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Effective chemical control of pollination would provide an alternative to the cytoplasmic male-sterile system in hybrid wheat (Triticum aestivum L.) production. The objective of this study was to determine concentration, formulation, growth stage of application and genotypic effects on levels of induced pollen suppression and subsequent natural out-crossing from foliar applications of promising Chemical Hybridization Agents (CHA's). Effective concentration ranges for each CHA were determined in preliminary trials. Growth stages of application were made at 1.5cm to 2.5cm mean primordia (spike) length of most advanced tillers.

Effective treatments were observed from both high and low concentrations and both growth stages of application with different CHA's. With specific concentrations and stages of application, ten of thirteen CHA's resulted in both high mean sterility (94-99.8 percent) relative to bagged control spikes and high plot grain yields (44.0 to

73.4 percent) relative to control mean plot yield. In some cases, highest CHA concentrations resulted in higher phytotoxicity and subsequent reduced grain yield suggesting some reduced female fertility and/or receptivity. Chemical Hybridization agents were subject to only small modifications with different formulations. Formulations which resulted in higher mean sterility also resulted in higher plant phytotoxicity, lower grain yield, and were generally effective at lower concentrations.

Large differences in genotype response were observed with a single concentration application of four CHA candidates. Concentrations high enough to induce high sterility (>95%) for all genotypes resulted higher phytotoxicity and subsequent lower yields of some treatments.

Screening genotypes with inherent capacities to receive wind-blown pollen, in addition to identifying superior pollinators, is needed. Assuming treatment genotypes can be efficiently screened for optimum concentrations and stages of application, the future of CHA technology as both a breeding tool and as a vehicle for development of hybrid wheat seems promising.

Chemically Induced Pollen Suppression and Subsequent Natural Outcrossing in Selected Wheat Cultivars (*Triticum aestivum* L. em Thell)

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

Table | Page

Table | Page

LIST OF APPENDIX TABLES

CHEMICALLY INDUCED POLLEN SUPPRESSION AND SUBSEQUENT NATURAL OUTCROSSING IN SELECTED WHEAT CULTIVARS (Triticum Aestivum L. em Thell)

INTRODUCTION

Because wheat comprises one of the worlds most important food, there is an incentive to improve its productivity on a per-hectare basis. Grain yield per unit of area can generally be equated to: Cultivar + Environment + cultivar x environmental interaction. Environment influences are the result of random effects of weather and the fixed effects that include cultural practices either of which can limit the genetic potential for yield of a cultivar.

Conventional breeding approaches for self-pollinating species, such as wheat, can utilize only the additive portion of the total genetic variability. Plant growth regulators, such as pollen suppressants, could have a significant impact on wheat productivity by improving the genetic potential by allowing the breeder to use the non-additive portion of the total genetic variability which is largely responsible for heterosis or hybrid vigor.

Hybrid wheat is currently being produced by chemical hybridization agents (CHA's) and by cytoplasmic male sterility (CMS). Some hybrid combinations have out-yielded self-pollinated lines, however, yield advantage has generally been low, indicating that the development

and identification of superior hybrid combinations to maximizeheterotic response requires further investigation.

When comparing CHA and CMS systems, both have advantages and disadvantages. The main advantage of the CMS system is that high levels of sterility can be achieved without chemical applications. Disadvantages include the necessity of incorporating specific cytoplasms and fertility restoring genes in agronomically acceptable lines which is costly and difficult. Also, the time lag required to convert promising new genotypes into CMS (female) inbreds, combined with relatively small yield improvements, has made CMS hybrids generally unacceptable for large scale commercial production.

Advantages of a CHA system are: a) there is no need to develope and maintain male sterility or identify fertility restorer lines, b) the relative ease of evaluating large numbers of combinations for general and specific combining ability that enhance the odds of identifying superior heterotic combinations, and c) with sudden changes in biotic stress, new and more resistant hybrids can be rapidly developed. There are several disadvantages inherent to the CHA system which include a) concentrations sufficient to induce 100% male sterility have generally led to some female sterility and subsequent reduced seed sets, b) inclement weather such as high winds and extended periods of rain can prevent CHA applications

and c) CHA x Genotype and CHA x Environment interactions can lead to inconsistent levels of male sterility, resulting in reduced hybrid purity and the loss of production fields.

Ultimately, both CHA and CMS systems may complement one another by utilizing their inherent strengths. The CHA system could be utilized for efficient screening and identification of superior heterotic combinations, and then apply the technology of the CMS system to develop CMS lines.

If CHA technology is to be of value, several problems must be overcome before commercially viable CHA hybrids become a reality. These are: a) the need to identify a superior CHA with minimal adverse effects on plant growth, particularly in selectively inducing male sterility without affecting female fertility, b) must induce complete or nearly complete sterility in both early and late tillers, and c) must have a broad window of effectiveness to overcome the effects of bad weather and the time required to treat large acreage.

The economic problems of hybrid seed production must also be overcome. These include: a) identification of significant heterotic combinations, b) overcome variable and low seed sets under natural conditions, and c) develop cultural practices for lowered seeding rates and improved precision planting of hybrids.

Chemical Hybridization Agent technology can resolve many of the problems stemming from the CMS system only if a superior CHA is identified that possesses the characteristics described above. The emphasis of this thesis research was to evaluate CHA candidates, concentrations, formulations, growth stages of application, and genotypes, to determine if the above criteria can be achieved.

LITERATURE REVIEW

Chemically-Induced Male Sterility In Wheat

The discovery of and subsequent problems associated with a biological system to induce selective male sterility in wheat spurred efforts to identify chemical agents that would act as selective pollen suppressants. Such compounds, it was believed, would avoid the sophisticated genetic intricacies associated with cytoplasmic-induced male sterility and subsequent fertility restoration (Virmani and Edwards, 1983). Chemically-induced pollen suppression would also provide an efficient method to produce large numbers of different hybrid combinations for evaluation. Finally, certain breeding strategies, such as polycross and recurrent selection, would be enhanced. However, to have practical applications chemically-induced pollen suppression must demonstrate complete effectiveness with minimal undesirable effects on other plant functions, particularly female fertility and receptiveness.

Significant progress has been achieved in identifying effective pollen suppression agents. The advanced state of the art of these chemical compounds stems from resolution of the early problems encountered: a) induction of selective pollen suppression b) retention of female

fertility and/or receptiveness c) association of chemical effectiveness with high levels of phytotoxicity. A chronological review of the literature that led to the development of current pollen suppression agents will be presented.

Porter and Wiese (1961) evaluated several compounds as potential selective male gametocides from 1958 to 1960. They reported that Maleic Hydrazide, FW-450 (sodium salt of 2,3-dichloroisobutyric acid), Potassium Giberellate, Dalapon (sodium 2,2 dichloropropanate), TIBA (triiodobenzoic acid), dimethylamine salt of Trichlorobenzoic Acid, Napthalene Acetic Acid, Ethanol and isopropanol series of amine salt of 2,4-D were not satisfactory. Greenhouse applications of FW-450 produced partially male-sterile plants when applied at the jointing stage. However, these treatments were vary phytotoxic. Field trials, conducted in 1959 on both spring and winter wheats using FW-450, resulted in severe damage to spring wheat at all stages of growth and moderate to severe damage to winter wheats. Applications of Malaic Hydrazide at 1.12 and 2.24kg/ha resulted in plant damage, but high levels of sterility occurred at both rates of application during late boot and early heading stages. Seed set at these stages was greatly reduced, attributed to both male and female sterility. Flowers that appeared to be male sterile did not appear to open normally. None

of the treatments caused an increase in cross pollination.

Ram and Rustagi (1966) reviewed the effectiveness of pollen suppressants prior to 1966. Prominent among these were Mendok (FW-450), Dalapon, Malaic Hydrazide, Napthaline Acidic Acid (NAA) and Triiodobenzoic acid (TIBA). The main objective of the treatments with these compounds was the selective induction of male sterility without undesirable side effects. They concluded that, although preliminary studies gave encouraging results, none of these compounds proved reliable.

Kaul and Singh (1967) evaluated the effectiveness of FW-450, Malaic Hydrazide and Dalapon to induce pollen suppression in wheat. At the highest concentrations of FW-450, growth was stunted, emergence of spikes were delayed, and pollen fertility was high. Higher concentrations of Maleic Hydrazide (0.025, 0.05 and 0.1 percent) induced complete pollen abortion, but resulted in considerable plant phytotoxicity. Female sterility was evident as hand pollinated spikes demonstrated poor seed sets (30-50 percent of the control). Treated plants also had restricted flower development. Dalapon treatments of 0.025 and 0.05 percent applied before and after initiation of floral primordia were found to be effective in producing complete pollen sterility; however, significant plant injury was noted with increased concentrations. Female sterility was evident as hand pollinations resulted

in an average seed set of 30 percent. Effective doses tended to resulted in the tendency of spikelets to remain fused.

Both greenhouse and field applications of Ethrel (2-chloroethyl phosphoric acid) to a soft white winter wheat proved effective in inducing male sterility with little side effect on plant development, including no apparent injury to female floral parts (Rowell and Miller 1971). Greenhouse applications of Ethrel at concentrations of 100 to 250ppm during the pre-boot stage of growth gave a significant reduction of seed set per spike. Concentrations of 500 to 2500ppm resulted in essentially in no seed set. Spikelets opened readily at anthesis. Earlier stages of application resulted in some adverse morphological effects, including shortening of internodes, reduced plant height, shortening of the peduncle, and poor spike emergence. Under field applications during early, mid, and late boot stages, Ethrel concentrations of 1000, 1500 and 2000ppm resulted in almost complete male sterility. Higher concentrations were required to induce high levels of male sterility at the boot stage of growth. Higher concentrations at pre-boot stages resulted in some abnormal spike emergence. Ethrel-treated florets revealed anther shrivelling, shortening of the filament, and lack of pollen release.

Encouraging observations by Rowell and Miller (1971)

spurred further investigations of Ethrel as a potential tool for developing hybrid wheat. Stoskopf and Law (1972), investigating Ethrel initially as a growth regulator and later as a pollen sterilant, reported that Ethrel may act more to retard anther development than as a pollen sterilant. Applications of 0.84 kg/ha Ethrel resulted in what appeared to be sterile florets which remained open for a 7 to 14 day period. Grain yield equal to or exceeding check plot yields was taken as evidence of outcrossing or selfing. Later experiments on Ethrel-treated spring wheat resulted in spikes that, while appearing to be male-sterile when bagged prior to flowering, contained 89 percent seed set at harvest. This suggested that selfing was a major barrier to the success of Ethrel as a tool for developing hybrid wheat. In addition, the requirements of precise rate and time of application, as well as critical time of pollination to ensure high percentages of outcrossed seed, made Ethrel a questionable agent for inducing male sterility.

Working with Chinese spring wheat, Bennett and Hughes (1972) demonstrated that abnormalities late in pollen development often involved the induction of additional nuclear divisions in pollen grains from Ethrel-treated plants. Florets with induced male sterility often resulted in anthers that were reduced in size. Anther extrusion and dehiscence frequently failed or sterile

pollen was released. Sterile pollen also differed from normal pollen at dehiscence by containing a) few or no starch granules b) no elongated sperm nuclei and c) occasionally more than three nuclei. Many anthers with abnormal pollen grains had still not extruded or dehisced ten days after the normal flowering time. Bennett and Hughes (1972) also determined that there was a critical period when Ethrel must be applied in order to observe these abnormalities and obtain subsequent induction of sterility. This period ends about the time meiosis is initiated in the pollen mother cells and includes the pre-meiotic interphase.

In greenhouse studies, Rowell and Miller (1974) reported that seed production from Ethephon (Ethrel)-treated winter wheat was predominately F1 for all concentrations and growth stages evaluated, except for the lowest concentrations applied at the heading stage. Pre-boot to late-boot stages, at concentrations of 250 to 3000ppm Ethrel resulted in 87 to 100 percent F1 seeds. Seed set from hand pollinated treatments were similar to those obtained from hand pollinated cytoplasmic male sterile lines for several concentrations and stages. Plants treated at the heading stage with low concentrations had high seed sets (the result of incomplete sterility) and correspondingly lower F1 seed percentage when compared to the male sterile checks.

Highest concentrations applied at the heading stage generally resulted in lower seed set than the male sterile check, suggesting some reduction in female fertility. Sterile spikes on plants treated at lower concentrations emerged from the flag leaf sheath with florets freely open. Higher concentrations showed increased peduncle shortening and spike retention in the flag leaf sheath.

Further field studies by Rowell and Miller (1974) supported the above greenhouse findings. Significant differences in seed set between bagged (prior to flowering) and non-bagged (open pollinated) spikes indicated good male sterility at all concentrations and stages except for 500, 1000 and 1500ppm concentrations at the pre-boot stage. However, at these concentrations and at this stage, F1 seed production ranged from 37 to 90 percent, even though seed set for bagged spikes was high. Plants treated at early and mid-boot stages with higher concentrations (2000-3000ppm) produced 95 to 100 percent F1 seed consistently over two years. The number of seeds produced on chemically-treated plants at pre, early, and mid-boot growth stages with these concentrations resulted in high levels of sterility which was significantly lower than in cytoplasmic male sterile controls. These observations indicated some female sterility or lack of female receptiveness. This work suggested that the stage of application was critical and for any given wheat

tiller, susceptibility to gametocidal effects of Ethrel occurred for a limited period of time.

Evaluating Ethrel as an agent for increasing tillering, reducing lodging and increasing yield, Brown and Earley (1973) reported that applications of 1.12 kg/ha on winter wheat at the early boot stage produced the fewest spikelets and seeds per spike, the heaviest kernels, and the lowest grain yield of any treatment when compared to other concentrations and stages of development. This was the only treatment that indicated any apparent sterility. To achieve these results required specific rates and stage of application. Although Brown and Earley (1973) did not bag spikes, they concluded that Ethrel may not be as effective a male gametocide as previously suggested by Rowell and Miller (1973).

Jan, et al (1974) reported on a promising new compound, RH-531 (sodium 1-(P-chlorophenyl)-1,2-dihydro-4,6-dimethyl-2-oxonicotinate), a growth retardant for use as a selective pollen suppressant. Cultivar differences in percentage of florets producing seeds on spikes treated with RH-531 were observed. Single applications of 2.0kg/ha at the pre-meiosis stage in the cultivar 'Anza' resulted in 98 percent of the florets failing to produce grain. Ninety percent of the florets failed to produce grain when applications were made during meiosis.

Similar treatments with 'Yecora 70' resulted in only 75 percent of the florets failing to produce grain at the pre-meiosis stage. Even though sterility was induced, florets did not open in a typical male-sterile fashion. Treatments resulted in a compaction of the spikes. Glumes, lemmas and paleas were reduced in size and apparently thickened, resulting in an unfavorable condition for receiving wind blown pollen. Of the more than 1500 plants observed, more than 98 percent of the progeny from RH-531 treatments resulted from sib or self-pollination.

Further evaluation of Ethrel by Hughes et al (1974) supported the findings of other researchers; that a positive correlation existed between concentration, growth stage, and degree of induced sterility. At the pre-meiosis stage, a 2000ppm concentration of Ethrel resulted in complete sterility on spring wheat tested under greenhouse conditions. At meiosis, only the highest concentration (8000ppm) of Ethrel resulted in complete sterility and when applied at the post-meiosis stage, even the highest concentration failed to induce full sterility. Delay in flowering also was correlated with stage and concentration. Earlier stages (pre-meiosis) and the highest concentrations resulted in delayed flowering up to six days. A reduced number of fully developed spikelets per spike were found. Treatments at pre-meiosis and

meiosis stages resulted in an average of five and four less spikelets per spike, respectively, for all Ethrel treatments. An average of 70 percent of the Ethrel-treated sterile florets set seed when hand-pollinated. Higher concentrations at the pre-meiosis and at meiosis resulted in a lower percentage florets setting seed. Ethrel treatments reduced plant height by preventing full elongation of the upper stem internodes. Again, earlier stages and higher concentrations resulted in the largest height reductions. Concentrations above 2000ppm shortened peduncle length and often resulted in failure of spikes to fully emerge, resulting in stigmas that could not be readily pollinated.

Hughes et al (1974) concluded that commercial application of Ethrel was not promising. Ethrel treatments that were most effective for inducing sterility also prevented spike emergence, resulting in 1/3 of the spike remaining in the flag leaf sheath. Ethrel treatments also resulted in a reduction of the number of spikelets per spike. Fewer spikelets were thus available for outcrossing. Spike emergence could be improved with later applications; however, such applications required higher concentrations to achieve complete sterility. This resulted in both reduced seed set and even further reduction of spikelets per spike. These observations led to the conclusion that timing of developmental stage and

concentration of Ethrel applications was critical, with less than satisfactory results even when conditions were optimized. This suggested that production of uniform stands on a large scale to facilitate sterilization of all primary tillers would be difficult, particularly if climactic conditions prevented timely applications. Finally, the cost of Ethrel at rates required to induce complete sterility was too high to make production of commercial hybrids economical. Ethrel would appear to be economical only in producing small quantities of F1 seed for yield trials and screening superior yielding combinations.

Recognizing the apparent limitations of foliar Ethrel application, Fairey and Stoskopf (1975) reported on greenhouse and field applications using granular Ethrel on three winter and six spring wheat cultivars. Sterility levels of up to 100 percent were achieved on individual spikes in some treatments with no apparent morphological or physiological abnormalities. A positive correlation between rate of application and induced male sterility was found. However, 548 to 822kg a.i./ha of Ethrel was required to induce the highest levels of sterility. Average sterility in any treatment was less than 100 percent. Levels of sterility ranged from 16.9 to 66.5 percent. Undesirable variability in levels of sterility and genotype x chemical interactions were observed with

granular Ethrel applications. Variation in levels of sterility in late formed tillers was most pronounced. Winter wheats at low concentration were more readily sterilized than spring cultivars. This was attributed to greater uptake from a more extensively developed root systems in winter wheat cultivars. Further variation in levels of sterility from year to year was observed.

Johnson and Brown (1976) reported on a promising pollen suppressing agent, DPX 3778 (3-(p-chlorophenyl)-6-meyhoxy-s-triazine-2,4-(1H,3H)dione, triethanolamine salt). Greenhouse applications of 9.0kg/ha DPX 3778 to 11 spring wheat cultivars at the early-boot stage reduced seed set to essentially zero on six cultivars and reduced seed set on the remaining cultivars as well. No phytotoxic effects were observed; however, anther extrusion was delayed by chemical treatments. Sterile florets failed to shed pollen. The lemma and palea opened, exposing the stamen and pistil in a typical male-sterile fashion. No noticeable affects on female fertility were observed as hand- pollinations of sterile spikes resulted in high seed set.

Johnson and Brown (1976) applied 4.5 to 27 kg/ha DPX 3778 to a hard red spring cultivar at two stages of growth under field conditions. An application rate of 9.0kg/ha was sufficient to prevent pollen shed at both early and late boot stages. Bagged secondary spikes generally

demonstrated higher levels of sterility than bagged primary spikes. Higher application rates had no effect on spikelet number or spike emergence, but outcrossed seed set was reduced to zero. Hand pollinations of the best treatments resulted in 50 to 80 percent seed set, indicating female fertility was not affected. Floret opening at anthesis was observed, but seed sets in non-bagged spikes were generally low. This observation was attributed to a delay in anther extrusion, resulting in treated plants not being receptive during peak pollen loads.

Four compounds, RH-531, 532, 2956 and 4667 (chemical identification not disclosed) were evaluated by Miller and Lucken (1977) as potential pollen suppressants. Initial evaluations involved three spring wheat cultivars treated at two stages of development. Cultivars differed in their response to rates and growth stages of treatment. All chemicals significantly reduced fertility of the three cultivars; however, only RH-352 reduced fertility to a low level (0-10 percent) in test cultivars. With fertility reduced from 0 to 10 percent, unacceptable yields of 7 to 26 percent of non-treatment control were observed; however, hybrid seed content of these treatments was over 75 percent. All treatments reduced plant height. Anthesis was delayed by three days and in some treatments a reduced spike length resulted. Although florets opened

sufficiently to facilitate cross pollination, growth of the stigma appeared retarded and did not become visible as in cytoplasmic male sterile lines.

