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SCIENTIFIC PERSPECTIVE

Combining xenia, male sterility, and synchronous pollination to improve maize grain yield and market value

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Abstract

Xenia (pollination of florets with genetically unrelated pollen), male sterility (removal of fertile pollen), and synchronous pollination (application of pollen when all silks are present) are well-documented processes that influence kernel formation and development in maize. There also have been numerous reports in which xenia and male sterility were combined in an attempt to increase kernel number, kernel weight, and/or grain yield. We present an analysis of published literature exploring impacts of xenia resulting from cross-pollination, male sterility, and synchronous pollination applied individually or in combination. Although synchronous pollination has had the most consistent and positive impact on seed formation, to our knowledge there are no reports combining all three effectors together. Recently developed technology to collect, preserve, and apply large volumes of maize pollen has provided the opportunity to evaluate the cumulative and potentially synergistic effects of synchronous pollination, xenia, and male sterility applied together on maize hybrids. We present the results of a field trial combining all three effectors in maize; this approach increased kernels per ear, kernel oil concentration, and grain yield per hectare. The results suggest synchronous pollination is essential to enable the greatest number of kernels to develop successfully. Male sterility supports kernel formation by providing greater assimilate supply per kernel, and xenia increases sink strength to attract the additional assimilates. The positive response and economic return when these effectors are applied in combination provides a novel opportunity for grain producers to manage kernel formation and composition proactively using pollen to deliver the trait of choice.

1 | CROSS POLLINATION (THE XENIA EFFECT)

Waller (1917) was among the early manuscripts describing the effect of foreign pollen on the endosperm of angiosperms –(i.e., the xenia effect). The term “foreign” refers generally to any pollen source that is not genetically identical to the female plant (allo-pollination), thereby resulting in predominantly heterozygous progeny rather than homozygous progeny. Waller concluded the xenia effect was expressed solely in the endosperm as a consequence of triple fusion of the (male) sperm nucleus with two polar nuclei in the (female) endosperm. Kiesselbach (1960) provided a more general definition of xenia as the immediate effects of a foreign pollen

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Abbreviations: CMS, cytoplasmic male sterility; GMS, genetic male sterility; HO1, HO2, two high oil populations; MF, male fertile; MS, male sterile; NO1, NO2, two normal oil inbreds.

parent on nonmaternal tissue of the kernel. Because the embryo of the maize kernel typically contributes 11% and the endosperm contributes 83% to the dry weight of the kernel (Tollenaar & Dwyer, 1999), the xenia phenotype is primarily associated with effects on endosperm metabolism and development. The xenia response often results in an increase in the kernel weight (Bulant & Gallais, 1998; Kiesselbach, 1924, 1932; Kiran & Wicks, 1990; Letchworth & Lambert, 1998; Lüders et al., 2008; Pinter et al., 1987; Seka & Cross, 1995; Westgate et al., 1999). The increase in kernel weight reflects both an increase in kernel growth rate (Seka & Cross, 1995; Seka et al., 1995) and/or duration of grain filling (Bulant & Gallais, 1998; Poneleit & Egli, 1983).

Few studies have actually confirmed the impact of xenia on harvested grain yield. Tsai et al. (1991) and Tsai and Tsai (1990) reported yield advantages as great as 15.1%. But negative responses for specific crosses have been reported as well (Weiland, 1992; Weingartner et al, 2002a, 2002b). Averaged over 38 published comparisons, the yield advantage from cross-pollinated hybrids has been about 3%, primarily reflecting the increase in kernel dry weight (Figure 1). This value clearly underestimates the benefit for grain production because any negative xenia response would not be commercially viable. The positive yield responses in these same studies averaged +7.3% (*n* = 22). The most favorable yield responses to cross-pollination are associated with greater genetic distance between the pollen source and female hybrid parent, with both hybrid parents having a positive impact on kernel growth (Bulant & Gallais, 1998; Westgate et al, 1999; Lüders et al, 2008).

