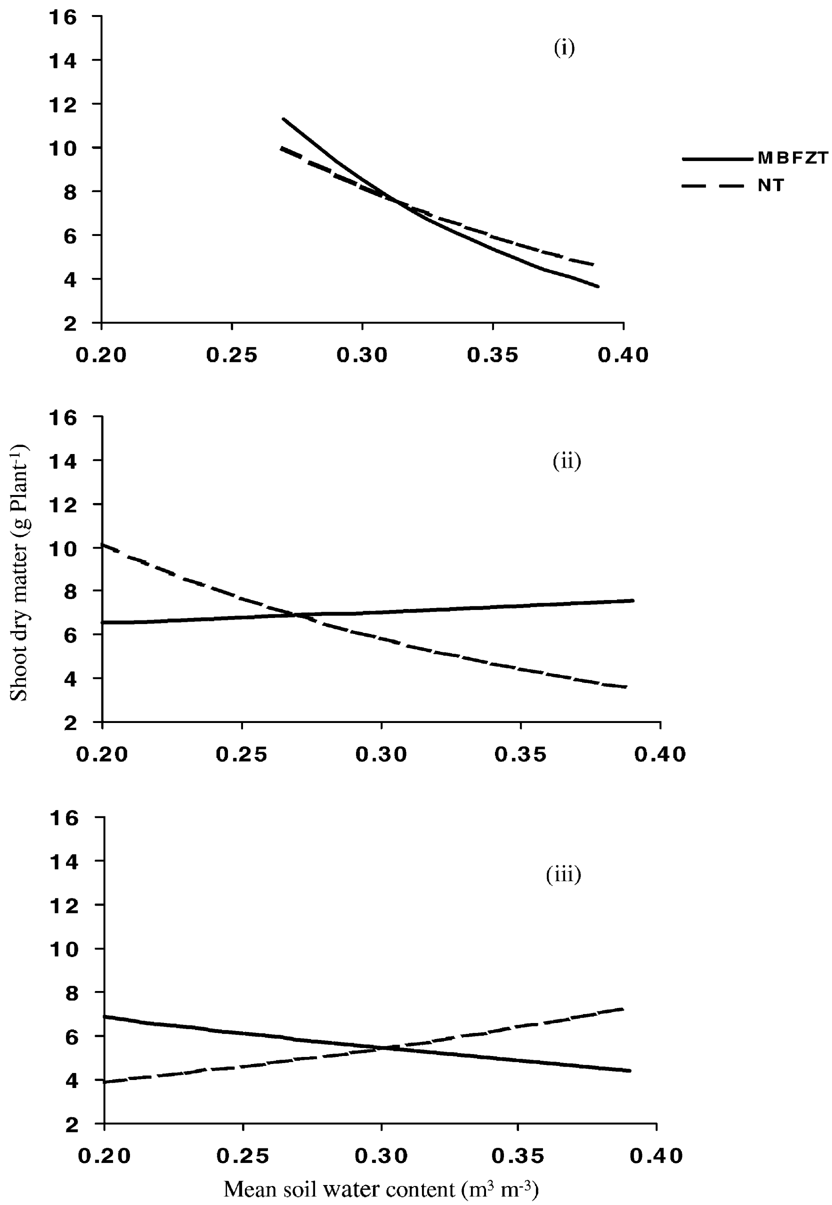

Fig. 6. Response surface slices of corn shoot dry matter near the appearance of the 12th leaf tip as a function of mean in-row temperature at 5 cm (A) and soil water content in the surface 10 cm (B). MBFZT = moldboard plow and FZT data combined: $Y = \exp(229 - 21.01A - 761.04B + 0.49A^2 + 70.79AB - 1.64A^2B)$; NT = no-till: $Y = \exp(-94 + 10.13A + 377.10B - 0.26A^2 - 38.89AB - 0.98A^2B)$. $R^2 = 0.91$, $n = 68$ (includes all tillage systems). (i) Mean soil temperature = 19 °C, (ii) mean soil temperature = 21 °C, (iii) mean soil temperature = 23 °C.

growth better in one tillage system than in another tillage system.

