

**Zinc Biofortification in Wheat Grains through Phosphorus and Zinc
Fertilization Strategies in Manitoba's Red River Valley**

by

Mathieu Proulx

A Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
of the University of Manitoba
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

Copyright © February, 2026 by Mathieu Proulx

ABSTRACT

Proulx, Mathieu. M.Sc., The University of Manitoba, February, 2026. Zinc Biofortification in Wheat Grains through Phosphorus and Zinc Fertilization Strategies in Manitoba's Red River Valley. Supervisor; Xiaopeng Gao.

Canada is one of the world's largest wheat exporters, yet zinc (Zn) concentrations in Canadian wheat often fall below levels considered optimal for human health. We evaluated agronomic biofortification options that align with Canadian prairie practices by testing phosphorus (P) and Zn fertilizer sources and placements, with or without foliar supplements. A plot-based four-year field study was conducted on calcareous soils of contrasting texture (clay vs. sandy loam) in southern Manitoba from 2023 to 2024. Treatments included monoammonium phosphate (MAP), MAP + granular ZnSO₄, and MicroEssentials[®] SZ[®] (MESZ) placed as broadcast-incorporated, side-band, or seed-row at seeding, with foliar Zn (\pm glycine betaine + salicylic acid; \pm K) applied at Zadok's growing stages Z12–15 and Z65. Plant nutrient concentrations were determined by ICP-MS following acid digestion, and Zn bioavailability was indexed by the phytic acid:Zn (PA:Zn) molar ratio.

Across site-years, Zn fertilization increased grain yield by 8.3% over the Zn unfertilized control, with the highest yields achieved when MESZ was banded at seeding. Grain Zn responses depended on formulation, placement, and foliar supplementation. Seed-row MESZ plus foliar Zn produced the largest and most consistent grain Zn gains, raising concentration by \sim 27–60% at the two most responsive site-years and achieving \sim 41–43 mg kg⁻¹. Depending on site-year, adding biostimulants

provided small non-significant additional gains, whereas adding K produced no clear effect (beneficial in some cases, neutral or slightly negative when combined with biostimulants). Independent of formulation, banding MAP and MESZ generally outperformed broadcast for yield and Zn uptake. Texture and weather moderated outcomes, with responses being strongest on the lighter sandy-loam site in the wetter year.

Grain PA:Zn ranged ~27–38 and improved (decreased ~25%) where foliar Zn was added to seed-row MESZ, but no treatment reduced PA:Zn below the conventional threshold of 15. Phosphorus fertilization did not systematically elevate grain phytic acid. Overall, integrating seed-row MESZ with well-timed foliar Zn is a practical path to raise grain Zn and improve PA:Zn under Prairie conditions, but achieving PA:Zn <15 will likely require higher Zn inputs than current recommendations. Further refinement should focus on foliar Zn rate/timing and context-dependent use of K and biostimulants to deliver consistent biofortification across soils and seasons. Future work should prioritize foliar Zn rate and timing and test compatibility with plant-health fungicides to simultaneously support yield and biofortification under Prairie conditions.

FOREWORD

This thesis is organized around a manuscript-format research chapter, following the guidelines of the Department of Soil Science at the University of Manitoba. Chapter 2 is prepared for future submission and publication to a relevant peer-reviewed journal. The research concept and funding were initiated by Dr. Xiaopeng Gao. Under Dr. Gao's supervision, Mathieu Proulx adapted the experimental design to equipment constraints, coordinated and conducted field and laboratory data collection, completed data curation and statistical analysis, and drafted the manuscript. Guidance on grain analysis methodology was provided by Dr. Gao, and suggestions from Dr. Joanne Thiessen-Martens and Dr. Mario Tenuta materially improved the quality of the work. Field trial execution and overall study management benefited from the support and advice of the supervisory committee. Thesis drafts were revised with the assistance of Dr. Gao, whose feedback shaped successive versions; final revisions by Dr. Thiessen-Martens and Dr. Tenuta were essential in refining the manuscript.

ACKNOWLEDGMENTS

I am grateful to my supervisor, Dr. Xiaopeng Gao, for his guidance, mentorship, patience, and steady encouragement throughout this work, and for the trust he placed in my experience and capacity to lead this project. I thank Dr. Joanne Thiessen-Martens and Dr. Mario Tenuta for insightful comments, methodological advice, and thorough reviews that strengthened this thesis. I am indebted to Curtis Carvers and his team at AAFC–Portage la Prairie for seeding the trials with the specific seeder configuration essential to this study. I also thank Dr. Maneka Malalgoda’s lab and Indhurathna Swaminathan for quantifying grain protein concentration, and Dr. Mario Tenuta’s lab for providing equipment for phytic-acid extraction and quantification. The technical support of Trevor Fraser was integral to the smooth execution of the field trials. Fernando Esposito provided invaluable assistance with laboratory analyses, and Anthony Buckley shared practical insight into laboratory procedures, and good company during long hours. I am grateful to Jing Hou and Elmira Parstesh, students supervised by Dr. Gao, for their help with field work and phytic-acid quantification, and to the summer students whose efforts made sample collection and processing possible. I also appreciate the field support of my friend Roger Kpankpari, and the encouragement and feedback from peers in the department. I thank our collaborating farmers, Myron Krahn and Curtis Hiebert, for hosting the four on-farm sites research trials. I am especially grateful to Curtis Hiebert and his family, as my employer, for the flexibility that allowed me to pursue my master’s degree over the past four years. Thanks to Dr. Abdel El-Hadrami (OMEX Agriculture Inc.), Karl Redekop (EZ-GRO Inc.), and Mosaic Canada for providing products essential to this study. Finally, I thank my parents for instilling a passion for agriculture and a curiosity about farming principles,

especially my mother, for teaching me to think outside the box. I dedicate this thesis to my wife and our three daughters for their love, patience, and unwavering support. To my wife, my soulmate, thank you for your patience through long hours, missed events, and the extra load you carried. Your expertise in animal nutrition broadened and deepened my perspective on grain nutritional value and mineral bioavailability. Without you, this thesis would not have been possible.

TABLE OF CONTENTS

	Page
ABSTRACT	ii
FOREWORD	iv
ACKNOWLEDGMENTS.....	v
TABLE OF CONTENTS	viii
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xii
1. INTRODUCTION	1
1.1 Wheat as a Biofortified Crop	2
1.1.1 Wheat as a Global Staple and Dietary Source.....	2
1.1.2 Canadian Wheat Production and Export Significance	3
1.2 Biofortification Challenges in Western Canada	3
1.2.1 Soil Constraints: Alkaline Soils and Micronutrient Availability	4
1.2.2 On-Farm Management Practices: The 4Rs Nutrient Stewardship	6
1.3 Agronomic Zinc Biofortification in Wheat	10
1.3.1 Physiological Roles and Critical Demand.....	10
1.3.2 Soil and Foliar Zinc Application: Limitations and Local Solutions.....	11
1.3.3 Biostimulants to Enhance Foliar Zinc Uptake	12
1.4 Grain Zinc Bioavailability.....	14
1.4.1 Zinc Storage Forms in Wheat Grain.....	15
1.4.2 Role of Phytic Acid in Mineral Bioavailability.....	15
Phytic Acid to Zinc Molar Ratio as a Bioavailability Indicator	16
1.4.4 Impact of the 4Rs Nutrient Stewardship on Grain Zinc Bioavailability	17
1.5 Objectives.....	18
1.6 Hypotheses	19

1.7	Structure of thesis.....	20
1.8	References	21
2.	IMPROVING ZINC BIOAVAILABILITY IN WHEAT GRAIN THROUGH FARM-TAILORED AGRONOMIC BIOFORTIFICATION STRATEGIES	34
2.1	Abstract	34
2.2	Introduction	36
2.3	Materials and Methods	40
2.3.1	Study Sites.....	40
2.3.2	Experimental Design	44
2.3.3	Plant densities , Biomass and Yield Determination	48
2.3.4	Plant Tissue Nutrient Analysis	48
2.3.5	Analysis of Grain Samples for Phytate Concentration and Zn Bioavailability.....	49
2.3.6	Relative Elevation Data Collection.....	50
2.3.7	Statistical Analysis	50
2.4	Results	52
2.4.1	Plant Establishment and Development.....	52
2.4.2	Yield and Grain Quality	56
2.4.3	Straw Nutrient Concentration and Uptake	58
2.4.4	Grain Nutrient Concentration and Uptake	64
2.4.5	Grain Zn Bioavailability.....	74
2.4.6	Relationships of Grain Zn and P Concentrations with Grain Yield, Protein, and Other Nutrients.....	77
2.5	Discussion	80
2.5.1	Effects of P and Zn Fertilization Strategy on Spring Wheat Establishment, Development, and Yield.....	80
2.5.2	Nutrient Uptake and PA Concentration	81
2.5.3	Wheat Zn Biofortification with Foliar Application.....	84
2.5.4	Impact of P and Zn Fertilization Strategy on Phytic Acid to Zn Molar Ratio	85
2.6	Conclusion.....	87
2.7	References	88
3.	SYNTHESIS.....	99

3.1	Key Findings	99
3.2	Project Accomplishments	100
3.3	Limitation and Analytical Gaps.....	100
3.4	Future Research.....	101
3.5	Recommendations	102
3.5.1	For Farmers	102
3.5.2	For Researchers	103
3.6	References	103
4.	APPENDICES	107
	Appendix A: Supplementary Material for Chapter 2	102

LIST OF TABLES

Table	Page
Table 2.1 Soil descriptions and chemical characteristics for the top 15 cm at Carman and Sperling site for 2023 and 2024.....	43
Table 2.2 Weather conditions at Carman and Sperling sites for the 2023 and 2024 wheat growing season (May-August) compared to the historical normal.	44
Table 2.3 Treatment list with detailed product with analysis, rate and timing of application, required placement and total nutrient applied for nitrogen (N), phosphorous (P), sulfur (S), and zinc (Zn).	47
Table 2.4 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on plant densities , aerial biomass, and straw yield at Carman and Sperling from 2023 to 2024.	53
Table 2.5 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on corrected grain yield, test weight, grain moisture and protein content at Carman and Sperling from 2023 to 2024.....	56
Table 2.6 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on grain concentrations and uptakes of P, Zn, phytic acid (PA), and phytic acid to Zn molar ratio (PA:Zn) at Carman and Sperling from 2023 to 2024.	64
Table A-1 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on straw nutrient concentrations and uptake of iron (Fe), P, potassium (K) and Zn at Carman and Sperling from 2023 to 2024.	109
Table A-2 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on grain nutrient concentrations and uptake of iron (Fe), and potassium (K) at Carman and Sperling from 2023 to 2024.	114

LIST OF FIGURES

Figure	Page
Figure 2.1 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat plant densities (A), dry matter biomass 10 days after herbicide application (B), and dry matter biomass 10 days after fungicide application (C) at Carman and Sperling, 2023–2024.	55
Figure 2.2 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain yield (A), and grain test weight (B) at Carman and Sperling, 2023–2024.	58
Figure 2.3 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw Zn concentration for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).	62
Figure 2.4 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw Zn uptake for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).	63
Figure 2.5 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain concentrations of P (A), Zn (B), phytic acid (PA) (C), and grain phytic acid to Zn molar ratio (PA:Zn) (D) at Carman and Sperling, 2023–2024.	69
Figure 2.6 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain uptake of P (A), Zn (B), and phytic acid (PA) (C) at Carman and Sperling, 2023–2024.	70
Figure 2.7 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain Zn concentration for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).	72

Figure 2.8 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain Zn uptake for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).73

Figure 2.9 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain phytic acid to Zn molar ratio (PA:Zn) for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).76

Figure 2.10 Linear relationships of grain Zn concentration with grain yield (A), protein (B), phytic acid (PA) (C), P (D), Fe (E), and K (F) concentrations.78

Figure 2.11 Linear relationships of grain P concentration with grain yield (A), phytic acid (PA) (B), Fe (C), and K (D) concentrations.79

Figure A-1 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat plant densities at Zadok’s stage Z11 for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).108

Figure A-2 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw concentrations of Zn (A), P (B), and iron (Fe) (C) at Carman and Sperling, 2023–2024.111

Figure A-3 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw uptake of Zn (A), and iron (Fe) (B) at Carman and Sperling, 2023–2024.112

Figure A-4 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw iron (Fe) concentrations for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D).113

Figure A-5 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain concentrations of iron (Fe) (A), and grain uptake of Fe (B), and potassium (K) (C) at Carman and Sperling, 2023–2024.115

1. INTRODUCTION

Since the Green Revolution, wheat yields worldwide have increased by more than threefold. However, this yield gain has coincided with a measurable decline in grain mineral concentrations. Studies report that for each ton increase in yield, grain zinc (Zn) and iron (Fe) concentrations fall by 3 to 5% (Fan et al., 2008; Mayer et al., 2008). This “yield dilution” effect means that modern high-yielding varieties often deliver less nutritional value per kilogram of grain.

Micronutrient malnutrition, often called hidden hunger, affects over two billion people despite adequate caloric intake (Bouis and Welch, 2010). Zinc deficiency alone impairs immune function, growth, and cognitive development in roughly 19% of the global population, with pregnant women, infants and young children at greatest risk (Ivanović et al., 2021). In severe cases, it contributes to stunting, increased diarrheal disease and delayed neurological maturation (WHO, 1996; Cakmak and Kutman, 2018)

Existing interventions to raise dietary Zn include dietary diversification, mineral supplements and industrial food fortification. Although these can be effective in controlled settings, they face major obstacles in low-income or remote regions. Dietary changes require cultural shifts and reliable markets; supplements incur ongoing costs and logistical challenges; and post-harvest fortification depends on centralized milling infrastructure, which is often absent or poorly regulated (Bouis, 2003; Stein et al., 2007; Mkambula et al., 2020).

Agronomic biofortification uses field-level fertilizer strategies to increase grain Zn concentration. By optimizing soil and foliar Zn applications, sometimes combined with biostimulants, this approach can deliver rapid, site-specific improvements in grain nutrient density (Cakmak, 2008). Unlike breeding, which can take years to develop high-Zn varieties, and fortification, which requires industrial infrastructure, agronomic biofortification can be implemented immediately on existing farms.

The remainder of this chapter is organized as follows. Section 1.1 explains why wheat is an ideal vehicle for agronomic biofortification, highlighting its role as a global staple and Canada's leadership in wheat production and export. Section 1.2 examines the specific challenges to Zn biofortification in Western Canada, including calcareous soils, phosphorus-zinc interactions, and common 4Rs management practices for fertilizer source, rate, placement, and timing. Section 1.3 reviews the physiological roles and critical demand for Zn in wheat and evaluates practical agronomic strategies, with emphasis on foliar Zn applications and the use of biostimulants. Section 1.4 describes the biochemical basis of Zn bioavailability in wheat grain, focusing on phytic acid and the phytic acid-to-Zn molar ratio as a nutritional indicator. Finally, Sections 1.5 and 1.6 state the objectives and hypotheses of this research, and Section 1.7 outlines the overall thesis structure.

1.1 Wheat as a Biofortified Crop

1.1.1 Wheat as a Global Staple and Dietary Source

Cereals provide more than 50% of daily calorie intake worldwide (Shiferaw et al., 2013). In 2022, wheat, barley, and oats accounted for 32.3% (989.7 million metric tons) of global cereal production and 37.7% (275.8 million ha) of cereal area (FAOSTAT, 2022). Wheat alone accounted for 29.8% of these cereals (FAOSTAT, 2022). Globally, wheat supplies roughly one-fifth of dietary energy,

with regional dependence reaching up to half of calories in parts of West/Central Asia and North Africa (Shiferaw et al., 2013). Between 2019 and 2023, Canada exported on average 7.5% of its wheat production to the Middle East and North Africa, and 10.6% to Central America and South Asia (Cereals Canada 2025). Many of these regions face widespread Zn deficiency in human nutrition (Akhtar, 2013; Cediél et al., 2015).

1.1.2 Canadian Wheat Production and Export Significance

Hard red spring wheat (*Triticum aestivum* L.) constitutes the majority of Canadian wheat production (Cereals Canada, 2024). From the 2023 harvest, Canada exported an estimated 23.3 million metric tons of spring wheat to 80 countries (Cereals Canada, 2024). Many of these importing nations rely on wheat as a key source of essential nutrients such as phosphorus (P) and Zn. However, wheat grain Zn concentrations typically range from 28 to 30 mg kg⁻¹, below the 40–50 mg kg⁻¹ target for optimal dietary intake (Cakmak and Kutman, 2018; HarvestPlus, 2025). In the Canadian Prairies, Zn concentrations in wheat grains often lie between 20 and 30 mg kg⁻¹ (Gawalko et al., 2002). Consequently, Canadian wheat exports may not meet the dietary Zn requirements of dependent populations, potentially jeopardizing health outcomes.

In summary, wheat's dietary importance and its prevalence as a crop in Zn-deficient regions make it an ideal candidate for biofortification. However, achieving the target grain Zn concentrations is challenging, especially in the Canadian Prairies where alkaline soils are widely distributed and limit Zn availability.

1.2 Biofortification Challenges in Western Canada

Interest in grain biofortification has grown among researchers and progressive farmers (e.g. in organic and regenerative systems), yet the market rarely pays premiums for nutrient-dense wheat.