In contrast to the variable yield response, the positive impacts of foreign pollen on the chemical composition of the maize kernel are well documented. These include altering protein, oil, and starch contents, amino and fatty acid compositions, and embryo/endosperm ratio (Curtis et al., 1956; Lambert et al., 1998; Letchworth & Lambert, 1998; Tsai & Tsai, 1990; Weingartner et al., 2004; Tanaka et al., 2009; Schaefer & Bernardo, 2013; Chen & Dong, 2017). Lambert et al. (1998) increased the average kernel oil concentration of normal oil hybrids by ∼38% when they were pollinated by a high oil hybrid pollinator. The increase in oil concentration resulted from a combination of greater embryo mass, increased embryo oil concentration, and decreased endosperm starch. Importantly, the increase in oil content per kernel did not decrease grain yield of the normal oil hybrids. Xenia was used successfully in the TopCross Blend (Dupont) system to elevate corn oil yield at the field level. But the yield penalty associated with increased kernel oil concentration and presence of a high-oil pollinator in the field limited its commercial potential (Thomison et al., 2002, 2003). Several alternative markets for biofuels and feedstocks have since emerged significantly increasing the demand for corn oil. These developments have made plant-based oils more competitive and attractive to ethanol and biodiesel processors as well as livestock producers.

Core Ideas

- Xenia, male sterility, and synchronous pollination each can impact maize kernel formation and development.
- Attempts to gain synergy by combining two of these effectors often produced variable or even negative results.
- Combing all three effectors increased kernel number, oil concentration, grain yield, and grain market value.

Jeff Taylor, Chairman of the Board Lincolnway Energy and a grain producer, offers a bioenergy industry perspective: “There is an unmet demand for corn oil in both the

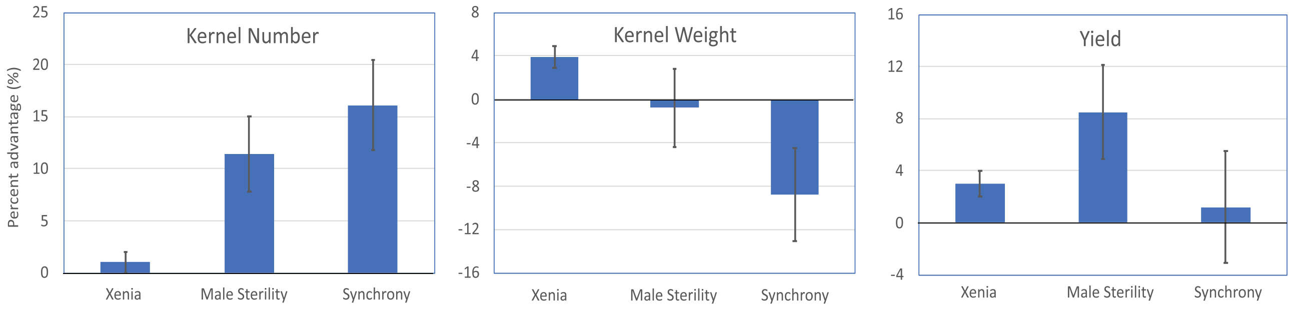

FIGURE 1 Impact of pollinating hybrid plants with non-isogenic pollen (xenia), pollinating male-sterile/detasseled plants (male sterility), and pollinating exposed silks at the same time (synchrony) on kernel number per plant, individual kernel weight, and grain yield per plant. Values are presented relative to their isogenically pollinated, male-fertile, or open pollinated counterparts. Data are pooled from Tsai and Tsai (1990), Tsai et al. (1991), Weiland (1992), Cárcova et al. (2000), Stamp et al. (2000), Cárcova and Otegui (2001, 2007), Balestre et al. (2007a, 2007b), Uribelarrea et al. (2008), Weingartner (2002a, 2002b), and Loussaert et al. (2017). Results for effectors applied in combination (e.g., male sterility + synchronous pollination) are not included. Values are the mean +/− SE for 8 to 38 hybrid-treatment combinations

ethanol and biodiesel industries. Delivering corn grain with greater oil content to a biorefinery facility greatly increases the extractable energy per bushel. It also decreases the carbon footprint of the entire process from grain production to energy extraction. It is a win for bio-based energy production, a win for the environment, and a win for agricultural producers" (personal communication, 30 November 2021).

