Occurrence and Proposed Cause of Hollow Husk in Maize
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ABSTRACT
In 2007, a maize (Zea mays L.) ear abnormality that we term here as “hollow husk” occurred in research trials designed to alter the level or the sensing of plant ethylene. The unique experimental conditions of 2007 enabled us to document the occurrence of hollow husk and propose a physiological mechanism for its cause. Ears exhibiting hollow husk have normal appearing husks that feel hollow due to an abrupt cessation in ear development and a concomitant lack of silk emergence. Hollow husk occurred when the foliage of actively growing plants was sprayed before the V11 growth stage with a chemical treatment that should either lower the level of plant ethylene (a strobilurin fungicide), or one that should decrease the plant’s sensitivity to ethylene (1-MCP). An attempt to increase ethylene status (via ethephon) led to virtually no hollow husk symptoms. The percentage of plants exhibiting hollow husk symptoms depended on the hybrid, the stage of plant growth when sprayed, and the combination of management conditions that promoted plant growth. Plants sprayed at V15 generally exhibited greater symptoms than those sprayed at V11, and hollow husk successively increased with increases in N supply and decreased with increases in plant population. Based on our data, we speculate that hollow husk is a physiological ear abnormality related to a perturbation in the level or the sensitivity of the plant to ethylene.

Ear abnormalities occur in maize due to a myriad of reasons that include mutations, pathogen infections, environmental perturbations, and chemical applications. There are obvious implications from a commercial standpoint, since malformed ears produce lower yields. Because of maize’s whorled phylotaxis and determinate reproductive development, many ear deformities are caused by events which occur during vegetative growth. One of the more common ear deformities found in production fields is the so-called “blunt ear” (also referred to as “beer-can” or “hand grenade” ear), which is smaller in length than normal ears, but often has a greater circumference due to larger individual kernels (Nielsen, 1996). This condition results from a perturbation in cell division in the apical meristem of the embryonic ear shoot when the primordia for kernel ovaules are being formed (Lejeune and Bernier, 1996). Blunt-ear originates from chemical or environmental stresses that occur between the V7 and V15 growth stages (Nielsen, 1996), and is distinctly different from stresses that disturb pollination, or that cause kernel abortion, which are typically manifested as kernel tip back (Thomison and Geyer, 2007). Blunt-ear occurred as a result of using certain combinations of soil insecticides and postemergence herbicides, cold temperature shock, or wide fluctuations in temperature during ear formation (Nielsen, 2007). Circumstantial evidence in some situations links the blunt ear symptoms to postemergence applications of herbicides, fungicides, insecticides, and/or assorted additives, while in other situations no obvious connection can be identified with postemergence applications (Nielsen, 2007).

Many growers noted a new widespread ear abnormality that occurred in a uniform manner in maize plants in 2007 that we term here as “hollow husk.” Because hollow husk was widespread in 2007, we believe that it is not just a localized or a random event within any one field (Nafziger, 2007) and the significance of when and where it occurs is of importance to growers. Hollow husk ears are characterized by a lack of silk elongation along with a reduction in the growth of the earshoot (i.e., the husk contains no ear or in the instances of ear growth, the ear is small and does not form grain, see the results section for visual estimations). We believe that the hollow husk abnormality is similar to the “remnant ear” observed in 2006 (Nafziger, 2007), or the baby “remnant ear” observed in some areas in 2007 (Nielsen, 2007), but is distinctly different from blunt ear as blunt ear is only a decrease in stature of the ear and kernel number and does not involve a lack of silk emergence.

A high degree of hollow husk symptoms occurred in 2007 in two of our ongoing research trials where the role of ethylene in the response of maize to growth conditions and stress were evaluated. Because of our unique experimental conditions in 2007, we were able to accurately document, pinpoint, and characterize the occurrence of hollow husk in maize and we propose here that ethylene may play a role in the physiological mechanism for its cause.