As a result, any strategy to raise grain Zn must integrate into existing operations and keep additional costs to a minimum. A second major hurdle is the alkaline, often calcareous, soils that dominate the Canadian Prairies. These soils strongly limit micronutrient availability, so only a small fraction of soil-applied Zn is recovered in the grain. In theory, boosting wheat grain Zn by 10 mg kg^{-1} in a $4,000 \text{ kg ha}^{-1}$ Canada Western Red Spring (CWRS) crop requires 40 g ha^{-1} of elemental Zn in the grain, equivalent to approximately 0.2 kg of zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) fertilizer applied. In practice, a meta-analysis from Hui et al. (2025) reported that, less than 1.5% of soil-applied Zn was available to wheat grown in calcareous soils. Across 174 observations, applying $>15 \text{ kg ha}^{-1}$ of elemental Zn ($\approx 68 \text{ kg}$ of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) increased crop grain Zn by only 7.5 mg kg^{-1} , regardless of soil pH. Likewise, Botoman et al. (2022) found that applying 30 kg ha^{-1} of elemental Zn ($\approx 136 \text{ kg}$ of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) increased maize grain Zn by just 40 g ha^{-1} over the 1 kg ha^{-1} rate treatment. These application rates are far above typical on-farm practices and are economically and logistically unrealistic for most wheat production systems.

These results suggest soil Zn alone is not cost-effective for biofortification, although a basal Zn supply remains important for early plant vigor. Consequently, agronomic Zn applications must be synchronized with standard seeding or spray operations to avoid extra passes and labor, and rate decisions should prioritize crop yield requirements while aiming to achieve the target $40\text{--}50 \text{ mg kg}^{-1}$ grain Zn. Understanding the mechanisms that restrict Zn availability in alkaline soils is critical for developing more efficient fertilizer placement and formulation strategies.

1.2.1 Soil Constraints: Alkaline Soils and Micronutrient Availability

Prairie soils are predominantly alkaline (pH 7.0–8.5) due to high evapotranspiration relative to precipitation, which concentrates cations such as Ca^{2+} , Mg^{2+} , K^+ , and Na^+ (Clark and Baligar,

2000). These soils typically contain a high proportion of 2:1 clay minerals (smectite-montmorillonite), giving them substantial cation-exchange capacity.

Calcareous soils, a subset of alkaline soils, contain high levels of calcium carbonate (CaCO_3) that significantly affect soil chemistry, nutrient dynamics, and plant growth. In the Canadian system, calcareous profiles fall within the Chernozemic order that dominates the Prairies (Pennock et al., 2011). Calcium carbonate levels in these soils range from barely detectable to over 90%, often accompanied by notable magnesium carbonate (Clark and Baligar, 2000; Marschner, 2012). Together, high pH and CaCO_3 content promote the precipitation of Zn and P minerals and the strong sorption of these nutrients onto soil components, sharply limiting their plant-available forms and posing a key challenge for agronomic biofortification.

1.2.1.1 Soil Phosphorus and Zinc Fertilization Practices. In alkaline prairie soils, P solubility declines markedly as it precipitates with calcium and magnesium to form sparingly soluble phosphates and becomes strongly adsorbed onto carbonate and clay surfaces (Clark and Baligar, 2000; Bertrand et al., 2003; Marschner, 2012). This limits the labile P pool available for root uptake.

Zn availability is similarly constrained: at high pH, Zn^{2+} ions are retained by soil carbonates and clay colloids and may precipitate as hydroxides or carbonates in calcareous conditions, sharply reducing its mobility to root surfaces (Marschner, 1993; Cakmak and Kutman, 2018). Moreover, high rates of P fertilizer can exacerbate Zn deficiency by promoting Zn-phosphate complex formation and increasing Zn sorption (Moraghan and Mascagni, 1991; Naeem et al., 2018). Cold, dry spring conditions further diminish both P and Zn diffusion in the soil solution (Grant et al., 2001), compounding nutrient limitations and underscoring the need for precise fertilizer placement, formulation, and timing.

1.2.1.2 Phosphorus-Zinc Interactions. P and Zn exert strong reciprocal effects in alkaline soils, often leading to P-induced Zn deficiency (Moraghan and Mascagni, 1991; Naeem et al., 2018). High soil P levels reduce Zn solubility, promote Zn-phosphate precipitation, and increase Zn sorption onto iron and aluminum oxides, all of which limit Zn diffusion to roots. Excessive P also suppresses arbuscular mycorrhizal colonization, further restricting Zn uptake (Marschner, 2012). Sacristán et al. (2021) found that when the soil Olsen-P/DTPA-Zn ratio exceeds 50, supplemental Zn becomes necessary to maintain adequate Zn in young maize tissues on calcareous soils. Interestingly, Naeem et al. (2018) found that co-applying K alleviated P-induced Zn deficiency, increasing grain Zn in K-treated plots.

At the plant-physiological level, Zn is a component of proteins that regulate phosphate uptake in root cells and is required for synthesizing phosphate transporters that mobilize P into the phloem (Huang et al., 2000; Marschner, 2012; Ding et al., 2021). Zn deficiency increases root-cell membrane permeability, undermining the root's control of P influx. Consequently, once phosphate enters the xylem, it can hyperaccumulate in leaf tissues, disrupting photosynthesis, sucrose production, and potentially enhancing phytic acid synthesis in grain.

Together, these chemical and physiological interactions underscore the necessity of balanced P and Zn fertilization. Fertility strategies must therefore avoid excessive P applications without concurrent Zn supplementation if the goal is to improve grain Zn concentration under alkaline, calcareous soil conditions.

1.2.2 On-Farm Management Practices: The 4R Nutrient Stewardship

Since the Green Revolution, prairie farmers have progressively shifted their fertilizer operations from simple surface broadcasting to broadcast incorporation and, more recently, to subsurface banding, to preserve soil health, improve economic returns and adapt to evolving seeding

equipment (Mooleki et al., 2010). Initially, fertilizer was broadcast on the soil surface and then incorporated by cultivation to a depth of 5–10 cm to achieve even distribution. In contrast, band application concentrates fertilizer in a narrow trench typically 10 to 15 cm below the soil surface, which is then closed with soil in the same operation. Traditional band application was performed with cultivators and planters, while today's modern seeders can apply fertilizer in a mid-row band and/or in a side-band position, usually 5 cm to the side and below the seed, or directly in the seed row. At the same time, fertilizer formulations have advanced: co-granulated P-S-Zn products have been in use for over two decades. With rising environmental and economic concerns, the 4R principles (right source, right rate, right place, right time) have been developed to guide nutrient management. They should be central to the biofortification program design.

1.2.2.1 Fertilizer Formulations and Products. Monoammonium phosphate (MAP) and ammonium polyphosphate (APP) remain the dominant P sources on alkaline prairie soils (Bertrand et al., 2003; Goh et al., 2013; Grant and Flaten, 2019). APP's polyphosphate chains can act as a slow-release P source under very high pH and calcareous conditions, whereas MAP may precipitate rapidly with calcium and become less available during critical growth stages (Bertrand et al., 2003). Under lower alkalinity and low calcareous conditions ($\text{CaCO}_3 < 5\%$), APP and MAP are recognized to perform similarly.

Co-granulated P-S-Zn fertilizers (for example, MicroEssentials® SZ®: MESZ) have been introduced to deliver Zn alongside P and S in a single granule. Common Zn sources include zinc sulfate (ZnSO_4), zinc oxysulfate, zinc oxide, zinc carbonate, zinc chloride, zinc nitrate, and zinc ethylenediaminetetraacetic (Zn-EDTA), with Zn-EDTA often blended into APP (Hergert et al., 1984; Mortvedt and Gilkes, 1993). Granular ZnSO_4 blended with MAP can result in uneven micronutrient distribution because P granules dominate application rates. Co-granulated

formulation is designed to improve micronutrient distribution. However, to date, peer-reviewed evaluations of co-granulated formulations in wheat production systems are lacking.

In corn, Caldwell et al. (2016) found equivalent yield and ear Zn concentration when comparing MESZ with MAP plus granular ZnSO₄ on acid soil, and Rogalsky et al. (2024) reported no yield or tissue Zn advantage for MESZ over MAP plus ammonium sulfate on alkaline prairie soils. Co-granulated product capacity to raise grain Zn in spring wheat, without compromising yield, remains to be demonstrated.

1.2.2.2 Fertilizer Rate. Adjusting P and Zn application rates offers one strategy to mitigate alkaline-soil nutrient constraints. However, the wheat response to P plateaus at relatively low rates, and a positive yield response often depends on co-application with nitrogen or potassium (Grant and Flaten, 2019). Economic-optimum and environmentally safe P rates typically lie below those that maximize crop uptake. In calcareous soils, high P rates accelerate phosphate precipitation, risk inducing magnesium deficiency (Sample et al., 1980), and can inhibit Zn uptake by disrupting Zn transporters (Ding et al., 2021). Consequently, in Zn-deficient alkaline soils, Zn application rates should be adjusted in proportion to P inputs (Dhaliwal et al., 2023).

Under sufficient P supply in a pot study, wheat yield increased with soil Zn concentration up to a threshold, beyond which excess Zn reduces yield (Hussain et al., 2013). This study also indicates that soil-applied Zn can effectively increase grain Zn concentration in wheat. However, the rate required to achieve biofortification targets was higher than what would be agronomically or economically sustainable at the field scale. In a field study, Wang et al. (2015) demonstrated that soil-applied ZnSO₄ increased grain Zn concentration, but only at application rates several times above economic and environmental safety rates.

1.2.2.3 Fertilizer Placement Methods. Placement of P and Zn fertilizers has a major influence on nutrient efficiency, crop yield and profitability (Grant and Bailey, 1998; Hopkins and Ellsworth, 2005; Grant and Flaten, 2019). Side-banding P beside and below the seed reduces soil contact. It enhances availability under cold conditions in the early season, whereas broadcast incorporation spreads P more thinly and increases the risk of surface runoff. Seed-row placement carries a risk of salt injury, particularly with high-salt fertilizers, but wheat tolerates seed-placed MAP better than canola or pulses (Grant and Flaten, 2019).

Previous studies on alkaline prairie soils showed that side-banding MAP increased wheat yield relative to broadcast incorporation (Grant and Bailey, 1998). MAP in the seed-row produced yields comparable to side-banding, with side-banding showing an advantage under dry conditions (Mooleki et al., 2010). In multi-site field work spanning from loam to clay soils, Karamanos et al. (2017) likewise reported that even at rates up to 60 kg P ha^{-1} , seed-placed MAP produced yields comparable to side-banding, with similar plant stands, contrasting with general recommendations where side-banding is usually preferred for higher fertilizer rates.

Zn is typically co-applied with P, but ZnSO_4 's salt index demands caution in seed-row placement (A&L Canada, 2013). Reviews indicate that broadcast-incorporated ZnSO_4 often results in greater Zn uptake than side-banding, likely because it increases root-soil contact and limits co-precipitation with P (Montalvo et al., 2016). By contrast, banded P is generally more effective than broadcast for wheat uptake (Strong and Soper, 1974). This divergence in uptake dynamics highlights the need to optimize placement strategies for co-granulated P-Zn products in calcareous soils.

While broadcasting can increase the risk of environmental losses, band application of granular Zn alone may leave many plants without adequate micronutrient access. To meet both yield and

biofortification objectives, alternative modes and timings, potentially integrating soil and foliar applications, should be investigated under realistic prairie conditions.

1.2.2.4 In-Crop Application Timing. Application timing critically influences both nutrient use efficiency and environmental losses (Grant and Flaten, 2019). Early-season P availability is essential for spring wheat seedling establishment (Grant et al., 2001), yet cold soil conditions can retard P solubilization even when soil test levels are adequate (Grant and Flaten, 2019). Seed-row or side-band placement at seeding thus remains the most reliable way to meet young plants' P demand. Broadcast applications in the fall increase the risks of surface residue and runoff. When a fall application is necessary, a deeper, sealed band application is recommended to reduce losses during winter freeze-thaw cycles and associated snowmelt runoff events (Yuan et al., 2020).

Zn is usually co-applied with macronutrients because its low rate, $<10 \text{ kg Zn ha}^{-1}$, prohibits a separate pass (Montalvo et al., 2016). While soil-applied Zn can meet basic developmental needs, it generally fails to raise grain Zn to biofortification targets ($40\text{--}50 \text{ mg kg}^{-1}$) when applied alone (Cakmak et al., 2010; Zou et al., 2012; Cakmak and Kutman, 2018). Foliar Zn spray during early grain filling (milk to soft-dough stage) has proven most effective to increase grain Zn concentration. Yet, this time window rarely coincides with standard disease-control fungicide applications. Integrating foliar Zn into existing spray schedules represents a major research need for practical implementation.

1.3 Agronomic Zinc Biofortification in Wheat

1.3.1 Physiological Roles and Critical Demand

Zn is essential throughout wheat development, supporting chlorophyll synthesis, leaf area expansion, photosynthesis, and stress tolerance (Qamari et al., 2023). Under abiotic stress, Zn

enhances germination and early root growth, improving seedling vigor (Seregina et al., 2023). Its uptake pattern parallels that of P; Miller et al. (1993) observed the greatest Zn accumulation rate at about 600 growing-degree days, coinciding with flag leaf emergence and spikelet maturation. As biomass increases rapidly after flag leaf emergence, and much of the soil-applied Zn remains unavailable for uptake, plants must remobilize Zn from older leaves to developing tissues, often leaving grain Zn below levels needed for human nutrition (Cakmak and Kutman, 2018). Consequently, the period from flag leaf emergence through the soft-dough stage represents the critical window for Zn supplementation to achieve effective grain biofortification.

1.3.2 Soil and Foliar Zinc Application: Limitations and Local Solutions

Combining soil and foliar Zn applications consistently outperforms soil-only applications for increasing grain Zn (Cakmak et al., 2010; Zou et al., 2012; Cakmak and Kutman, 2018). Foliar sprays during early grain filling are especially effective, but most studies have used high water volumes (250–800 L ha⁻¹) or timings that do not align with standard pesticide applications. Lower-volume sprays (approximately 250 L ha⁻¹) typically require multiple applications at seven-day intervals, whereas higher-volume spray solutions of 800 L ha⁻¹ or more often need only two passes. ZnSO₄·7H₂O is the preferred source due to its low cost, with rates ranging from 1.5 to 8 kg ha⁻¹ per season (0.34–1.82 kg Zn ha⁻¹).

In Canadian Prairies, Zn can be co-applied during one herbicide pass at tillering and one fungicide pass at anthesis, both usually applied at <100 L ha⁻¹. This approach is feasible provided tank-mix compatibility is confirmed by the foliar fertilizer product label. However, additional precautions are needed to prevent leaf burn from the ZnSO₄ solutions (Xu et al., 2022). Most commercial foliar Zn products are 9% Zn solutions (density 1.3 kg L⁻¹) applied at 1.25–5 L ha⁻¹ (0.15–0.58 kg Zn ha⁻¹).

¹). EDTA-chelated Zn formulations are also available and generally tank-mix compatible with pesticides but are more expensive than sulfate formulations.

Kamran et al. (2023) demonstrated that only 0.9% of foliar-applied Zn was absorbed by the leaf and just 43% of that was translocated into the grain. In theory, if 100% were absorbed and translocated, only 40 g Zn ha⁻¹ would be needed to boost grain Zn by 10 mg kg⁻¹ in a 4,000 kg ha⁻¹ wheat crop. Based on their uptake efficiencies, achieving that would require over 10 kg Zn ha⁻¹ in the spray solution, which is far above rates used in Cakmak et al. (2010) and Zou et al. (2012). Most foliar Zn never reaches the grain, highlighting the need to understand and improve uptake and translocation mechanisms, possibly through the use of biostimulants.

1.3.3 Biostimulants to Enhance Foliar Zinc Uptake

Plant biostimulants are defined by the European Biostimulants Industry Council as “products which stimulate plant nutrition processes independently of the product’s nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, or the availability of confined nutrients in soil or rhizosphere” (EBIC, 2025). They offer a promising route to boost foliar Zn absorption and remobilization to grain. Three classes are of particular interest: phytohormones, osmolytes and osmoticants.

Phytohormones are compounds, such as salicylic acid (SA), acting as antioxidants and signaling molecules that support stress-response pathways (Dubey et al., 2021). When applied foliarly, they can stimulate antioxidant enzyme activity, reduce membrane lipid peroxidation, and sustain phloem loading, conditions that may enhance Zn mobility from leaves to developing kernels. Zarea

(2025) reported that SA sprays, alone or combined with Zn, increased winter wheat yield under rain-fed conditions, though their direct effect on grain Zn concentration remains to be tested.

Osmolytes are small, highly soluble organic compounds (e.g., glycine betaine) that help maintain cell turgor under water deficit by balancing cytosolic osmotic pressure (Dubey et al., 2021). By preserving membrane integrity and the function of nutrient transporters, osmolytes may prolong leaf function and enhance remobilization of foliar-applied Zn to the grain. Aldesuquy et al. (2012) showed that glycine betaine improved wheat yield and mineral (Ca, Mg, Cl, Na) status under non-stress conditions, while its impact on Zn remains to be evaluated.

Osmotocants are inorganic solutes, primarily K^+ , but also Ca^{2+} , Mg^{2+} , and Si, that raise phloem-sap osmolality (Ahmad et al., 2018; Tränkner et al., 2018; Wang et al., 2021). By increasing the driving force for mass flow in the phloem, these osmotocants can accelerate the bulk transport of Zn and other nutrients toward the grain. Naeem et al. (2018) demonstrated that soil K application improved grain Zn concentration, underscoring the role of sap osmolality in micronutrient translocation.