Although the xenia effect for oil concentration can be manipulated by the pollen parent, protein concentration appears to be less flexible (Gilbert, 1960; Letchworth & Lambert, 1998; Lambert et al., 1998; Pixley & Bjarnason, 1994). Kahriman et al. (2015a, 2015b) compared the effect of open pollination, self-pollination, and bulk pollination (pollen combined from several pollinators) on several ear and kernel traits (ear weight, kernel weight, kernel number, mean kernel weight) as well as grain composition (protein, oil, carbohydrate, and carotenoid content). Pollination treatment had a significant effect ($P < 0.05$) on all traits except oil content. Combining foreign pollen sources confounded xenia effects on composition, but open and bulk pollinations consistently enhanced kernel weight relative to self-pollination. As in the commercial TopCross system, the greatest benefit of xenia on kernel composition depended on the combination of male and female hybrid parents (Thomison et al., 2002, 2003; Vanćetović et al., 2014).

2 | MALE STERILITY

Numerous publications over the past 70 yr document the yield advantage of eliminating the male component of the maize reproductive system either physically via tassel removal (male-less) or genetically via cytoplasmic (CMS) or genetic (GMS) male sterility (Rogers & Edwardson, 1952; Grogan, 1956; Chinwuba et al., 1961; Hunter et al., 1969; Kalman et al., 1985; Loussaert et al., 2017; Gao et al., 2020). The yield advantage of male-less plants can be quite dramatic. Grogan (1956) reported a 97% yield increase in response to de-tasseling plants grown under poor fertility. Chinwuba et al. (1961) reported yield advantages as great as 41% for GMS hybrids over their male fertile (MF) counterparts planted together in alternate rows. And Loussaert et al. (2017) observed a 40% yield advantage of a GMS hybrid grown under favorable conditions, and a 70% yield advantage of the same GMS hybrid exposed to drought stress during seed formation. The yield advantage under drought stress reflected a 48% decrease in barren plants and 57% increase in kernels per ear.

In most comparisons, however, the yield advantage of male sterile (MS)/male-less hybrids over their MF counterparts has been more modest (Duvick, 1958; Gao et al., 2020; Loussaert et al., 2017; Stamp et al., 2000; Weingartner et al., 2002a, 2002b), and occasional negative values have been reported (Uribelarrea et al., 2008). Weingartner et al. (2002a, 2002b), for example, evaluated the yield response of three CMS hybrids in non-restored T-cytoplasm, pollinated by their male--fertile isogenic counterparts. The three CMS hybrids tested across six environments yielded 11.8 Mg ha^{-1} on average and had 7.4% greater grain yields than their male-fertile counterparts. The cytoplasm × genotype and cytoplasm × environment interactions were not significant indicating the effect of male sterility on grain yield was relatively consistent across hybrids and environments. Averaged over 25 published comparisons, MS/male-less hybrids yielded about 8.6% more than their MF counterparts (Figure 1), reflecting a large increase in kernels ear$^{\mathbf{-1}}$. In most cases, the MS plants supported the initiation and growth of additional kernels with a minimal decrease in individual kernel weight.

The grain yield advantage displayed by male-sterile plants is associated with greater ear biomass at pollination, which reflects increased supply of nutrients allocated for female floral development particularly under stressful growing conditions (Loussaert et al., 2017; Gao et al., 2020). The current supply of photo-assimilate to the developing female rachis and female flowers is well established as a critical factor controlling the success of seed formation in maize (Schussler & Westgate, 1991, 1995; Borrás et al., 2007). Maintaining a constant assimilate supply per kernel also is required to ensure the additional kernels achieve their potential mass (Borrás & Otegui, 2001; Gambín et al., 2006).

A 27-year seed industry veteran offers the perspective that "it is generally accepted across the corn seed industry that a male sterile hybrid will yield up to 7% more than its male fertile counterpart. There are a multitude of location-years of seed industry data on male sterile genotypes to substantiate this phenomenon" (Todd Krone, personal communication, 15 November 2021).

3 | SYNCHRONOUS POLLINATION

Stressful growing conditions such as high plant populations or drought increase the interval in functional maturity (observed outwardly as silking) between ovaries at the base and tip of the ear. Pollination of apical florets may lag behind that of basal florets by 5 d or more even under favorable growing conditions (Bassetti & Westgate, 1993a, 1993b). Such a delay in pollination inhibits kernel formation and development on later-pollinated florets (Cárcova & Otegui, 2001; Freier et al., 1984; Wang et al., 2007). The reason for the failure of later-formed kernels to develop has been related to competition for assimilates, inhibitory hormonal signals from early-formed kernels, and primigenic dominance (Bangerth, 1989; Brugière et al., 2003; Schussler & Westgate, 1991).