A second trial conducted by Miller and Lucken (1977) involved applications of RH-352 on one cytoplasmic male (CMS) sterile wheat line and three spring wheat cultivars at four rates of application and at two growth stages. Increasing dosage significantly decreased fertility, seed yield, plant height and spike length when averaged across cultivars. Stage of development and concentration affected cultivars differently, resulting in the need to screen genotypes for the optimum growth stage and concentration of application to optimize hybrid yields. Increasing concentrations at earlier stages had a more pronounced influence on pollen suppression than increasing rates at later stages. When low seed sets under bagged spikes were achieved (0-10 percent), yields of outcrossed spikes were significantly reduced (1-21 percent) and percentages of hybrid seed were low (2-55 percent). Grain yield of the CMS sterile check was 57 percent of the male fertile check, indicating an adequate cross pollination potential. All chemical treatments of RH-352 on the CMS male sterile line significantly reduced the yield of hybrid seed. This reduction was most pronounced at earlier stages of application. Miller and Lucken (1977) concluded that RH-352 has inhibitory properties

that suppress the fertilization potential of treatment plants. This, combined with an inability to achieve consistent results from year to year with similar treatments, made commercial application of RH-352 unlikely.

Dotlacil and Apltauerova (1978) utilized a spring wheat line grown under field conditions to assess pollen suppression of Ethrel over a two year period. Appropriate applications of Ethrel resulted in nearly complete sterility. Treatment spikes were well developed and female fertility remained high. During the first year of experiments, the highest levels of sterility were achieved with two applications of 3000ppm and applications of 3000ppm followed by 2000ppm split-applications of Ethrel at a stage corresponding to the appearance of the ligule of the flag leaf. Fertility levels were reduced to 5 percent of control with percentages of completely sterile spikes ranging from 68.3 to 75 percent with the above treatments. Reduced plant height, and spike retention in the flag leaf sheath, were correlated with increased concentrations. Repeat applications of 3000ppm Ethrel at the booting stage of the flag leaf did not produce the highest levels of sterility, but did result in the greatest average number of hybrid seeds per spike (4.6). This treatment resulted in 50 percent F1 seed. Similar results were observed in the

second year of testing. The proportion of open pollinated F1 ranged from 28 to 55.8 percent over all treatments. Repeat applications of 2000ppm Ethrel at the stage prior to meiosis resulted in 55.8 percent hybrid progeny (9.9 hybrid seeds per spike) from open pollinated spikes. Higher concentrations resulted in higher levels of sterility, but fewer F1 seed per spike. Dotlacil and Apltauerova (1978) stressed the importance of adequate pollen loads at critical times, as well as appropriate dose and stage of Ethrel applications to optimize percentage of hybrid seeds.

Applications of Ethrel to produce F1 seed and assess yield performances of the resulting hybrid seed produced was evaluated by Hughes and Bodden (1978). Two isolated crossing blocks, each with a different male pollinator, but with the same nine female treatment cultivars, were used to generate F1 seed. Applications of 10.86kg(a.i.)/ha Ethrel to induce pollen suppression and 1.1kg(a.i.)/ha Gibberellic acid to ensure emergence of spikes from the leaf sheath were applied to nine winter wheat cultivars. Applications were made when the anthers of the earliest treatment plots had reached the stage of development just prior to meiosis. Seed per spikelet ranged from 0.02 to 0.52 following Ethrel treatments. Improved spike emergence from gibberellic acid applications in semi-dwarf wheats was less than in

cultivars of conventional height, and some spikes failed to emerge. Seed set was low in open pollinated spikes, but sufficient quantities of seed were produced to assess yield potential. Proportion of hybrid seed harvested from treatment plots ranged from 62.6 to 97.3 percent, when quantities of seed in bagged spikes were compared with a similar sample of non-bagged spikes. Hughes and Bodden (1978) concluded that Ethrel could prove useful for providing sufficient quantities of F1 seed to screen superior yielding hybrid combinations.

After three more years of experimentation with DPX-3778 utilizing three spring wheat cultivars as female treatments and one male pollinator, Johnson and Brown (1978) observed seed set in bagged spikes was essentially zero with concentrations above 9.0kg/ha applied at the boot stage in two out of the three years. High levels of sterility were associated with reduced plant height and delayed anther extrusion of 4 to 5 days. The best results were obtained in 1975 where the proportion of harvested seed from open pollinated spikes ranged from 83 to 98 percent F1 seed and plot yields ranged from 33 to 80 percent of control. In 1977, several treatments resulted in good pollen suppression, but the proportion of hybrid seed did not exceed 85 percent. The low proportion of hybrid seed was attributed to low seed set, resulting from high temperatures during pollination. In 1976, the

proportion of hybrid seed from treatment plots did not exceed 55 percent which was associated with lower levels of induced pollen suppression and plant wilting from drought conditions at the time of chemical applications. Failure of DPX-3778 to reduce plant height and delay anther extrusion in 1976 was taken as evidence of chemical uptake failure, causing subsequent lower levels of induced pollen suppression and a resulting low proportion of hybrid seeds. Highest rates (18.0 and 27.0kg/ha) of DPX-3778 applied at late boot stage resulted in reducing seed set to essentially zero in bagged spikes. However, these treatments reduced seed yields and were associated with a lower proportion of F1 seed. This observation was taken as evidence that higher concentrations reduced female fertility or the fertilization process. Johnson and Brown (1978) concluded that inconsistent results achieved with DPX-3778 were the result of environment x chemical interactions. High proportion of hybrid seed and high seed set were dependent on favorable environments that prolonged pollen viability and stigma receptivity.

Jan and Rowell (1981) reported on the comparative pollen suppression effects of Ethrel, RH-532 and RH-2956 on different stages of development in primary and secondary tillers using two spring wheat cultivars grown under greenhouse conditions. Ethrel was found to be much less effective than RH-352 and RH-2956 in reducing seed

set. Ethrel treatments of 2.0kg/ha resulted in plants producing an average of one seed per spikelet compared to an average of 2.2 seeds per spikelet when compared to the control. At the 2.0kg/ha rate, Ethrel treatments affected only secondary tillers when applied at or before meiosis. No sterility was observed in primary tillers treated after meiosis. Early Ethrel treatments also resulted in poor spike emergence.

Several chemical treatments resulted in differences in cultivar response. Yecora 70 produced more seeds per spike than Anza in most treatments. Applications of RH-532 to Yecora 70 treated at or before meiosis resulted in higher fertility in secondary tillers than primary tillers. Anza demonstrated less variation among tillers and resulted in good sterility even with late applications. Both cultivars treated with RH-2956 demonstrated only small differences in fertility between early and late tillers, particularly at higher rates of application.

In recent years, significant advances have taken place in chemically-induced male sterility with minimal side effects. While chemical hybridization agents have improved, published literature on chemical performance has been limited due to the proprietary nature of much of the current research.

At the twelfth annual meeting of the Plant Growth

Regulator Society Of America, Warner (1985) reported that Rohm and Haas Seeds, Incorporated's product, Fenridazon-K, trademark HYBREX (potasium 1-(p-chlorophenyl)-1,4-dihydro-6-methyl-4-oxopyridazine-3-carboxylate) was currently producing both experimental and commercial wheat hybrids. Data from cultivar x Fenridazon-K interaction trials indicated that the effective doses for 100 percent male sterility ranged from 0.2kg/ha, for the most sensitive hard red spring cultivars, to 2.0kg/ha for the most tolerant.

Present at the same meeting, Foster and Kaplin (1985) reported that CHA-811, developed by Shell, was effective at doses greater than 0.7kg/ha in inducing complete sterility in both hard red winter and soft red winter wheats. Seed sets from CHA-811 treatments were generally low in open pollination situations, however, hand pollinations revealed that female fertility was unimpaired. Fifty-two of the 350 evaluated genotypes produced no seed in bagged spikes when treated with CHA-811, but yielded 60 percent or greater seed set in open pollinated situations, compared to untreated checks. Phytotoxic side effects of CHA-811 were not significant, even at doses three times the effective dose required to produce adequate pollen suppression. CHA-811 also possessed a wide range in terms of effective growth stages for effective application. Good responses as early as a

primordia length of 3.0cm and as late as late boot or awn emergence (interval of stage 34-49 on Zadoks scale) were found with CHA-811 treatments.

Porter et al (1985) compared seed sets of five cultivars with induced male sterility under greenhouse conditions. Three sources of sterility were a) hand-emasculation b) cytoplasmic male sterile A-lines and c) 84811 CHA male sterile B-lines. Mean seed set on male sterile spikes following hand pollinations resulted in 92, 92, and 87 percent for the respective sources of sterility. These observations suggest that CHA SD 84811 causes negligible female sterility.

Recently developed compounds clearly demonstrate a) induction of selective pollen suppression, b) retention of female fertility and/or receptiveness and c) chemical effectiveness with low levels of phytotoxicity. Further advancement of this technology will depend on the identification of superior yielding hybrid combinations and the economic feasibility of producing hybrid combinations through chemically-induced pollen suppression (McRae, 1985).

MATERIALS AND METHODS

Preliminary Studies

Initial studies evaluated pollen suppression properties of sixty potential Chemical Hybridization Agents (CHA's) and determined the capacity of chemically emasculated plants to outcross. The specific objectives of these early investigations were to determine gross chemical efficacy under field conditions and to identify effective stages of application and concentrations. Logarithmic dilution foliar applications were used to evaluate effective concentration ranges. Specific concentrations along the logarithmic gradient were identified. Glassine bags were placed on emerged spikes prior to flowering to prevented outcrossing and determined levels of induced sterility for various target concentrations. Female fertility and/or receptiveness, was evaluated by collecting non-bagged spikes near the bagged spikes at harvest. Measurements of sterility and outcrossed seed (bagged and non-bagged samples) from treatments were based on relative performance to control plots treated in a similar manner. Superior CHA candidates and concentrations were then examined in a more detailed advanced studies, the results serving as basis for this thesis.

Advanced Studies

The above preliminary studies combined with observations made by other researchers placed promising CHA candidates into two categories: a) New CHA candidates: eight compounds which demonstrated, from a single season of preliminary trial, effective concentrations equal or superior in performance characteristics to more advanced candidates and b) Advanced CHA candidates: four compounds that have emerged from several years experimentation and demonstrate the best overall characteristics to date. Different formulations of this later group were explored to determine if performance could be enhanced. In addition, genotype x chemical interactions were evaluated for advanced CHA candidates to identify the best CHA efficacy across a broader genetic background.

Field trials to evaluate new and advanced CHA candidates were established near Amity, Oregon on Woodburn silty clay loam soil (Aqualtic Argixeroll fine-silty mixed mesic), on 12 October 1986. Male pollinator and female treatment plots were planted in a one to one ratio and oriented in a north-south direction to minimize border effects and optimize cross pollination from prevailing west and northeast winds. Individual plots were 1.5M x 4.8M with seven rows spaced 18cm apart. A seeding rate of

96 kg/ha was used. Fertilizer amendments included 185 kg/ha granular Ammonia Phosphate Sulfate (16-20-0-15) applied with the seed at planting time, followed by a single application of 200 kg/ha granular Urea (46-0-0) in early spring. Weeds were controlled with 2.3 kg/ha Diuron (pre-emergence), followed by a tank mixture of 2.9 L/ha Diclofop-methyl with 35.5 gm/ha Chlorsufuron applied in early spring.

All compounds[1] were put into solutions with deionized water and appropriate surfactant (see individual studies). Treatments were then applied as foliar spray and delivered by a tractor-mounted nitrogen-pressurized sprayer. The sprayer boom was fitted with eight T-Jet twin-fan 60-8002 nozzles. A double-overlap pattern was used to optimize coverage. Solutions were delivered at a rate of 600L/ha with a nozzle pressure of 30 k.p.a.. Uniform applications, even under windy conditions, were made possible by completely enclosing the sprayer boom with a shroud that extended to the crop surface. Drop shields adapted to the end of the shroud extended into the crop canopy to further shield pollinator plots from spray drift. An electronic flow indicator monitored uniform delivery. Three individual studies conducted in the 1986-87 growing season will be presented individually.

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[1] All compounds were provided by Sogetal, Inc., Hayward, CA.

STUDY 1: New Chemical Hybridization Agents (CHA's)

One test cultivar, two stages of growth, and three concentrations were employed to evaluate the effectiveness of eight new CHA candidates and four advanced CHA candidates against a standard (1058). The standard 1058 has previously demonstrated the capacity to produce sufficient sterility and subsequent quantities of pure F1 seed to facilitate hybrid yield evaluations. However, concentrations sufficient to ensure near-complete pollen suppression have generally resulted in low seed sets due to reduced female fertility and/or receptivity. Phytotoxic effects and subsequent reduced seed set with 1058 applications generally results from incomplete spike emergence, primarily manifested in secondary and tertiary tillers. These observations suggest that 1058 is not a suitable CHA for commercial hybrid development. Four advanced CHA candidates (2053, 1168, 1271 and 1244) were also included to evaluate the relative performance levels of the eight new CHA candidates. Chemical Hybridization Agents (CHA,s) and concentrations were as follows:

Standard	1058 -	0.4, 0.5, 0.6kg/ha
Advanced	2053 -	0.2, 0.4, 0.6kg/ha
	1168 -	1.0, 1.2, 1.4kg/ha
	1244 -	1.0, 1.2, 1.4kg/ha
	1271 -	0.2, 0.4, 0.6kg/ha

New	1260 -	0.6, 0.9, 1.2kg/ha
	1272 -	0.1, 0.3, 0.5kg/ha
	2029 -	0.1, 0.2, 0.3kg/ha
	2099 -	0.3, 0.5, 0.7kg/ha
	2129 -	0.5, 0.8, 1.0kg/ha
	2157 -	1.0, 1.2, 1.4kg/ha
	2168 -	0.6, 0.9, 1.2kg/ha
	2186 -	0.2, 0.4, 0.6kg/ha

Malcolm, a soft white winter wheat, served as test cultivar for female treatment plots (Appendix Table 1). Malcolm was selected as the treatment cultivar to nick with the male pollinator. The male pollinator (Blue Norco; Appendix Table 1) posses a small telocentric chromosome from an Agropyron species which carries a dominant allele that conditions blue color. One dose of this allele is sufficient to convey a blue xenia effect to F1 seeds. Outcrossed seed can then be visually evaluated at harvest. A split plot design with three replications was used. Main plots consisted of growth stages of application. Subplots consisted of CHA candidates and concentrations and were randomized within the main plots.

Two growth stages of plant development were defined by mean primordia (spike) length of the earliest formed tillers. Growth Stage one applications were made when the primordia length reached 1.5cm in length. This growth stage corresponded to the appearance of the flag leaf (stage 37 on Zadoks' scale). Growth Stage two applications were made at 2.5cm spike length and corresponded to flag leaf sheath becoming visible to the flag leaf sheath extending (stages 39 to 41 on Zadoks'

scale). Stage one applications were made on 22 April 1987 and stage two on 27 April 1987. All CHA solutions contained 0.2 percent non-ionic surfactant (Triton AG-98: Octyl Phenoxy Polyethoxy ethenol).

Glassine bags were placed on a total of 21 spikes prior to flowering to determine the degree of induced sterility and/or the amount of selfing. Bags were randomly placed on both primary and secondary tillers of all rows at three locations to ensure complete sampling of all plots. Percent sterility was derived from a comparison with control plots (no chemical application) which were bagged in a similar manner and defined as STERILITY:

$$1 - \frac{\text{(Av. No. Seeds From 21 Trt. Bagged Spikes)}}{\text{(Av. No. Seeds From 21 Bagged Control Spikes)}} \times 100$$

Twenty one additional spikes for each treatment and from the same proximity as bagged spikes within plots were collected at harvest to determine the amount of outcrossed seed on an individual spike basis. Percent outcrossed seed was also derived from a comparison with control plots (no chemical application), and 'adjusted' for level of sterility to give a more accurate assessment of outcrossed seed, and defined as MICRO OUTCROSS or OUTCROSSED SEED:

$$\frac{\text{(Av. No. Seeds From 21 Unbagged Trt Spikes)}}{\text{(Av. No. Seeds From 21 Unbagged Control Spikes)}} \times \%\text{STERILITY}$$

In addition to OUTCROSSED SEED observations, all

plots were combine-harvested and subsequent yield served as an additional measure of the amount of outcrossed seed. Grain yields were given as percentage of mean control plot yields (three replications with no chemical application) and defined as MACRO OUTCROSS or GRAIN YIELD:

$$\frac{\text{(Wt. of Trt. Plot)}}{\text{(Av. Wt. of Control Plots)}} \times \%\text{STERILITY}$$

The proportion of harvested seed resulting from pollination by the male plots was determined by the number of blue seeds from each treatment compared to the total number of seeds harvested from the above 21 open-pollinated (blue seed vs total seed). This was defined as MICRO PURITY:

$$\frac{\text{(No. of Blue Seeds From 21 Trt. Spikes)}}{\text{(Total No. Seeds From 21 Trt. Spikes)}}$$

An additional assessment of purity was based on a one-hundred seed sample taken from combined treatments which resulted in sterility of greater than 95 percent of control and was defined as MACRO PURITY:

$$\frac{\text{(No. of Blue Seeds in 100 Seed Combine Sample)}}{100}$$

Chemical Hybridization Agent phytotoxic effects for all treatments were recorded using a scale from 0 to 100. Treatments with a score of 0 demonstrated no visible symptoms as compared to control. Phytotoxic scores

greater than 40 were considered significantly damaging. Scores were based on overall plant appearance and combined several factors, emphasizing those effects that influence floral characteristics: 1)foliar damage 2)imperfect heading 3)delayed heading and 4)indications of reduced female fertility and/or receptivity. Plant height was recorded for each treatment and was also incorporated into the phytotoxic score; however, plant height was not considered a significant factor in overall CHA evaluations (see Appendix Table 2 for complete scoring system).

Seed quality was visually evaluated for any treatment demonstrating greater than 95 percent sterility. Aberrant appearing seeds were scored on a scale from 1 to 5; scores of greater than 3 were considered significantly damaged, generally expressed as wrinkled or shriveled seed (see Appendix Table 2 for complete scoring system).

Analysis of variance for a split plot design was used to determine differences between CHA candidates, concentrations and growth stages of application for observations of Sterility, Micro Outcrossed Seed, Macro Outcrossed Seed, Micro Purity and Phytotoxicity. Superior treatments were identified based on treatments resulting in both high sterility and high yield with corresponding low phytotoxicity and low seed quality observations.

Study 2: Chemical Hybridization Agent x Formulation Evaluation

Five Formulations plus a water control for four advanced CHA candidates (1168, 2053, 1271 and 1244), two growth stages of application, and two concentrations of each CHA/formulation were used to determined the effects of formulation on individual CHA effectiveness. As in Study 1, a split plot design with three replications was employed. Main plots were plant growth stages of application. Subplots contained CHA/Formulation combinations and concentrations and were randomized within the main plots. Cultivars, growth stages of application, and assessment of treatment performance were identical to the protocol established in Study 1. Formulations and CHA concentrations were as follows:

CHA	Concentrations
1168 -	1.0 and 1.4kg/ha
2053 -	0.3 and 0.5kg/ha
1271 -	0.3 and 0.6kg/ha
1244 -	1.0 and 1.4kg/ha

	Formulation
A=	0.2% Triton AG-98
B=	0.2% Triton AG-98 plus 2.0% Glycerol
C=	0.75% Tween-20 plus 0.1% Aresol GPG
D=	0.2% Flomo
E=	0.2% Flomo plus 2.0% Glycerol
F=	Water Control

Study 3: Chemical Hybridization Agent x Genotype Evaluations

The best estimation of a superior single concentration of four advanced CHA candidates (1168, 1244, 1271 and 2053) were applied to 25 genetically diverse soft white winter wheats (Appendix Table 1). The cultivar 'Stephens', also a soft white winter wheat, served as the male pollinator (Appendix Table 1). A completely randomized design was used to identify differences between mean CHA performance across genotypes for observations of: a)sterility, b)micro and macro outcrossed seed, c)seed quality, d)plant phytotoxicity, e)growth stage of application and f)heading date. Chemical Hybridization Agent applications were made to each genotype when mean primordia length of the most advanced tillers reached 2.0cm (stages 39 to 41 according to Zadoks' scale). Concentrations were as follows: 1168, 1.4kg/ha; 1244, 1.4kg/ha; 1271, 0.5kg/ha; 2053, 0.3kg/ha. Assessments of sterility, micro outcross seed, macro outcross seed, seed quality, and plant phytotoxicity were similar to the protocol established in Studies 1 and 2. Cloth cages, supported by iron frames covering an area of 30cm x 30cm, and extending to the soil surface, replaced the use of glassine bags to assess degree of sterility. Cages were placed in the center of both treatment and control plots and removed when the plots were no longer receptive to pollen. Twenty previously caged spikes were collected at

harvest and the amount of sterility relative to control plots was determined. Twenty additional open pollinated spikes were randomly collected from plots at harvest to assess degree of outcrossed seed relative to control plots treated in a similar manner (micro outcross). Treatment plots were then combine-harvested and subsequent plot grain yields served as an additional measure of outcrossed seed.