Recent studies support pairing biostimulants with foliar Zn applications. In a field trial in Iran, Zarea (2025) reported a synergistic yield response of durum wheat when foliar Zn ($0.3\% w v^{-1}$: 6 kg $ZnSO_4 \cdot 7H_2O$) was combined with SA ($50 mmol w v^{-1}$). In a pot experiment under controlled environmental conditions, Aldesuquy et al. (2012) found that SA (50 mmol, seed presoaking solution) plus glycine betaine (10 mmol) was the most effective treatment for increasing wheat yield and inorganic P in grain under non-stress conditions. Under field conditions, the combination of 1.5% foliar K with 100 mmol glycine betaine was the best for increasing yield (Raza et al., 2014) and improving P uptake (Raza et al., 2015). Taken together, these results suggest that combining SA or betaine with foliar Zn, and in some cases K, can enhance conditions that favor

Zn uptake and remobilization to the grain. However, direct effects on grain Zn concentration and bioavailability remain to be confirmed in systematic field trials.

To our knowledge, no field study has tested the combined use of foliar Zn, glycine betaine, SA, and K on productivity and nutrient bioavailability in crops on Canadian Prairies. Given their complementary roles (osmotic protection, antioxidant signaling, and phloem osmoregulation), a combination has strong potential to produce additive or synergistic improvements in both wheat yield and grain Zn concentration. Field evaluation is warranted to assess its impact on Zn bioavailability.

1.4 Grain Zinc Bioavailability

Few biofortification studies have evaluated not only total grain Zn, but also its bioavailability, which is the fraction absorbable by the human digestive system. The primary barrier to Zn absorption from cereal grain is phytic acid (phytate when bound to minerals), an anti-nutritional compound concentrated in the aleurone layer and, to a lesser extent, in the bran and germ (Sanz-Penella and Haros, 2014). Tran et al. (2021) showed in durum wheat that raising soil P fertility also increases grain phytic acid, which can counteract gained Zn. Since Canadian hard red spring wheat is predominantly milled into white flour, the extraction rate has a major impact on residual phytic acid, and thus on Zn bioavailability, in the final product (Turksoy et al., 2010). Foliar Zn sprays have been demonstrated to improve estimated Zn bioavailability in wheat flour using a trivariate prediction model for human nutrition (Yu et al., 2021). Moreover, foliar applications increase Zn levels more effectively in the endosperm (the part retained in white flour), whereas soil-applied Zn tends to accumulate in the bran and germ (Govindan et al., 2022). The following subsections will elaborate further into Zn speciation, the role of phytic acid in grain, the main

indicator of Zn bioavailability in grain, and the potential impact of the 4R nutrient principles on grain Zn bioavailability.

1.4.1 Zinc Storage Forms in Wheat Grain

Zn in the wheat endosperm partitions into two primary pools: a water-soluble fraction ($\approx 24\%$ of total Zn) and a water-insoluble fraction ($\approx 76\%$) (Persson et al., 2016). The water-soluble Zn pool consists mainly of low-molecular-weight complexes, including Zn-peptide associations (Schjoerring et al., 2009), and chelates with transport ligands such as nicotianamine and deoxymugineic acid (Xue et al., 2012; Grujic et al., 2018). These forms are readily mobilized during digestion and thus more bioavailable. The water-insoluble Zn pool is bound to high-molecular-weight components: primarily gluten proteins (e.g., glutenin), starch matrices, and phytic acid complexes (Persson et al., 2016; Balk et al., 2019). Higher Zn concentrations are associated with an increase in gluten proteins, which can enhance dough quality and loaf volume (Peck et al., 2008; Rehman et al., 2018). However, the largest share of this fraction is Zn-phytic acid, which strongly reduces intestinal Zn absorption.

1.4.2 Role of Phytic Acid in Mineral Bioavailability

Phytic acid (myo-inositol hexakisphosphate) serves as the primary P storage compound in seeds, accounting for 60–90% of total seed P depending on plant species (Silva et al., 2021). In wheat, 74–96% of the seed's P can be bound as phytic acid (Froese et al., 2020), with 87% localized to the aleurone layer in structures called globoids (Madsen and Brinch-Pedersen, 2020). Despite representing only 1% of the total grain dry weight in many crop seeds (Lott et al., 2001), phytic acid is a powerful anti-nutrient compound for monogastric animals and humans because it chelates essential cations, including Zn, Fe, Mn, Cu, Ca and Mg, rendering them largely unavailable during

digestion (Silva et al., 2021). The strength of these metal-phytic acid bonds varies by ion: Cu^{2+} binds most tightly, followed by Zn^{2+} , Co^{2+} , Mn^{2+} , Fe^{2+} and Ca^{2+} (Weaver and Kannan, 2001). This “chelation strength” reflects how readily phytic acid binds a metal under given conditions. By contrast, the stability constant describes the thermodynamic favorability of the complex at equilibrium and decreases in the order of $\text{Zn}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+} > \text{Ca}^{2+}$ (Weaver and Kannan, 2001). For example, at pH 5, enzymatic-hydrolysis degraded 84% of Ca-phytate within a 20-hour period, compared with only 23% of Zn-phytate (Sun et al., 2021). Because Zn forms among the strongest complexes with phytic acid, it is especially prone to sequestration and resists common household processes (e.g., cooking, fermenting, soaking) that can reduce phytic acid's binding to other minerals (Sathe and Venkatachalam, 2001; Gibson et al., 2018). Consequently, achieving a sufficiently high grain Zn concentration and/or reducing phytic acid concentration are critical to overcome phytic acid's inhibitory effects and ensure adequate Zn bioavailability in wheat-based diets.

1.4.3 Phytic Acid to Zinc Molar Ratio as a Bioavailability Indicator

The molar ratio of phytic acid to zinc (PA:Zn) in grain is widely used to estimate Zn bioavailability, with values below 15 considered indicative of adequate Zn uptake in humans (WHO, 1996; Bouis and Welch, 2010). Because phytic acid accumulates in the developing seed and binds Zn, three general approaches can improve Zn bioavailability in wheat grain: limiting phytic acid accumulation, increasing grain Zn concentration, or combining both strategies.

1.4.3.1 Timing of Phytic Acid Accumulation. Shukla et al. (2016) demonstrated that phytic acid in developing wheat grain rises sharply during the first 14 days after anthesis and then plateaus until maturity. During this time window, inorganic phosphate (Pi) is still being taken up by the roots, and actively translocated into developing grain from both roots and leaves. Once root Pi

uptake declines (around two weeks post-anthesis), starch accumulation drives simultaneous phytic acid synthesis, with phytic acid becoming the main Pi sink in the grain. Consequently, any agronomic intervention aimed at reducing phytic acid should target this immediate post-anthesis period.

1.4.3.2 Role of Zinc in Phytic Acid Regulation. Zn itself contributes to Pi homeostasis by modulating phosphate transporter activity in roots and leaves (Marschner, 2012; Ding et al., 2021). Yang et al. (2011) conducted two-season trials in which foliar application of zinc sulfate (2.5 kg ha⁻¹) after jointing reduced grain phytic acid by 14%. Similar reductions have been reported by Bharti et al. (2013) and Saha et al. (2020), indicating that timely Zn supplementation can reduce phytic acid synthesis in the seed.

1.4.3.3 Practical Implications. Because excessive soil P can drive phytic acid accumulation, foliar Zn treatments will be most effective when P fertility is high. Integrating a foliar Zn spray into the early grain filling period thus offers dual benefits, elevating grain Zn concentration while constraining phytic acid levels, and can be a key component of an agronomic biofortification program designed to improve PA:Zn ratio to level beneficial to human nutrition.

1.4.4 Impact of the 4R Nutrient Stewardship on Grain Zinc Bioavailability

Applying the 4R nutrient stewardship to Zn biofortification means choosing the right fertilizer source, at the right rate, placed in the right position, and applied at the right time to maximize both yield and grain Zn bioavailability.

In early-season, cold conditions of the Canadian Prairies, growers favor highly soluble P sources (MAP, APP). However, when ZnSO₄ is co-banded with MAP, insoluble Zn-P precipitates can form in the furrow, sharply reducing Zn available for uptake during grain filling. Co-granulated P-S-Zn

products (e.g., MESZ) are designed to limit that precipitation, but peer-reviewed data on their performance in wheat remains scarce. Choosing a Zn source that stays available in calcareous soils is therefore critical.

Because banded P fertilizers are generally more effective than broadcast, high P uptake during grain filling can increase phytic-acid synthesis and further immobilize any available Zn unless soil Zn fertility is adequate. In contrast, foliar Zn sprays timed from flag leaf emergence through soft-dough stages bypass soil fixation and deliver Zn directly to leaves and indirectly to roots via wash-off, reducing both soil and tissue immobilization. This timing also helps balance P uptake by developing grain, constraining phytic-acid accumulation while enhancing Zn bioavailability.

Most producers apply P at rates that replace what the crop will remove ($\approx 17 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ for a $4,000 \text{ kg ha}^{-1}$ wheat yield), so P-induced Zn deficiency is unlikely if soil Zn fertility is maintained at adequate levels. However, situations that either call for building soil P reserves, manure applications based on N needs, or multi-year P replacement plans may require supplementary Zn to avoid imbalances. An agronomically and economically optimal Zn rate for biofortification on the Prairies has yet to be established.

Ultimately, optimizing source, rate, placement, and timing of Zn, especially via well-timed foliar sprays, will be the key strategy to overcoming both soil fixation and phytic-acid binding, thereby ensuring that wheat grain is not only enriched in total Zn but also provides Zn in a form that is highly bioavailable for human nutrition.

1.5 Objectives

Building on the agronomic and physiological context above, this thesis aims to evaluate practical fertilization strategies under field conditions. Specifically, it assesses the effects of different

combinations of P and Zn fertilizer sources and placements, along with foliar biostimulants, on wheat productivity, grain Zn concentration, and grain Zn bioavailability at two sites in Manitoba's Red River Valley.

1.6 Hypotheses

The following hypotheses were tested:

- 1) Banding Zn fertilizer increases grain Zn concentration and Zn uptake more than broadcast application because plant-available Zn is higher in the fertilizer band, where co-precipitation with MAP is assumed to be less limiting than immobilization by carbonates when Zn is broadcast and mixed through the soil.
- 2) Combining soil and foliar Zn applications produces higher grain Zn concentrations and Zn bioavailability than soil application alone as late foliar Zn is taken up more efficiently by the plant and translocated directly to the developing grain during the filling period. Consequently, if a greater amount of Zn is uptaken by plants, uptake and distribution of other nutrients could also be affected.
- 3) P fertilization increases grain phytic acid concentration as greater P uptake is stored predominantly as phytic acid, the main P sink in the grain, thereby reducing Zn bioavailability.
- 4) Fertilization strategies that include foliar Zn will have a negligible impact on grain yield and crop development as prairie soils are able to provide more nutrients later in the season due to increased temperature and microorganism's activities.
- 5) Seed-row placement of granular zinc sulfate will reduce plant establishment because localized salt injury to germinating seeds adjacent the zinc sulfate granule is more likely, given its high salt index.

1.7 Structure of thesis

This thesis is organized around a manuscript-format research chapter, following the guidelines of the Department of Soil Science at the University of Manitoba. Chapter 1 provides a general overview of the challenges and key factors influencing wheat grain Zn biofortification. Chapter 2 presents the field study conducted at two locations in the Manitoba Red River Valley over two growing seasons. It investigates the effects of P and Zn fertilizer sources and placements on wheat grain Zn concentration and bioavailability, and evaluates soil-applied P and Zn in combination with foliar Zn, used alone or with biostimulants. The study compares broadcast-incorporated, side-banded, seed-row, and foliar Zn applications, using granular or co-granulated products for soil application and solution formulations for foliar application, and examines fertilizer treatment effects on plant development, yield, and nutrient concentrations and bioavailability in wheat grain. The final chapter synthesizes the key findings, outlines study limitations, and identifies directions for future research aimed at providing actionable recommendations for farmers and industry.

1.8 References

- Ahmad, Z., Waraich, E.A., Akhtar, S., Anjum, S., Ahmad, T., Mahboob, W., Hafeez, O.B.A., Tapera, T., Labuschagne, M., and Rizwan, M. 2018. Physiological responses of wheat to drought stress and its mitigation approaches. *Acta Physiol. Plant.* **40**: 1–13. doi:10.1007/s11738-018-2651-6.
- Akhtar, S. 2013. Zinc status in South Asian populations—An update. *J. Health Popul. Nutr.* **31**. doi:10.3329/jhpn.v31i2.16378.
- A&L Canada 2013. Fertilizer salt index: A&L fact sheet 141. [Online] Available: www.alcanada.com.
- Aldesuquy, H.S., Abbas, M.A., Abo-Hamed, S.A., Elhakem, A.H., and Alsokari, S.S. 2012. Glycine betaine and salicylic acid induced modification in productivity of two different cultivars of wheat grown under water stress. *J. Stress Physiol. Biochem.* **8**: 72–89. [Online] Available: <https://www.researchgate.net/publication/233916194> [Accessed 6 Aug. 2025].
- Balk, J., Connorton, J.M., Wan, Y., Lovegrove, A., Moore, K.L., Uauy, C., Sharp, P.A., and Shewry, P.R. 2019. Improving wheat as a source of iron and zinc for global nutrition. *Nutr. Bull.* **44**: 53–59. doi:10.1111/nbu.12361.
- Bertrand, I., Holloway, R.E., Armstrong, R.D., and McLaughlin, M.J. 2003. Chemical characteristics of phosphorus in alkaline soils from southern Australia. *Aust. J. Soil Res.* **41**: 61–76. doi:10.1071/SR02021.

Bharti, K., Pandey, N., Shankhdhar, D., Srivastava, P.C., and Shankhdhar, S.C. 2013. Improving nutritional quality of wheat through soil and foliar zinc application. *Plant Soil Environ.* **59**: 348–352. doi:10.17221/158/2013-PSE.

Botoman, L., Chimungu, J.G., Bailey, E.H., Munthali, M.W., Ander, E.L., Mossa, A., Young, S.D., Broadley, M.R., Lark, R.M., and Nalivata, P.C. 2022. Agronomic biofortification increases grain zinc concentration of maize grown under contrasting soil types in Malawi. *Plant Direct.* **6**: e458. doi:10.1002/pld3.458.

Bouis, H.E. 2003. Micronutrient fortification of plants through plant breeding: Can it improve nutrition in man at low cost? *Proc. Nutr. Soc.* **62**: 403–411. doi:10.1079/PNS2003262.

Bouis, H.E., and Welch, R.M. 2010. Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci.* **50**: S20. doi:10.2135/cropsci2009.09.0531.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil.* **302**: 1–17. doi:10.1007/s11104-007-9466-3.

Cakmak, I., and Kutman, U.B. 2018. Agronomic biofortification of cereals with zinc: A review. *Eur. J. Soil Sci.* **69**: 172–180. doi:10.1111/ejss.12437.

Cakmak, I., Pfeiffer, W.H., and McClafferty, B. 2010. Review: Biofortification of durum wheat with zinc and iron. *Cereal Chem.* **87**: 10–20. doi:10.1094/CCHEM-87-1-0010.

Caldwell, M., Nelson, K.A., and Nathan, M. 2016. Co-granulated and blended zinc fertilizer comparison for corn and soybean. *J. Agric. Sci.* **8**: 9–26. doi:10.5539/jas.v8n12p9.

Cediel, G., Olivares, M., Brito, A., Cori, H., and López de Romaña, D. 2015. Zinc deficiency in Latin America and the Caribbean. *Food Nutr. Bull.* **36**: S129–S138. doi:10.1177/0379572115585781.

Cereals Canada 2024. Canada's 2023 new wheat crop report. [Online] Available: <https://cerealscanada.ca/2023-wheat-crop> [Accessed 27 Mar. 2024].

Cereals Canada 2025. Key insights report: Global markets for Canadian wheat. [Online] Available: https://cerealscanada.ca/wp-content/uploads/2025/03/250311_Global-Markets-for-Cdn-Wheat_Key-Insights-Report_FIN-1.pdf [Accessed 28 Jun. 2025].

Clark, R.B., and Baligar, V.C. 2000. Acidic and alkaline soil constraints on plant mineral nutrition. Pages 133–177. in R.E. Wilkinson (Ed.). *Plant-Environment Interactions*, 2nd edition. Marcel Dekker, New York, NY. U.S.A. doi:10.1201/9780824746568.

Dhaliwal, S.S., Sharma, V., Shukla, A.K., Behera, S.K., Verma, V., Kaur, L., and Verma, G. 2023. Optimization of phosphorus (P) induced zinc (Zn) deficiency in low and high P soils and monitoring their effect on productivity, Zn and P uptake by wheat (*Triticum aestivum* L.). *J. Plant Nutr.* **46**: 917–928. doi:10.1080/01904167.2022.2144365.

Ding, J., Liu, L., Wang, C., Shi, L., Xu, F., and Cai, H. 2021. High level of zinc triggers phosphorus starvation by inhibiting root-to-shoot translocation and preferential distribution of phosphorus in rice plants. *Environ. Pollut.* **277**: 116778. doi:10.1016/j.envpol.2021.116778.

Dubey, A., Kumar, A., Malla, M.A., Chowdhary, K., Singh, G., Ravikanth, G., Harish, Sharma, S., Saati-Santamaria, Z., Menéndez, E., and Dames, J.F. 2021. Approaches for the amelioration of adverse effects of drought stress on crop plants. *Front. Biosci.-Landmark.* **26**: 928–947. doi:10.52586/4998.

EBIC 2025. Plant biostimulants. [Online] Available: <https://biostimulants.eu/plant-biostimulants/> [Accessed 15 Jul. 2025].

Fan, M.-S., Zhao, F.-J., Fairweather-Tait, S.J., Poulton, P.R., Dunham, S.J., and McGrath, S.P. 2008. Evidence of decreasing mineral density in wheat grain over the last 160 years. *J. Trace Elem. Med. Biol.* **22**: 315–324. doi:10.1016/j.jtemb.2008.07.002.

FAOSTAT 2022. Crop and livestock products. [Online] Available: <https://www.fao.org/faostat/en/#data/QCL> [Accessed 6 Feb. 2024].