MATERIALS AND METHODS
Research Approach
Two separate but adjacent experiments in the same field in 2007 were designed to alter the level or the perception of

Abbreviations: 1-MCP, 1-methylcyclopropene; ACC, 1-aminocyclopropane-1-carboxylic acid; a.i., active ingredient; DKC, DeKalb; DOY, day of year; R, reproductive; UTC, untreated control; V, vegetative.
plant ethylene. One experiment consisted of a single hybrid and combinations of N level and plant population to achieve varying degrees of cultural stresses (hereafter referred to as the cultural stress experiment), while another examined 10 different commercial hybrids grown at the optimal N level and plant population (hereafter referred to as the hybrid comparison experiment). To both experiments, foliar treatment applications were made to either decrease or increase the plant’s sensitivity to ethylene. We used the strobilurin-based fungicide Headline (containing the active ingredient pyraclostrobin; BASF, Florham Park, NJ) to decrease ethylene levels, since strobilurin fungicides decrease the activity of ACC (1-aminocyclopropane-1-carboxylic acid) synthase (Grossmann and Retzlaff, 1997; Ypema and Gold, 1999), the rate limiting enzyme in ethylene synthesis. We enhanced ethylene levels with ethephon (2-chloroethylphosphonic acid; Rhone-Poulenc Agriculture Company, Research Triangle Park, NC; Warner and Leopold, 1969), and we decreased the plant’s sensitivity to ethylene by using the competitive ethylene inhibitor 1-MCP (Sisler and Serek, 1999; Sisler et al., 1996; AgroFresh Inc., Spring House, PA). In both experiments, an untreated control (UTC) that was not sprayed with any compound was used for comparison.

For both experiments, Headline was applied at the commercial rate of 100 g/ha with the inclusion of the non-ionic surfactant, Intimidator (Crop Production Services, Galesburg, IL) at 350 mL/ha. 1-MCP was applied at a rate of 25 g a.i./ha with the crop oil concentrate, Dyne-Amic (Helena Chemical Company, Collierville, TN) at 0.38% by volume (as per recommendation from AgroFresh Inc.), and ethephon was applied at 250 g a.i./ha without any additional additives (Langan and Oplinger, 1987). All applications were made between 1030 and 1300 central standard time using a CO2 pressurized back-pack sprayer at a rate of 200 L/ha using XR TeeJet (Wheaton, IL) 1102 vs. nozzles on a 114 cm 4-nozzle spray boom which delivered a 152 cm swath. Applications were made to the cultural stress experiment on 2 July 2007 (V15 growth stage; Ritchie et al., 1992). Plants were considered at a particular growth stage when greater than half of the plants in a plot were exhibiting characteristics of that stage.

Field Experiments

Both experiments were located on the Fisher Farm at the Crop Sciences Research and Education Center in Savoy, IL. The soil at this site is a Drummer-Flanagan soil association (fine-silty, mixed, superactive, mesic Endoaquolls) typical of East-Central Illinois, with the proper pH and drainage, and with adequate levels of P, K, and other nutrients for high productivity. Precipitation and daily temperature extremes were measured on site. Both experiments were overplanted with a machine cone planter on 3 May 2007 and thinned at V6 to achieve the desired plant populations. Fertilizer N treatments were hand applied in a diffuse band between the center of the rows as ammonium sulfate, and followed by cultivation, when the crop was between the V2 and V3 growth stages.

For the cultural stress experiment, treatments consisted of a factorial combination of three N levels (45, 90, and 180 kg N/ha), three plant populations (74, 99, and 123 thousand plants/ha), and the three ethylene treatments (strobilurin fungicide, 1-MCP, and ethephon) arranged in a randomized complete block experimental design with four replications. An untreated control (nonsprayed) was included for each N by plant population combination. An experimental unit was a four row plot (rows 5.3 m long spaced 0.76 m apart) with the center two rows receiving the treatments. A high-yielding commercial hybrid (DKC 63-74) was used.