RESULTS AND DISCUSSION

STUDY 1: New Chemical Hybridization Agents (CHA's)

Sterility (pollen suppression)

Bagged spikes from control plots (no treatment) contained an average of 14.4 and 24.7 percent fewer seeds per spike than non-bagged control spikes for growth stage one and two applications, respectively. Reduced and variable seed set observed in both control and treatment bagged spikes may be largely attributed to: a) small sample size, and b) unusually high temperatures during grain filling ultimately resulting in some light and shriveled seed lost during the threshing process. Despite these observations, bagged control spikes served as the base seed set level to evaluate relative treatment performance. Further reduction in seed set was assumed to be the result of chemically induced pollen suppression and subsequent male sterility and not the result of bagging or bag x treatment interaction.

All first-order interactions for observations of sterility were significant (Table 1; Appendix Table 4). This suggests that main effects (CHA candidates, concentrations and growth stage of application) were not acting independently in affecting sterility and nothing

statistically meaningful inferences can be concluded regarding main effects. Generally, each CHA candidate did not respond the same to changes in concentration and growth stages of application and required a unique range of concentrations and growth stages of application to induce highest levels of sterility (Table 2; Appendix Table 3).

Higher concentrations generally resulted in higher mean sterility values (Table 3). Deviations from this observation can largely be attributed to unique CHA responses to different stages of application. Applications of 1058, 1271, 2029, 2053, 2099, and 2186 resulted in higher mean sterility with growth stage one applications; conversely, applications of 1168, 1244, 1260, 1272, 2129, 2157, and 2168 resulted in higher mean sterility with growth stage two applications (Table 4). However, within growth stage one and growth stage two applications, mean sterility values generally increased with increased concentration (Table 5). Again, deviations from this observation can largely be attributed to unique CHA responses to different growth stages of application.

Ten of 13 CHA candidates resulted in greater than 95 percent sterility with specific concentrations and growth stages of application evaluated (Appendix Table 3). Some CHA candidates demonstrated larger concentration and growth stage of application ranges where high levels of

sterility were observed.

Growth stage one applications of 1058 resulted in near-perfect sterility (>99%) at 0.4 to 0.6kg/ha. Growth stage two applications resulted in 92.6 to 94.5 percent sterility with similar concentrations (Table 2, Appendix Table 3). These observations were consistent with previous findings; high levels of sterility could be achieved with 1058 applications, however, these applications resulted in corresponding lower percent of outcrossed seed suggesting reduced female fertility and/or receptivity.

Individual treatment means are summarized in Appendix Table 3. Of the four advanced CHA candidates (1168,1244,1271,2053), 1168 applications resulted in the highest and most consistent mean sterility across all concentrations and growth stages of application (Tables 3, 4). Growth stage one applications at 1.0 to 1.4kg/ha resulted in mean sterility of 95.7 to 99.4 and 97.7 to 99.3 percent at similar concentrations with growth stage two applications. Applications of 1244 also resulted in consistently high levels of sterility at all concentrations and growth stages of application evaluated. Concentrations between 1.0 and 1.4kg/ha resulted in sterility of 92.6 to 95.7 percent and 91.2 to 96.8 percent with growth stage one and two applications, respectively. Applications of both 1271 and 2053 resulted in higher

levels of sterility with growth stage one applications (Table 4). Sterility ranged from 97.2 percent at 0.2kg/ha to 99.2 percent at 0.6kg/ha with growth stage one applications of 1271. Growth stage two applications resulted in sterility of 64.5 to 83.8 percent with similar concentrations. Growth stage one applications of 2053 resulted in sterility ranging from 97.2 to 99.2 percent at 0.2 and 0.6kg/ha, respectively, and 74.4 to 88.2 percent sterility at similar concentrations with growth stage two applications.

None of the eight new CHA candidates demonstrated higher mean levels of sterility across concentrations and growth stages of application than 1168 applications (Tables 3, 4, Appendix Table 3). However, high sterility at the highest concentrations with corresponding low levels of phytotoxicity suggests optimum concentrations may not have been used and larger ranges may be obtained with some newer CHA candidates. Five of the eight new CHA candidates resulted in sterility greater than 95 percent at some concentration and stage of application evaluated (Appendix Table 3). These five CHA candidates (1260, 2099, 2168, 2186, and 2129) will be discussed in more detail.

Applications of 1260 resulted in near perfect sterility (>99%) at all concentrations evaluated (0.6, 0.9 and 1.2kg/ha) with growth stage two applications. Growth

stage one applications resulted in sterility ranging from 80.7 percent at 0.6gk/ha to 95.3 percent at 0.9kg/ha. Conversely, growth stage one applications of 2099 resulted in near-perfect sterility at all concentrations evaluated (0.3 to 0.7kg/ha). Growth stage two applications resulted in sterility of 66.0 percent at 0.3kg/ha to 83.3 percent with applications of 0.7kg/ha. Growth stage two applications of 2168 at 0.6kg/ha and 1.2kg/ha resulted in 93.9 to 99.0 percent sterility, respectively. Growth stage one applications at 0.6kg/ha resulted in 41.5 percent sterility and 80.0 percent sterility at 0.9kg/ha. Applications of 2186 were most effective at growth stage one. Applications of 0.2kg/ha to 0.6kg/ha resulted in 84.4 and 97.8 percent sterility, respectively. Growth stage two applications at 0.2kg/ha and 0.6kg/ha resulted in sterility of 75.4 to 87.1 percent, respectively. Applications of 2129 resulted in only slightly higher levels of sterility with growth stage two applications. Growth stage one application sterility ranged from 79.4 percent at 0.5kg/ha to 83.6 percent at 1.0kg/ha. Growth stage two applications ranged from 81.7 percent sterility at 0.5kg/ha to 96.8 percent at 1.0kg/ha. Growth stage one applications of 2029 ranged from 56.2 percent sterility at 0.1kg/ha to 93.8 percent at 0.2kg/ha and reduced sterility of 87.6 percent at 0.3kg/ha. Growth stage two applications resulted in highly variable and lower

sterility at similar concentrations. Concentrations of 0.1kg/ha resulted in only 4.6 percent sterility and 81.7 percent at 0.3kg/ha.

The above observations suggest that each CHA candidate demonstrated relatively unique characteristics in response to concentrations, growth stages of application, and levels of induced sterility. Similar concentrations for individual CHA's were used for both growth stages of application, however, optimum concentration ranges were apparently not the same with different growth stages. As a result, despite preliminary performance observations, optimum concentration ranges for all CHA candidates and growth stages of application may not have been used and requires further investigation.

Outcrossed Seed (Yield)

High levels of sterility are only useful if corresponding female fertility and/or receptivity is retained and high levels of outcrossed seed can be achieved. Two assessments of outcrossed seed were evaluated: a) random spike sample and b) plot grain yields. Mean values for seed set obtained from 21 random open pollinated spikes from each treatment plot were compared to the over-all mean seed set value from three control plots (no treatment; 21 spikes collected from each

control plot) and designated Micro Outcrossed seed or Outcrossed Seed. Each treated plot was then combine-harvested. Weight of treated plots were compared to the over-all mean weight from the above three control plots and designated Macro Outcrossed Seed or Grain Yield. These two assessments generally resulted in similar mean observations (Table 1; Appendix Table 3). Both assessments were 'adjusted' by multiplying corresponding percent sterility to give a more accurate assessment of actual outcrossed seed. The value of this adjustment factor becomes less meaningful as sterility is reduced. Despite the adjustment, low levels of sterility invariable result in higher percent outcrossed seed and can be misleading without consideration of corresponding observations of sterility.

Evidence that main effects were not acting independently in effecting observations of outcrossed seed and grain yield was observed in two of three significant first order interactions (Table 1, Appendix Tables 5 and 6). Due to these complex interactions of CHA candidates, concentrations and growth stages of applications, no statistically meaningful inferences can be concluded regarding main effects. However, some general observations can be made.

The highest sterility was generally not correlated with the highest grain yield performance. Where high and

increasing levels of sterility with increased concentration were observed (Table 3), mean outcrossed seed and grain yield values were generally reduced (Tables 6 and 8). Decreased outcrossed seed and grain yield with corresponding higher sterility values, resulting from higher concentrations, was observed despite 'adjustment' of the observations. However, concentrations higher than required to achieve near-perfect sterility (>99%) generally resulted in further reductions of outcrossed seed and grain yield (Appendix Table 3). Increasing sterility from 95 to 100 percent results in disproportionately larger decrease in outcrossed seed and grain yield. These observations suggest some reduced female fertility and/or receptivity. Where a relatively high mean sterility (>90%) was observed (Table 4), growth stage one applications generally resulted in higher mean outcrossed seed and grain yield than growth stage two applications (Tables 7 and 9). The degree of reduced seed set and grain yield with growth stage two applications was largely dependent on individual CHA responses to concentrations and growth stages of application evaluated.

Each CHA candidate generally required a fairly specific concentration and stage of application to induce both high levels of sterility and outcrossed seed (grain yield). These concentrations and growth stages were generally not the same for each CHA candidate (Table 2,

Appendix Table 3). Twenty-one individual treatments demonstrated both high levels of sterility (>93.8%) and high levels of grain yield (>44%). These treatments included all four advanced CHA candidates, six new CHA candidates, growth stage 1 and 2 applications and both high and low concentrations (Table 2). Many treatments of both advanced and new CHA treatments demonstrated a three-fold increase in outcrossed seed and grain yield over the standard 1058, while maintaining high levels of sterility. Outcrossed seed and grain yield measurements were predictably variable, however, both measurements were not significantly different and percent grain yield (Macro outcrossed seed) for treatments resulting in sterility greater than 95 percent will be discussed in more detail.

The following individual grain yields presented are given as mean performance across three replications and summarized in Table 2 and Appendix Table 3. The highest grain yield of 1058 with growth stage one applications was only 19.7 percent of mean control plot grain yield at 0.4kg/ha and 13.0 percent at 0.6kg/ha, where near-perfect sterility was observed. The highest grain yield of growth stage two applications was 17.2 percent at 0.5kg/ha (Table 2). This observation confirms earlier observations suggesting that 1058 applications which result in high levels of sterility also results in some reduced female fertility and/or receptiveness.

All advanced CHA candidates (1168, 1244, 1271, and 2053) resulted in significantly higher grain yields than 1058 applications. Applications of both 1168 and 1244 resulted in high levels of sterility with both growth stage one and growth stage two applications. However, growth stage two applications resulted in reduced grain yield. Growth stage one applications of 1168 resulted in 56.1 to 65.9 percent grain yield at 1.4 to 1.0kg/ha, respectively. Growth stage two application grain yields ranged from 19.8 to 31.5 percent with similar concentrations. Growth stage one applications of 1244 resulted in grain yields of 59.0 to 38.0 percent at 1.0 to 1.4kg/ha. Growth stage two application grain yields ranged from 24.8 to 23.5 percent with similar concentrations.

While flowering of the male pollinator was observed one day after control plots, accurate flowering notes were not recorded for treatments. Whether reduced grain yields with growth stage two applications were the result of reduced female fertility or by delayed receptive periods at critical pollen availability periods is not clear. Many treatments that resulted in higher sterility were observed to delay plant heading by up to 4 days. Delay of plant development was generally accompanied by other plant phytotoxic affects such as imperfect heading. High levels of sterility and outcrossed seed were generally associated

with low levels of phytotoxicity (Table 2; Appendix Table 3). Further evaluations of these treatments will be required to determine whether reduced female fertility and/or receptivity, or delayed plant development, was responsible for reduced grain yields with increased concentrations and different growth stages of application.

Applications of both 1271 and 2053 resulted in both higher sterility and higher grain yields with growth stage one applications. No growth stage two application resulted in sterility greater than 95 percent. Growth stage one applications of 1271 grain yields ranged from 59.3 to 35.1 percent at 0.2 to 0.6kg/ha respectively. Growth stage one applications of 2053 grain yields ranged from 63.0 percent at 0.2kg/ha to 44.3 percent at 0.4kg/ha.

Six of eight new CHA candidates resulted in sterility of greater than 95 percent and equal or greater grain yields than advance CHA candidates with specific concentrations and growth stages of application (Table 2). Growth stage one application of 1260 resulted in a grain yield of 64.9 percent at 0.9kg/ha. Growth stage two applications, where near perfect sterility was observed, 57.9 to 37.9 percent grain yield was observed with 0.6 to 1.2kg/ha respectively. Near-perfect sterility was observer with growth stage one applications of 2099. Grain yields ranged from 56.7 to 33.7 at 0.5 to 0.7kg/ha, respectively. No growth stage two application resulted in

sterility of greater than 95 percent. No growth stage one application of 2168 resulted in sterility greater than 95 percent, however, growth stage two applications resulted in grain yields ranging from 67.3 to 47.1 percent at 0.9 to 1.2kg/ha, respectively. Growth stage one applications of 2186 resulted in a grain yields of 61.3 percent at 0.4kg/ha to 72.8 percent with 0.6 kg/ha. No growth stage two application exceeded 95 percent sterility. No growth stage one application of 2129 exceeded 95 percent sterility, however, a grain yield of 59.0 percent was observed with growth stage two application at 1.9kg/ha (Appendix table 3).

Purity

Proportion of harvested seed from treatment and control plots resulting pollination from adjacent male pollinator plots was assessed with use of a genetic marker that conditions a dominant blue xenia affect on outcrossed seed. General agreement between analysis of 21 random outcrossed spike samples (Micro Purity) and one-hundred seed samples from bulk-harvested treatments (Macro Purity) was observed (Appendix Table 3). Micro Purity assessment will be used in more detailed discussion as only selected treatments were assessed for Macro Purity and no formal analysis was performed. The following individual purity

observations presented represent mean percentage of blue seeds from three replications and is summarized in Appendix Table 3. Purity assessment of three control plots (no treatment) resulted in an average of 1.6 percent blue seeds from 21 random open-pollinated spikes. Higher percentages of blue seed was attributed to induced pollen suppression and subsequent pollination by the blue marker.

The first order interaction CHA x stage of application was significant suggesting no statistically meaningful inferences can be concluded regarding these main effects (CHA and stage of application). However, first order interactions CHA x concentration and concentration x stage of application were not significant (Table 1; Appendix Table 7). Increased concentration generally resulted in increased sterility and, as anticipated, generally resulted in increased purity, independent of CHA candidates and growth stages of application. Mean purity values achieved with different CHA candidates was dependent on interactions of specific CHA candidates with growth stages of application and concentrations.

Large variation between CHA candidates, stage of application and purity was generally observed. Mean percent purity values for all concentrations ranged from 1.9 percent for 2157 to 59.4 percent for 2053 with growth stage one applications. Purity with growth stage two

applications ranged from 3.5 percent with 2157 to 52.7 percent with 1244 applications (Table 10).

Low purity values were observed in many treatments where high purity values were expected (high sterility). Growth stage one applications of 1058, where near perfect sterility was observed, purity values were relatively high and ranged from 46.9 to 58.2 percent. Growth stage two applications, where sterility ranged from 92.6 to 94.5 percent, purity values were significantly reduced and ranged from 22.3 to 29.6 percent. Of the ten CHA candidates and 21 individual treatments that demonstrated both high levels of sterility (>93.4%) and grain yield, purity values ranged from 13.4 to 68.8 percent. With the exception of growth stage one application of 1168 at 1.0kg/ha, six treatments with near-perfect sterility (>99%), purity values ranged from 50.8 to 68.8 percent (Table 2). This observation suggests small decreases in sterility result in disproportionately larger decreases in purity. However, this observation was not demonstrated for some CHA candidates and individual treatments. Growth stage two applications of 2053 resulted in relatively low sterility (74.4 to 88.2 percent) and relatively high purity values (39.5 to 50.6 percent). Observations of high sterility and high plot grain yields with corresponding low purity is particularly troublesome. Growth stage one applications of 1168 resulted in high

sterility (95.7 to 99.4 percent) and relatively high grain yields (56.0 to 65.9 percent) with purity values of 22.3 to 29.6 percent (Appendix Table 3). Several factors may have contributed to observations which deviate from expected purity in addition to overall low proportions of blue seeds.

Low over-all proportions of blue seed from treatments demonstrating high sterility may be largely attributed to white pollen sources. However, transmission of the chromosome which results in blue xenia effect may also be in question. Despite removal of all white seed from planted male plot seed, five random 1000 seed samples from bulk harvested male plots revealed 6 to 15 percent white seeds. The source of this white seed results from the heterozygote condition of a small portion of planted blue seed. Segregation and the subsequent possible loss of the telocentric chromosome containing the dominant blue gene could have resulted in some white pollen production and subsequent white seeds. This white pollen source may have contributed to pollination of male-sterile treatments, ultimately resulting white outcrossed seeds. Further white pollen sources were produced by six control plots (three for each stage of application), in addition to ineffective CHA candidates, concentrations and growth stages of application. Female treatment plots generally flowered prior to male plots. Ineffective treatments and

subsequent white pollen resulted in an advantageous environment for male-sterile treatments to be pollinated with white pollen sources. Micro-environment fluctuations of white pollen load undoubtedly contributed to highly variable observations of purity. Further, a disproportionately larger amounts of pollination of sterile florets from a small percentage of fertile florets within a treatment may have occurred.

Treatment influence of plant development, particularly with respect to relative periods of female flowering/receptiveness periods, may ultimately provide explanations of troublesome purity observations. Flowering was generally determined by first appearance of extruded anthers. Male pollinator plots were observed to flowered one day after female control plots. However, male-sterile plots did not generally result in extruded anthers and relative flowering dates were somewhat difficult to determine. However, many treatments resulted in up to five days delay in heading. Treatments resulting in delays in heading were generally reflected in higher phytotoxicity observations and higher phytotoxicity was generally correlated to lower grain yields. Treatments resulting in high sterility and high grain yields with corresponding low phytotoxicity (least delay) may have been subject to larger proportions of the above white pollen sources and subsequent low purity observations.

Treatments resulting in moderate delay (moderately low phytotoxicity) may have escaped this period of higher white pollen proportions and resulted in relatively high grain yields and purity. Finally, treatments resulting in the largest delay (highest phytotoxicity) undoubtedly resulted in low grain yields due to low pollen loads and, in many instances, relatively high purity.

This conclusion does not explain all troublesome observations. Weaknesses may be the result of the visual observations of phytotoxicity that took into account foliage damage, imperfect heading, delayed heading and indications of female sterility. Further studies will be required to fully understand complex interactions of individual CHA candidates with different concentrations, growth stages of application and subsequent influence on delayed development and purity observations. Given the intricacies associated with the use of the dominant blue marker in this kind of study, only the above broadest generalizations can be made.

Phytotoxicity

Visual assessments of phytotoxicity was first made at heading with final assessments at physiological maturity. Index used to assess severity was based on a scale from 0 to 100 relative to control plots (0=control) and is

summarized in Appendix Table 2. Measurements above 40 were considered significantly damaging.

The first order interaction CHA x concentration was significant. As a result, no statistically meaningful inferences can be made regarding main effects of CHA candidates, concentrations, and growth stages (Table 1; Appendix Table 8).