Froese, S., Wiens, J.T., Warkentin, T., and Schoenau, J.J. 2020. Response of canola, wheat, and pea to foliar phosphorus fertilization at a phosphorus-deficient site in eastern Saskatchewan. *Can. J. Plant Sci.* **100**: 642–652. doi:10.1139/cjps-2019-0276.

Gawalko, E.J., Garrett, R.G., and Nowicki, T.W. 2002. Cadmium, copper, iron, manganese, selenium, and zinc in Canadian spring wheat. *Commun. Soil Sci. Plant Anal.* **33**: 3121–3133. doi:10.1081/CSS-120014510.

Gibson, R.S., Raboy, V., and King, J.C. 2018. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutr. Rev.* **76**: 793–804. doi:10.1093/nutrit/nuy028.

Goh, T.B., Karamanos, R.E., and Lee, J. 2013. Effects of phosphorus form on short-term solubility and availability in soils. *Commun. Soil Sci. Plant Anal.* **44**: 136–144. doi:10.1080/00103624.2013.736162.

Govindan, V., Michaux, K.D., and Pfeiffer, W.H. 2022. Nutritionally enhanced wheat for food and nutrition security. Pages 195–214. In M.P. Reynolds and H.-J. Braun (Eds.). *Wheat Improvement*.

1st edition. Springer International Publishing, Cham, Switzerland. doi:10.1007/978-3-030-90673-3_12.

Grant, C.A., and Bailey, L.D. 1998. Nitrogen, phosphorus and zinc management effects on grain yield and cadmium concentration in two cultivars of durum wheat. *Can. J. Plant Sci.* **78**: 63–70. doi:10.4141/P96–189.

Grant, C.A., and Flaten, D.N. 2019. 4R management of phosphorus fertilizer in the northern Great Plains. *J. Environ. Qual.* **48**: 1356–1369. doi:10.2134/jeq2019.02.0061.

Grant, C.A., Flaten, D.N., Tomasiewicz, D.J., and Sheppard, S.C. 2001. The importance of early season phosphorus nutrition. *Can. J. Plant Sci.* **81**: 211–224. doi:10.4141/P00-093.

Grujcic, D., Hansen, T.H., Husted, S., Drinic, M., and Singh, B.R. 2018. Effect of nitrogen and zinc fertilization on zinc and iron bioavailability and chemical speciation in maize silage. *J. Trace Elem. Med. Biol.* **49**: 269–275. doi:10.1016/j.jtemb.2018.02.012.

HarvestPlus 2025. Zinc wheat - HarvestPlus. [Online] Available: <https://www.harvestplus.org/crop/zinc-wheat/> [Accessed 7 Aug. 2025].

Hergert, G.W., Rehm, G.W., and Wiese, R.A. 1984. Field evaluations of zinc sources band applied in ammonium polyphosphate suspension. *Soil Sci. Soc. Am. J.* **48**: 1190–1193. doi:10.2136/sssaj1984.03615995004800050048x.

Hopkins, B., and Ellsworth, J. 2005. Phosphorus availability with alkaline/calcareous soil. *Western Nutrient Management Conf.* **6**: 88–93. Salt Lake City, UT. U.S.A. [Online] Available: <https://www.researchgate.net/publication/237734366>.

Huang, C., Barker, S.J., Langridge, P., Smith, F.W., and Graham, R.D. 2000. Zinc deficiency up-regulates expression of high-affinity phosphate transporter genes in both phosphate-sufficient and -deficient barley roots. *Plant Physiol.* **124**: 415–422. doi:10.1104/pp.124.1.415.

Hui, X., Luo, L., Chen, Y., Palta, J.A., and Wang, Z. 2025. Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis. *Nat. Commun.* **16**: 3913. doi:10.1038/s41467-025-58397-y.

Hussain, S., Maqsood, M.A., Aziz, T., and Basra, S.M.A. 2013. Zinc bioavailability response curvature in wheat grains under incremental zinc applications. *Arch. Agron. Soil Sci.* **59**: 1001–1016. doi:10.1080/03650340.2012.701732.

Ivanović, D., Dodig, D., Đurić, N., Kandić, V., Tamindžić, G., Nikolić, N., and Savić, J. 2021. Zinc biofortification of bread winter wheat grain by single zinc foliar application. *Cereal Res. Commun.* **49**: 673–679. doi:<https://doi.org/10.1007/s42976-021-00144-2>.

Kamran, A., Ghazanfar, M., Khan, J.S., Pervaiz, S., Siddiqui, M.H., and Alamri, S. 2023. Zinc absorption through leaves and subsequent translocation to the grains of bread wheat after foliar spray. *Agriculture.* **13**: 1775. doi:10.3390/agriculture13091775.

Karamanos, R., Flore, N., and Harapiak, J. 2017. The impact of phosphorus fertilizer placement on crop production. *Agric. Res. Technol.* **11**: 555817. doi:10.19080/ARTOAJ.2017.11.555817.

Lott, J.N.A., Ockenden, I., Raboy, V., and Batten, G.D. 2001. A global estimate of phytic acid and phosphorus in crop grains, seeds, and fruits. Pages 23–40. in N.R. Reddy and S.K. Sathe (Eds.). *Food Phytates*. 1st edition. CRC Press, Boca Raton, FL. U.S.A. [Online] Available: <https://www-taylorfrancis-com.uml.idm.oclc.org/chapters/edit/10.1201/9781420014419-4/> [Accessed 6 Aug. 2025].

- Madsen, C.K., and Brinch-Pedersen, H. 2020. Globoids and phytase: The mineral storage and release system in seeds. *Int. J. Mol. Sci.* **21**: 7519. doi:10.3390/ijms21207519.
- Marschner, H. 1993. Zinc uptake from soils. Pages 59–77. in A.D. Robson (Ed.). *Zinc in Soils and Plants*. Springer Netherlands, Dordrecht. doi:10.1007/978-94-011-0878-2_5.
- Marschner, P. (Ed.). 2012. *Marschner's Mineral Nutrition of Higher Plants*. 3rd edition. Elsevier. San Diego, CA. U.S.A. 651 pp. doi:10.1016/C2009-0-63043-9.
- Mayer, J.E., Pfeiffer, W.H., and Beyer, P. 2008. Biofortified crops to alleviate micronutrient malnutrition. *Curr. Opin. Plant Biol.* **11**: 166–170. doi:10.1016/j.pbi.2008.01.007.
- Miller, R.O., Jacobsen, J.S., and Skogley, E.O. 1993. Aerial accumulation and partitioning of nutrients by hard red spring wheat. *Commun. Soil Sci. Plant Anal.* **24**: 2389–2407. doi:10.1080/00103629309368963.
- Mkambula, P., Mbuya, M.N.N., Rowe, L.A., Sablah, M., Friesen, V.M., Chadha, M., Osei, A.K., Ringholz, C., Vasta, F.C., and Gorstein, J. 2020. The unfinished agenda for food fortification in low- and middle-income countries: Quantifying progress, gaps and potential opportunities. *Nutrients*. **12**: 354. doi:10.3390/nu12020354.
- Montalvo, D., Degryse, F., da Silva, R.C., Baird, R., and McLaughlin, M.J. 2016. Agronomic effectiveness of zinc sources as micronutrient fertilizer. Pages 215–267. in D.L. Sparks (Ed.). *Advances in Agronomy*. **139**. Elsevier. San Diego, CA. U.S.A. doi:10.1016/bs.agron.2016.05.004.
- Mooleki, S.P., Malhi, S.S., Lemke, R.L., Schoenau, J.J., Lafond, G., Brandt, S., Hultgreen, G.E., Wang, H., and May, W.E. 2010. Effect of form, placement and rate of N fertilizer, and placement of P fertilizer on wheat in Saskatchewan. *Can. J. Plant Sci.* **90**: 319–337. doi:10.4141/CJPS09075.

- Moraghan, J.T., and Mascagni, H.J. 1991. Environmental and soil factors affecting micronutrient deficiencies and toxicities. Pages 371–425. in J.J. Mortvedt (Ed.). *Micronutrients in Agriculture*. 2nd edition. SSSA, Madison, WI. USA. doi:10.2136/sssabookser4.2ed.c11.
- Mortvedt, J.J., and Gilkes, R.J. 1993. Zinc fertilizers. Pages 33–44. in A.D. Robson (Ed.). *Zinc in Soils and Plants*. Springer Netherlands, Dordrecht. doi:10.1007/978-94-011-0878-2_3.
- Naeem, A., Aslam, M., and Lodhi, A. 2018. Improved potassium nutrition retrieves phosphorus-induced decrease in zinc uptake and grain zinc concentration of wheat. *J. Sci. Food Agric.* **98**: 4351–4356. doi:10.1002/jsfa.8961.
- Peck, A.W., McDonald, G.K., and Graham, R.D. 2008. Zinc nutrition influences the protein composition of flour in bread wheat (*Triticum aestivum* L.). *J. Cereal Sci.* **47**: 266–274. doi:10.1016/j.jcs.2007.04.006.
- Pennock, D., Bedard-Haughn, A., and Viaud, V. 2011. Chernozemic soils of Canada: Genesis, distribution, and classification. *Can. J. Soil Sci.* **91**: 719–747. doi:10.4141/cjss10022.
- Persson, D.P., de Bang, T.C., Pedas, P.R., Kutman, U.B., Cakmak, I., Andersen, B., Finnie, C., Schjoerring, J.K. and Husted, S. 2016. Molecular speciation and tissue compartmentation of zinc in durum wheat grains with contrasting nutritional status. *New Phytol.* **211**: 1255–1265. doi:10.1111/nph.13989.
- Qamari, P., Shekari, F., Afsahi, K., Tavakoli, A., Samimifard, R., Shekari, K., and Mastinu, A. 2023. Response of wheat cultivars to zinc application for seed yield and quality improvement. *J. Agric. Sci.* **161**: 549–562. doi:10.1017/S0021859623000473.

- Raza, M.A.S., Saleem, M.F., and Khan, I.H. 2015. Combined application of glycinebetaine and potassium on the nutrient uptake performance of wheat under drought stress. *Pak. J. Agric. Sci.* **52**: 19–26. [Online] Available: <http://www.pakjas.com.pk>.
- Raza, M.A.S., Saleem, M.F., Shah, G.M., Khan, I.H., and Raza, A. 2014. Exogenous application of glycinebetaine and potassium for improving water relations and grain yield of wheat under drought. *J. Soil Sci. Plant Nutr.* **14**: 348–364. doi:10.4067/S0718-95162014005000028.
- Rehman, A., Farooq, M., Ozturk, L., Asif, M., and Siddique, K.H.M. 2018. Zinc nutrition in wheat-based cropping systems. *Plant Soil.* **422**: 283–315. doi:10.1007/s11104-017-3507-3.
- Rogalsky, M., Tiessen, K.H.D., Flaten, D., Lawley, Y., Tenuta, M., and Heard, J. 2024. Response to side-banded phosphorus and zinc fertilizer for corn grown after canola or soybean in southern Manitoba. *Can. J. Soil Sci.* **104**: 54–71. doi:10.1139/cjss-2023-0051.
- Sacristán, D., González-Guzmán, A., Torrent, J., and Campillo, M.C. 2021. Optimum Olsen phosphorus/zinc DTPA ratio for the initial growth of maize in agricultural soils of the mediterranean region. *J. Sci. Food Agric.* **101**: 3056–3064. doi:10.1002/jsfa.10940.
- Saha, B.N., Saha, S., Saha, S., Roy, P.D., Bhowmik, A., and Hazra, G.C. 2020. Zinc (Zn) application methods influences Zn and iron (Fe) bioavailability in brown rice. *Cereal Res. Commun.* **48**: 293–299. doi:10.1007/s42976-020-00038-9.
- Sample, E.C., Soper, R.J., and Racz, G.J. 1980. Reactions of phosphate fertilizers in soils. Pages 263–310. in F.E. Khasawneh et al. (Eds.). *The Role of Phosphorus in Agriculture*. 1st edition, ASA, Madison, WI. U.S.A. doi:10.2134/1980.roleofphosphorus.c12.

Sanz-Penella, J.M., and Haros, M. 2014. Whole grain and phytate-degrading human bifidobacteria. Pages 17–31. in R.R. Watson et al. (Eds.). *Wheat and Rice in Disease Prevention and Health*. 1st edition. Elsevier, San Diego, CA. U.S.A. doi:10.1016/B978-0-12-401716-0.00002-7.

Sathe, S.K., and Venkatachalam, M. 2001. Influence of processing technologies on phytate and its removal. Pages 157–188. in N.R. Reddy and S.K. Sathe (Eds.). *Food Phytates*. 1st edition. CRC Press, Boca Raton, FL. U.S.A. [Online] Available: <https://www.taylorfrancis.com/chapters/edit/10.1201/9781420014419-12/> [Accessed 6 Aug. 2025].

Schjoerring, J.K., Persson, D.P., Hansen, T.H., Laursen, K.H., and Husted, S. 2009. Zinc and iron speciation in the cereal grain. Pages 1–5. in *The Proceedings of the International Plant Nutrition Colloquium XVI*. [Online] Available: <https://escholarship.org/uc/item/31h065fh#author> [Accessed 6 Aug. 2025].

Seregina, I., Akhmetzhanov, D., Trukhachev, V., Belopukhov, S., Dmitrevskaya, I., and Islamgulova, R. 2023. Effect of different zinc concentrations on the growth functions of spring wheat seedlings. *BIO Web Conf.* **67**: 02026. doi:10.1051/bioconf/20236702026.

Shiferaw, B., Smale, M., Braun, H.-J., Duveiller, E., Reynolds, M., and Muricho, G. 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Secur.* **5**: 291–317. doi:10.1007/s12571-013-0263-y.

Shukla, V., Kaur, M., Aggarwal, S., Bhati, K.K., Kaur, J., Mantri, S., and Pandey, A.K. 2016. Tissue specific transcript profiling of wheat phosphate transporter genes and its association with phosphate allocation in grains. *Sci. Rep.* **6**: 39293. doi:10.1038/srep39293.

- Silva, V.M., Putti, F.F., White, P.J., and Reis, A.R. dos. 2021. Phytic Acid accumulation in plants: Biosynthesis pathway regulation and role in human diet. *Plant Physiol. Biochem.* **164**: 132–146. doi:10.1016/j.plaphy.2021.04.035.
- Stein, A.J., Nestel, P., Meenakshi, J., Qaim, M., Sachdev, H., and Bhutta, Z.A. 2007. Plant breeding to control zinc deficiency in india: How cost-effective is biofortification? *Public Health Nutr.* **10**: 492–501. doi:10.1017/S1368980007223857.
- Strong, W.M., and Soper, R.J. 1974. Phosphorus utilization by flax, wheat, rape, and buckwheat from a band or pellet-like application. I. reaction zone root proliferation 1. *Agron. J.* **66**: 597–601. doi:10.2134/agronj1974.00021962006600050001x.
- Sun, M., He, Z., and Jaisi, D.P. 2021. Role of metal complexation on the solubility and enzymatic hydrolysis of phytate. *PLoS One.* **16**: 1–16. doi:10.1371/journal.pone.0255787.
- Tran, B.T.T., Cavagnaro, T.R., Able, J.A., and Watts-Williams, S.J. 2021. Bioavailability of zinc and iron in durum wheat: A trade-off between grain weight and nutrition? *Plants, People, Planet.* **3**: 627–639. doi:10.1002/ppp3.10151.
- Tränkner, M., Tavakol, E., and Jákli, B. 2018. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. *Physiol. Plant.* **163**: 414–431. doi:10.1111/ppl.12747.
- Turksoy, S., Ozkaya, B., and Akbas, S. 2010. The effect of wheat variety and flour extraction rate on phytic acid content of bread. *J. Food Agric. Environ.* **8**: 178–181. [Online] Available: <https://www.wfpublisher.com/Abstract/1597> [Accessed 9 Apr. 2023].

Wang, M., Wang, R., Mur, L.A.J., Ruan, J., Shen, Q., and Guo, S. 2021. Functions of silicon in plant drought stress responses. *Hortic. Res.* **8**: 1–13. doi:10.1038/s41438-021-00681-1.

Wang, Z., Liu, Q., Pan, F., Yuan, L., and Yin, X. 2015. Effects of increasing rates of zinc fertilization on phytic acid and phytic acid/zinc molar ratio in zinc bio-fortified wheat. *Field Crops Res.* **184**: 58–64. doi:10.1016/j.fcr.2015.09.007.

Weaver, C., and Kannan, S. 2001. Phytate and mineral bioavailability. Pages 227–240. in N.R. Reddy and S.K. Sathe (Eds.). *Food Phytates*. 1st edition. CRC Press, Boca Raton, FL. U.S.A. [Online] Available: <https://www.taylorfrancis.com/um/oclc.org/chapters/edit/10.1201/9781420014419-15/> [Accessed 6 Aug. 2025].

WHO 1996. Trace elements in human nutrition and health. 1st edition. World Health Organization, Geneva, Switzerland. 343 pp. [Online] Available: <https://www.who.int/publications/i/item/9241561734>

Xu, M., Liu, M., Si, L., Ma, Q., Sun, T., Wang, J., Chen, K., Wang, X., and Wu, L. 2022. Spraying high concentrations of chelated zinc enhances zinc biofortification in wheat grain. *J. Sci. Food Agric.* **102**: 3590–3598. doi:10.1002/jsfa.11705.

Xue, Y.-F., Yue, S.-C., Zhang, Y.-Q., Cui, Z.-L., Chen, X.-P., Yang, F.-C., Cakmak, I., McGrath, S.P., Zhang, F.-S., and Zou, C.-Q. 2012. Grain and shoot zinc accumulation in winter wheat affected by nitrogen management. *Plant Soil.* **361**: 153–163. doi:10.1007/s11104-012-1510-2.