Numerous studies document the benefits of synchronous pollination of exposed silks on primary and secondary ears as a means to overcome the disadvantage of late-pollinated flowers to produce kernels (Cárcova et al., 2000; Cárcova & Otegui, 2001; Motto & Moll, 1983; Sarquís et al., 1998; Uribelarrea et al., 2008). In these studies, primary and secondary ears (if present) are pollinated by hand to ensure all exposed silks received pollen at the same time. This intraplant approach for imposing pollination synchrony differs from altering the anthesis-silking interval between pollen shed and silking of the plant population that primarily impacts pollination synchrony between plants. Cárcova et al. (2000) reported synchronous pollination of primary and secondary ears of four hybrids grown at a low population density increased kernel set on secondary ears from 35 to 535% (250% on average), while maintaining kernel set on primary ears. Synchronous pollination of the same hybrids grown at commercial population densities (7.5–9.0 pl m^{-2}) to eliminate prolificacy increased kernel set on primary ears from 8 to 31% (20% on average). Kernel set responses to synchronous pollination as great as 29 and 31% at these plant densities also were reported by Cárcova and Otegui (2007) and Uribelarrea et al. (2008), although Uribelarrea et al. (2008) occasionally observed a decrease in kernels per ear on MF plants as well. Averaged across 15 published hybrid combinations, synchronous pollination of primary ears increased kernels per ear about 16.1% relative to their open pollinated hybrid counterparts (Figure 1).

To our knowledge, Uribelarrea et al. (2008) is the only study to couple the response to synchronous pollination with final grain yield per plant. In that study comparing the response of MS and MF versions of two hybrids over two seasons, only three of eight comparisons resulted in a yield advantage from synchronous pollination. In all cases, average kernel weight decreased dramatically with the increase in kernel set. Although this study was limited in scope and results for yield and yield components were not consistent across years, it does underscore the importance of the plants having sufficient assimilate supply per kernel during seed filling, as indicated by Borrás and Otegui (2001) and Gambín et al. (2006). Consistent with the trends shown in Figure 1, the MS plants in Uribelarrea et al. (2008) had a clear advantage over the MF plants in this regard. Averaged across hybrids and years the advantage of MS plants for yield per plant was +7.3% MS vs. −4.9% MF, for kernels ear^{-1} was +12.9% MS vs. +9.7% MF, and for kernel weight was −5.9% MS vs. −11.7% MF. Evidently, the MS versions of these hybrids provided sufficient assimilate per kernel to net a positive yield response per plant, despite the decrease in kernel weight.

4 | COMBINING THE BENEFITS OF XENIA, MALE STERILITY, AND SYNCHRONOUS POLLINATION

Several studies have explored the potential for combining the individual benefits of cytoplasmic male sterility and xenia on grain yield and/or composition (Weingartner et al., 2002a, 2002b, 2004; Vanćetović et al., 2009; Munch et al., 2010; Bozinovic et al., 2015). The approach, referred to as the Plus-hybrid system, is deployed similarly to a TopCross field in that it mixes a CMS hybrid with an unrelated male-fertile hybrid, which acts as a pollinator for the field (Munsch et al., 2010). The Plus-hybrid advantage is calculated as the difference in yield between the cross-pollinated CMS hybrid and that of the isogenic self-pollinated fertile hybrid. Because the advantage over CMS depends on pollination heterosis, the greater the difference in genetic background between the pollinator and CMS hybrid, the greater the expected Plus-hybrid effect.