Increasing concentration generally resulted in increased phytotoxicity (Table 11). The overall level and degree of increase in phytotoxicity with increased concentration was generally dependent on specific CHA candidates and subsequent unique interactions with stage of application and range of concentrations used (Appendix Table 3).

Growth stage one applications of 1058 resulted in phytotoxicity values ranging from 30.0 to 40.0 at 0.4 to 0.6kg/ha, respectively. Growth stage two applications resulted in values ranging from 36.7 to 43.3 with similar concentration ranges. These relatively high phytotoxicity values undoubtedly contributed to low grain yields of 1058 applications (Table 2, Appendix Table 3).

Ten CHA candidates and 21 treatments that demonstrated both high sterility (>93.8%) and grain yield, resulted in phytotoxicity values ranging from 3.3 to 30 (Table 2). These superior treatments included all four advanced CHA candidates and five of six new CHA

candidates. In six observations were near-perfect sterility was observed, phytotoxicity ranged from 5 to 30. These observations suggest that high levels of sterility can be achieved without association of high levels of phytotoxicity, as observed with 1058 applications. A nearly three-fold increase in grain yield over 1058 applications with many superior treatments can largely be attributed to significantly reduced plant phytotoxicity.

Seed Quality

Harvested seed from any treatment that demonstrated greater than 95 percent sterility was visually evaluated for seed quality. Assessments were based on a scale from one to five relative to control with values of one equal to the control. Values greater than three were considered significantly damaging. Such damage was generally expressed as wrinkled and shrivelled seed. Because only selected treatments were assessed, no formal analysis of variance was performed. Visual assessments represent average values across three replications and are summarized in Appendix Table 3.

Values were generally variable and inconclusive for individual treatments. Seed quality values of 1058 applications ranged from 2.3 to 3.3. Measurements ranged

from 1.0 to 2.7 among the ten CHA candidates and 21 treatments that demonstrated both high sterility and grain yield (Table 2). Generally, treatments that resulted in lowest plant phytotoxicity also resulted in superior seed quality.

Summary

Several CHA candidates were found to be superior to 1058. Superior treatments were the result of both high levels of induced male sterility with minimal effects on plant development resulting in high levels of female fertility and subsequent high grain yields from treatment plots. Assessment of hybrid purity using the dominant male marker proved unreliable in this experiment which contained several additional and unexplained sources of white pollen. In addition to white pollen sources, inconsistent and troublesome observations resulted in variable and generally low purity assessments. Individual CHA candidates demonstrated relatively unique characteristics and generally did not respond the same to changes in concentrations and growth stages of application. Three experimental factors (CHA candidates, concentrations, and stage of application) and subsequent significant interactions resulted in the inability to conclude statistical inferences for main affects.

Table 1
Level of significance for percent sterility, percent outcrossed seed, percent grain yield, percent blue seed (purity), and plant phytotoxicity for Malculm wheat treated with thirteen CHA candidates at three concentrations and two growth stages of application grown at Amity Oregon in 1986-87.

Source of Variation	df	(1) Sterility	(2) Outcrossed Seed	(3) grain Yield	(4) Micro Purity	(5) Phytot-oxicity
Growth Stage	1	NS	**	**	*	NS
Error M.S.		383.8	127.4	172.4	139.3	74.8
CHA	12	**	**	**	**	**
Conc.	2	**	NS	NS	**	**
CHA x Conc.	24	**	**	**	NS	**
CHA x Stage	12	**	**	**	**	NS
Conc. x Stage	2	**	NS	NS	NS	NS
CHA x Conc. x Stage	24	NS	NS	NS	NS	NS
Error M.S.		123.6	137.6	134.9	175.3	64.0

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Table 2

Superior treatments demonstrating both high percent sterility and percent grain yield, relative to 1058 applications, for Malculm wheat treated with thirteen CHA candidates at three concentrations and two growth stages of application grown at Amity Oregon in 1986-87: 1)growth stage of application 2)percent sterility 3)percent outcrossed seed from 21 spike sample compared to mean non-bagged control spikes 4)percent grain yield compared to mean control plot grain yield 5)percent blue seed from 21 open-pollinated spike sample 6)percent blue seed from 100 seed sample from bulk-harvested treatment plot 7)plant phytotoxicity (scale 0-100; 0=control) 8)visual evaluation of seed quality (scale 1-5;1=control).

CHA	CONC kg/ha	(1) GS	(2) STERIL	(3) OUTCR SEED	(4) GRAIN YIELD	(5) MICRO PUR	(6) MACRO PUR	(7) PHYTOT-OXICITY	(8) SEED QUAL
2029	0.2	1	93.8	80.1	73.4	13.4	27.0	20.0	1.3
2168	0.9	2	97.8	63.6	67.3	40.2	44.7	8.3	2.0
2186	0.6	1	93.8	54.3	68.5	37.3	40.0	6.7	2.3
2186	1.0	1	97.8	62.4	72.8	52.0	41.3	3.3	1.3
1168	1.0	1	99.4	55.1	65.9	26.7	23.7	10.0	2.7
1260	1.0	1	95.3	61.4	64.9	21.3	23.7	6.7	1.7
2053	0.2	1	97.2	57.2	63.0	58.6	46.0	3.3	2.0
2186	0.4	1	94.8	65.5	61.3	35.4	33.0	3.3	2.0
1260	1.2	1	93.4	55.8	60.8	34.7	35.3	6.7	1.7
1168	1.4	1	96.1	45.1	60.6	23.4	25.3	16.7	2.3
1271	0.2	1	97.2	58.9	59.3	30.9	40.0	6.7	1.0
2129	1.0	2	96.8	46.8	59.0	42.1	40.3	6.7	1.0
1244	1.0	1	95.7	70.5	59.0	27.4	33.0	16.6	1.3
1260	0.6	2	99.8	55.5	57.9	52.2	48.0	5.0	1.7
1168	1.2	1	95.7	54.8	56.1	21.1	26.0	16.7	2.0
2099	0.3	1	98.2	46.9	56.0	31.9	48.7	10.0	1.7
2168	1.2	2	99.0	43.4	47.1	51.8	38.7	23.3	1.7
2053	0.6	1	99.2	40.8	46.3	68.8	50.7	30.0	2.3
2099	0.5	1	99.4	43.2	45.8	54.9	47.0	16.7	2.0
2053	0.4	1	99.8	28.4	44.3	50.8	48.7	10.0	2.0
1271	0.4	1	99.2	42.6	44.1	51.8	53.0	26.7	2.3
1058	0.4	1	99.1	23.6	19.7	51.4	46.3	30.0	3.0
1058	0.5	1	100.0	15.5	19.9	46.9	50.7	36.7	2.3
1058	0.6	1	99.6	15.9	13.0	58.2	52.3	40.0	3.0
1058	0.4	2	92.6	15.2	27.2	24.1	40.0	36.7	2.7
1058	0.5	2	94.0	18.1	17.2	29.6	43.3	36.7	2.7
1058	0.6	2	94.5	12.7	12.9	22.3	34.7	43.3	3.3

Table 3
Chemical Hybridization Agent (CHA) x Concentration interaction treatment means for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Concentration		
	(1)	(2)	(3)
CHA			
1058	92.6	97.0	97.1
1168	99.2	96.7	98.1
1244	93.5	94.1	95.9
1260	90.3	97.4	96.6
1271	80.9	81.3	91.8
1272	18.6	56.6	58.4
2029	30.4	67.1	84.7
2053	85.5	92.9	93.7
2099	82.1	84.6	91.4
2129	80.6	81.5	90.2
2157	24.2	10.4	14.2
2168	67.7	88.9	84.1
2186	79.9	89.5	92.5

[1] see Materials and Methods for specific CHA concentrations

Table 4
Chemical Hybridization Agent (CHA) x Growth Stage of application interaction treament means for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Stage	
	(1)	(2)
CHA		
1058	99.6	93.7
1168	97.3	98.7
1244	94.4	94.5
1260	89.8	99.6
1271	98.7	70.5
1272	33.5	55.4
2029	79.2	42.2
2053	98.7	82.9
2099	99.0	73.0
2129	80.5	87.6
2157	15.5	17.0
2168	63.6	96.9
2186	92.3	82.3

[1] see Materials and Methods for specific CHA concentrations

Table 5
Concentration x Concentration for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

		Concentration (1)	(2)	(3)
Stage	1)	75.1	83.3	82.2
	2)	67.8	76.3	85.2

[1] see Materials and Methods for specific CHA concentrations

Table 6
Chemical Hybridization Agent (CHA) x Concentration interaction treatment means for observations of percent outcrossed seed (micro outcross), significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Concentration		
	(1)	(2)	(3)
CHA			
1058	19.4	16.8	14.3
1168	38.3	32.4	29.9
1244	49.3	47.8	50.4
1260	60.6	44.3	44.7
1271	45.7	34.1	39.4
1272	16.7	52.1	41.5
2029	29.5	50.2	56.0
2053	41.7	27.8	34.0
2099	41.1	33.5	32.5
2129	53.5	58.8	58.2
2157	21.9	11.2	13.8
2168	48.5	64.8	53.2
2186	62.2	54.5	53.1

[1] see Materials and Methods for specific CHA concentrations

Table 7
Chemical Hybridization Agent (CHA) x Growth Stage of application interaction treament means for observations of percent outcrossed seed (micro outcross), significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Stage	
	(1)	(2)
CHA		
1058	18.3	15.3
1168	51.7	15.4
1244	68.8	29.3
1260	60.9	38.7
1271	48.2	31.2
1272	31.9	41.6
2029	66.6	23.9
2053	42.1	26.8
2099	43.2	28.2
2129	67.9	45.7
2157	15.3	15.9
2168	57.2	53.8
2186	66.2	47.0

[1] see Materials and Methods for specific CHA concentrations

Table 8
Chemical Hybridization Agent (CHA) x Concentration interaction treatment means for observations of percent grain yield (macro outcross), significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Concentration		
	(1)	(2)	(3)
CHA			
1058	23.5	18.6	13.0
1168	48.7	40.8	36.8
1244	41.9	32.3	30.8
1260	63.8	53.3	49.4
1271	48.3	33.2	31.5
1272	18.7	49.2	52.1
2029	29.0	49.8	52.0
2053	49.1	27.4	35.5
2099	46.6	36.0	26.9
2129	52.9	47.7	59.4
2157	22.0	11.5	8.2
2168	55.2	67.1	48.4
2186	67.8	58.1	62.3

[1] see Materials and Methods for specific CHA concentrations

Table 9

Chemical Hybridization Agent (CHA) x Growth Stage of application interaction treament means for observations of percent grian yield (macro outcross), significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Stage	
	(1)	(2)
CHA		
1058	17.5	19.1
1168	60.9	25.6
1244	46.6	23.5
1260	65.1	45.8
1271	46.2	29.1
1272	33.2	46.7
2029	61.8	25.4
2053	51.1	29.5
2099	45.2	27.8
2129	57.3	49.3
2157	15.1	12.4
2168	52.8	61.0
2186	69.5	56.0

[1] see Materials and Methods for specific CHA concentrations

Table 10
Chemical Hybridization Agent (CHA) x Growth Stage of application interaction treament means for observations of percent blue seed (micro purity), significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Stage	
	(1)	(2)
CHA		
1058	52.2	25.3
1168	23.7	52.1
1244	21.9	52.7
1260	22.3	52.0
1271	45.2	34.6
1272	4.5	16.9
2029	10.6	19.4
2053	59.4	45.7
2099	51.4	31.4
2129	9.1	29.0
2157	1.9	3.5
2168	7.5	42.9
2186	35.4	39.4

[1] see Materials and Methods for specific CHA concentrations

Table 11
Chemical Hybridization Agent (CHA) x Concentration interaction treatment means for observations of plant phytotoxicity (scale: 0-100;0=control), significant at the one percent level of probability for Malculm wheat treated with thirteen CHA candidates at three concentrations (low, medium, and high)[1] and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87.

	Concentration		
	(1)	(2)	(3)
CHA			
1058	33.4	36.7	41.7
1168	13.4	15.0	13.4
1244	25.0	30.0	41.7
1260	2.5	10.9	11.7
1271	10.0	30.0	33.4
1272	0.0	4.2	1.7
2029	1.7	13.4	21.7
2053	6.7	16.7	34.4
2099	11.7	21.7	36.7
2129	11.7	15.0	8.4
2157	0.0	0.0	0.0
2168	3.4	6.7	16.7
2186	0.0	4.2	6.7

[1] see Materials and Methods for specific CHA concentrations

STUDY 2: Chemical Hybridization Agents (CHA's) x Formulations

Sterility (Pollen Suppression)

Bagged spikes from control plots (no treatment) contained an average of 23.6 and 33.5 percent fewer seeds per spike than non-bagged control spikes for growth stage one and two applications, respectively. Reduced seed set in bagged control spikes was greater than reduced seed set in bagged control spikes observed in Study 1 (mean of 14.4 and 24.7 percent fewer seeds/spike with growth stage one and two applications, respectively). This further reduction was observed despite the use of similar genotypes. Variable seed set observed in both control and treatment bagged spikes may be largely attributed to: a) small sample size and b) unusually high temperatures during grain filling, ultimately resulting in some light and shriveled seed which could have been lost during the threshing process. Despite this observation, bagged control spikes served as the base seed set level to evaluate relative treatment performance. Further reduction in seed set was assumed to be the result of chemically induced pollen suppression and subsequent male sterility and not the result of bagging or bag x treatment interaction.

Significant three and four-way interactions were

evidence that main effects (CHA candidates, concentrations, stages of application and formulations) were not acting independently in affecting observations of sterility (Table 12; Appendix table 10). Significant first order interactions (CHA x concentration, CHA x formulation, CHA x stage of application and formulation x stage of application) further suggested that nothing statistically meaningful can be concluded regarding main effects and observations of sterility. However, some generalized observations can be made.

Similar to observations made in Study 1, higher concentrations generally resulted in higher sterility for all CHA candidates (Table 14). Higher mean levels of sterility were observed with increased concentration regardless of formulation (Tables 14 and 17). The high 'F' value associated with formulations (Appendix Table 10) can largely be attributed to inclusion of water control (formulation F) in the analysis. Small differences were observed between formulations A-E (Table 15). Also similar to Study 1 observations, applications of 1168 and 1244 resulted in higher mean levels of sterility with growth stage two applications. Additionally, applications of 1271 and 2053 resulted in higher mean levels of sterility with growth stage one applications (Table 16). Higher mean levels of sterility were observed with growth stage one applications than growth stage two applications

regardless of formulation (Table 18). Formulations B and C resulted in the highest mean level of sterility (94.8 and 94.5 percent, respectively) and formulation D resulted in the lowest mean level of sterility (89.0 percent). It is possible that the higher mean levels of sterility observed with formulations B and C resulted from enhanced CHA penetration.

Generally, individual CHA/formulation combinations did not respond the same to changes in concentration and growth stage of application and required fairly specific concentrations and growth stages of application to induce consistently high sterility values. Individual treatment means presented represent mean values across three replications and are summarized in Appendix Table 9. Optimum concentration ranges were generally not the same for different growth stages of application. When both high and low concentrations resulted in high and similar sterility values in one growth stage of application, wider and generally lower sterility values were observed between high and low concentrations with another growth stage of application. This observation was consistent regardless of formulation.

Applications of 1168 demonstrated the highest and most consistent mean values of sterility for all formulations (A-E), concentrations and growth stages of application evaluated. Sterility ranged from 93.2 to 99.3

percent with formulations A-E applications (Table 15). Growth stage one applications at concentrations of 0.3 to 0.6kg/ha with formulations A-E resulted in a mean sterility of 97.1 percent. Growth stage two applications with similar concentrations and formulations resulted in a mean sterility of 99.1 percent (Appendix Table 9).

Mean sterility ranged from 90.9 to 97.4 percent with applications of 1244 with formulations A-E (Table 15). Growth stage one applications at concentrations of 1.0 to 1.4kg/ha with formulations A-E resulted in a mean sterility of 92.4 percent. Growth stage two applications resulted in a mean sterility of 94.8 percent with similar concentrations and formulations (Appendix Table 9).

Applications of 1271 with formulations A-E resulted in mean sterility values of 82.8 to 90.0 percent (Table 15). Lower mean sterility values were the result of lower sterility with growth stage two applications. Growth stage one applications at concentrations of 0.3 to 0.6kg/ha with formulations A-E resulted in a mean sterility of 98.9 percent. However, growth stage two applications with similar concentrations and formulations resulted in a mean sterility of only 69.0 percent (Appendix table 9).

Applications of 2053 with formulations A-E resulted in mean sterility of 86.3 to 95.8 percent (Table 15). As with 1271 applications, lower mean sterility was the

result of lower sterility observed with growth stage two applications. Growth stage one applications at concentrations of 0.3 to 0.5kg/ha with formulations A-E resulted in a mean sterility of 99.4 percent. Growth stage two applications with similar concentrations and formulations resulted in a mean sterility of 85.3 percent (Appendix table 9)

Fourteen of 96 treatments that demonstrated both the highest levels of sterility (93.9-100%) and grain yield (43.3-66.5%) included all four CHA candidates, both high and low concentrations and formulations A-E (Table 13). However, these superior treatments were observed with growth stage one applications only. These observations suggests that CHA candidates can be optimized with varying windows of effective concentrations and growth stages of application, subject to some modification with different formulations. Similar performance of individual CHA candidates found in Study 1 further support this conclusion.

Outcrossed Seed (grain yield)

Two assessments of outcrossed seed were evaluated: a) mean seed set from 21 random open pollinated spikes from each treatment plot were compared to the over-all mean seed set from three control plots (no treatment; 21 spikes

collected from each control plot) and designated Micro Outcrossed seed or Outcrossed Seed. b) each (treated) plot was then combine-harvested. Weight of treated plots were compared to the mean weight from three control plots and designated Macro Outcrossed seed or Grain Yield. These two assessments generally resulted in similar mean observations, however, outcrossed seed values generally resulted in more variation (Table 12). Both assessments were 'adjusted' by multiplying corresponding percent sterility to give a more accurate assessment of actual outcrossed seed.

Significant first order interactions suggested main effects (CHA, concentration, formulation and growth stage of application) were not acting independently in affecting outcrossed seed (Table 12; Appendix Table 11). Statistically meaningful inferences cannot be made regarding main effects. However, general observation merit consideration

Higher mean sterility values were generally associated with lower mean outcrossed seed. Mean percent outcrossed seed ranged from 19.6 percent with applications of 1168/formulation C to 48.3 percent with applications of 1244/formulation D (Table 19). This corresponded to mean sterility of 99.3 and 91.5 percent, respectively (Table 15). All CHA candidates, with the exception of 1271/formulation E, resulted in the highest mean level of

outcrossed seed with formulation D (Table 19). However, formulation D resulted in the lowest mean percent sterility (Table 15).

Applications of 1168 resulted in more than two-fold increase in mean outcrossed seed values (19.6 to 45.1 percent) with different formulations. Remaining CHA candidates ranged from 10.6 to 14.7 percent differences in mean percent outcrossed seed values with different formulations (Table 19). This suggests greater enhancement of outcrossed seed with some formulations in combination with specific CHA candidates. However, higher mean levels of outcrossed seed can at least be partially attributed to lower sterility. Individual treatments strongly suggest that small increases in sterility generally result in disproportionately larger decreases in outcrossed seed (Appendix Table 9).

Growth stage one applications resulted in higher mean levels of outcrossed seed than growth stage two applications for all CHA candidates (Table 20). Growth stage one applications resulted in higher mean outcrossed seed regardless of formulation (Table 22). Growth stage one applications of all treatments with formulations A-E also resulted in higher mean sterility suggesting the overall superior performance of growth stage one applications. The above observation is consistent with applications of 1168 and 1244 which resulted in lower mean

sterility and higher mean outcrossed seed values with growth stage one applications. However, applications of 1271 and 2053 resulted in higher mean sterility with growth stage one applications (Table 16), suggesting an overall advantage with these CHA candidates and treatments. Higher mean sterility and lower mean outcrossed seed values with growth stage two applications of 1168 and 1244 suggests reduced female fertility and/or receptiveness for these applications. Tables 16 and 20 further suggest that lower mean outcrossed seed values with growth stage two applications of 1271 and 2053 was the result of lower mean sterility and not reduced fertility; indicating higher concentrations could have resulted in both higher sterility and higher outcrossed seed values.