Yang, X.-W., Tian, X.-H., Lu, X.-C., Gale, W. J., and Cao, Y.-X. 2011. Foliar zinc fertilization improves the zinc nutritional value of wheat (*L.*) grain. *Afr. J. Biotechnol.* **10**: 14778–14785. doi:10.5897/AJB11.780.

Yu, B.-G., Liu, Y.-M., Chen, X.-X., Cao, W.-Q., Ding, T.-B., and Zou, C.-Q. 2021. Foliar zinc application to wheat may lessen the zinc deficiency burden in rural Quzhou, China. *Front. Nutr.* **8**: 697817. doi:10.3389/fnut.2021.697817.

Yuan, M., Fernández, F.G., Pittelkow, C.M., Greer, K.D. and Schaefer, D. 2020. Soil and crop response to phosphorus and potassium management under conservation tillage. *Agron. J.* **112**: 2302–2316. doi:10.1002/agj2.20114.

Zarea, M.J. 2025. Foliar application of *Azospirillum brasilense*, salicylic acid and zinc on wheat performance under rain-fed condition. *Cereal Res. Commun.* **53**: 1073–1090. doi:10.1007/s42976-024-00570-y.

Zou, C.Q., Zhang, Y.Q., Rashid, A., Ram, H., Savasli, E., Arisoy, R.Z., Ortiz-Monasterio, I., Simunji, S., Wang, Z.H., Sohu, V., Hassan, M., Kaya, Y., Onder, O., Lungu, O., Mujahid, M.Y., Joshi, A.K., Zelenskiy, Y., Zhang, F.S., and Cakmak, I. 2012. Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant Soil.* **361**: 119–130. doi:10.1007/s11104-012-1369-2.

2. IMPROVING ZINC BIOAVAILABILITY IN WHEAT GRAIN THROUGH FARM-TAILORED AGRONOMIC BIOFORTIFICATION STRATEGIES

2.1 Abstract

Canada is a major wheat exporter, yet grain zinc concentrations often fall below levels needed for human nutrition. This study tested practical agronomic biofortification to raise grain zinc and its bioavailability and evaluated yield impacts in Canadian context. Over two years at four site-years on calcareous clay and sandy loam in southern Manitoba, we compared phosphorus and zinc sources (MAP, MAP + granular zinc sulfate, co-granulated phosphorus-sulfur-zinc; MicroEssentials® SZ®) and placements (broadcast-incorporation, side-band, seed-row) with or without foliar zinc, potassium, and biostimulants applied at 2-leaf and anthesis stages. Grain and straw nutrients were quantified after acid digestion, and zinc bioavailability was indexed by the phytic acid to zinc molar ratio. Across site-years, zinc fertilization increased wheat yield by 8.3% compared with the Zn unfertilized control, with banding at seeding out-yielding broadcast-incorporation. The largest and most consistent gains in grain zinc concentration were achieved when the co-granulated product was placed in the seed-row and supplemented with foliar zinc, raising grain zinc by ~27–60% at the two most responsive site-years (to ~41–43 mg kg⁻¹). Adding potassium or the biostimulant mix produced small effects but were not significant. The phytic acid to zinc ratio ranged ~27–38 and improved by about 25% with foliar zinc application, while

phosphorus fertilization did not systematically increase phytic acid. Integrating seed-row co-granulated with well-timed foliar zinc application is a practical strategy to improve grain zinc bioavailability in Canadian prairie wheat systems. However, stronger and more consistent gains may require higher foliar zinc rates.

2.2 Introduction

Canada is one of the world's largest grain producers and exporters, with cereals accounting for approximately 25% of total cropping acreage (FAOSTAT, 2022). Wheat is the most prominent crop, covering about two-thirds of the cereal area (FAOSTAT, 2022). Hard red spring wheat (*Triticum aestivum* L.) makes up the majority of wheat grown in Canada, and in 2023, 23.3 million metric tons were exported to 80 countries (Cereals Canada, 2024). Many of these nations rely on wheat as a dietary staple food and a source of essential nutrients such as phosphorus (P) and zinc (Zn).

Ivanović et al. (2021) emphasized the global significance of Zn deficiency, which affects roughly one-third of the world's population, especially vulnerable groups such as pregnant women, infants, and children. Cakmak and Kutman (2018) noted that the typical Zn concentrations in wheat grain range from 28 to 30 mg kg⁻¹, below the desired levels of 40 to 50 mg kg⁻¹ for human health (HarvestPlus, 2025). Gawalko et al. (2002) and Gao et al. (2011) found a similar concentration range in wheat grain grown on the Canadian Prairies. This indicates that Canadian wheat, despite its global importance, may lack sufficient Zn to meet nutritional needs.

Several strategies have been proposed to address dietary Zn deficiency, including dietary diversification, food supplementation, nutrient enrichment during processing, and crop biofortification (Borrill et al., 2014). However, most of these approaches often lack feasibility for vulnerable populations (Bouis, 2003; Stein et al., 2007). Therefore, agronomic biofortification, which refers to enhancing the nutritional bioavailability of food crops through agronomic practices, is considered the most cost-effective and sustainable solution for improving food Zn content (Cakmak, 2008).

Phosphorus and Zn are essential nutrients for plant development. P is a key structural component of nucleic acids and energy carriers, such as ATP, and is critical for starch synthesis (Marschner, 2012). Zinc plays diverse roles, including enzyme activation, protein synthesis, and maintaining membrane integrity (Marschner, 2012). Both nutrients contribute significantly to wheat yield through supporting starch biosynthesis (Thitisaksakul et al., 2012; Zhang et al., 2021). However, P and Zn can interact antagonistically. High P supply can suppress mycorrhizal colonization, restrict fine root development, and reduce Zn solubility, all of which limit plant Zn uptake (Marschner, 2012). This interaction is particularly problematic in alkaline soils, where both nutrients tend to form insoluble compounds (Clark and Baligar, 2000). A further constraint on the Canadian Prairies is cold soils, which limit early P availability and thus wheat seedling establishment (Grant et al., 2001). These challenges have shaped the application of 4R nutrient stewardship for P management on the Canadian Prairies for the last few decades (Grant and Flaten, 2019).

The 4R nutrient management principles consist of applying fertilizer at the right time, right rate, right place, and with the right source. P rates are usually based on annual crop removal, or on multi-year removal when salt-sensitive crops (e.g., canola, flax, soybean) are in the rotation, so that extra P can be applied in wheat years to minimize the potential toxicity of seed-row or side-band P on sensitive crops (Grant and Flaten, 2019). Because of its early season demand, wheat often responds to P even when soil test P is adequate (Alessi and Power, 1980). To maximize P availability, the best time for P fertilizer application is at seeding in spring (Grant et al., 2001). Yet, high P supply may reduce plant Zn uptake. Over recent decades, fertilizer placement practices in the Canadian Prairies have shifted from broadcast, usually incorporated in the top 5 cm, to banded P application, driven by concerns for soil health, economic efficiency, and equipment capabilities

(Mooleki et al., 2010). These improvements have allowed a reduction in tillage intensity and reduced P in water runoff serving both soil and environmental health. Equipment advancements now allow for precise placement methods, such as side-banding (SB) and seed-row (SR) application. In SB, fertilizers are usually placed in a small trench 5 cm beside and below the seed, while in SR, fertilizers are placed directly in the seed row. Wheat is very efficient at taking up P from a band (Strong and Soper, 1974; Grant et al., 2001) while Zn often performs better when broadcast-incorporated, because Zn may precipitate with P when co-banded (Montalvo et al., 2016). Thus, co-banding P and Zn may reduce grain Zn and increase grain P associated with phytic acid, thereby limiting Zn bioavailability. Fertilizer formulations have also evolved. Previous strategies consisted of blending monoammonium phosphate (MAP), a fertilizer form effective in alkaline prairie soils (Bertrand et al., 2003; Goh et al., 2013; Grant and Flaten, 2019), with granular zinc sulfate when soils lacked Zn. However, uneven fertilizer distribution at low Zn rates often limited crop response (Montalvo et al. 2016). New co-granulated products, such as MicroEssentials[®] SZ[®] (MESZ), incorporate P, sulfur (S), and Zn in each granule to improve nutrient distribution. These products are commercially available and increasingly used by producers. However, data remain limited on the efficacy of these products in increasing crop productivity and grain Zn bioavailability across varying field and climate conditions.

Many successful Zn biofortification studies have employed combined soil and foliar Zn applications to enhance Zn in crop grains (Cakmak et al., 2010; Yang et al., 2011b; Zou et al., 2012). However, these studies often used Zn rates or foliar application timings that do not align with standard Canadian Prairies wheat production practices, which typically include an herbicide spray at early growth stages and a fungicide spray at anthesis, using water volumes less than 100 L ha⁻¹. Integrating biostimulants with foliar Zn could enhance Zn uptake and accumulation to grain

while mitigating risks such as foliar burn (Xu et al., 2022). Biostimulants such as osmolytes and phytohormones offer additional potential benefits for plant growth and metabolism. For example, Dubey et al. (2021) reported that osmolytes like betaine improved osmotic regulation and protected cellular structures during drought stress, potentially sustaining nutrient uptake. Similarly, phytohormones such as salicylic acid (SA) act as antioxidants and support stress-response. Studies have also shown that combining biostimulants with fertilizers can maximize benefits. For instance, Safar-Noori et al. (2018) demonstrated increased Zn concentrations in wheat when potassium (K) and SA were applied together. Aldesuquy et al. (2012) reported that SA and betaine improved wheat yield under non-stress conditions. Field studies in Pakistan showed that foliar K with betaine applications enhanced crop yield and P uptake (Raza et al., 2014, 2015). However, to our knowledge, no study has examined the effectiveness of simultaneous applications of K, betaine, and SA.

Another critical yet often overlooked aspect of Zn biofortification is bioavailability. Phytic acid, which constitutes 60–90% of seed P, is an antinutritional compound that binds cations like Zn and reduces mineral absorption in humans (Silva et al., 2021). The phytic acid to Zn molar ratio (PA:Zn) is a common indicator of Zn bioavailability, with ratios below 15 indicating adequate Zn bioavailability (WHO, 1996; Gibson et al., 2018). Excessive P fertilization may increase phytic acid levels, potentially reducing Zn bioavailability (Tran et al., 2021).

Given these knowledge gaps and agronomic constraints, this study was conducted to evaluate practical fertilization strategies under real-world field conditions. Specifically, it assessed the effects of various fertilization strategies, including different combinations of P and Zn fertilizer placements and sources, as well as foliar biostimulants, on productivity and grain Zn concentrations and bioavailability in wheat. The following hypotheses were tested: 1) Banding Zn

fertilizer increases grain Zn concentration and Zn uptake more than broadcast application because plant-available Zn is higher in the fertilizer band, where co-precipitation with MAP is assumed to be less limiting than immobilization by carbonates when Zn is broadcast and mixed through the soil; 2) Combining soil and foliar Zn applications produces higher grain Zn concentrations and Zn bioavailability than soil application alone as late foliar Zn is taken up more efficiently by the plant and translocated directly to the developing grain during the filling period. Consequently, if a greater amount of Zn is uptaken by plants, uptake and distribution of other nutrients could also be affected; 3) P fertilization increases grain phytic acid concentration as greater P uptake is stored predominantly as phytic acid, the main P sink in the grain, thereby reducing Zn bioavailability; 4) Fertilization strategies that include foliar Zn will have a negligible impact on grain yield and crop development as prairie soils are able to provide more nutrients later in the season due to increased temperature and microorganism's activities; 5) Seed-row placement of granular zinc sulfate will reduce plant establishment because localized salt injury to germinating seeds adjacent the zinc sulfate granule is more likely, given its high salt index. The findings of this study aim to provide practical recommendations for integrating Zn biofortification into Canadian Prairies wheat production systems, contributing to improved nutritional quality in wheat and addressing Zn deficiency in vulnerable populations.

2.3 Materials and Methods

2.3.1 Study Sites

The study was conducted in the Red River Valley of southern Manitoba, a region characterized by near-level topography and a humid-continental climate. Plot-based field trials were established at two sites over two years: Carman_2023 (49°33'40"N, 98°2'44"W) and Carman_2024 (49°32'26"N, 98°4'28"W) on sandy loam lacustrine soils, and Sperling_2023 (49°29'51"N, 97°39'50"W) and

Sperling_2024 (49°30'12"N, 97°39'54"W) on clay lacustrine soils. According to the Canadian Soil Classification System, the dominant soil series at each site (AgriMaps, 2025) is reported as imperfectly drained Gleyed Black Chernozem (Kronstal) for Carman_2023, imperfectly drained Gleyed Rego Black Chernozem (Reinland) for Carman_2024, drained Gleysolic Humic Vertisol (Osborne) for Sperling_2023, and imperfectly drained Gleyed Humic Vertisol (Red River, Scanterbury) for Sperling_2024. At each site, different field locations were selected each year to avoid cumulative effects of fertilizer treatments.

Prior to field establishment each year, 6 soil core samples (0–15 cm) were collected using hand augers, composited into one sample per site, and analyzed by a third-party laboratory for basic chemical properties. Methods followed Goldberg and Suarez (2014) for the DTPA-sorbitol extraction and NCERA-13 (2015) for all the other determinations. Analyses included pH (1:1 water), soil organic matter (loss-on-ignition), cation exchange capacity (ammonium displacement), Olsen-P (NaHCO_3), DTPA-sorbitol extractable micronutrients (B, Cu, Fe, Mn, Zn), exchangeable K, Ca, Mg, Na (1.0 M NH_4OAc), sulfate-S (0.2 M KCl), chloride (0.25 M K_2SO_4), electrical conductivity (1:1 water), and calcium carbonate equivalent (15% HCl). Results are summarized in Table 2.1. Briefly, Olsen-P was similar at three sites (11–12 mg kg^{-1}) and lower at Sperling_2024 (9 mg kg^{-1}). Cation exchange capacity averaged 9.2 cmol kg^{-1} at Carman and 48.6 cmol kg^{-1} at Sperling. DTPA-Zn (mg kg^{-1}) ranked: Sperling_2023 (1.54) > Sperling_2024 (1.18) > Carman_2023 (0.64) > Carman_2024 (0.34). Soil organic matter was ~2.1% at Carman and ~7.1% at Sperling. Calcium carbonate equivalent was higher at Sperling (2023: 2.4%, 2024: 4.1%) and negligible at Carman. Exchangeable K, S, B, Fe, Mg, and Ca were several-fold higher at Sperling than Carman. Another difference between sites was the previous crop where only one site

was seeded on canola stubble, which is known to reduce naturally occurring arbuscular mycorrhizal fungi. This could potentially interfere with nutrient uptake at Sperling_2023 sites.

Weather data (mean monthly air temperature, total monthly growing degree days (GDD), and total monthly precipitation) were obtained from the Manitoba Agrometeorology Department (Table 2.2). For both growing seasons, data for the Carman and Sperling sites were taken from the on-station Carman and nearby Brunkild stations, respectively. The 1991–2020 long-term normal was taken from the Carman station, the nearest to both sites. GDD were calculated as °C·days (base 5 °C). Weather conditions differed sharply between 2023 and 2024. In 2023, a drought year, the two sites accumulated ~115% of normal GDD but only ~39% of normal precipitation; hot and dry conditions in May and June hastened development and maturity. In 2024, GDD was near normal, while precipitation was ~130% of normal, with excess rainfall in May and June causing waterlogged conditions that restricted wheat development in depressional areas which was around 5 cm below land mean level.

Table 2.1 Soil descriptions and chemical characteristics for the top 15 cm at Carman and Sperling sites for 2023 and 2024.

Site	Carman_2023	Sperling_2023	Carman_2024	Sperling_2024
Soil Texture	Sandy Loam	Heavy Clay	Sandy Loam	Heavy Clay
Soil Series	KOT ^a	OBO8-SCY2 ^b	RLD ^c	RIV7-OBO3 ^d
Previous Crop	soybean	canola	corn	soybean
pH-H ₂ O	6.8	7.7	6.9	7.8
Organic Matter (%)	2.0	7.3	2.2	6.8
Cation Exchange Capacity (cmol kg ⁻¹)	8.3	52.2	10.0	41.3
Phosphorus-Olsen (mg kg ⁻¹)	11.0	12.0	11.0	9.0
Zn (mg kg ⁻¹)	0.6	1.5	0.3	1.2
Potassium (mg kg ⁻¹)	120	668	118	463
Chloride (kg ha ⁻¹)	53.8	67.2	3.4	6.7
Sulfur (kg ha ⁻¹)	11.2	42.6	15.7	51.5
Boron (mg kg ⁻¹)	0.2	2.1	0.3	1.9
Iron (mg kg ⁻¹)	74	17	35	12
Manganese (mg kg ⁻¹)	5.4	5.0	3.8	1.7
Copper (mg kg ⁻¹)	0.3	1.7	0.1	1.3
Magnesium (mg kg ⁻¹)	159	1041	252	847
Calcium (mg kg ⁻¹)	1275	8305	1465	6574
Sodium (mg kg ⁻¹)	14	66	9	41
Calcium Carbonate Equivalent (%)	<0.0	2.4	0.2	4.1
Electrical Conductivity (mS cm ⁻¹)	0.11	1.2	0.1	1.3

^a Kronstal, ^b Osborne drained 80% - Scanterbury 20%, ^c Reinland, ^d Red River 70% - Osborne 30%

Table 2.2 Weather conditions at Carman and Sperling sites for the 2023 and 2024 wheat growing season (May-August) compared to the historical normal.