Weingartner et al. (2002a, 2002b) tested the Plus-hybrid approach on three CMS-T cytoplasm hybrids pollinated by five non-isogenic hybrids. The choice of pollinator hybrid had a significant effect on grain yield, kernel weight, and kernel number. Averaged across six environments and five pollinators, the three CMS hybrids out-yielded their MF, self/sib-pollinated counterparts by 2.1, 9.3, and 15.8%. Six of 15 Plus-hybrid combinations increased grain yield by more than 10%, relative to the male-fertile hybrids. The best combination of a CMS hybrid and non-isogenic pollinator increased grain yield by 21.4%. The yield advantages of the CMS hybrids were due primarily to increases in kernel number, as there were only minor changes in kernel weight. In a more extensive study, Munsch et al. (2010) investigated the yield response of five CMS hybrids to eight pollinator hybrids across 12 environments in four countries over two years. The CMS hybrids included all three male-sterile cytoplasms - C, S, and T. Plus-hybrid combinations increased grain yield by 10% or more, on average, and by up to 20% in specific environments. The Plus-hybrid response reflected increases in both yield contributors; CMS leading mainly to a greater number of kernels per ear, and xenia causing mainly an increase in kernel weight. It was not possible to resolve whether the CMS-C, CMS-S, or CMS-T cytoplasm was superior in their Plus-hybrid system, primarily due to variation in yield across environments. The xenia effect also was variable but consistently positive across environments. As in other studies, the greatest advantage accrued with specific combinations of highly responsive CMS hybrids and 'good' pollinators. A case in point is Bozinovic et al (2015) who reported variable results with the Plus-hybrid approach. Yield responses ranged from −6 to +6% for the cross-pollinated MS hybrids relative to their fertile counterparts receiving the same pollen. They suggested the inconsistent response to cross pollination was due to their use of 3-way CMS-S hybrids and the poor pollinating ability of the fertile hybrids. Such variable results reinforce earlier conclusions by Duvick (1958) and Liu et al. (2010) that the advantage of combining male sterility and foreign pollen on kernel formation and metabolism depends on the genetic distance between the female and pollinator.

Efforts to combine CMS and xenia to impact kernel composition in the Plus-hybrid system have been inconsistent at best. Weingartner et al. (2004) reported that CMS did not significantly affect grain nitrogen content, but xenia effects on nitrogen content were positive. Vanćetović et al. (2009) observed a significant effect of CMS on kernel protein, oil, and starch concentration in some Plus-hybrid combinations. But differences in composition values between the CMS and MF hybrids were generally within 0.5% of the isogenic fertile hybrid. Likewise, xenia effects were evident for kernel protein, oil, and starch concentration across several of the 10 Plus-hybrid combinations, but the greatest differences were less than 1%. The combined analysis of CMS+xenia effects showed similarly small effects. To our knowledge, there are no reports in the scientific literature combining all three effectors together to impact seed formation and/or grain composition.

5 | IMPLICATIONS FOR IMPROVING GRAIN YIELD AND MARKET VALUE

Average yield advantages associated with xenia (+3%), male sterility (+8.5%), and synchronous pollination (+1.2%) presented in Figure 1 included a wide range of positive and negative results from unrelated studies in which each effector was applied individually. When applied in combination (e.g., xenia + male sterility), responses are more often positive and consistent, but not for all hybrid combinations. What might reasonably be expected if xenia, male sterility, and synchronous pollination were applied in combination to affect kernel set and/or kernel composition? To our knowledge, such an experiment has never been attempted, possibly because the short life of maize pollen and managing the volume of viable pollen required to make timely pollinations across treatments and replications confound experimental design and interpretation. Technologies developed recently to collect, preserve, and apply large volumes of maize pollen (Cope et al., 2016, 2017, 2019) has provided the opportunity to evaluate the cumulative and potentially synergistic effects of synchronous pollination, xenia, and male sterility applied together on maize hybrids. These technologies were tested in a replicated field trial near Ames, IA, in 2021 in which plants of a public hybrid consisting of a mixed stand of 50% MF and 50% MS plants were pollinated with preserved 'foreign' pollen collected from two normal oil inbreds (designated NO1, NO2) and from two high oil populations (designated HO1, HO2). Ears on randomly selected plants were covered prior to silk emergence to prevent self- of sib-pollination. Individual plants with silk emergence initiated on the same day were identified as MF or MS at the onset of pollen shed. Pollen was collected mechanically from NO1 NO2, HO1, and HO2 plants grown at a separate field location and preserved for 1 or 2 d prior to application. Mechanical pollen application involved delivering the preserved pollen-matrix in an air stream onto all exposed silks. All plants were pollinated on two occasions corresponding to 4 and 6 d after first silk emergence to minimize the impact of delayed fertilization on seed set (Cárcova et al., 2000; Cárcova & Otegui, 2001). Ears were covered to prevent cross- or sib/self-pollination between pollinations. After the second pollen application, silks were left uncovered to allow open pollination of any remaining unpollinated silks. The two mechanical pollinations successfully fertilized 85–90% of the female flowers as determined by the distribution of high-oil kernels on the ears.