Higher concentrations resulted in lower mean percent outcrossed seed values (Table 21). This is consistent with the general observation that increased concentration results in increased sterility and subsequent decreased outcrossed seed. Individual treatment means generally support the above observations (Appendix Table 9). Variations from this general response can be at least partly attributed to relatively small sample size and subsequent error in estimating treatment means. Additionally, small errors in CHA foliar delivery could have significantly altered mean values.

Plot grain yield values (Macro Outcrossed seed) were generally similar to outcrossed seed observations (Table 12; Appendix Tables 12 and 13). Little additional insight can be gained by a more detailed analysis. All but one first order interaction was significant, indicating main affects were not acting independently in affecting treatment grain yield.

Similar to mean outcrossed seed observations, formulation D resulted in the highest mean grain yield and corresponding lowest mean sterility for all CHA candidates (Table 23). Regardless of formulation, higher mean treatment grain yield values were observed with lower concentrations (Table 24). However this corresponded to lower mean sterility (Table 18). Growth stage one applications resulted in higher mean treatment grain yields than growth stage two applications for all CHA candidates (Table 25). Higher mean treatment grain yields with growth stage one applications were observed regardless of formulation (Table 27). Higher mean sterility for formulations A-E with growth stage one applications suggest overall superior performance with growth stage one applications. Lower concentrations and earlier stages of application resulted in highest mean grain yield (Table 26). However, lower concentrations generally resulted in lower mean sterility (Table 17) and subsequently higher mean grain yields.

Nineteen individual treatments that resulted in higher sterility (>94%) resulted in mean treatment grain yields ranging from 43.4 to 66.5 percent. The above treatments included all CHA candidates, both high and low concentrations, formulations A-E, and resulted from growth stage one applications only (Table 13). Each CHA candidate required fairly specific concentrations, formulations, and stage of application to result in superior overall performance. Formulations appear to only slightly modify inherent CHA charactoristics with respect to grain yields. These modifications appear to be primarily manifested in affecting sterility and subsequently influencing treatment grain yields.

Purity (Percent Blue Seed)

As in Study 1, the proportion of harvested seed from both treatment and control plots resulting from pollination by the adjacent male pollinator was assessed with the use of genetic marker that conditions a dominant blue xenia affect on outcrossed seed. General agreement between analysis of 21 random outcrossed spike samples (Micro Purity) and one-hundred seed sample from bulk-harvested treatments (Macro Purity) was observed (Appendix Table 9). Micro Purity assessment will be used in a more detailed discussion as only selected treatments were

assessed for Macro Purity and no formal analysis was performed. Purity observations of three control plots (no chemical treatment) resulted in an average of 1.6 percent blue seeds from 21 random open-pollinated spikes. Higher percentages of blue seed observed in treatment plots was attributed to induced pollen suppression and subsequent pollination by the blue genetic marker.

The three-way interaction and several first order interactions were not significant, suggesting some main effects were acting independently in influencing levels of purity (Table 12; Appendix Table 13).

Higher concentrations resulted in higher mean purity values than lower concentrations with growth stage one applications. However, similar mean purity with higher concentrations with both growth stage one and growth stage two applications in addition to lower concentrations with growth stage two applications was observed. Further, at lower concentrations, the lowest mean purity was observed with growth stage one applications (Table 28). These observations are consistent with mean sterility values (Tables 14 and 18), suggesting higher sterility would result in higher purity.

Applications of both 1168 and 1244 resulted in higher mean purity values with growth stage two applications. Conversely, applications of 1271 and 2053 resulted in the highest mean purity values with growth stage one

applications (Table 29). Growth stage two applications of 1168 and 1244 resulted in the highest mean sterility values (Table 16) and, despite lower mean outcrossed seed, high mean purity. Conversely, growth stage one applications of 1271 and 2053 resulted in highest mean sterility (Table 16), highest mean outcrossed seed (Table 20), and the highest mean purity, suggesting an overall superiority of these treatments. Despite lower sterility associated with growth stage two applications of 2053, purity remained high suggesting some advantage to over-all performance of this CHA candidate.

Analysis of varience suggests that changes in formulation were acting independently to changes in CHA candidates, concentrations and growth stages of application for observations of purity (Table 12; Appendix Table 13). The large 'F' value associated with formulations can largely be attributed to the inclusion of water control (formulation F) in the analysis. Formulations resulting in highest mean sterility generally resulted in highest mean purity (Table 15; Appendix Table 9). Formulation E resulted in highest mean purity (47.3 percent) with corresponding third highest mean sterility (92.5 percent). Formulations B and C resulted in mean purity of 45.9 and 44.0 percent, respectively, with corresponding mean sterility of 94.7 and 94.5 percent, respectively. Formulation D resulted in the lowest mean

purity (37.0 percent) and lowest mean sterility (89.0 percent). Applications of 2053 resulted in the highest mean purity (52.3 percent) and applications of 1244 resulted in the lowest mean purity of 36.8 percent (Appendix Table 9). These CHA comparisons could be biased as optimum concentrations and stage of applications were probably not used for individual CHA candidate.

Despite higher mean sterility values with growth stage one applications with formulations A-E (Table 18), higher purity values were observed with growth stage two applications with all formulations (except formulation C; Appendix Table 9). This was observed despite higher mean percent outcrossed seed with growth stage one applications with formulations A-E (Table 22). This may be attributed to the charactoristic responses of individual CHA candidates with respect to growth stage of application and mean sterility (Table 16), with corresponding mean outcrossed seed (Table 20).

Nineteen individual treatments that demonstrated both high mean sterility and outcrossed seed, resulted in mean purity ranging from 19.5 to 63.1 percent (Table 13). Six of seven of these treatments that demonstrated a mean sterility greater than 99.0 percent, resulted in mean purity values ranging from 50.6 to 63.1 percent. This observation suggests that small changes in sterility can result in disproportionately larger decreases in purity.

Inconsistent and over-all low purity observations were discussed in greater detail in Study 1.

General observations suggest that purity is closely correlated to sterility with higher sterility generally resulting in higher purity values. Formulations generally influenced purity in response to modification of sterility. Within the scope of this experiment, applications of 2053 resulted in superior over-all observations of seed purity.

Phytotoxicity

The index used to assess phytotoxic severity was based on a scale from 0 to 100 relative to control plots (0=control) and summarized in Appendix Table 2. Values above 40 were considered significantly damaging. Individual values presented represent mean values across three replications and are summarized in Appendix Table 9.

All but one first-order interaction was significant suggesting main effects (CHA candiates, concentrations, and growth stage of application) were not acting independently in effecting phytotoxicity values (Table 12; Appendix Table 14). As such, no statistically meaningful inferences can be made regarding main effects, however, some general observations merit consideration. Formulations and CHA candidates resulting in high mean

sterility (Table 15) generally resulted in higher phytotoxicity values. The degree of phytotoxicity was largely dependent on charactoristic individual CHA response (Table 30). Many treatments did result in both high sterility and low phytotoxicity; particularly those treatments associated with high mean outcrossed seed (Table 13).

Higher concentrations, that generally resulted in higher mean sterility (Table 14), resulted in higher mean phytotoxicity (Table 32). The magnitude of increased mean phytotoxicity was again largely dependent on charactoristic individual CHA responses, combined with interactions with concentrations and growth stage of applications evaliated. Higher mean phytotoxicity values with higher concentrations was observed for all formulations (Table 33). This observation was consistent with observations of mean sterility (Table 17). Formulation D resulted in the lowest increase in mean phytotoxicity with increased concentration, however, formulation D resulted in lowest mean sterility. Conversely, formulations B and C resulted in the highest increases in phytotoxicity with higher concentrations with corresponding higher mean sterility values.

Growth stage two applications resulted in a higher mean phytotoxicity than growth stage one applications for all CHA candidates (Table 32). This was not consistent

with sterility observations. Applications of both 1271 and 2053 resulted in higher mean sterility with growth stage one applications (Table 15). Formulations B-E resulted in higher mean phytotoxicity with growth stage two applications (Table 34), however, formulations A-E applications resulted higher mean sterility with growth stage one applications (Table 18). These observations strongly suggest that higher phytotoxicity is more closely associated with lower percent outcrossed seed than the association of increased phytotoxicity with increased sterility. Formulations A-E resulted in lower mean outcrossed seed with growth stage two applications (Table 22). This observation was observed with all CHA candidates (Table 20).

Nineteen individual treatments demonstrating both high sterility and outcrossed seed values resulted in phytotoxicity values ranging from 3.3 to 26.7. These superior treatments resulted from only growth stage one applications and contained all CHA candidates, formulations A-E and both high and low concentrations (Table 13). This suggests that high phytotoxicity is strongly associated with reduced outcrossed seed and a tendency to be associated with higher sterility. Changes in phytotoxicity with changes in formulations generally resulted from modification of sterility and subsequent modification of outcrossed seed.

Seed Quality

Seed quality assessments were based on a scale from one to five relative to control plots with scores of one equal to control. Scores greater than three were considered particularly damaged which was generally expressed as wrinkled and shrivelled seed. Because only selected treatments were assessed, no formal analysis was performed. Values presented represent average assessment across three replications and are summarized in Appendix Table 9.

Assessments were generally higher than the control, however, observations tended to be variable and inconclusive for individual treatments. Treatments which resulted in high outcrossed seed and low phytotoxicity, generally resulted improved seed quality. Values ranged from 1.3 to 2.3 among the nineteen treatments that demonstrated both high sterility and grain yield (Table 13).

Summary

Significant interactions of main effects (CHA candidates, concentrations, formulations and stages of application) for observations of sterility, outcrossed

seed, grain yield, purity, and phytotoxicity resulted in few statistically meaningful inferences regarding main effects. Specific treatments which resulted in both high sterility and percent outcrossed seed included all CHA/formulations (A-E) combinations with specific concentrations and growth stages of application. Individual CHA candidates demonstrated relatively characteristic responses with specific concentrations and groeth stages of application, subject to some modifications with changes in formulation. Modification of performance with different in formulation was ultimately expressed by modification of sterility. This was thought to result from capacity of different formulations to penetrate leaf surfaces.

Despite higher variability associated with outcrossed seed assessment, general agreement between outcrossed seed and plot grain yield was observed. No formulation resulted in both higher sterility and outcrossed seed for all CHA candidates. Growth stage one applications generally resulted in a higher mean percent outcrossed seed set than mean growth stage two applications. Higher concentrations, and formulations resulting in higher sterility, generally resulted in a lower mean percent outcrossed seed. Formulations resulting in higher mean sterility were also generally effective at lower concentrations. Higher phytotoxicity was associated with

lower percent outcrossed seed and generally associated with higher sterility. High sterility generally resulted in higher purity. The use of the dominant genetic marker proved unreliable for this kind of study. Variable and overall low purity resulted in inconsistent and some troublesome observations.

This study suggests that formulations can play an important role in optimizing CHA performance, but the inherent properties of CHA candidates are largely responsible for performance levels.

Table 12
Level of significance for percent sterility, percent outcrossed seed, percent grain yield, percent blue seed (purity), and plant phytotoxicity for Malculm wheat treated with four CHA candidates at two concentrations, two growth stages of application and six formulations grown at Amity Oregon in 1986-87.

Source of Variation	df	(1) Sterility	(2) Outcrossed Seed	(3) Grain Yield	(4) Micro Purity	(5) Phytot-oxicity
Growth Stage(GS)	1	**	**	**	NS	**
Error M.S.		69.0	69.0	53.6	169.5	31.6
CHA	3	**	**	NS	**	**
Conc.	1	**	**	**	*	**
Form.	5	**	**	**	**	**
CHA x Conc	3	**	NS	NS	NS	**
CHA x Form	15	**	*	**	NS	**
Form x Conc	5	NS	NS	**	NS	**
CHA x GS	3	**	**	**	**	**
Conc x GS	1	NS	*	*	*	NS
Form x GS	5	**	**	**	NS	**
CHA x Form x conc	15	**	NS	NS	NS	NS
CHA x Form x Conc x GS	38	**	NS	*	*	*
Error M.S.		42.6	94.3	38.2	153.9	57.5

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Table 13

Superior treatments demonstrating both high percent sterility and percent grain yield for Malculm wheat treated with four CHA candidates at two concentrations, two growth stages of application, and six formulations grown at Amity Oregon in 1986-87: 1)CHA/Formulation 2)growth stage of application 3)percent sterility 4)percent outcrossed seed from 21 spike sample compared to mean non-bagged control spikes 5)percent grain yield compared to mean control plot grain yield 6)percent blue seed from 21 open-pollinated spike sample 7)percent blue seed from 100 seed sample from bulk- harvested treatment plot 8)plant phytotoxicity (scale:0-100; 0=control) 9)visual evaluation of seed quality (scale 1-5;1=control).

(1) CHA/ FORM	CONC Kg/ha	(2) GS	(3) % STERI- LITY	(4) % OUTCR. SEED	(5) % GRAIN YIELD	(6) % MICRO PUR	(7) % MACRO PUR	(8) PHYTO	(9) SEED QUAL
D2053	0.3	1	99.8	50.8	66.5	35.6	38.8	3.3	2.3
D1168	1.4	1	98.9	51.6	57.0	31.4	34.3	10.0	2.0
E1271	0.3	1	99.3	48.5	56.6	52.9	43.7	13.3	2.0
E1168	1.0	1	96.8	60.6	56.1	19.5	21.3	16.7	1.7
D2053	0.5	1	99.8	47.5	55.8	50.6	41.7	16.7	2.3
A1168	1.4	1	98.6	43.1	55.1	5.8	5.7	13.3	1.7
B1244	1.0	1	98.8	57.5	52.7	33.5	30.0	26.7	1.3
E1244	1.4	1	98.8	60.5	52.6	29.8	45.0	20.0	1.7
A1271	0.3	1	98.0	46.4	51.6	41.5	48.7	26.7	1.7
B2053	0.3	1	100.0	30.6	50.3	63.1	60.0	20.0	2.0
A2053	0.3	1	96.7	39.7	48.9	58.6	41.3	6.7	2.0
B1271	0.3	1	100.0	43.0	47.1	52.3	43.7	23.3	2.0
B1168	1.4	1	97.8	33.1	47.0	32.0	28.3	20.0	1.7
C1168	1.0	1	97.3	46.4	46.9	29.3	31.0	13.3	1.3
C2053	0.3	1	99.5	38.4	45.2	58.7	51.0	21.7	2.7
A2053	0.5	1	100.0	34.5	43.4	61.4	57.3	20.0	2.7
D1168	1.0	1	95.8	62.9	68.4	19.9	22.0	8.3	1.7
D1244	1.4	1	95.8	53.1	51.5	29.3	20.7	16.7	2.0
B1168	1.0	1	93.9	48.1	56.7	31.1	26.3	6.7	2.0

Table 14
Chemical Hybridization Agent (CHA) x Concentration interaction treatment means for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Concentration	
	(1)	(2)
CHA		
1168	96.9	99.3
1244	91.1	96.5
1271	80.9	86.9
2053	90.0	94.7

[1] see Materials and Methods for specific CHA concentrations

Table 15

Chemical Hybridization Agent (CHA) x Formulation interaction treatment means for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
CHA						
1168	97.0	97.7	99.3	93.2	96.0	0.7
1244	90.9	97.4	95.8	91.5	94.5	10.9
1271	83.6	90.0	87.0	76.4	82.8	4.1
2053	86.3	95.3	95.8	89.9	94.5	3.7
Mean	89.4	94.7	94.5	89.0	92.5	6.0

[1] see Materials and Methods for specific CHA concentrations

Table 16
Chemical Hybridization Agent (CHA) x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Stage of Application	
	(1)	(2)
CHA		
1168	97.1	99.1
1244	92.8	94.8
1271	98.9	69.0
2053	99.4	85.3

[1] see Materials and Methods for specific CHA concentrations

Table 17

Concentration x Formulation interaction treatment means for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Areso GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol,F=Water Control) grown at Amity Oregon in 1986-87.

		Formulations					
		(A)	(B)	(C)	(D)	(E)	(F)
Concentration	(1)	86.8	94.1	92.7	85.8	89.3	5.8
	(2)	92.0	95.4	96.3	92.3	95.7	6.1
Mean		90.9	94.8	94.5	89.1	92.5	6.0

[1] see Materials and Methods for specific CHA concentrations

Table 18
Growth Stage of Application x Formulation interaction treatment means for observations of percent sterility, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
Stage of Application (1)	95.9	98.1	97.7	95.7	97.8	10.5
(2)	82.9	91.4	91.3	85.5	87.1	1.5
Mean	89.4	94.8	94.5	90.6	92.5	6.0

[1] see Materials and Methods for specific CHA concentrations

Table 19
Chemical Hybridization Agent (CHA) x Formulation interaction treatment means for observations of percent outcrossed seed, significant at the five percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
CHA	(A)	(B)	(C)	(D)	(E)	(F)
1168	37.5	25.2	19.6	45.1	28.6	5.0
1244	45.6	39.8	33.6	48.3	43.1	11.4
1271	36.2	33.8	29.3	37.5	39.9	4.4
2053	33.8	28.3	32.5	41.0	28.8	3.6

* STERILITY = $\frac{\text{(Av. No. Seeds From 21 Unbagged Treatment Spikes)}}{\text{(Av. No. Seeds From 21 Unbagged Control Spikes)}}$ x

[1] see Materials and Methods for specific CHA concentrations

Table 20
Chemical Hybridization Agent (CHA) x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent outcrossed seed, significant at the five percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Stage of Application	
	(1)	(2)
CHA		
1168	57.5	20.6
1244	66.9	38.3
1271	52.1	36.1
2053	46.8	34.9

[1] see Materials and Methods for specific CHA concentrations

Table 21

Concentration x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent outcrossed seed, significant at the five percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

		Concentration (1)	Concentration (2)
Stage of Application	(1)	66.2	49.4
	(2)	35.1	30.1

[1] see Materials and Methods for specific CHA concentrations

Table 22
Formulation x Growth Stage of Application interaction treatment means for observations of percent outcrossed seed, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
Stage of (1)	46.7	39.4	38.9	52.7	46.0	10.7
Application (2)	30.1	24.2	18.7	33.3	24.3	1.5

[1] see Materials and Methods for specific CHA concentrations

Table 23
Chemical Hybridization Agent (CHA) x Formulation interaction treatment means for observations of percent grain yield, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
CHA						
1168	39.4	34.9	30.5	46.9	34.5	5.4
1244	30.5	33.5	26.9	40.7	38.3	9.5
1271	34.6	33.4	28.7	36.2	38.2	3.6
2053	37.9	30.8	30.2	46.6	29.0	3.5

[1] see Materials and Methods for specific CHA concentrations

Table 24

Concentration x Formulation interaction treatment means for observations of percent grain yield, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

		Formulations					
		(A)	(B)	(C)	(D)	(E)	(F)
Concentration	(1)	39.4	38.6	33.3	44.9	38.9	5.3
	(2)	34.0	27.7	24.9	41.3	31.1	5.7

[1] see Materials and Methods for specific CHA concentrations

Table 25
Chemical Hybridization Agent (CHA) x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent grain yield, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Stage of Application	
	(1)	(2)
CHA		
1168	52.2	22.2
1244	46.4	23.4
1271	43.7	24.7
2053	44.6	25.2

[1] see Materials and Methods for specific CHA concentrations

Table 26

Concentration x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent grain yield, significant at the five percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

		Concentration (1)	Concentration (2)
Stage of Application	(1)	51.4	26.6
	(2)	42.1	21.1

[1] see Materials and Methods for specific CHA concentrations

Table 27
Formulation x Growth Stage of Application interaction treatment means for observations of percent grain yield, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
Stage of (1)	46.7	44.3	40.2	55.6	46.5	9.9
Application (2)	26.5	22.0	17.9	29.6	23.5	4.6

[1] see Materials and Methods for specific CHA concentrations

Table 28
Concentration x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent blue seed, significant at the five percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

		Concentration (1)	Concentration (2)
Stage of Application	(1)	34.7	45.5
	(2)	44.6	44.3

[1] see Materials and Methods for specific CHA concentrations

Table 29
Chemical Hybridization Agent (CHA) x Growth Stage of Application interaction treatment means (formulations A-E) for observations of percent blue seed, significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Stage of Application	
	(1)	(2)
CHA		
1168	32.6	45.5
1244	25.6	47.9
1271	49.9	38.7
2053	57.7	45.7

[1] see Materials and Methods for specifis CHA concentrations

Table 30
Chemical Hybridization Agent (CHA) x Formulation interaction treatment means for observations of plant phytotoxicity (scale: 0-100;0=control), significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween-20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
CHA						
1168	17.1	20.0	20.8	15.4	22.5	0.0
1244	32.5	37.5	47.5	21.7	23.3	0.0
1271	29.2	32.5	36.7	15.4	19.2	1.7
2053	14.2	35.9	29.6	19.2	30.8	0.0

[1] see Materials and Methods for specific CHA concentrations

Table 31
Chemical Hybridization Agent (CHA) x Concentration interaction treatment means (formulations A-E) for observations of plant phytotoxicity (scale: 0-100;0=control), significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween- 20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Concentration	
	(1)	(2)
CHA		
1168	16.7	21.7
1244	27.3	37.7
1271	22.4	32.8
2053	18.2	35.7

[1] see Materials and Methods for specific CHA concentrations

Table 32
Chemical Hybridization Agent (CHA) x Growth Stage of Application interaction treatment means (formulations A-E) for observations of plant phytotoxicity (scale: 0-100;0=control), significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween- 20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Stage of Application	
	(1)	(2)
CHA		
1168	14.3	24.0
1244	25.7	39.3
1271	26.0	29.2
2053	21.5	32.3

[1] see Materials and Methods for specific CHA concentrations

Table 33

Concentration x Formulations treatment means for observations of plant phytotoxicity (scale: 0-100;0=control), significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween- 20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

		Formulations					
		(A)	(B)	(C)	(D)	(E)	(F)
Concentration	(1)	18.6	24.2	27.7	16.5	18.7	0.0
	(2)	27.9	38.8	42.1	20.6	30.4	1.2

[1] see Materials and Methods for specific CHA concentrations

Table 34
Formulation x Growth Stage of Application treatment means for observations of plant phytotoxicity (scale:0-100;0=control), significant at the one percent level of probability for Malculm wheat treated with four CHA candidates at two concentrations (low and high)[1], two growth stages of application (1.5cm and 2.5cm spike length at treatment), and six formulations (A=0.2% Triton AG-98, B=0.2% Triton AG-98 plus 2.0% Glycerol, C=0.75% Tween- 20 plus 0.1% Aresol GPG, D=0.2% Flomo, E=0.2% Flomo plus 2.0% Glycerol, F=Water Control) grown at Amity Oregon in 1986-87.