Measure	May	June	July	August	May-Aug.
Mean air temperature, °C					
Carman_2023	16.1	21.0	18.4	18.9	18.5
Sperling_2023	14.8	20.5	18.5	19.0	18.2
Carman_2024	11.8	16.4	20.7	18.6	16.9
Sperling_2024	12.0	16.5	20.6	18.9	17.0
Total growing degree days (°C days)^a					
Carman_2023	343	460	414	430	1647
Sperling_2023	305	464	418	433	1620
Carman_2024	199	343	486	421	1449
Sperling_2024	218	345	484	430	1477
Total precipitations, mm					
Carman_2023	17.4	25.1	23.9	59.1	125.5
Sperling_2023	11.3	15.3	45.1	33.3	105.0
Carman_2024	130.3	143.9	38.3	73.7	386.2
Sperling_2024	127.6	129.0	71.0	74.5	402.1
Normal (1991-2020)^b					
Mean air temperature, °C	11.4	17.1	19.3	18.5	16.6
Total growing degree-days (°C days)	197	362	443	418	1420
Total precipitations, mm	75.9	88.4	70.0	67.8	302.1

^a Total growing degree days are calculated on a 5 °C base. ^b Normals are from Carman, MB.

2.3.2 Experimental Design

The field trial was conducted across four site-years in commercial fields, surrounded by spring wheat or oats to facilitate pest management. A randomized complete block design (RCBD) was used at each site-year, with each of the 12 treatments replicated four times. Blocks were aligned perpendicular to the main slope gradient and oriented along the predominant direction of farm equipment traffic.

Twelve treatments (Table 2.3) were evaluated, spanning three soil placements and three soil fertilizer formulations. The soil fertilizer placements were: 1) side-band (SB, 5 cm beside and 5 cm below the seed furrow at seeding); 2) broadcast surface-incorporated (BC, in the top 10 cm of soil), and 3) seed-row (SR). The soil fertilizer sources were: 1) monoammonium phosphate (MAP,

11-52-0); 2) MAP blended with Nexus Zinc Sulfate Granular (MAP + Zn granular (0-0-0-18S-35.5Zn); NexusBioAg, Downers Grove, IL); and 3) MicroEssentials® SZ® (MESZ (12-40-0-10S-1Zn); The Mosaic Company, Tampa, FL). SB and SR fertilizers were applied during seeding using a plot seeder (R-Tech Industries Ltd., Homewood, MB) equipped with independent fertilizer and seed delivery systems, and Pillar Mark III opener (Pillar AG, Warman, SK) featuring a single disc opener with a double-shoot for accurate side-band placement. In contrast, BC fertilizers were applied using two passes with a disk cultivator, oriented lengthwise and in opposite directions across the block.

Treatment 1 was the control without P and Zn additions. Treatments 2–4 were SB at seeding: T2, MAP only (SB MAP); T3, MAP + granular Zn (SB MAP + Zn granular); T4, MESZ (SB MESZ). Treatments 5–7 were BC incorporated before seeding: T5, MAP only (BC MAP); T6, MAP + granular Zn (BC MAP + Zn granular); T7, MESZ (BC MESZ). Treatments 8–12 used SR placement of MESZ: T8, SR MESZ only; T9, SR MESZ + foliar ZnSO₄ (SR MESZ + Fol(+Zn)); T10, SR MESZ + foliar ZnSO₄ + K (SR MESZ + Fol(+Zn,+K)); T11, SR MESZ + foliar ZnSO₄ + biostimulants (SR MESZ + Fol(+Zn,+Bio)); T12, SR MESZ + foliar ZnSO₄ + K + biostimulants (SR MESZ + Fol(+Zn,+Bio,+K)).

Foliar spray treatments were: 1) Fol(+Zn), a 9% w w⁻¹ Zn solution prepared from zinc sulfate monohydrate (ZnSO₄·H₂O; Nexus Zinc Sulfate Granular); 2) Fol(+Zn,+K), where K was applied as Liquid K (0-0-10; Omex Agriculture Inc., Oak Bluff, MB); 3) Fol(+Zn,+Bio), where Bio was a biostimulant blend of EZ-GRO BETAINE (99% glycine betaine) and EZ-GRO SA-L 10% (10% salicylic acid and 4.6% soluble K₂O; Hicrocorp Inc., Kingston, ON); and 4) Fol(+Zn,+Bio,+K), which combined all foliar products above. Sprays were applied at 490 L ha⁻¹ using a bike sprayer (R-Tech Industries Ltd., Homewood, MB) in a water solution at two Zadoks stages: Z12–

Z15 (early leaf stage) and Z65 ($\approx 50\%$ anthesis), aligning with typical herbicide and fungicide timings in Prairie wheat production systems

All plots (including the control) received a uniform N rate of 168 kg ha^{-1} from N and N+P sources. Sulfur was applied at 6.25 kg ha^{-1} as ammonium sulfate, except in MESZ treatments where S was supplied by the product (half sulfate, half elemental S). Phosphorus was applied at $21.8 \text{ kg P ha}^{-1}$ ($\approx 50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$) in all fertilized treatments. The MESZ Zn rate was set by the product ($1.25 \text{ kg Zn ha}^{-1}$ at the above P_2O_5 rate). In the MAP + Zn treatments, Zn was added at 2.5 kg ha^{-1} , reflecting local recommendations for granular zinc sulfate (Rahman et al. 2022). Foliar Zn only applied to MESZ treatment as they were providing the lowest actual Zn. However, this uneven distribution of foliar treatment across fertilizer sources required particular attention during statistical analysis as it brings confounding effects that can mislead the results interpretation.

At the Sperling sites, anhydrous ammonia (82-0-0) was fall-applied in both 2022 and 2023 by the cooperating farmer using a knife-type applicator using a John Deere 2430 TrueSet chisel plow (John Deere, Moline, IL) equipped with Bourgault 200-ASY-7523 knives (Bourgault Tillage Tools Ltd., St. Brieux, SK) to deliver bands every 30 cm at 10 cm depth. At the Carman sites, urea was surface-applied using a Valmar 2055 pneumatic granular applicator (Salford Group Inc., Salford, ON) and incorporated 3 to 5 days before seeding, coinciding with the timing of broadcast-incorporation treatments to minimize soil disturbance.

The wheat cultivar used was AAC Hodge VB, a Canada Western Red Spring (CWRS) variety. CWRS wheat is widely grown and exported and is well-suited for bread and flour production. The target plant population was $330 \text{ plants m}^{-2}$. Each seeder pass planted four rows spaced 30 cm apart, and seeding depth was adjusted at each site to ensure seed placement into moist soil, typically between 30–45 mm. Each plot consisted of 12 rows, each 6 m long. A 50 cm buffer was maintained

between plots within each block, and a 2 m alley was left between blocks to reduce cross-contamination between treatments. Rows 5 to 8 were designated for yield assessment using a plot combine, while rows 2, 3, 10, and 11 were used for in-season sampling.

Table 2.3 Treatment list with detailed product with analysis, rate and timing of application, required placement and nutrients nitrogen (N), phosphorus (P), sulfur (S), and zinc (Zn) applied with the treatment products.

Treatments		Products:	Products Analysis	Rate kg ha ⁻¹ (L ha ⁻¹)	Time	Place	Nutrient applied rate kg ha ⁻¹				
#	Name						N	P	K	S	Zn
1	Control (-P, -Zn) ^a	AMS	20-0-0-24	26.00	Pre-seeding	BC	5.2	0	0	6.25	0
2	SB ^b MAP ^c	MAP	11-52-0	96.20	At-seeding	SB	15.8	21.8	0	6.25	0
		AMS	20-0-0-24	26.00	At-seeding	SB					
3	SB MAP + Zn granular	MAP	11-52-0	96.20	At-seeding	SB	14.7	21.8	0	6.25	2.50
		Zinc sulfate	0-0-0-18S-35.5Zn	7.04	At-seeding	SB					
		AMS	20-0-0-24	26.00	At-seeding	SB					
4	SB MESZ ^d	MESZ	12-40-0-10S-1Zn	125.00	DS	SB	15.0	21.8	0	6.25	1.25
5	BC ^e MAP	MAP	11-52-0	96.20	Pre-seeding	BC	15.8	21.8	0	6.25	0
		AMS	20-0-0-24	26.00	Pre-seeding	BC					
6	BC MAP + Zn granular	MAP	11-52-0	96.20	Pre-seeding	BC	14.7	21.8	0	6.25	2.50
		Zinc sulfate	0-0-0-18S-35.5Zn	7.04	Pre-seeding	BC					
		AMS	20-0-0-24	26.00	Pre-seeding	BC					
7	BC MESZ	MESZ	12-40-0-10S-1Zn	125.00	Pre-seeding	BC	15.0	21.8	0	6.25	1.25
8	SR ^f MESZ	MESZ	12-40-0-10S-1Zn	125.00	At-seeding	SR	15.0	21.8	0	6.25	1.25
9	SR MESZ + Fol(+Zn)	MESZ	12-40-0-10S-1Zn	125.00	At-seeding	SR	15.0	21.8	0	6.49	1.72
		Zn 9% sol.	0-0-0-4.56S-9Zn	(2.00)	Z12 & Z65 ^h	Foliar					
10	SR MESZ + Fol ^g (+Zn,+K)	MESZ	12-40-0-10S-1Zn	125.00	At-seeding	SR	15.0	21.8	0.52	6.49	1.72
		Zn 9% sol.	0-0-0-4.56S-9Zn	(2.00)	Z12 & Z65	Foliar					
		Liquid K	0-0-10	(4.00)	Z12 & Z65	Foliar					
11	SR MESZ + Fol(+Zn,+bio)	MESZ	12-40-0-10S-1Zn	125.00	At-seeding	SR	15.0	21.8	0	6.49	1.72
		Zn 9% sol.	0-0-0-4.56S-9Zn	(2.00)	Z12 & Z65	Foliar					
		SA-L 10%	0-0-4.5	(0.05)	Z12 & Z65	Foliar					
		Betaine	10% solution	(0.50)	Z12 & Z65	Foliar					
12	SR MESZ + Fol(+Zn, +bio,+K)	MESZ	12-40-0-10S-1Zn	125.00	At-seeding	SR	15.0	21.8	0.52	6.49	1.72
		Zn 9% sol.	0-0-0-4.56S-9Zn	(2.00)	Z12 & Z65	Foliar					
		SA-L 10%	0-0-4.5	(0.05)	Z12 & Z65	Foliar					
		Betaine	10% solution	(0.50)	Z12 & Z65	Foliar					
		Liquid K	0-0-10	(4.00)	Z12 & Z65	Foliar					

^a The '+' or '-' refers to "added" or "not added", respectively. ^b Side-banded at 5 cm beside and below the seed row. ^c Monoammonium phosphate. ^d MicroEssentials® SZ®. ^e Broadcast surface-incorporated. ^f Seed-row. ^g Foliar application. ^h wheat Zadok's growing stages Z12 and Z65. N was added using 82-0-0 (Sperling) or 46-0-0 (Carman) to reach 162 kg N ha⁻¹ applied.

2.3.3 Plant densities , Biomass and Yield Determination

Early plant density , used as an indicator of potential salt toxicity, was assessed at the Z11 stage using a 30 cm × 30 cm quadrat at six locations per plot, each at least one meter from the plot border. A second determination was conducted 10 days after the first foliar treatment application done between Z12–Z15 growth stage, coinciding with the first biomass sampling. For biomass, whole plants were dug using a spade, from a 17 cm row segment at one location per plot within the dedicated sampling rows. Individual plants were separated, counted, and the aboveground portion was cut at ground level. A second biomass sampling was taken 10 days after the Z65 foliar application. Aboveground biomass samples from both sampling events were oven-dried at 35°C for 7 days, then at 65°C for 3 days to determine dry mass. At physiological maturity, straw biomass was sampled by cutting a 1-m standing wheat row at ground level from four locations in each plot, avoiding previous sampling points and harvest rows. Samples were dried at 35°C for 7 days, weighed, and then threshed. Straw biomass was calculated by subtracting the grain weight from the total dry biomass. Biomasses were assessed

Grain yield was determined by harvesting the middle four rows of each plot using a research plot combine (Classic, Wintersteiger Inc., Saskatoon, SK). Fresh grain moisture at harvest was measured using a HarvestMaster GrainGages™ system (Logan, UT). Final yield was adjusted to 13.5% moisture content. Grain samples were then dried at 35°C for 14 days and cleaned using a Clipper Office Tester (A.T. Ferrell Company Inc., Bluffton, IN). Test weight of dried grain was determined according to Canadian Grain Commission procedures (CGC 2025). Grain protein content was analyzed with PerkinElmer DA 7250 diode array NIR analyzer (PerkinElmer, Shelton, CT) and reported on a 13.5% moisture basis.

2.3.4 Plant Tissue Nutrient Analysis

At physiological maturity, plant tissue samples were collected from three locations within each plot by cutting plants at ground level. Wheat heads were separated near the junction of the peduncle and rachis. All samples were washed with reverse osmosis water to minimize contamination from dust and soil particles. Following washing, samples were dried at 35°C for 7 days. The heads were hand-threshed, and the grain was separated from the chaff using the Clipper Office Tester. Straw, chaff, and grain samples were ground using a Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ) equipped with a 2 mm sieve. For digestion, 0.4 g of dry tissue was placed into a Kjeldahl digestion tube, and 4.4 mL of freshly prepared piranha solution (a mixture of concentrated sulfuric acid and hydrogen peroxide) was added, following the protocol of Westerman (1990). The tubes were left at room temperature for 1 h, then heated in a digestion block at 100°C for 1 h, followed by 350°C for 2 h. Concentrations of P, K, Fe, and Zn in digest solution were then quantified using an inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7850, Agilent Technologies, Santa Clara, CA). Plant nutrient uptake was determined by summing the nutrient content of each tissue, calculated as tissue nutrient concentration multiplied by its corresponding dry biomass.

2.3.5 Analysis of Grain Samples for Phytate Concentration and Zn Bioavailability

A subsample of harvested grain was finely ground using a Cyclone Sample Mill (Seedbuo Equipment Company, Des Plaines, IL). Grain phytic acid concentration was determined using a modified protocol of Haug and Lantzsch (1983). Briefly, 0.15 g of whole wheat flour was mixed with 25 mL of 0.2 N HCl, and 2 mL of the suspension was transferred to 1 mL of ferric solution. Samples were heated in a 100°C water bath for 30 min, then cooled in an ice bath for 15 min, and equilibrated to room temperature. The mixture was then centrifuged at 3,000 g for 30 minutes. A 1 mL aliquot of the resulting supernatant was combined with 1.5 mL of 2,2'-bipyridine solution. Absorbance was measured at 519 nm using a UV/visible spectrophotometer (Ultrospec 2100 pro,

GE Healthcare, Chicago, IL), and phytic acid concentration was quantified using a standard curve prepared with known concentrations of phytic acid.

The molar ratio of phytic acid to zinc (PA:Zn) in wheat grain was calculated as an indicator of Zn bioavailability, with lower values reflecting greater nutritional accessibility.

$$\text{PA:Zn molar ratio} = \frac{\text{Phytic acid (mg kg}^{-1}\text{)} / 660.04}{\text{Zn (mg kg}^{-1}\text{)} / 65.38}$$

2.3.6 Relative Elevation Data Collection

Relative elevation was used to assess within block microtopography variations that affect plant development and yield. For example, during drought year, higher yield was observed in the depression while during wet years, yield was superior on higher areas. Relative elevation was derived from digital surface models (DSM) created using drone imagery captured by a Mavic 3M drone (DJI Agriculture, Shenzhen, China) at a 5 cm resolution. Orthomosaics and DSM files were processed with DJI SmartFarm software, and plot-level elevation values were extracted using spatial analysis tools in R Studio (version 2023.12.0). Plot boundaries were digitized, and relative elevation was calculated by subtracting the minimum trial elevation from each plot's minimum elevation. Key steps were implemented using spatial and raster data packages (e.g., *FIELDimageR*, *sf*, *terra*).

2.3.7 Statistical Analysis

Data analysis was performed using PROC GLIMMIX in SAS Studio 3.81. Analysis of covariance (ANCOVA) was conducted for all studied variables. Specifically, data for plant biomass, grain and straw yield, nutrient concentrations (Zn, P, K, Fe), phytic acid concentration, nutrient export in

grain and straw, and the molar ratio of phytic acid to Zn were analyzed using a log-normal distribution. Back-transformation was done using the Omega method as described by Edwards (2017). Plant densities were analyzed with a Poisson distribution using the log link function. The homogeneity of variance was tested with a graphical distribution. The Satterthwaite correction for the denominator degrees of freedom was applied to improve the normality of residuals when the homogeneity of variance appeared non-homogenous. The Chi-square statistic, divided by the degrees of freedom, was used to determine the most appropriate statistical model.

Covariance analysis included relative elevation and the interaction of relative elevation with site-year as covariates, as they best captured soil condition variability within and between blocks. A biomass gradient was observed within some blocks that was not visible at plot establishment. This variability suggests site-level environmental stressors could influence nutrient uptake and translocation. Due to contrasting conditions between years, particularly in precipitation and growing degree day accumulation, a site-year analytical approach was adopted. This approach accounts for variability in soil moisture availability and its influence on crop development, nutrient uptake and translocation. When the covariate effect was non-significant, the covariate was retained to ensure consistent adjustment and comparability of treatment effects across variables.