For the hybrid comparison, the four ethylene treatments were the main plots and the 10 hybrids the subplots of a split plot experimental design with four replications. The hybrids represented a range of brands, biotechnology trait combinations, relative maturities, and endosperm end-use segments and included (with relative maturity in parenthesis): DKC60-18 (110), DKC 63-74 (113), DKC 57-79 (107), DKC 61-66 (111), DKC 58-13 (108), Pioneer 33N11(114), Asgrow RX837 (113), Agrigold 6467 (110), Becks 5827 (111) and FS 6996 (115). Plots were two rows (5.3 m long and 0.76 m apart) with each row receiving the ethylene treatment. A constant plant population of 74,000 plants/ha with an N level of 180 kg N/ha was used for all hybrids.

Plant Measurements and Statistical Analysis

Flowering dynamics were estimated in the cultural stress and hybrid variation experiment by counting the proportion of plants with visible silks and anthers daily starting on 10 July 2007 which was when the crop reached tasseling. The occurrence of hollow husk ears in these trials was quantified at the R3 growth stage (grain milk stage) by counting the number of plants that had ears lacking any visible silks, yet still had normally developed husks.

The corresponding statistical analysis was done using PROC MIXED in SAS (SAS Institute, 2000). Plant population, N rates, and product applications were considered fixed effects, and mean separation was performed using the PDIFF option in the MIXED procedure. The significance level used in all of the experiments was 5%.

RESULTS

The early part of the 2007 growing season was dry with only 41 mm of precipitation between planting and 1 wk before the V11 treatment application (Fig. 1). An especially dry and warm period (0 mm of precipitation and maximum temperatures up to 4°C higher than the 10-yr average for that month) began 30 d after planting [day of year (DOY) 148] when the crop exhibited severe leaf rolling during the day. This condition persisted until DOY 172 when an 8-d rainy period began totaling 95 mm of precipitation. Afterward, the crop exhibited extremely rapid growth progressing through five leaf stages (from V11–V15) in only 5 d. This highly unusual growth spurt occurred directly before a V11 or a V15 ethylene-treatment application (Fig. 1).

The first visual indication of the hollow husk phenomenon in 2007 was the detection of fully developed ear husks with no silk emergence (Fig. 2A). These husks had a hollow feeling due to arrested ear and silk development. Excision of the husk revealed ears with varying degrees of arrested development ranging from no visual ovule development to a decrease in the number of ovules per kernel row (Fig. 2B). Kernel row number,
however, was not affected (data not shown). Kernel ovules that failed to develop exhibited a staminate-like appearance, and did not produce silks. Hollow husk symptoms were most abundant on strobilurin-treated plants (sprayed at V11 or V15), but also occurred with 1-MCP when treated at V15 (Table 1). In neither experiment did increasing the ethylene levels with ethephon cause more hollow husk plants than in the UTC.

Associated with the hollow husk symptom was a drastic reduction in silk emergence and in the total number of plants with exposed silks (Fig. 3). This effect was more pronounced for plants grown at the optimal (74,000 plants/ha) than the supra-optimal plant population (123,000 plants/ha), although high population delayed the onset of silking in UTC plants. High plant population also delayed the onset and the degree of pollen shed, but pollen shed was not altered by the strobilurin fungicide (Fig. 3) or any of the other ethylene treatments (data not shown).

The magnitude of hollow husk was closely associated with cultural conditions conducive to maximum plant growth; symptoms increased with increasing N rate and decreased with increasing plant population (Table 2). Sixty-two percent of strobilurin-treated plants exhibited hollow husk symptoms when grown at the lowest population and with the highest N rate (i.e., 74,000 plants/ha with 180 kg/N), compared to 23% of plants grown at the highest population and with the lowest N (123,000 plants/ha with 45 kg N/ha). Although much lower in magnitude, a similar pattern was observed for 1-MCP-treated plants with 5% of ears exhibiting hollow husk under the best N/plant population combination, and only 1% under the poorest (data not shown).