	Formulations					
	(A)	(B)	(C)	(D)	(E)	(F)
Stage of (1)	22.3	26.7	26.5	11.5	21.3	0.8
Application (2)	20.4	36.3	43.3	25.6	26.7	0.4

[1] see Materials and Methods for specific CHA concentrations

STUDY 3: Chemical Hybridization Agents (CHA) x Genotype interaction

Sterility (pollen suppression)

Glassine bags were replaced with cloth cages supported by iron frames covering an area 30cm x 30cm and placed in the center of plots. Hoods were placed on plots prior to pollen shed and removed following the pollination period. Both treatment and control (no chemical treatment) plots were treated in a similar manner. Twenty spikes previously covered from each control genotype contained an average of 3.0 to 55.0 percent fewer seeds than twenty random open-pollinated spikes from each control genotype. Reduced and variable seed set observed in treatments may be largely attributed to: a) small sample size and b) unusually high temperatures during grain filling period and, c) variable response of different genotypes to covered environment and subsequent variable levels of light and shriveled seed that may have been lost during the threshing process. Despite this observation, covered control spikes served as the base seed set level to compare relative treatment performance. Further reduction in seed set was assumed to be the result of chemically induced pollen suppression and subsequent male sterility and not the result of hoods or hood x treatment interaction.

Individual treatment values are summarized in Appendix Table 15. Based on previous studies, a single concentration for each CHA candidate was used. Mean level of sterility for all 25 genotypes ranged from 91.2 percent with 2053 applications to 98.0 percent with 1244 applications (Table 35). However, mean level of sterility between these CHA candidates did not differ significantly (Table 35; Appendix Table 16). Applications of 1168 resulted in sterility values from 43 to 100 percent across 25 genotypes. Eighteen of 25 genotypes resulted in greater than 95 percent sterility. Applications of 1244 resulted in 77 to 100 percent sterility across 25 genotypes. Twenty-two of 25 genotypes had sterility values greater than 95 percent. Applications of 1271 resulted in 82 to 100 percent sterility across 25 genotypes. Sixteen of 25 genotypes resulted in greater than 95 percent sterility. Applications of 2053 resulted in 16 to 100 percent sterility values across 25 genotypes. Sixteen of 25 genotypes resulted in greater than 95 percent sterility (Appendix Table 15).

The target growth stage of application for all genotypes was when mean primordia (spike) length of the most advanced tillers reached 2.0cm. Previous studies suggested that the growth stage of application could influence individual CHA sterility performance. No statistically significant differences were observed between

CHA candidates and mean growth stage of applications (Table 35; Appendix Table 21).

Despite lack of statistically significant differences between CHA candidates and sterility observations, the above observations suggest large differences between genotypes in their response to a single CHA concentration.

Outcrossed seed (yield)

High levels of sterility are only useful if corresponding female fertility and/or receptivity is retained and high levels of outcrossed seed can be achieved. Two assessments of outcrossed seed were made: a) mean seed set from 20 random open pollinated spikes from each treatment were compared to mean seed set from one control plot (no treatment; 20 spikes collected from control plot) and designated Micro Outcrossed seed or Outcrossed Seed and b) each treatment plot was combine-harvested. Weight of treatment plots were compared to the weight from the control plot and designated Macro Outcrossed Seed or Grain Yield. Both assessments were 'adjusted' by multiplying corresponding sterility values to give a more accurate assessment of actual outcrossed seed. The value of this adjustment factor becomes less meaningful as sterility is reduced. Despite the adjustment, low levels of sterility invariable result in higher percent

outcrossed seed and can be misleading without corresponding sterility values.

Due to inherent capacities of different genotypes to receive wind-blown pollen, and differences in flowering/receptive periods, some variability of outcrossed seed and grain yield between genotypes was expected. No statistical difference between CHA candidates and outcrossed seed set was observed (Tables 35; Appendix Table 17). Statistical differences between CHA candidates and mean grain yields were observed. Lack of statistically significant differences between CHA candidates and mean outcrossed seed values may be attributed to higher variation associated with outcrossed seed observations (Appendix Tables 17 and 18). This higher variation may be attributed to small sample size and lack of random sampling with outcrossed seed values. Higher phytotoxicity was generally manifested in secondary and tertiary tillers. Primary tillers with lower phytotoxic effects and subsequent higher percent outcrossed seed may have been favored in sampling of some treatments. Grain yield values avoided biased sampling, and will be used in more detailed discussion.

Individual treatment observations are summarized in Appendix Table 15. Applications of 2053 and 1168 resulted in higher mean grain yields than 1271 and 1244 applications, however, mean grain yield with 1168

applications was not significantly greater than 1271 and 1244 applications. Mean grain yields ranged from 41.6 to 34.9 percent with 2053 and 1168 applications and 29.4 to 29.8 percent with 1271 and 1244 applications, respectively (Table 35). Similar to Study 1 and 2 observations, higher mean grain yields were generally associated with lower mean sterility. Applications of 2053 and 1168 resulted grain yields from 13 to 62 and 15 to 87 percent grain yield with corresponding mean sterility of 94.0 and 91.2 percent, respectively. Applications of 1271 and 1244 resulted in grain yields from 10 to 65 and 12 to 75 percent with corresponding mean sterility of 92.8 and 98.0 percent, respectively (Table 35).

Previous studies have indicated that different CHA candidates interacting with specific concentrations and growth stages of application may alter heading dates differently. No statistical differences were observed between CHA candidates and mean heading dates of the twenty-five genotypes (Table 35; Appendix Table 22). Small differences in mean heading dates were observed and may have influenced grain yields due to rapidly changing climactic conditions and optimum pollination periods.

The above observations suggest that small changes in mean sterility may result in disproportionately larger influence on grain yields. Additionally, large differences between genotypes and their capacity to receive wind-blown

pollen was observed.

Phytotoxicity

Visual assessments of phytotoxicity were based on a scale from 0 to 100 relative to control plots (summarized in Appendix Table 2). Values above 40 were considered significantly damaging. Individual observations are summarized in Appendix Table 15.

Mean levels of phytotoxicity were generally dependent on individual CHA candidate characteristic performance combined with concentration used. For individual CHA candidates, higher phytotoxicity generally resulted in lower grain yields and usually higher sterility (Table 35). Some treatments resulted in both high sterility and high grain yield values. Highest grain yields were almost always associated with lower phytotoxicity (Appendix Table 15). Applications of 1271 and 1244 resulted in significantly higher phytotoxicity than 1168 and 2053 applications (Appendix Table 20). Applications of 1271 and 1244 resulted in mean phytotoxicity of 37.6 and 42; 1271 and 2053 applications resulted in mean phytotoxicity of 15.2 and 10.0, respectively (Table 35). Ranges of phytotoxicity values for these two groups were similar. Phytotoxicity ranged from 10 to 70 and 10 to 80 for 1271 and 1244 applications, respectively, and 0 to 60 and 0 to 40 for 1271 and 2053 applications, respectively (Appendix

Table 15). Higher mean phytotoxicity values with 1271 and 1244 applications resulted in some delay in heading that may be partially responsible for reduced mean grain yields for these treatments (Table 35).

Wide ranges of phytotoxicity, particularly with 1271 and 1244 applications, suggests large genotypic differences in response to applications. Higher phytotoxicity was not always associated with higher sterility further suggesting genotypic differences in response to CHA applications. Observations made in Study 1 and 2 suggest that higher concentrations result in higher sterility. Higher concentrations also frequently resulted in higher phytotoxicity and subsequent lower grain yields. Lower phytotoxicity and sterility values associated with 1168 and 2053 applications suggest that comparable concentrations for individual CHA candidates may not have been used. Regardless of CHA candidate, concentrations capable of inducing high levels of sterility (>95%) for all genotypes will undoubtedly result in higher phytotoxicity and lower grain yields for many treatments.

Low phytotoxicity with 2053 applications resulted in higher contribution of secondary and tertiary tillers to total grain yield and subsequent higher grain yields than other CHA candidates. Due to lower phytotoxicity values and despite lower mean sterility, 2053 appears to have a greater potential as a pollen suppressant across genotypes.

Seed Quality

Bulk seed harvested from all treatments was visually evaluated for seed quality. Assessments were based on a scale from one to five relative to control plots with scores of one equal to the control. Scores greater than three were considered particularly damaging. Such damage was generally expressed as wrinkled and shrivelled seed. Individual seed quality assessments are summarized in Appendix Table 15.

Seed quality observations were variable and generally inconclusive for individual treatments. Higher phytotoxicity generally resulted in lower seed quality. Applications of 1244 resulted in highest mean phytotoxicity and significantly lowest mean seed quality (Table 35; Appendix Table 19).

Summary

No statistical differences between CHA candidates and observations of mean sterility, outcrossed seed, growth stage of application and heading date of genotypes were observed. Statistical differences between CHA candidates and mean grain yield, phytotoxicity and seed quality were observed. Applications of 1244 and 1271 resulted in higher

phytotoxicity and were associated with lower mean grain yields, lower seed quality and delayed heading date. It is possible that optimum concentrations for individual CHA candidates to induce optimum mean sterility with acceptable grain yields were not identified. Still, observations suggest large genotypic differences in response to treatments and subsequent sterility achieved. A single concentration capable of inducing high mean levels of sterility (>95%) for all genotypes, regardless of CHA candidate, resulted in high phytotoxicity and subsequent low grain yields for many genotypes. Concentrations can undoubtedly be optimized for most genotypes.

Table 35

Mean CHA performance for twenty-five genotypes treated with a single concentration (1168, 1.4kg/ha; 1244, 1.4kg/ha; 1271, 0.5kg/ha; 2053, 0.3kg/ha) grown at Amity Oregon in 1986-87: 1)mean percent sterility 2)mean percent outcrossed seed from 20 spike sample compared to mean non-bagged control spikes 3)mean percent plot grain yield compared to control plot grain yield 4)mean visual evaluation of seed quality (scale 1-5; 1=control) 5)mean visual evaluation of plant phytotoxicity (scale: 0-100; 0=control) 6)mean growth stage of application (spike length at treatment in cm.) 7)mean number of days to heading from January 1, 1986.

CHA	(1) % STERIL	(2) % MICRO OUTCR	(3) % MACRO OUTCR	(4) SEED QUAL	(5) PLANT PHYTO	(6) SPIKE LENGTH AT TRT	(7) HEADING DATE OF TRTS.
1168	94.0	35.5	34.1	2.2	15.2	2.1cm	130.4
1244	98.0	40.2	29.8	2.9	42.0	2.1cm	131.3
1271	92.8	28.2	29.4	2.5	37.6	2.1cm	131.2
2053	91.2	30.0	41.6	2.5	10.0	2.1cm	130.3
Sig.	NS	NS	*	*	**	NS	NS

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

CONCLUSIONS

The objective of this study was to evaluate the performance of several promising CHA cadidates and determine: a) capacity of CHA candidates to selectively induce male-sterility, while maintaining a high level of female fertility, b) determine the relative magnitude of effective windows of concentration and growth stage of application of individual CHA candidates, c) evaluate CHA performance through improved formulations and d) evaluate CHA efficacy over a wide range of genotypes. The results would hopefully lead to CHA technology applied both as a breeding tool and as a vehicle to develop hybrid wheat for commercial production.

The following observations were made regarding the feasibility of CHA technology based on the results of this study: a) CHA candidates did not generally respond the same to changes in concentrations, formulations and growth stages of application. Subsequent complex interactions resulted in few statistically meaningful inferences regarding main effects.

b) Several CHA candidates demonstrated both high levels of sterility (>95%) and high outcrossed seed (>60%) with specific ranges of concentration and growth stages of application.

c) Optimum concentrations were generally not the same

for different stage of application, suggesting the need to develop sliding scales of concentrations for specific genotypes.

d) CHA candidates were subject to small modifications of performance with different formulations. These modifications were generally manifested in changes of sterility and subsequent modification of outcrossed seed and grain yield observations. Enhanced sterility with superior formulations may have been the result of enhanced CHA uptake by treated plants. Treatments resulting in highest sterility generally resulted in some reduction of outcrossed seed and subsequent grain yields.

e) Moderately large CHA x genotype interactions were observed. Treatment of 25 genotypes with a single concentration generally resulted in some distribution of sterility values and a wide distribution of phytotoxicity values. Single concentrations, high enough to result in high mean sterility (>95%), resulted in higher phytotoxicity and subsequent low yields of many genotypes.

Assuming genotypes can be efficiently screened for effective concentrations and growth stages of application, current CHA technology would seem to be an efficient method to screen large numbers of combinations for yield assessments, eventually obtaining general and specific combining ability estimates for specific traits. A ten-day window where effective treatments can be made would

appear to be obtainable. Such a window would facilitate larger scale hybrid production fields. If superior combinations can be identified, further hybrid development seems most promising using current CHA technology.

BIBLIOGRAPHY

Bennett, M. D. and W. L. Hughes. 1972. Additional mitosis in wheat pollen induced by ethrel. Nature 240:566-568.

Brown, C. M. and E. B. Earley. 1973. Response of one winter wheat and two spring oat varieties to foliar applications of 2-chloroethyl phosphonic acid (ethrel). Agron. J. 65:829-832.

Dotlacil, L. and M. Apltouerova. 1978. Pollen sterility induced by ethrel and its utilization in hybridization of wheat. Euphytica 27:353-368.

Fairey, D. T. and N. C. Stoskopf. 1975. Effects of granular ethephon on male sterility in wheat. Crop Sci. 15:29-32.

Foster, J. P. and S. L. Kaplin. 1985. CHA-811 hybridization agent for small grains. Proceedings, twelfth annual meeting, plant growth regulator society of america. July 28 - August 1, 1985.

Hughes, W. G., M. D. Bennett, J. J. Bodden and S. Galanopoulou. 1974. Effects of time of application of ethrel on male sterility and ear emergence in wheat (Triticum aestivum). Annals of Applied Biology 76 (2):243-252.

Hughes, W. G. and J. J. Bodden. 1978. Production and yield assessment of F1 hybrid wheats using ethephon induced male sterility. Z. Pflanzenzuchtg 81:17-23.

Jan, C. C., C. O. Qualset and H. E. Vogt. 1974. Chemical induction of male sterility in wheat. Euphytica 23:78-85.

Jan, C. C., and P. L. Rowell. 1981. Response of wheat tillers at different growing stages to gametocide treatment. Euphytica 30:501-504.

Johnson, R. R. and C. M. Brown. 1976. Chemical control of pollination in wheat and oats. Crop Sci. 16:584-587.

Johnson, R. R. and C. M. Brown. 1978. Use of DPX 3778 to produce hybrid wheat seed. Crop Sci. 18:1026-1028.

Kaul, C. L. and S. P. Singh. 1967. On induced male sterility in wheat, sunn-hemp and onion. Indian J. Pl. Physiol. 10:112-118.

Mcrae, D. H. 1985. Advances in chemical hybridization. Plant Breeding Reviews. 3:169-191.

Miller, J. F. and K. A. Lucken. 1977. Gametocidal properties of RH-531, RH-532, RH-2956, and RH-4667 on spring wheat (Triticum aestivum). Euphytica 26:103-112.

Porter, K. B. and A. F. Wiese. 1961. Evaluation of certain chemicals as selective gametocides for wheat. Crop Sci. 1:381-382.

Porter, K. B., J. P. foster, G. L. Peterson, and W. D. Worrel. 1985. Evaluation of chemical hybridization agent SD 84811 in winter wheat. Agronomy abstracts annual meetings, Dec. 1-6, 1985.

Ram, H. Y. Mohan and P. N. Rustagi. 1966. Phytogametocidal compounds. Science and culture. 32:286-291.

Rowell, P. L. and D. G. Miller. 1971. Induction of male sterility in wheat with 2-chloro ethylphosphonic acid (ethrel). Crop Sci. 11:629-631.

Rowell, P. L. and D. G. Miller. 1974. Effect of 2-chloro ethylphosphonic acid (ethephon) on female fertility of two wheat varieties.

Stoskopf, N. C. and J. Law. 1972. Some observations of ethrel as a tool for developing hybrid cereals. Can. J. Plant Sci. 52:680-683.

Virmani, S. S. and I. B. Edwards. 1983. Current status and future prospects for breeding hybrid rice and wheat. Adv. in Agronomy. 36:145-214.

Warner, H. L. 1985. Chemical hybridization agent interactions in wheat. Proceedings, twelfth annual meeting, plant growth regulator society of america. July 28 - August 1, 1985.

APPENDICES

Appendix Table 1:
Pedigree and description of test cultivars/genotypes

STUDIES 1 and 2:

Treatment (female): MALCULM; SPN//63-189-66-7/BEZ
Awned Semi-dwarf soft white winter wheat cultivar released by Oregon State University.

Pollinator (male): BLUE NORCO; AGROPYRON/BAART/NORCO
Tall, awned genetic marker line developed by USDA scientist at Oregon State University. Possesses a telocentric chromosome from Agropyron which was backcrossed into it from blue Baart to obtain the blue aleurone color.

STUDY 3:

Treatment (female): Twenty-five diverse experimental soft white winter wheat selections provided by Oregon State University.