In the mixed model, treatment and site-year were treated as fixed effects, while blocks within site-year were treated as random effects. The main effects of treatment and site-year were evaluated for all dependent variables. Least square means (LSMEANS) were calculated for treatment and site-year combinations. Multiple comparisons of the means were done using orthogonal contrasts were applied using the LSMESTIMATE statement to compare a maximum of N-1 planned comparisons for both treatment and site-year, based on study hypotheses and differences in fertilizer placement and formulation. When significant treatment by site-year interactions were

detected, the same set of contrasts was applied within each site-year to assess the uncombined responses. Pearson's correlation analysis (PROC CORR) was conducted on log-transformed data to evaluate relationships among dependent variables and assess their impacts on grain P and Zn concentrations, and Zn bioavailability. For all analyses, differences were considered statistically significant at $P < 0.05$.

2.4 Results

2.4.1 Plant Establishment and Development

The relative elevation had a significant effect on aerial biomass at 10 days after herbicide foliar treatment (DAH) and on straw yield (Table 2.4). Additionally, plant count, biomass, and straw yield exhibited significant relative elevation by site-year interactions. These interactions were mainly attributable to contrasting weather conditions: in 2023, dry conditions favored plant growth in lower-lying areas, whereas in 2024, excessive moisture caused waterlogging in depressions, which reduced plant growth.

Fertilizer treatment significantly affected aerial biomass, and plant densities at Z11. Compared to broadcast incorporation, side-banded MAP and MESZ increased biomass by 20.3% and 16.7%, respectively (**Error! Reference source not found.**). Other contrasts at this stage were not significantly different. At 10 days after fungicide foliar treatment (DAF), Zn addition significantly increased biomass by 18.1%. Side-banded MAP significantly increased biomass by 39.4% compared with broadcast-incorporation. Surprisingly, in the late biomass sampling, seed-row MESZ with added K increased biomass by 32.8% compared with foliar Zn solution alone. However, these biomass gains did not translate into significant changes in straw yield.

Table 2.4 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on plant densities , aerial biomass, and straw yield at Carman and Sperling from 2023 to 2024.

Treatment	Plant densities		Aerial biomass		Straw
	Z11 ⁱ	10DAH ^j	10DAH	10DAF ^k	Yield
	# plant m ⁻¹		kg DM ^l ha ⁻¹		
			Means (SEM) ^m		
1: Control (-P,-Zn) ^a	291 (10)	343 (22)	1248 (89)	4677 (348)	2542 (105)
2: SB ^b MAP ^c	292 (10)	363 (35)	1563 (132)	5392 (364)	2510 (121)
3: SB MAP+ Zn granular	284 (9)	375 (29)	1589 (110)	4804 (408)	2738 (161)
4: SB MESZ ^d	283 (9)	353 (27)	1475 (128)	5357 (530)	2598 (177)
5: BC ^e MAP	284 (9)	396 (38)	1299 (117)	3868 (372)	2711 (149)
6: BC MAP+ Zn granular	282 (9)	355 (26)	1200 (125)	5395 (442)	2493 (123)
7: BC MESZ	313 (10)	369 (26)	1278 (84)	4989 (430)	2401 (150)
8: SR ^f MESZ	283 (9)	335 (31)	1416 (131)	5808 (353)	2769 (118)
9: SR MESZ + Fol(+Zn) ^g	296 (10)	373 (29)	1479 (141)	4767 (393)	2657 (117)
10: SR MESZ + Fol(+Zn,+K)	278 (9)	367 (30)	1639 (135)	6331 (536)	2761 (116)
11: SR MESZ + Fol(+Zn,+bio ^h)	293 (10)	340 (28)	1560 (137)	6273 (484)	2749 (146)
12: SR MESZ + Fol(+Zn,+bio,+K)	289 (9)	360 (26)	1385 (109)	5674 (566)	2684 (232)
Site-Year					
Carman_2023	276 (15)	395 (28)	1416 (97)	2909 (166)	1192 (63)
Sperling_2023	309 (16)	319 (19)	1385 (76)	3615 (171)	1462 (65)
Carman_2024	241 (13)	357 (21)	1292 (69)	6007 (267)	4759 (213)
Sperling_2024	338 (24)	374 (76)	1626 (333)	11968 (2324)	5811 (713)
ANCOVA					
Effect			P value		
Relative elevation	0.3310	0.4982	0.0001	0.1999	0.0003
Rel. elev. x Site-Year	0.0228	0.0337	0.0004	0.0008	0.0006
Site-Year	0.0445	0.0054	<.0001	0.1238	<.0001
Treatment	<.0001	0.7723	0.0079	0.0028	0.1090
Site-Year x Treatment	<.0001	0.3891	0.4629	0.1516	0.1562
Contrast Site-Year					
2023 vs. 2024	0.7012	0.7967	0.8228		<.0001
2023: Carman vs. Sperling	0.1625	0.0394	0.8130		0.8025
2024: Carman vs. Sperling	0.0028	0.8267	0.3171		0.0649

^a The '+' or '-' refers to "added" or "not added", respectively. ^b Side-banded at 5 cm beside and below the seed furrow. ^c Monoammonium phosphate. ^d MicroEssentials[®] SZ[®]. ^e Broadcast surface-incorporated. ^f Seed-row. ^g Foliar application at wheat Zadok's growing stages Z12 and Z65. ^h Biostimulants blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. ⁱ Zadok's growing stages Z11. ^j 10 days after herbicide foliar treatment. ^k 10 days after fungicide foliar treatment. ^l Dry matter. ^m Means and standard error (Treatment, n=16; Site-Year, n=48) were calculated with the LSMEANS function, and contrast p-values with the LSMESTIMATE function, both in PROC GLIMMIX in SAS. Contrasts for treatment effects are presented in **Error! Reference source not found.** and interaction in Figure A-6.

Plant densities were affected by the fertilizer treatment only at the Z11 stage. Plant densities ranged between 278 and 313 plants m⁻² (**Error! Reference source not found.**). Compared to MESZ, the MAP + Zn treatment significantly decreased counts by 2.7%. Compared to broadcast-incorporation, banding MESZ also significantly decreased counts by 7.5%. Other soil-applied treatment contrasts were not significantly different. Treatments with foliar applications were excluded from comparison, as they were applied after the early plant count stage.

According to the interaction contrast analysis (Appendix Figure A-6), MESZ resulted in higher plant densities than MAP + Zn at Carman 2023, while this difference was not observed at other site-years. At Carman 2024, MAP significantly decreased plant densities compared to the control, whereas no significant effects were observed at other sites. Compared to broadcast-incorporation, side-banding of MAP and MAP+Zn increased plant densities at Carman 2024, while decreased counts at Sperling 2024. Interestingly, MESZ placement had the opposite trend: broadcast-incorporation resulted in higher plant densities than banded at Carman 2024, with no significant effects at other sites. Overall, plant count varied across treatments and sites without a clear trend with fertilizer placement or soil type.

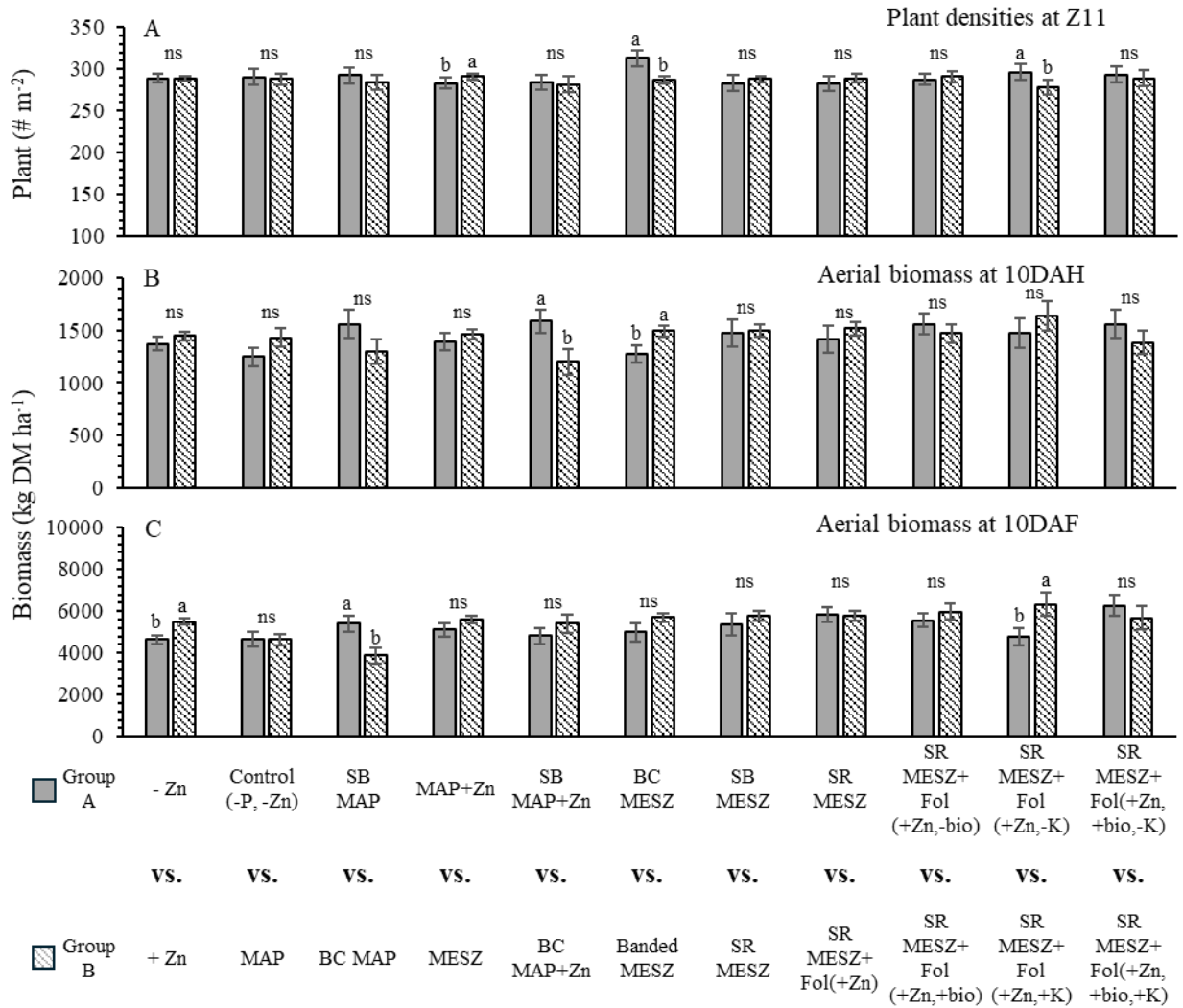


Figure 2.1 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat plant densities (A), dry matter biomass 10 days after herbicide application (B), and dry matter biomass 10 days after fungicide application (C) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant differences. Error bars represent standard errors of the means ($n=16$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials® SZ®. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium. Z11 = Zadok’s growing stages Z11.

2.4.2 Yield and Grain Quality

The relative elevation and its interaction with site-year significantly affected both grain yield and grain moisture (Fertilizer treatments significantly affected both test weight and grain yield. Broadcast-incorporated MESZ slightly (0.5%) but significantly increased test weight compared to banded MESZ (Figure 2.2), though such a difference is unlikely to affect commercial grading at elevators. Compared to the control, Zn fertilizer application significantly increased yield by 253 kg ha⁻¹ (8.3%). Compared to broadcast-incorporation, banded MESZ increased yield by 315 kg ha⁻¹ (10.8%). Similarly, banded MAP increased grain yield by 13.0% over broadcast-incorporation. Compared to side-banding, seed-row placement of MESZ with or without foliar applications resulted in comparable grain yield. Grain protein ranged between 16.5–17.6% and was not affected by fertilizer treatment or site-year. All variables including grain yield, test weight, grain moisture, and protein did not show a significant site-year × treatment interaction.

Table 2.5). This interaction was attributable to field topography modified soil moisture content under contracting weather conditions between years. In contrast, test weight and grain protein content in all site-years were not significantly affected by field elevation.

Despite those observations, dry grain test weight and fresh grain moisture were affected by site-year effects. Dry grain test weight was 2.1–3.6% higher at Sperling than Carman, even though the differences were considered negligible and did not affect quality class grading. Dried grain test weight values for all treatments ranged from 81.2–84.1 kg hl⁻¹, well above the minimum export classification threshold of 79 kg hl⁻¹. Grain moisture was consistently higher in 2024 than 2023, reflecting wetter conditions.

Fertilizer treatments significantly affected both test weight and grain yield. Broadcast-incorporated MESZ slightly (0.5%) but significantly increased test weight compared to banded MESZ (Figure 2.2), though such a difference is unlikely to affect commercial grading at elevators. Compared to the control, Zn fertilizer application significantly increased yield by 253 kg ha⁻¹ (8.3%). Compared to broadcast-incorporation, banded MESZ increased yield by 315 kg ha⁻¹ (10.8%). Similarly, banded MAP increased grain yield by 13.0% over broadcast-incorporation. Compared to side-banding, seed-row placement of MESZ with or without foliar applications resulted in comparable grain yield. Grain protein ranged between 16.5–17.6% and was not affected by fertilizer treatment or site-year. All variables including grain yield, test weight, grain moisture, and protein did not show a significant site-year × treatment interaction.

Table 2.5 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on corrected grain yield, test weight, grain moisture, and protein content at Carman and Sperling from 2023 to 2024.

Treatment	Grain yield	Test	Grain	
	at 13.5%	weight	moisture	protein at 13.5%
	kg ha ⁻¹	kg hl ⁻¹	%	
	Means (SEM) ⁱ			
1: Control (-P,-Zn) ^a	2964 (127)	83.2 (0.2)	11.5 (0.3)	17.2 (0.3)
2: SB ^b MAP ^c	3212 (149)	82.7 (0.2)	11.1 (0.3)	16.9 (0.3)
3: SB MAP+ Zn granular	3296 (138)	83.0 (0.2)	10.8 (0.2)	17.3 (0.4)
4: SB MESZ ^d	3246 (133)	82.8 (0.3)	10.9 (0.2)	17.3 (0.3)
5: BC ^e MAP	2918 (124)	82.9 (0.2)	11.2 (0.3)	17 (0.3)
6: BC MAP+ Zn granular	3102 (161)	82.7 (0.3)	11.4 (0.4)	17.1 (0.3)
7: BC MESZ	2924 (117)	83.1 (0.2)	11.4 (0.2)	17.1 (0.3)
8: SR ^f MESZ	3343 (103)	82.9 (0.2)	10.6 (0.2)	16.8 (0.3)
9: SR MESZ + Fol(+Zn) ^g	3328 (114)	82.6 (0.2)	11.8 (0.5)	17.3 (0.3)
10: SR MESZ + Fol(+Zn,+K)	3369 (139)	82.6 (0.2)	10.9 (0.2)	16.8 (0.2)
11: SR MESZ + Fol(+Zn,+bio ^h)	3246 (183)	82.4 (0.2)	9 (0.2)	16.8 (0.3)
12: SR MESZ + Fol(+Zn,+bio,+K)	3299 (198)	82.7 (0.3)	11.1 (0.2)	17.1 (0.2)
Site-Year				
Carman_2023	2183 (112)	82.1 (0.3)	9.4 (0.1)	16.6 (0.4)
Sperling_2023	2498 (112)	83.8 (0.3)	9.3 (0.1)	16.5 (0.3)
Carman_2024	3514 (160)	81.2 (0.3)	11.2 (0.1)	17.6 (0.3)
Sperling_2024	5381 (529)	84.1 (0.6)	15.9 (0.8)	17.6 (0.7)
ANCOVA				
Effect	P value			
Relative elevation	0.0001	0.9050	0.0077	0.7636
Rel. elev. x Site-Year	0.0001	0.0537	0.0242	0.3185
Site-Year	0.0829	0.0001	<.0001	0.2568
Treatment	0.0037	0.0110	0.0568	0.3436
Site-Year x Treatment	0.3465	0.3462	0.6218	0.2220
Contrast Site-Year				
	P > t 			
2023 vs. 2024		0.4454	<.0001	
2023: Carman vs. Sperling		0.0032	0.6719	
2024: Carman vs. Sperling		0.0001	<.0001	

^a The '+' or '-' refers to "added" or "not added", respectively. ^b Side-banded at 5 cm beside and below the seed furrow. ^c Monoammonium phosphate. ^d MicroEssentials[®] SZ[®]. ^e Broadcast-incorporation. ^f Seed-row. ^g Foliar application at wheat Zadok's growing stages Z12 and Z65. ^h Biostimulants blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. ⁱ Means and standard error (Treatment, n=16; Site-Year, n=48) have been calculated using the LSMEANS function in PROC GLIMMIX in SAS. Contrasts for treatment effects are presented in Figure 2.2.