TABLE 1 Impact of combining a foreign pollen source, male sterility, and synchronous pollination on percent change in grain yield, kernels per ear, and grain value of an open pollinated public hybrid

Male pollinator	Grain yield	Kernels ear^{-1}	Grain yield	Gain in value
	——% change——		Mg ha^{-1}	\$ ha^{-1}
NO1	+8.0*	+15.4*	13.1	+204
NO2	+9.7*	+10.9*	13.3	+241
HO1	+7.3*	+14.8*	13.0	+184
HO2	+6.4†	+13.6*	12.9	+161
Average	+7.9*	+13.7*	13.1	+198

Note. Plants were sown on two dates at approximately 8 pl m^{-2} in two-row plots, 76 cm rows × 21 m long replicated four times. Percent change was calculated relative to values for open pollinated hybrid plants within the same plots. Open pollinated hybrid plants produced 722 kernels plant^{-1}, on average. Grain yield and net gain in value were calculated against the average grain yield for open pollinated hybrid plants across all plots, which was approximately 12.1 Mg ha^{-1}. Grain value was taken from the USDA Daily Ethanol Report for Eastern Corn Belt, 20 Oct 2021. HO1 and HO2 are two high oil populations; NO1 and NO2 are two normal oil inbreds.

*Difference from open pollinated significant at the .05 probability level. †Difference from open pollinated significant at the .07 probability level. Effect of male sterility was nonsignificant at the .05 probability level. Therefore, data for male sterile and male fertile plants were combined for analysis. Values are the mean of 8 reps of 12 plants each.

As observed in other studies, male sterility did not have a significant effect ($P \leq 0.05$) on grain yield. Therefore, we assessed the impact of synchronous-xenia pollinations against the combined data for open pollinated MF and MS plants. Synchronous pollination increased kernels per ear 10.9–15.4% and increased grain yield 6.4–9.7% across the four pollinators (Table 1). On average, synchronous pollination with foreign pollen increased kernels per ear by 13.7% and grain yield by 7.9% relative to the open pollinated hybrid plants. Based on the price of maize grain in October 2021 when grain harvest was taken, the increase in yield translated to an additional \$198 ha^{-1}. Although the cost to implement a system relying on preserved pollen will vary by producer and as the technology evolves, a reasonable commercial target for implementation is \$125 ha^{-1}. Using this value, the average net gain would be \$73 ha^{-1}, based on yield alone.

TABLE 2 Impact of combining a foreign pollen source, male sterility, and synchronous pollination on proximate analysis values of an open pollinated public maize hybrid

Male pollinator	Protein	Oil	Starch	Test weight	Gain in value
oil%		——% change relative to open pollinated——			$ ha^{-1}
HO1 (12.0%)	+2.6, ns	+53.8*	−4.2*	−2.1*	+258
HO2 (11.5%)	+4.1*	+67.0*	−5.4*	−1.2, ns	+462

Note. Percent change was calculated relative to open pollinated hybrid plants within the same plots. Average protein, oil, and starch concentrations and test weight of open pollinated hybrid plants were 9.4%, 4.0%, 59.7%, and 59.1 lb bu^{-1}, respectively, as measured by NIR. Prices for oil, ethanol, and DDGs used for Net Gain in Value are from the USDA Daily Ethanol Report for Eastern Corn Belt, 20 Oct 2021. HO1 and HO2 are two high oil populations.

*Difference from open pollinated significant at the .05 probability level. ns, difference from open pollinated nonsignificant at the .05 probability level. Effect of male sterility was nonsignificant at the .05 probability level. Therefore, data for male sterile and male fertile plants were combined for analysis. Values are the mean of eight reps of approximately 2,000 g each.