1)7C/CNO//CAL/3/YMH
2)RMN F3-7//TORIM
3)KVZ/3/HD/ON//BB/4/VPOPR/3/1744//SV/GNS
4)KVZ/JUACA, F1//KVZ
5)6720/3/CNO/INIA//RFN
6)6720/3/CNO/INIA//RFN
7)TJB729-1278/SPN
8)HYS/4/ND/WW/3/LEE//FN/N,F1/5/AVC
9)ND/P101//KAL/BB
10)NOR/6720//YMH/3/ZZ,F1/4/ASP
11)HYS/4/ND/WW/3/LEE//FN/N,F1/5/AVT
12)RMNF 3-71/TORIM
13)6720/HYS//R37/GHL1,F1/3/SPN
14)KVZ/3/HD/ON/BB/4/YBOPR/3/55-1744//SU/GNS
15)HYS//R37/GHL 1
16)KVZ/JUACA,F1//KVZ
17)TJB801-12795/SPN
18)6720-11//MDA38/WRM
19)V6707/BNN
20)CLEO/PCH//ZZ
21)CLEO/PCH//ZZ,F1/4/F1,AVC/3/DJ/BEZ//WA5204
22)RDL/6720//HYS/CD,F1/4/1523/DRC
23)VPM/MOS83-11-4-8//YMH/HYS,F1/F3/ASP
24)69-153/YMH//YMHDW

Appendix Table 1: (cont.)

25)SPN/CROW

Pollinator (male): STEPHENS; ND/PULLMAN 101
Awned semi-dwarf soft white winter wheat cultivar released by Oregon State University.

Appendix Table 2:
Treatment phytotoxicity and seed quality index.

Phytotoxicity:	0	-equal to control
	20	-uniform; all tillers fully emerged from flag leaf sheath, tertiary tillers may be reduced in length and may be slightly delayed in maturity. Plot may be slightly off-color in appearance.
	40*	-Primary tillers uniform; secondary tillers fully emerged, but may be reduced in length and delayed in maturity resulting in lower seed set. Tertiary tillers may be significantly reduced in length and may not be fully emerged; some may be chlorotic.
	60	-Stand may not be uniform; primary tillers may be variably reduced and delayed in maturity. Secondary tillers may not be fully emerged. Seed set visibly reduced, suggesting reduced female fertility and/or receptivity. Foliage damage moderate resulting in some regrowth.
	80	-All tillers may not be fully emerged. Reduced female fertility and/or receptivity obvious with very low seed sets. Treatments generally significantly delayed in maturity. Foliar damage significant with considerable regrowth.
	100	-Plants necrotic.
Seed Quality:	1	-Equal to control
	2	-Seeds generally slightly more shriveled and aberrant appearing
	3*	-Higher proportion of Seeds noticeably more shriveled and wrinkled.
	4	-Seeds severely shriveled with low test weight. Portion of seeds may be green from plot regrowth. Viability questionable.
	5	-Dry seeds severely shriveled with vary low test weight and generally darker in color. High portion of green seeds. purity and viability questionable.

* Highest level for acceptable treatment.

Appendix Table 3

Three-way interaction treatment means for Malculm wheat treated with thirteen CHA candidates at three concentrations and two growth stages of application (1.5cm and 2.5cm spike length at treatment) grown at Amity Oregon in 1986-87: 1)growth stage of application 2)percent sterility 3)percent outcrossed seed from 21 spike sample compared to mean non-bagged control spikes 4)percent grain yield compared to mean control plot grain yield 5)percent blue seed from 21 open-pollinated spike sample 6)percent blue seed from 100 seed sample from bulk-harvested treatment plot 7)plant phytotoxicity (scale 0-100; 0=control)8)visual evaluation of seed quality (scale 1-5;1=control).

CHA	CONC Kg/Ha	(1) GS	(2) % STER-ILITY	(3) % OUTCR. SEED	(4) % GRAIN YIELD	(5) % MICRO PUR	(6) % MACRO PUR	(7) PLANT PHYTO	(8) SEED QUAL
1058	0.4	1	99.1	23.6	19.7	51.4	46.3	30.0	3.0
1058	0.5	1	100.0	15.5	19.9	46.9	50.7	36.7	2.3
1058	0.6	1	99.6	15.9	13.0	58.2	52.3	40.0	3.0
1058	0.4	2	92.6	15.2	27.2	24.1	40.0	36.7	2.7
1058	0.5	2	94.0	18.1	17.2	29.6	43.3	36.7	2.7
1058	0.6	2	94.5	12.7	12.9	22.3	34.7	43.3	3.3
1168	1.0	1	99.4	55.1	65.9	26.7	23.7	10.0	2.7
1168	1.2	1	95.7	54.8	56.1	21.1	26.0	16.7	2.0
1168	1.4	1	96.9	45.1	60.6	23.4	25.3	16.7	2.3
1168	1.0	2	99.0	21.5	31.5	46.7	29.3	16.7	1.7
1168	1.2	2	97.7	10.0	25.4	52.7	38.7	13.3	2.0
1168	1.4	2	99.3	14.7	19.8	56.8	39.3	16.7	2.7
1244	1.0	1	95.7	70.5	59.0	27.4	33.0	16.7	1.3
1244	1.2	1	92.6	67.5	42.3	10.6	27.3	6.7	1.7
1244	1.4	1	95.0	68.8	38.0	27.8	40.0	0.0	2.0
1244	1.0	2	91.2	28.1	24.8	60.7		3.3	
1244	1.2	2	95.6	28.0	22.3	44.7	40.3	3.3	2.7
1244	1.4	2	96.8	31.9	23.5	52.6	48.0	3.3	2.7
1260	0.6	1	80.7	65.6	69.7	11.0		0.0	
1260	0.9	1	95.3	61.4	64.9	21.3	23.7	6.7	1.7
1260	1.2	1	93.4	55.8	60.8	34.7	35.3	6.7	1.7
1260	0.6	2	99.8	55.5	57.9	52.2	48.0	5.0	1.7
1260	0.9	2	99.4	27.1	41.6	47.4	39.0	5.0	2.0
1260	1.2	2	99.5	33.5	37.9	56.3	48.7	6.7	2.0
1271	0.2	1	97.2	58.9	59.3	30.9	40.0	6.7	1.0
1271	0.4	1	99.2	42.6	44.1	51.8	53.0	6.7	2.3
1271	0.6	1	99.7	43.2	35.1	52.9	44.3	0.0	2.0
1271	0.2	2	64.5	32.4	37.2	35.3		3.3	
1271	0.4	2	63.3	25.5	22.2	20.6		3.3	
1271	0.6	2	83.8	35.6	27.8	47.8	42.3	6.7	2.3
1272	0.1	1	15.6	14.0	15.8	1.9		0.0	

Appendix Table 3 continued

CHA	CONC Kg/Ha	(1) GS	(2) % STER-ILITY	(3) % OUTCR. SEED	(4) % GRAIN YIELD	(5) % MICRO PUR	(6) % MACRO PUR	(7) PLANT PHYTO	(8) SEED QUAL
1272	0.3	1	45.8	45.6	41.3	4.4		1.7	
1272	0.5	1	39.2	36.1	42.5	7.1		0.0	
1272	0.1	2	21.5	19.3	21.5	2.9		0.0	
1272	0.3	2	67.3	58.6	57.0	23.8		6.7	
1272	0.5	2	77.5	46.9	61.6	23.9		3.3	
2029	0.1	1	56.2	54.7	54.0	4.4		3.3	
2029	0.2	1	93.8	80.1	74.3	13.4	27.0	20.0	1.3
2029	0.3	1	87.6	64.9	57.0	14.0	29.0	20.0	2.0
2029	0.1	2	4.6	4.2	4.0	8.6		0.0	
2029	0.2	2	40.3	20.3	25.3	14.5		6.7	
2029	0.3	2	81.7	47.1	47.0	35.0		23.3	
2053	0.2	1	97.2	57.2	63.0	58.6	46.0	3.3	2.0
2053	0.4	1	99.8	28.4	44.3	50.8	48.7	10.0	2.0
2053	0.6	1	99.2	40.8	46.0	68.8	50.7	30.0	2.3
2053	0.2	2	74.4	26.2	35.1	39.5		10.0	
2053	0.4	2	86.0	27.1	29.5	47.1		23.3	
2053	0.6	2	88.2	27.1	24.0	50.6		36.7	
2099	0.3	1	98.2	46.9	56.0	31.9	48.7	10.0	1.7
2099	0.5	1	99.4	43.2	45.8	54.9	47.0	16.7	2.0
2099	0.7	1	99.5	39.6	33.7	67.3	50.3	33.3	3.0
2099	0.3	2	66.0	35.3	37.2	30.8		13.3	
2099	0.5	2	69.8	23.8	26.1	25.2		26.7	
2099	0.7	2	83.3	25.4	20.1	38.2		40.0	
2129	0.5	1	79.4	61.3	55.7	9.6	16.0	10.0	2.0
2129	0.8	1	78.6	73.0	56.4	9.7		16.7	
2129	1.0	1	83.6	69.5	59.8	8.1		10.0	
2129	0.5	2	81.7	45.7	50.0	20.9		13.3	
2129	0.8	2	84.3	44.6	39.0	24.1	30.3	13.3	2.3
2129	1.0	2	96.8	46.8	59.0	42.1	40.3	6.7	1.0
2157	1.0	1	31.2	28.0	29.3	3.9		0.0	
2157	1.2	1	8.1	9.3	8.9	0.7		0.0	
2157	1.4	1	7.2	8.5	7.0	1.1		0.0	
2157	1.0	2	17.2	15.8	14.7	3.9		0.0	
2157	1.2	2	12.6	13.0	14.0	3.9		0.0	
2157	1.4	2	21.1	19.0	9.4	2.6		0.0	
2168	0.6	1	41.5	42.7	41.9	3.5		0.0	
2168	0.9	1	80.0	65.9	66.9	6.6		5.0	
2168	1.2	1	69.2	63.0	49.6	12.5		10.0	
2168	0.6	2	93.9	54.3	68.5	37.3	40.0	6.7	2.3
2168	0.9	2	97.8	63.6	67.3	40.2	44.7	8.3	2.0
2168	1.2	2	99.0	43.4	47.1	51.1	38.7	23.3	1.7
2186	0.2	1	84.4	70.7	74.4	18.8		0.0	
2186	0.4	1	94.8	65.5	61.3	35.4	33.0	3.3	2.0
2186	0.6	1	97.8	62.4	72.8	52.0	41.3	3.3	1.3

Appendix Table 3 continued

CHA	CONC Kg/Ha	(1) GS	(2) % STER- ILITY	(3) % OUTCR. SEED	(4) % GRAIN YIELD	(5) % MICRO PUR	(6) % MACRO PUR	(7) PLANT PHYTO	(8) SEED QUAL
2186	0.2	2	75.4	53.6	61.2	26.9	34.3	0.0	2.3
2186	0.4	2	84.2	43.5	54.9	32.6	36.3	5.0	2.3
2186	0.6	2	87.1	43.8	51.8	58.8	47.0	10.0	1.3

Appendix Table 4
Analysis of Variance for the dependent variable sterility (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr >F
Growth Stage Stage (GS)	1	802.7	802.7	2.1	0.29
Rep. x GS	2	767.6	383.8		
(Error A)					
CHA	12	124760.6	10396.7	84.2	0.01**
Conc.	2	6132.4	3066.2	24.8	0.01**
CHA x Conc	24	13286.6	553.6	4.5	0.01**
CHA x GS	12	21534.5	1794.5	14.5	0.01**
Conc. x GS	2	1364.0	682.0	5.5	0.01*
CHA x Conc. x GS	24	4165.9	173.6	1.4	0.11
Error B	152	18779.0	123.6		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) $1 - \frac{\text{(av. no. seeds from 21 trt. bagged spikes)}}{\text{(av. no. seeds from 21 bagged control spikes)}} \times 100$

Appendix Table 5
Analysis of Varience for the dependent variable Micro Outcrossed Seed or Outcrossed Seed (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr >F
Growth Stage (GS)	1	17655.8	17655.8	138.3	0.01**
Rep x GS	2	254.7	127.4		
(Error A)					
CHA	12	40240.2	3353.7	24.4	0.01**
Conc.	2	16.3	8.1	0.1	0.94
CHA x Conc.	24	10504.2	437.7	3.2	0.01**
CHA x GS	12	13541.8	1128.5	8.2	0.01**
Conc. x GS	2	282.3	141.1	1.0	0.36
CHA x Conc. x GS	24	4215.7	175.7	1.3	0.19
Error B	152	20919.9	137.6		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) MICRO OUTCR= Average Percent Outcrossed Seed from individual spikes:

$$\frac{\text{(av.\# seeds from 21 unbagged trt. spikes)}}{\text{(av. \# seeds from 21 unbagged control spikes)}} \times \%\text{Sterility}$$

Appendix Table 6
Analysis of Varience for the dependent variable Macro Outcrossed Seed or Grain Yield (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr > F
Growth Stage (GS)	1	10075.9	10075.9	58.5	0.02*
Rep.x GS	2	344.7	172.4		
(Error A)					
CHA	12	43466.4	3622.2	27.0	0.01**
Conc.	2	789.0	394.5	2.9	0.06
CHA x Conc.	24	12250.4	510.4	3.8	0.01**
CHA x GS	12	12566.4	1047.2	7.8	0.01**
Conc. x GS	2	234.7	117.4	0.9	0.42
CHA x Conc. x GS	24	3935.1	164.0	1.2	0.23
Error B	152	20425.8	134.9		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) Macro Outcrossed seed= Average Percent weight of trt. plots compared to untreated control plots x %Sterility

Appendix Table 7
Study 1 Analysis of Varience for the dependent variable Micro Purity (1).

Source of VAriation	DF	Anova SS	Mean Square	F Value	Pr > F
Growth Stage (GS)	1	3443.1	3443.1	27.7	0.04*
Rep.x GS	2	278.5	139.3		
(Error A)					
CHA	12	45375.2	3781.3	21.6	0.01**
Conc.	2	5605.4	2802.7	16.0	0.01**
CHA x Conc.	24	4510.1	187.9	1.0	0.38
CHA x GS	12	23280.4	1940.0	11.1	0.01**
Conc. x GS	2	74.9	37.4	0.2	0.81
CHA x Conc. x GS	24	4021.7	167.6	1.0	0.53
Error B	152	26651.0	175.3		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) Micro Pur= Average Percent blue seed from individual spikes:

$$\frac{\text{(av. no. of blue seeds from 21 trt. spikes)}}{\text{(total no of seeds from 21 trt. spikes)}}$$

Appendix Table 8
Analysis of Varience for the dependent variable Plant phytotoxicity (scale 0-100; 0=control).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr >F
Growth Stage Stage (GS)	1	558.0	558.0	7.8	0.11
Rep.x GS	2	149.6	74.8		
(Error A)					
CHA	12	29208.8	2434.1	38.0	0.01**
Conc.	2	5619.4	2809.7	43.9	0.01**
CHA x Conc.	24	4544.4	189.4	3.0	0.01**
CHA x GS	12	1140.0	95.0	1.5	0.14
Conc. x GS	2	52.8	26.4	0.4	0.66
CHA x Conc. x GS	24	1588.9	66.2	1.0	0.43
Error B	152	9726.5	64.0		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Appendix Table 9

Four-way interaction treatment means for Malculm wheat treated with four CHA candidates at two concentrations, two growth stages of application (1.5cm and 2.5cm spike length at treatment, and six formulations grown at Amity Oregon in 1986-87: 1)Formulation/CHA 2) growth stage of application 3)percent sterility 4)percent outcrossed seed from 21 spike sample compared to mean non-bagged control spikes 5)percent grain yield compared to mean control plot grain yield 6)percent blue seed from 21 open-pollinated spike sample 7)percent blue seed from 100 seed sample from bulk-harvested treatment plot 8)plant phytotoxicity (scale:0-100; 0=control) 9)visual evaluation of seed quality (scale 1-5;1=control).

(1) FORM/ CHA	CONC Kg/Ha	(2) GS	(3) % STER-ILITY	(4) % OUTCR. SEED	(5) % GRAIN YIELD	(6) % MICRO PUR.	(7) % MACRO PUR.	(8) PLANT PHYTO	(9) SEED QUAL.
A1168	1.0	1	92.2	60.9	53.4	20.8	29.7	15.0	2.0
A1168	1.4	1	98.6	43.1	55.1	35.8	35.7	13.3	1.7
B1168	1.0	1	93.9	48.1	56.7	31.1	26.3	6.7	2.0
B1168	1.4	1	97.8	33.1	47.0	32.0	28.3	20.0	0.7
C1168	1.0	1	97.3	46.4	46.9	29.3	31.0	13.3	1.3
C1168	1.4	1	99.8	21.9	41.5	49.6	26.0	20.0	2.0
D1168	1.0	1	95.8	62.9	68.4	19.9	22.0	8.3	1.7
D1168	1.4	1	98.9	51.6	57.0	31.4	34.3	10.0	2.0
E1168	1.0	1	96.8	60.6	56.1	19.5	21.3	16.7	1.7
E1168	1.4	1	99.7	31.1	40.0	56.7	21.7	20.0	2.0
F1168	1.0	1	15.0	13.9	15.8	0.9		0.0	
F1168	1.4	1	3.5	3.6	4.0	1.6		0.0	
A1168	1.0	2	98.8	30.9	29.7	47.9	33.7	16.7	2.0
A1168	1.4	2	98.3	15.0	19.3	43.4	35.0	23.3	2.0
B1168	1.0	2	99.2	11.2	18.6	42.2	35.3	26.7	2.7
B1168	1.4	2	99.8	8.4	17.1	46.3	31.0	26.7	2.7
C1168	1.0	2	00.0	6.8	18.5	54.8	30.7	23.3	1.7
C1168	1.4	2	00.0	3.4	15.0	29.3	31.7	26.7	2.7
D1168	1.0	2	99.0	39.9	33.2	45.4	31.0	20.0	2.3
D1168	1.4	2	99.6	26.1	29.0	50.7	40.3	23.3	2.3
E1168	1.0	2	96.2	12.7	24.2	55.5	43.0	20.0	2.0
E1168	1.4	2	00.0	10.1	17.5	39.3	29.3	33.3	2.3
F1168	1.0	2	0.0	0.0	0.0	0.4		0.0	
F1168	1.4	2	2.6	2.6	1.8	3.6		0.0	
A1244	1.0	1	82.8	62.9	44.4	12.6	19.7	16.7	1.3
A1244	1.4	1	99.1	45.8	41.7	34.0	32.3	46.7	2.0
B1244	1.0	1	98.8	57.5	52.7	33.5	30.0	26.7	1.3
B1244	1.4	1	94.4	44.0	36.5	24.3	25.7	43.3	2.0
C1244	1.0	1	93.5	59.2	48.6	25.2	33.7	16.7	2.3
C1244	1.4	1	93.6	41.8	33.4	33.0	43.7	40.0	2.3
D1244	1.0	1	82.2	60.2	51.7	16.4		10.0	
D1244	1.4	1	95.8	53.1	51.5	29.3	20.7	16.7	2.0