As soil temperature increased in all tillage systems, shoot growth responded less to changes in soil water content and air-filled porosity. Corn shoot growth was reduced by up to 20% in all tillage systems as the soil water content increased beyond 0.30 $m^3\ m^{-3}$. Shoot growth was reduced most in NT with a mean soil temperature of 19 °C during the first 4 weeks after emergence. Lizaso and Ritchie (1997) also reported

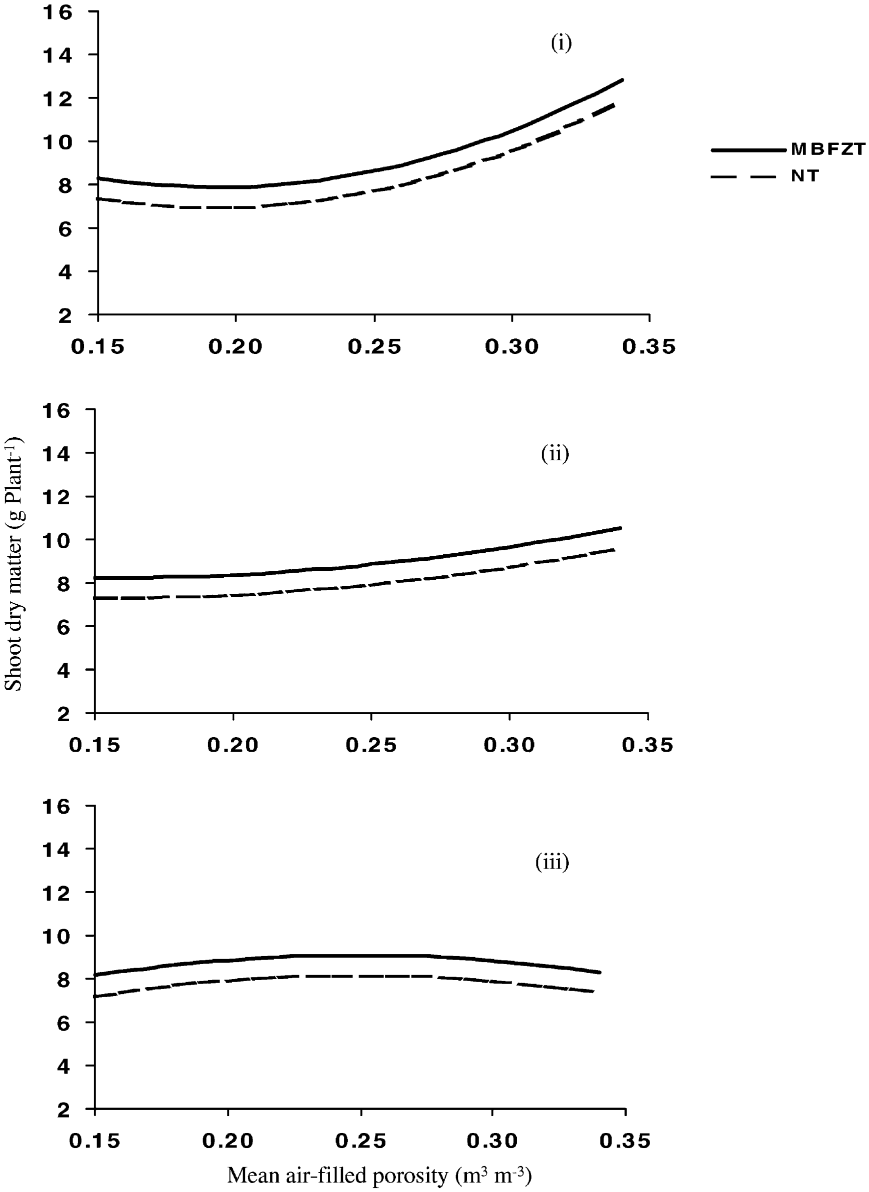

Fig. 7. Response surface slices of corn shoot dry matter near the appearance of the 12th leaf tip as a function of mean in-row temperature at 5 cm (A) and air-filled porosity in the surface 10 cm (B). MBFZT = moldboard plow and FZT data combined: $Y = 79 - 3.29A - 728.67B + 1758.42B^2 + 33.75AB - 80.60AB^2$; NT = no-till: $Y = 78 - 3.29A - 728.67B + 1758.42B^2 + 33.75AB - 80.60AB^2$. $R^2 = 0.90$, $n = 68$ (includes all tillage systems).(i) Mean soil temperature = 19 °C, (ii) mean soil temperature = 21 °C, (iii) mean soil temperature = 23 °C.

reduced corn leaf surface area and photosynthesis following soil saturation with water, but the interactive effects of low air-filled porosity (or soil saturation) and soil temperature on corn response has heretofore not been quantified in the literature. Corn growth in the MB and FZT systems was usually more stable across a range of soil water contents and soil temperatures than corn in the NT system.

A key advantage of the multivariable analysis using a mixed-model approach is accounting for the variability associated with random effects within the experiment, and for identifying multiple stresses that possibly interact with tillage. In essence, NT corn plants in early developmental stages are negatively affected by high soil water levels when they also experience cool soil conditions. On the other hand, the combination of cool and dry soil conditions resulted in root and shoot growth comparable to those with mean soil temperatures that were 4 °C higher in the first 4 weeks of development.

Mitigation efforts to enhance stress tolerance in corn need to consider the complexity of the interactions among stress factors affecting corn development at various stages. Although plant breeders may very well feature most prominently in the future enhancement of abiotic stress tolerance in plants (Duncan, 2000), their rate of progress will be affected by the extent to which their programs on genotype improvement involve multiple stresses. There may, therefore, be some doubt about the appropriateness of selection strategies in field environments with a single known stress condition. Agronomists and physiologists might be able to provide more guidance into which critical stresses limit yields if relevant data is analyzed using this multivariable statistical approach.

6. Conclusion