Hybrids exhibited considerable variation in their sensitivity to hollow husk and in their response to the ethylene treatments (Table 3). Although the strobilurin fungicide generally resulted in the highest levels of hollow husk (range of 9–89%), 1-MCP also caused substantially more hollow husk ears (range 2–19%), compared to the UTC, with some hybrids being particularly susceptible. There was no relationship between a hybrid’s susceptibility to hollow husk induced by 1-MCP and its susceptibility to hollow husk induced by the strobilurin fungicide (Table 3). There was also no relationship between a hybrid’s relative maturity and the occurrence of hollow husk. A very
DISCUSSION

The hollow husk symptoms observed in our study occurred in plants that were actively growing following relief from a prolonged period of vegetative water stress (Fig. 1); a condition under which ethylene is an important factor (Young et al., 2004; Morgan and Drew, 1997). The unique growth conditions during 2007 are unlikely to be exactly repeatable in a field setting and therefore we believe that hollow husk symptoms may be hard to repeat in a replicated fashion in subsequent years. However, we believe our replicated trials in 2007 serve to help us understand hollow husk and its impact, as ear deformities have severe implications for grain yield.

Actively growing plants produce or perceive optimal ethylene levels (Davies, 1987; Morgan and Drew, 1997), and as such, a sudden decrease in level (i.e., from the strobilurin fungicide) or perception (i.e., from 1-MCP) would be inhibitory to growth. The higher occurrence of hollow husk ears in chemically treated plants grown with high N and low plant population (the cultural stress experiment, which should be exhibiting the most rapid growth and should have higher endogenous ethylene levels before treatment; Morgan and Drew, 1997) demonstrates that ethylene levels and perception are important to maintain proper growth and reproductive viability. Conversely, the plants that were chemically treated under high population and low N levels (which we believe have less ethylene produced endogenously before treatment) show less susceptibility to hollow husk, again demonstrating that proper ethylene levels are required for optimal growth and when altered by a chemical agent, can severely affect reproductive capacity.

Other evidence is mounting showing that ethylene does not act strictly as a growth inhibitor, but under some circumstances can also stimulate plant growth (Pierik et al., 2006). Various growth aspects for several plant species follow a biphasic response model with both stimulatory and inhibitory responses depending on the ethylene concentration and the tissue (Pierik et al., 2006). Ethylene stimulates internode or hypocotyl elongation in germinating seedlings of wheat (Triticum aestivum L.) and Arabidopsis, respectively (Smalle et al., 1997; Suge et al., 1997), and may be needed for silk elongation which did not occur in hollow husk ears (Pinthus and Jackson, 1994; Fig. 3). Ethylene has also been shown to change the sexual orientation of individual flowers (Davies, 1987) or of whole dioecious plants (Byers et al., 1972), in agreement with our observation of staminate-like flowers at the tip of hollow husk (Fig. 2B).

Hollow husk conditions were exacerbated with strobilurin-treated plants, and strobilurin fungicides inhibit the activity of ACC synthase, a key enzyme in ethylene production (Grossmann and Retzlaff, 1997; Ypema and Gold, 1999). Strobilurin fungicides also have a multitude of other noted effects besides ethylene production, including impacting photosynthesis and chlorophyll degradation (Grossmann and Retzlaff, 1997) and we cannot discount that strobilurin fungicides may impact other key regulatory processes in plants that affect yield under unique weather conditions. Also, we cannot discount the possibility that adjuvants included in the strobilurin fungicide application may also play a role in affecting ethylene levels as well as producing hollow husk.

Table 3. Hybrid variation in the percentage of plants with hollow husk symptoms induced by treatments designed to alter the status or sensitivity of the plant to ethylene in 2007. The UTC represents untreated control plants. Treatments were applied at the V15 growth stage.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Strobilurin</th>
<th>1-MCP</th>
<th>Ethephon</th>
<th>UTC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of plants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agrigold 6467</td>
<td>78.8</td>
<td>18.7</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Asgrow RX837</td>
<td>35.0</td>
<td>12.0</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Becks 5827</td>
<td>88.8</td>
<td>6.6</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td>DKC 57-79</td>
<td>50.6</td>
<td>13.0</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DKC 58-13</td>
<td>35.5</td>
<td>1.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DKC 60-18</td>
<td>15.0</td>
<td>14.7</td>
<td>1.9</td>
<td>0.0</td>
</tr>
<tr>
<td>DKC 61-66</td>
<td>9.2</td>
<td>1.9</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td>DKC 63-74</td>
<td>64.0</td>
<td>8.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>FS 6996</td>
<td>71.6</td>
<td>9.4</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Pioneer 33N1</td>
<td>62.6</td>
<td>2.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>20.6</td>
<td>4.1</td>
<td>ns †</td>
<td>ns</td>
</tr>
</tbody>
</table>