Appendix Table 9 continued

(1) FORM/ CHA	CONC Kg/Ha	(2) GS	(3) % STER- ILITY	(4) % OUTCR. SEED	(5) % GRAIN YIELD	(6) % MICRO PUR.	(7) % MACRO PUR.	(8) PLANT PHYTO	(9) SEED QUAL.
E1244	1.0	1	88.8	50.1	51.0	18.0		20.0	
E1244	1.4	1	98.8	60.5	52.6	29.8	45.0	20.0	1.7
F1244	1.0	1	15.5	16.9	13.5	1.7		0.0	
F1244	1.4	1	19.0	19.1	17.8	1.4		3.3	
A1244	1.0	2	88.1	35.7	29.5	54.6		30.0	
A1244	1.4	2	93.7	38.0	24.5	49.6		36.7	
B1244	1.0	2	94.2	32.0	25.8	58.2	48.7	33.3	2.3
B1244	1.4	2	97.5	25.7	19.0	54.6	42.0	46.7	2.7
C1244	1.0	2	97.0	18.4	14.4	35.8	33.0	63.3	3.0
C1244	1.4	2	99.2	14.8	11.1	38.6	33.7	70.0	2.7
D1244	1.0	2	92.3	36.1	30.9	34.9	29.7	33.3	2.0
D1244	1.4	2	95.7	43.9	28.7	49.9	39.0	26.7	2.7
E1244	1.0	2	93.1	27.9	27.3	58.2	45.7	23.3	2.3
E1244	1.4	2	97.1	33.9	22.4	45.0	44.7	30.0	2.3
F1244	1.0	2	9.1	9.6	6.5	0.5		0.0	
F1244	1.4	2	0.0	0.0	0.0	0.6		0.0	
A1271	0.3	1	98.0	46.4	51.6	41.5	48.7	26.7	1.7
A1271	0.6	1	100.0	38.2	37.0	51.4	46.3	33.3	1.7
B1271	0.3	1	100.0	43.0	47.1	52.3	43.7	23.3	2.0
B1271	0.6	1	99.9	30.5	33.8	54.9	48.3	36.7	2.3
C1271	0.3	1	99.5	37.1	38.9	64.2	62.3	26.7	2.0
C1271	0.6	1	99.7	32.7	35.4	49.4	52.0	36.7	2.3
D1271	0.3	1	93.3	55.1	50.9	28.8	29.7	16.7	2.3
D1271	0.6	1	99.8	40.0	42.9	42.5	51.3	20.0	2.0
E1271	0.3	1	99.3	48.5	56.6	52.9	43.7	13.3	2.0
E1271	0.6	1	99.4	45.4	42.4	61.2	49.3	26.7	2.3
F1271	0.3	1	4.7	5.1	4.0	0.4		0.0	
F1271	0.6	1	11.5	12.6	10.4	1.0		3.3	
A1271	0.3	2	77.1	31.8	27.5	32.5		26.7	
A1271	0.6	2	59.1	28.2	22.3	36.3		30.0	
B1271	0.3	2	77.3	32.4	31.1	42.8		26.7	
B1271	0.6	2	82.2	29.1	21.5	37.1		43.3	
C1271	0.3	2	63.6	28.4	27.0	49.5		30.0	
C1271	0.6	2	85.3	19.1	13.4	37.2		53.3	
D1271	0.3	2	43.8	19.2	21.7	34.2		16.7	
D1271	0.6	2	68.7	35.5	29.2	34.1		18.3	
E1271	0.3	2	57.3	34.0	27.2	37.0		16.7	
E1271	0.6	2	75.2	31.7	26.4	46.1		30.0	
F1271	0.3	2	0.0	0.0	0.0	0.2		0.0	
F1271	0.6	2	0.0	0.0	0.0	0.6		3.3	
A2053	0.3	1	96.7	39.7	48.9	58.6	41.3	6.7	2.0
A2053	0.5	1	100.0	34.5	43.4	61.4	57.3	20.0	2.7
B2053	0.3	1	100.0	30.6	50.3	63.1	60.0	20.0	2.0
B2053	0.5	1	100.0	28.1	30.5	59.8	52.0	36.7	2.7

Appendix Table 9 continued

(1) FORM/ CHA	CONC Kg/Ha	(2) GS	(3) % STER- ILITY	(4) % OUTCR. SEED	(5) % GRAIN YIELD	(6) % MICRO PUR.	(7) % MACRO PUR.	(8) PLANT PHYTO	(9) SEED QUAL.
C2053	0.3	1	99.5	38.4	45.2	58.7	51.0	21.7	2.7
C2053	0.5	1	98.9	33.3	32.0	56.0	63.0	36.7	3.0
D2053	0.3	1	99.8	50.8	66.5	35.6	38.3	3.3	2.3
D2053	0.5	1	99.8	47.5	55.8	50.6	41.7	16.7	2.3
E2053	0.3	1	100.0	37.1	42.0	66.6	58.0	16.7	3.0
E2053	0.5	1	99.6	34.5	31.4	66.4	57.0	36.7	2.3
F2053	0.3	1	2.5	2.6	2.5	0.5		0.0	
F2053	0.5	1	12.4	11.7	11.3	1.5		0.0	
A2053	0.3	2	60.9	29.4	30.4	30.8		10.0	
A2053	0.5	2	87.4	31.7	28.8	49.4		20.0	
B2053	0.3	2	89.3	34.7	26.6	51.4	49.7	30.0	2.0
B2053	0.5	2	91.7	19.7	15.9	50.1	48.3	56.7	3.0
C2053	0.3	2	91.1	36.2	26.5	33.6	46.0	26.7	3.0
C2053	0.5	2	93.8	22.1	17.1	50.6	60.7	53.3	3.3
D2053	0.3	2	79.9	37.1	35.5	41.4		23.3	
D2053	0.5	2	80.2	28.4	28.6	47.2		33.3	
E2053	0.3	2	82.7	27.1	26.5	51.7		23.3	
E2053	0.5	2	95.5	16.6	16.1	51.1	50.7	46.7	3.0
F2053	0.3	2	0.0	0.0	0.0	0.3		0.0	
F2053	0.5	2	0.0	0.0	0.0	1.6		0.0	

Appendix Table 10
Analysis of Varience for the dependent variable sterility (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr > F
Growth	1	6851.2	6851.2	99.4	0.01**
Stage (GS)					
Rep.x GS	2	137.9	68.9	1.6	0.20
(Error A)					
CHA	3	5573.5	1857.8	43.6	0.01**
Conc.	1	1159.9	1159.9	27.2	0.01**
Form.	5	297308.7	59461.7	1394.6	0.01**
CHA x Conc.	3	142.1	47.4	1.1	0.35
CHA x Form.	15	2090.4	139.4	3.3	0.01**
Form x Conc.	5	378.8	75.8	1.8	0.12
CHA x GS	3	8662.0	2887.3	67.7	0.01**
Conc. x GS	1	16.2	16.2	0.4	0.54
Form x GS	5	648.1	129.6	3.0	0.01**
CHA x Form. x Conc.	15	1661.6	110.8	2.6	0.01**
CHA x Form. x Conc. x GS	38	5283.8	139.1	3.3	0.01**
Error B	187	7973.4	42.6		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) $1 - \frac{\text{(av. no. seeds from 21 trt. bagged spikes)}}{\text{(av. no. seeds from 21 bagged control spikes)}} \times 100$

Appendix Table 11
Analysis of Varience for the dependent variable Micro Outcross Seed or Outcrossed Seed (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr > F
Growth Stage (GS)	1	20295.9	20295.9	294.7	0.01**
Rep. x GS	2	137.7	68.9	0.7	0.48
(Error A)					
CHA	3	4718.0	1572.7	16.7	0.01**
Conc.	1	2323.9	2323.9	24.6	0.01**
Form.	5	39697.8	7939.6	84.2	0.01**
CHA x Conc.	3	695.6	231.9	2.5	0.06
CHA x Form.	15	2556.3	170.4	1.8	0.03*
Form. x Conc.	5	885.6	177.1	1.9	0.10
CHA x GS	3	3065.7	1021.9	10.8	0.01**
Conc. x GS	1	369.1	369.1	3.9	0.05*
Form. x GS	5	1384.1	276.8	2.9	0.01**
CHA x Form. x Conc.	15	1010.8	67.4	0.7	0.77
CHA x Form. x Conc. x GS	38	4724.2	124.3	1.3	0.11
Error B	187	17642.5	94.3		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) MICRO OUTCR= Mean Percent Outcrossed Seed from individual spikes:

$$\frac{\text{(av. no. seeds from 21 unbagged trt. spikes)}}{\text{(av. no. seeds from 21 unbagged control spikes)}} \times \%\text{Sterility}$$

Appendix Table 12
Analysis of Varience for the dependent variable Macro Outcross Seed or Grain Yield (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr > F
Growth Stage (GS)	1	30020.4	30020.4	560.2	0.01**
Rep.x GS (Error A)	2	107.2	53.6	1.4	0.25
CHA	3	272.2	90.7	2.4	0.07
Conc.	1	2571.4	2571.4	67.3	0.01**
Form.	5	40084.8	8017.0	210.0	0.01**
CHA x Conc.	3	77.3	25.8	0.7	0.57
CHA x Form.	15	1979.4	132.0	3.5	0.01**
Form. x Conc.	5	965.9	193.2	5.1	0.01**
CHA x GS	3	1058.3	352.8	9.2	0.01**
Conc. x GS	1	188.1	188.1	4.9	0.03*
Form. x GS	5	2271.2	454.2	11.9	0.01**
CHA x Form. x Conc.	15	759.6	50.6	1.3	0.19
CHA x Form x Conc. x GS	38	2146.4	56.5	1.5	0.05*
Error B	187	7140.5	38.2		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) Macro Outcrossed seed= Mean percent trt weight of mean untreated control plot weight x % Sterility

Appendix Table 13
Analysis of Varience for the dependent variable Micro Purity (1).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr >F
Growth Stage (GS)	1	426.4	426.4	2.5	0.3
Rep. x GS (Error A)	2	339.0	169.5	1.1	0.34
CHA	3	6488.4	2162.8	14.1	0.01**
Conc.	1	788.8	788.8	5.1	0.03*
Form.	5	73442.3	14688.5	95.5	0.01**
CHA x Conc.	3	125.4	41.8	0.3	0.84
CHA x Form.	15	3091.3	206.1	1.3	0.18
Form. x Conc.	5	1332.3	266.5	1.7	0.13
CHA x GS	3	11189.7	3729.9	24.2	0.01**
Conc. x GS	1	833.5	833.5	5.4	0.02*
Form. x GS	5	1445.3	289.1	1.9	0.10
CHA x Form. x Conc.	15	1297.5	86.5	0.6	0.90
CHA x Form x Conc. x GS	38	8766.6	230.7	1.5	0.04*
Error B	187	28769.4	153.9		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) Micro Pur= Average Percent blue seed from individual spikes:

$$\frac{\text{(av. no. of blue seeds from 21 trt. spikes)}}{\text{(total no of seeds from 21 trt. spikes)}}$$

Appendix Table 14
Analysis of Varience for the dependent variable Plant Phytotoxicity (scale 0-100;0=control).

Source of Variation	DF	Anova SS	Mean Square	F Value	Pr > F
Growth Stage (GS)	1	4353.7	4353.7	137.6	0.01**
Rep. x GS (Error A)	2	63.3	31.7	0.6	0.58
CHA	3	4832.6	1610.9	28.0	0.01**
Conc.	1	6236.0	6236.0	108.5	0.01**
Form.	5	35146.2	7029.3	122.3	0.01**
CHA x Conc.	3	952.9	317.6	5.5	0.01**
CHA x Form.	15	5678.6	378.6	6.6	0.01**
Form. x Conc.	5	1801.0	360.2	6.3	0.01**
CHA x GS	3	764.7	254.9	4.4	0.01**
Conc. x GS	1	56.9	56.9	1.0	0.32
Form. x GS	5	2391.7	478.3	8.3	0.01**
CHA x Form. x Conc.	15	1027.2	68.5	1.2	0.28
CHA x Form x Conc. x GS	38	3613.0	95.1	1.7	0.02*
Error B	187	10745.8	57.5		

* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Appendix Table 15

Treatment means for twenty-five genotypes treated with four CHA candidates at single concentrations and a single 'target' growth stage of application planted in Amity Oregon on 1986-87: 1)treatment genotype 2)growth stage of application (spike length at treatment in cm) 3)pollinator heading date 4)treatment heading date 5)mean percent sterility from 20 spike sample compared to hooded control 6)mean percent outcrossed seed from 20 spike sample compared to non-bagged control 7)percent plot yield compared to control 8)visual evaluation of seed quality (scale 1-5; 1=control) 9)visual evaluation of plant phytotoxicity (scale 0-100; 0=control.

(1) TRT	CHA	(2) GROWTH STAGE cm@TRT	(3) MALE H.D.	(4) FEMALE H.D.	(5) % STER-ILITY	(6) % OUTCR. SEED	(7) % GRAIN YIELD	(8) SEED QUAL	(9) PLANT PHYTO
1	1168	1.8	132	131	99	21	28	2	10
1	1244	2.3	132	132	100	17	20	3	50
1	1271	1.8	132	133	97	11	20	2	60
1	2053	1.8	132	133	99	25	29	3	20
2	1168	1.9	132	130	88	62	50	1	20
2	1244	2.3	132	132	97	20	15	4	60
2	1271	2.2	132	132	97	11	20	2	30
2	2053	2.3	132	133	97	25	29	3	10
3	1168	2.0	132	133	97	22	26	2	20
3	1244	1.8	132	133	98	83	36	4	40
3	1271	2.3	132	133	83	38	20	2	60
3	2053	1.9	132	131	83	65	43	3	0
4	1168	2.2	132	131	100	30	32	2	10
4	1244	2.0	132	131	94	66	27	3	40
4	1271	1.5	132	133	100	7	18	3	70
4	2053	2.0	132	133	97	20	40	1	0
5	1168	1.6	132	132	100	19	44	4	20
5	1244	1.9	132	134	100	6	19	3	50
5	1271	1.7	132	135	100	6	19	3	50
5	2053	2.1	132	133	100	8	20	3	10
6	1168	1.8	132	130	100	60	35	3	40
6	1244	2.0	132	130	98	15	24	3	50
6	1271	2.0	132	133	98	3	10	4	70
6	2053	2.0	132	130	97	24	38	3	40
7	1168	2.4	132	130	96	28	29	2	20
7	1244	2.0	132	130	89	68	31	2	20
7	1271	2.2	132	130	100	18	16	2	20
7	2053	2.2	132	131	89	8	24	3	10
8	1168	2.1	132	130	100	27	13	3	10
8	1244	2.0	132	133	100	9	15	3	60
8	1271	2.2	132	131	100	11	20	2	50
8	2053	2.0	132	130	100	23	27	2	0

Appedix Table 15 (continued)

(1) TRT	CHA	(2) GROWTH STAGE cm@TRT	(3) MALE H.D.	(4) FEMALE H.D.	(5) % STER-ILITY	(6) % OUTCR. SEED	(7) % GRAIN YIELD	(8) SEED QUAL	(9) PLANT PHYTO
9	1168	2.7	132	127	100	55	48	1	10
9	1244	3.8	132	126	100	62	45	4	30
9	1271	2.2	132	128	92	25	30	3	30
9	2053	2.3	132	125	16	16	15	1	0
10	1168	1.8	132	130	84	51	62	3	0
10	1244	2.0	132	128	98	59	56	3	40
10	1271	1.8	132	126	85	80	65	1	20
10	2053	2.2	132	130	90	38	88	3	10
11	1168	2.0	132	130	100	23	17	3	10
11	1244	2.1	132	130	100	42	25	3	20
11	1271	2.2	132	131	91	24	18	2	20
11	2053	2.0	132	130	91	24	26	4	0
12	1168	2.0	132	132	83	55	37	1	10
12	1244	1.8	132	135	100	2	12	2	70
12	1271	1.8	132	132	99	23	15	3	40
12	2053	2.7	132	131	83	19	24	3	10
13	1168	1.9	132	132	99	36	43	2	0
13	1244	1.9	132	135	100	5	21	3	50
13	1271	1.6	132	130	92	33	28	2	10
13	2053	2.0	132	133	99	36	41	4	0
14	1168	2.0	132	130	100	11	21	2	30
14	1244	2.2	132	132	100	18	32	3	50
14	1271	2.2	132	132	99	36	30	3	50
14	2053	2.0	132	132	93	21	44	3	0
15	1168	2.4	132	131	100	16	31	3	30
15	1244	2.1	132	135	100	35	19	3	70
15	1271	2.4	132	132	97	13	30	2	50
15	2053	1.8	132	130	100	38	44	3	40
16	1168	2.1	132	131	100	14	47	2	10
16	1244	2.0	132	131	77	54	28	3	10
16	1271	2.3	132	132	98	21	38	1	10
16	2053	2.0	132	127	96	73	86	1	0
17	1168	2.0	132	132	88	32	29	4	30
17	1244	1.9	132	130	100	79	49	2	20
17	1271	2.0	132	133	99	39	46	2	20
17	2053	2.0	132	130	100	61	51	3	0
18	1168	2.1	132	132	99	11	20	2	10
18	1244	2.2	132	132	100	25	36	2	30
18	1271	2.2	132	131	100	31	32	3	10
18	2053	2.0	132	125	100	25	37	3	0
19	1168	2.1	132	131	100	46	25	3	10
19	1244	1.8	132	132	100	51	26	3	10
19	1271	2.0	132	131	100	21	21	2	10
19	2053	2.2	132	132	100	24	35	3	10

Appendix Table 15 (continued)

(1) TRT	CHA	(2) GROWTH STAGE cm@TRT	(3) MALE H.D.	(4) FEMALE H.D.	(5) % STER- ILITY	(6) % OUTCR. SEED	(7) % GRAIN YIELD	(8) SEED QUAL	(9) PLANT PHYTO
20	1168	1.8	132	125	100	55	53	1	10
20	1244	1.8	132	126	100	15	20	2	40
20	1271	3.0	132	126	41	31	19	3	50
20	2053	1.8	132	125	96	17	39	1	0
21	1168	2.8	132	126	100	8	21	2	10
21	1244	2.5	132	127	100	54	29	3	50
21	1271	2.6	132	129	96	26	38	1	60
21	2053	2.4	132	127	100	11	34	3	40
22	1168	2.1	132	130	92	35	26	1	60
22	1244	2.0	132	132	100	29	14	3	70
22	1271	2.4	132	132	96	49	35	3	60
22	2053	2.2	132	129	96	19	30	3	10
23	1168	2.3	132	129	82	67	50	3	0
23	1244	2.1	132	135	100	6	25	3	80
23	1271	2.1	132	131	84	42	39	2	60
23	2053	2.2	132	131	71	26	33	1	40
24	1168	2.0	132	129	100	60	48	1	0
24	1244	2.5	132	128	100	86	46	2	10
24	1271	1.6	132	129	93	47	60	1	10
24	2053	1.9	132	132	89	42	76	1	0
25	1168	1.4	132	135	43	43	39	3	0
25	1244	1.3	132	134	99	99	75	2	30
25	1271	1.4	132	135	82	60	49	3	20
25	2053	1.5	132	134	99	61	87	1	0

Appendix Table 16
Analysis of Variance for assessment of mean sterility (1) and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	628.9	209.6	1.3	.26 NS
Treatments	96	14905.1	155.2		
Total	99	15534.0			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) $1 - \frac{\text{(Av. No. Seeds From 20 Trt. Covered Spikes)}}{\text{(Av. No. Seeds From 20 Covered Control Spikes)}} \times 100$

Appendix Table 17
Analysis of Variance for assessment of mean Micro Outcrossed Seed or Outcrossed Seed (1) and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	2225.2	741.7	1.6	.19 NS
Treatments	96	44613.8	464.7		
Total	99	46838.9			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) $\frac{\text{(Av. No.Seeds From 21 Unbagged Trt. Spikes)}}{\text{(Av. No. Seeds From 21 Unbagged Control Spikes)}} \times$ STERILITY

Appendix Table 18
Analysis of Variance for assessment of mean Macro outcrossed seed or Grain Yield (1) and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	2427.6	809.2	3.2	.03*
Treatments	96	24471.1	254.9		
Total	99	26898.8			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

(1) $\frac{\text{(Wt. of Trt. Plot)}}{\text{(Wt. of Control Plot)}} \times$ STERILITY

Appendix Table 19
Analysis of Variance for assessment of mean seed quality (scale 1-5; 1=control) and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	5.6	1.9	2.6	.05*
Treatments	96	69.2	0.7		
Total	99	74.8			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Appendix Table 20
Analysis of Variance for assessment of mean plant phytotoxicity (scale 0-100; 0=control) and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	19076.0	6358.7	20.0	.01**
Treatments	96	30480.0	317.5		
Total	99	49556.0			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Appendix Table 21
Analysis of Variance for assessment of mean growth stage of application (spike length at treatment) and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	0.02	0.008	0.06	.98 NS
Treatments	96	11.3	6.1		
Total	99	11.3			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability

Appendix Table 22
Analysis of Variance for assessment of mean genotype heading date and CHA candidates.

Source of Variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
CHA candidates	3	22.4	7.5	1.2	.30 NS
Treatments	96	584.2	6.1		
Total	99	606.6			

NS: Not Significant
* : Significant at the five percent level of probability
**: Significant at the one percent level of probability