Impacts of abiotic stress factors such as cold temperatures on the response of corn have typically been assessed through simple effects of individual variables, yet corn plants are frequently responding to multiple stresses simultaneously during the growing season. We suggest that interactions among individual stress factors are usually ignored in field experiments because: (1) some individual stresses are not "of interest" and therefore not measured; (2) interactive effects among individual stress factors are assumed to be negligible; or (3) the power for testing interactions is low when significant random effects are pooled into the error variance of a fixed effects, correlation, or multiple regression analysis in a structured experimental design. The failure to acknowledge interactions among stress factors results in misdirected efforts (in research and management) to ameliorate the effects of a single stress or a combination of stress factors. Crop physiologists and agronomists can contribute to a better understanding of the interactions among multiple stresses on corn plants at various growth stages.

Multivariable statistical approaches can assist in the pursuit of understanding the response of corn to multiple stress conditions. In the specific examples provided for the response of corn to conservation tillage systems, we identified relationships among multiple soil factors limiting the growth of corn in the field using simple procedures within SAS PROC MIXED. Corn growth in the conventional tillage system was more stable across a range of soil water contents and temperatures because the intensity of multiple stresses was lower in conventional tillage. The characteristic response of corn to soil physical variables across tillage systems could be explained better by considering interactions among soil variables, rather than considering only the simple effects.

Field research efforts must, of course, be appropriately established and properly instrumented so that the response of corn, along with multiple stress factors, is quantified during sensitive periods of corn development. That imperative is especially important in tillage experiments, where interactions among soil physical factors are well known but poorly documented. However, the latter imperative is also important in other field experiments involving corn response to management and environmental variables. In addition, corn hybrids varying in susceptibility to stress factors of interest should be investigated in such experiments where possible. Integration of team approaches involving a continuum of skills, ranging from agronomy to molecular genetics, may be essential to accelerate the progress in overcoming individual and combinations of multiple stresses that limit corn yields. However, identification of the most important stress(es) to address in both agronomic and genetic research endeavors are considerably assisted by implementing multivariable statistical approaches like we used in SAS PROC MIXED.

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