† ns indicates nonsignificant differences among hybrids.
In addition to strobilurin fungicides, 1-MCP also decreases the plant’s sensitivity to ethylene (by inactivating ethylene receptors), we believe we lowered the effective ethylene status of the plant, explaining the hollow husks that were observed from 1-MCP application at V15. There are clearly growth-stage and environment specific instances where plants produce excess ethylene that is detrimental to growth (Cheng and Lur, 1996; Sun et al., 2005; Pierik et al., 2006), and in some cases this can be protected or alleviated with 1-MCP (Hays et al., 2007). However, in our experiments, we believe that the 1-MCP treatments occurred under a unique set of weather conditions and at a crucial time for continued ear development. In contrast to the chemical treatments designed to lower ethylene status with strobilurin fungicides and ethylene perception with 1-MCP, we observed virtually no hollow husk symptoms where the ethylene level was likely enhanced by ethephon, although these plants were noticeably shorter (data not shown). This observation that high rates of applied exogenous ethylene with ethephon did not contribute to the occurrence of hollow husk demonstrates that it is the lowering of ethylene levels that leads to the incidence of hollow husk, although further experimentation is needed to verify and validate this hypothesis.

The large variation among hybrids exhibiting hollow husk (Table 3) is indicative of genetic variation in the response to plant growth regulators. Varieties of rice (Oryza sativa L.) and wheat differ in their production and/or sensitivity to ethylene (Klassen and Bugbee 2002) and we speculate that maize hybrids also differ in their responses to ethylene. Moreover, the lack of a relationship between a hybrid’s hollow husk symptoms induced by the strobilurin fungicide (a decrease in synthesis) or by 1-MCP (a decrease in sensitivity) shows that these two mechanisms can act independently to alter plant ethylene status (i.e., at the enzymatic level or the receptor level) and variation differs among hybrids (i.e., hybrids may have varying numbers of ethylene receptors). Collectively, the variation among the hybrids in response to the treatments demonstrates that ethylene perception differs among commercial hybrids and may contribute to fitness and/or susceptibility to hollow husk as well as other stresses.

Ear development of maize is also clearly associated with the integration of the other plant hormones, such as abscisic acid and gibberellin, which can directly interact with ethylene (Ross and O’Neill, 2001). We do not discount that other hormones may play a role in the occurrence of hollow husk in addition to that of ethylene. The ease with which growth regulators can hinder, rather than enhance ear development was well documented by Earley et al. (1974) who showed the hybrid, the stage of plant growth, the part of the plant treated, and the type of growth regulating chemical all affected the degree of earshoot response. Similarly, we believe the specific environmental conditions experienced at our site in 2007, in conjunction with the application of chemicals that should alter ethylene level or sensitivity caused hollow husk.

SUMMARY

Our unique set of experiments during the summer of 2007 allowed us to speculate that ethylene is a potential causal agent in the occurrence of the hollow husk ear abnormality. We believe that the environmental conditions during our Midwest growing season in 2007 may have caused perturbations in maize ethylene levels and that coupled with the spraying of ethylene altering compounds caused hollow husk to occur. Although further molecular and biochemical evidence is needed to verify the alterations in the ethylene biosynthesis and perception pathways in plants exhibiting hollow husk, the documentation of its occurrence and causal factors is worth examining due to its negative impact on grain yield. We could benefit by understanding how ethylene plays a role in maize growth conditions as well as understanding when best to apply ethylene altering compounds.

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