Pollinating the public hybrid with pollen preserved from the high oil populations, HO1 and HO2, increased the kernel oil concentration by 53.8 and 67.0%, respectively (Table 2). Based on USDA values for biorefinery products derived from maize grain, these increases in kernel oil concentration are worth an additional $258 and $462 per hectare. This gain in value takes into account losses due to lower kernel starch content for ethanol and lower test weight. Combined with the net gain from yield, synchronous-xenia pollinations with preserved HO1 and HO2 pollen would have netted producers and additional $331 and $535 ha^{-1} over the open pollinated plants.

The realized benefit of combining xenia, synchronous pollination, and male sterility for increased kernel set will depend on hybrid response to environmental conditions known to impact seed set as well as the plant's capacity to maintain growth of additional kernels and/or altered kernel composition. An important observation in Cárcova et al. (2000) was that synchronous pollination increased the floret fertility index (i.e., the ratio of kernels formed to silks exposed for pollination). This result confirms that later-emerging silks are fully capable of supporting pollen germination, pollen tube growth, and ovary fertilization. Likewise, apical florets are capable of forming kernels if ovary fertilization is not delayed. In a related study, Cárcova and Otegui (2001), documented the impact of a pollination delay on kernel set within a maize ear. Delaying pollination of tip florets by as few as 2 d reduced kernels per ear 16%. A 4-d gap in pollination timing decreased kernel number 45%, the maximum interference they observed for kernel formation on later-pollinated florets. In this regard, it is interesting that other reproductive phenomena that promote more synchronous pollination among early and late-formed florets such as rapid ear and floret growth, fewer florets per kernel row, and loss of basal floret receptivity all have proven advantageous for maintaining grain yield under stressful growing conditions (Otegui, 1997; Otegui & Melón, 1997; Pagano et al., 2007; Shen et al., 2018).

Assimilate supply per kernel is a maternally determined characteristic. Numerous studies have demonstrated that establishment of kernel growth and metabolism after pollination are regulated by current assimilate supply (Sarquís et al., 1998; Walker et al., 1988; Borrás & Otegui, 2001; Borrás et al., 2002). The hydroponic field study by Walker et al. (1988) is particularly instructive in this regard. They temporarily decreased the plant population around flowering and kernel set, which increased kernels per plant by nearly 50% due to seed set on secondary ears. There was no impact on total grain yield per plant, however, because average seed size decreased by about 35%. Evidently, the plants returned to the original plant density during seed filling did not provide sufficient assimilate per kernel to establish the same potential kernel size or maintain kernel fill for such a large increase in kernel number. Uribelarrea et al. (2008) also concluded failure to maintain kernel weight could negate the advantage of increased kernel set resulting from synchronous pollination. The MF hybrids in their study lacked the capacity to support growth of kernels added by synchronous pollination. Their MS counterparts, however, supported the additional kernels often resulting in a positive yield response. A second perhaps less critical issue is variation in ear morphology and silking dynamics that might limit the potential for adding kernels, even if the plants could support development of additional kernels (Kaeser et al., 2003; Rossini et al., 2020). Nonetheless, the results of our initial field study clearly indicate that hybrid plants can support additional kernels with much greater oil content without a yield penalty if multiple pollination effectors are applied together. Of the three positive effectors discussed herein, synchronous pollination has by far the greatest potential for increasing the number of kernels that develop successfully. Male sterility supports the formation and establishment of additional kernels by limiting competition with the tassel for assimilates. And xenia increases kernel sink strength to attract the additional assimilates during grain filling. But only synchronous pollination ensures all receptive female florets have an equal opportunity to initiate kernel development and take advantage of the additional maternal support and sink strength. The positive yield response and economic return when these effectors are applied in combination provides a novel opportunity for grain producers to manage kernel formation and composition proactively using pollen to deliver the trait of choice.

AUTHOR CONTRIBUTIONS

Mark Westgate:Conceptualization; Data curation; Methodology; Writing – original draft; Writing – review & editing. Rachel Halbach: Conceptualization; Data curation; Formal analysis; Writing – review & editing. Mike Lauer: Conceptualization; Data curation; Formal analysis; Investigation; Writing – review & editing. Bradley VanDeWoestyne: Conceptualization; Data curation; Formal analysis; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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