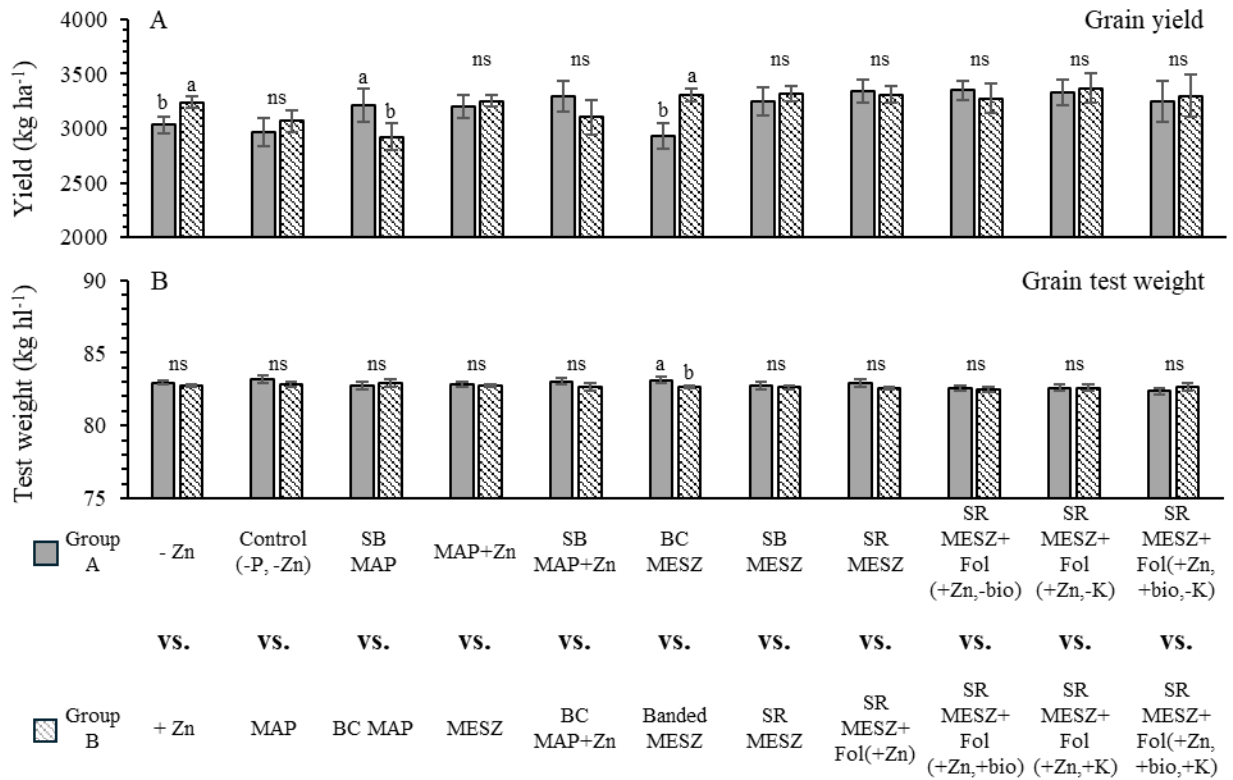


Figure 2.2 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain yield (A), and grain test weight (B) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=16$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

2.4.3 Straw Nutrient Concentration and Uptake

The relative elevation did not significantly affect straw nutrient, but it did affect nutrient uptake (Appendix Table A-1). Straw uptake of Fe, K, and P decreased with increasing relative elevation. Significant interactions between relative elevation and site-year were detected for straw concentrations of K and Zn, and for straw uptake of K, P, and Zn. Straw K concentration increased with elevation at three site-years, except at Sperling_2024, where it decreased. Straw Zn

concentration increased with elevation at Sperling_2023, was unchanged at Carman_2024, and decreased at Carman_2023 and Sperling_2024. Straw K uptake was stable at both Carman site-years and increased with elevation at both Sperling site-years. Straw P uptake was unchanged at both sites in 2023, decreased with elevation at Carman_2024, and increased at Sperling_2024. Straw Zn uptake was unchanged at Carman_2024, decreased at Carman_2023, and increased with elevation at Sperling in both years. These patterns suggest that elevation- and weather-driven differences in soil moisture strongly influence both straw biomass and nutrient uptake.

Straw P concentrations were highest at Carman_2024 and lowest at Sperling_2023, while Zn concentrations were consistently higher at Carman than Sperling in both years. In contrast, compared to 2023, greater straw yields in 2024 resulted in 7.0–8.8 times and 3.9–5.0 times higher straw P and Zn uptake at Carman_2024 and Sperling_2024, respectively. Similarly, straw Fe and K uptake were approximately 4–5 times higher in 2024 than in 2023, irrespective of sites.

Fertilizer treatments significantly affected straw Zn, P, and Fe concentrations, as well as Zn and Fe uptake. Contrast analysis showed that foliar Zn increased by approximately 50% straw Zn concentration and uptake (Appendix Figure A-7 and Figure A-8). Adding K to the foliar Zn + biostimulant reduced straw Zn concentration by 20.6% and uptake by 22.9% relative to Zn + biostimulant without K. Contrast analysis indicated apparent advantages of MESZ over MAP + Zn, banding over broadcast incorporation, and seed-row over side-band. However, these effects were confounded by the treatment structure, as foliar Zn was applied only with seed-row MESZ. Indeed, on average MESZ lowered straw Zn concentration and uptake by 13–14% compared with MAP + Zn (Appendix Table A-1). For MESZ, banding generally increased straw Zn concentration and uptake by 11.9% and 23.6%, respectively, compared to broadcast incorporation. Side-banded MESZ did not affect straw Zn concentration compared with seed-row application, but increased

uptake by 4.8%. For MAP+Zn treatments, compared with broadcast incorporation, side-banding increased straw Zn concentration and uptake by 16.3% and 28.2%, respectively. MAP alone did not differ from the control for straw Zn concentration or uptake.

Among site-years, Carman_2024 was the only site where adding Zn significantly increased straw Zn concentration and uptake (by 48.1% and 74.9%, respectively), and where MESZ outperformed MAP+Zn (by 77.7% and 85.6%, respectively), reflecting the greater Zn contribution from foliar applications (Figure 2.3 and Figure 2.4). Compared to broadcast-incorporation, side-banded MAP increased straw Zn concentration at Carman_2023 (41.6%), whereas increasing Zn uptake only at Sperling_2024 (49.9%). For MAP+Zn, side-banding increased straw Zn concentration relative to broadcast incorporation at Carman_2023 (35.3%) and Sperling_2023 (37.9%), while increasing uptake only at Sperling_2023. For MESZ, banding plus foliar application increased straw Zn concentration by 30.1–106.9% and uptake by 53.3–116.8% across all site-years, compared to broadcast-incorporation. Even compared to MESZ alone in the seed row, adding foliar Zn increased straw Zn concentration and uptake at all site-years, with the strongest response at Carman_2024 (89–96%). Foliar applications of biostimulants and K had limited effects at all site-years, except at Sperling_2023 where biostimulants alone or plus K reduced straw Zn concentration and uptake by approximately 20–50%.

For straw P, compared to broadcast-incorporation, side-banded MAP decreased P concentration by 14.1%. Banded MESZ resulted in similar P concentrations as broadcast-incorporation, while side-banded MESZ increased P concentration by 8.7% compared to seed-row placement. Surprisingly, MAP applications did not increase straw P concentrations above the control, and MAP+Zn had no impact on straw P concentrations and produced similar yields as MESZ. Overall, Zn addition

reduced straw P concentrations numerically by 6%, with the strongest reductions observed in foliar Zn treatments.

For straw Fe, Zn fertilization significantly reduced concentration and uptake by 10–12%. The strongest effect occurred in the SR-MESZ + foliar Zn treatment, where concentration and uptake declined by approximately 30%. In contrast, adding K to the foliar Zn + biostimulant spray increased straw Fe concentration and uptake by 35–39%. P fertilization had no effect. Among site-years, Carman_2024 was the only one where soil Zn applications significantly decreased straw Fe concentration, and where banded MESZ resulted in lower Fe concentration than broadcast-incorporation (Appendix Figure A-9). Foliar Zn also reduced Fe concentration at both sites in 2024. Placement effects for MAP+Zn were not consistent. Side-banding increased Fe concentration over broadcast-incorporation at Sperling_2023 but decreased it at Carman_2024. It had no effect at the other two site-years.

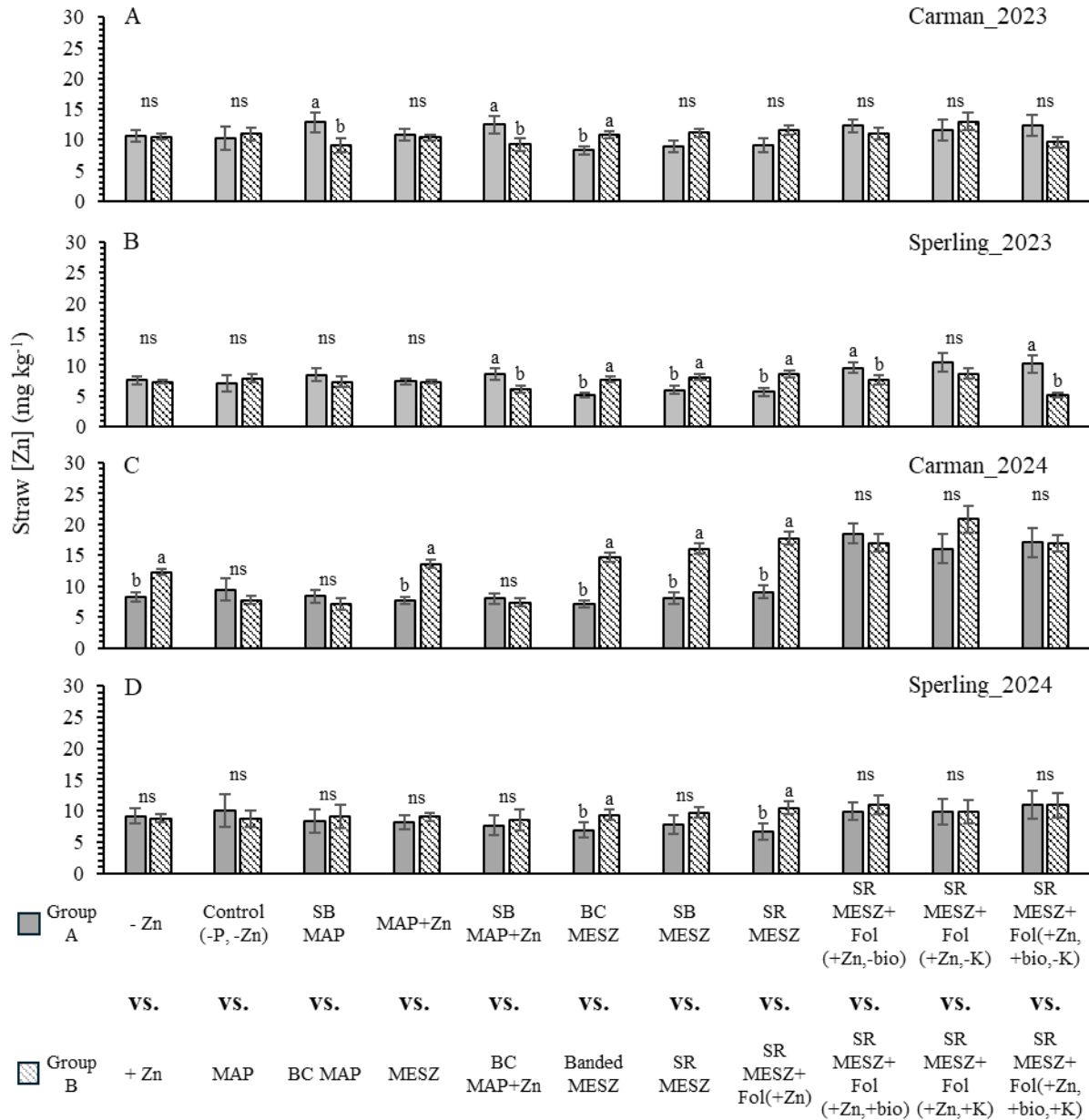


Figure 2.3 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw Zn concentration for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The '+' or '-' refers to "added" or "not added", respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok's growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

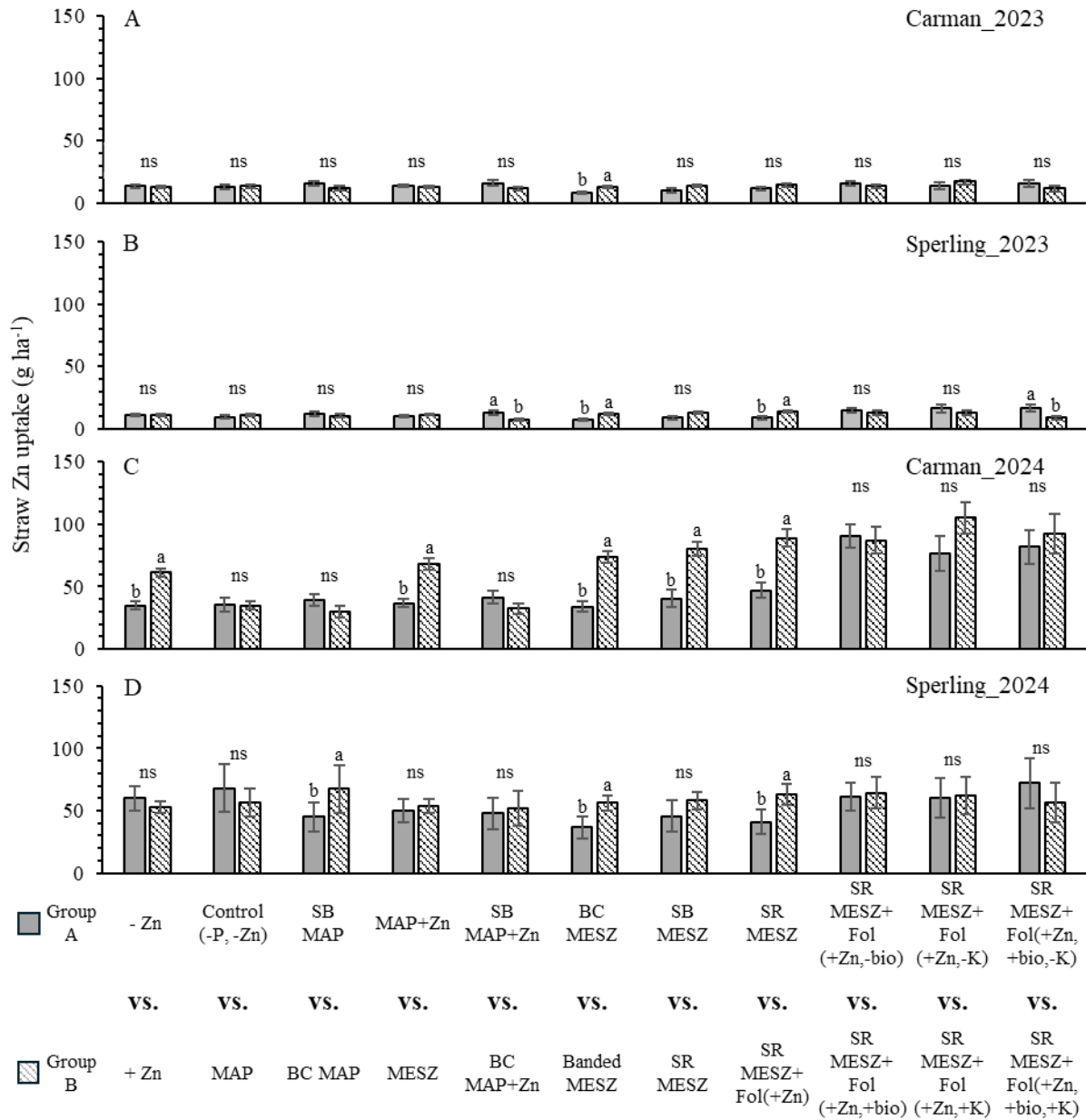


Figure 2.4 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw Zn uptake for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant differences. Error bars represent standard errors of the means ($n=4$). The '+' or '-' refers to "added" or "not added", respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok's growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

2.4.4 Grain Nutrient Concentration and Uptake

The relative elevation did not significantly influence grain concentrations of K, P, Zn, or phytic acid (PA) but did for Fe (Appendix Table A-2 and . Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6). In contrast, it significantly affected grain uptake of Fe, K, P, Zn, and PA, with uptake generally declining as elevation increased. A significant relative elevation \times site-year interaction was detected for grain PA concentration and for grain uptake of Fe, K, P, and Zn. In both years, grain PA concentration increased with elevation at Carman but decreased at Sperling. Uptake of Zn, P, and K increased with elevation at Sperling_2023 and Sperling_2024, decreased at Carman_2023, and remained relatively stable at Carman_2024. Grain Fe uptake increased with elevation at Carman_2023 and Sperling_2024, decreased at Carman_2024, and was relatively unchanged at Sperling_2023. These patterns likely reflect elevation-driven differences in soil

moisture and resulting yield variability. Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on grain concentrations and uptakes of P, Zn, phytic acid (PA), and phytic acid to Zn molar ratio (PA:Zn) at Carman and Sperling from 2023 to 2024.

Treatment	Grain concentration			Molar ratio	Grain uptake		
	P mg kg ⁻¹	Zn mg kg ⁻¹	PA g kg ⁻¹	PA:Zn	P kg ha ⁻¹	Zn g ha ⁻¹	PA kg ha ⁻¹
				Means (SEM) ⁱ			
1: Control (-P,-Zn) ^a	3789 (101)	33.1 (1.0)	10.6 (0.4)	31.9 (1.1)	11.3 (0.6)	99.8 (5.2)	31.2 (1.7)
2: SB ^b MAP ^c	3700 (87)	34.7 (1.5)	11.3 (0.7)	32.1 (2.0)	11.9 (0.6)	113.3 (6.9)	35.8 (2.6)
3: SB MAP+ Zn granular	3620 (93)	35.9 (1.0)	11.7 (0.6)	32.6 (1.9)	12.0 (0.6)	120.4 (5.9)	38.3 (2.4)
4: SB MESZ ^d	3860 (87)	30.7 (1.0)	11.7 (0.3)	38.0 (1.6)	12.5 (0.5)	101.4 (5.1)	37.6 (1.8)
5: BC ^e MAP	3987 (108)	33.3 (1.4)	10.9 (0.3)	32.8 (1.5)	11.6 (0.6)	99.0 (5.5)	31.7 (1.5)
6: BC MAP+ Zn granular	3991 (103)	33.2 (1.1)	11.4 (0.4)	34.2 (1.7)	12.4 (0.7)	104.9 (6.4)	35.0 (2.0)
7: BC MESZ	3792 (88)	30.9 (0.9)	11.1 (0.3)	36.3 (1.0)	11.1 (0.5)	91.5 (3.8)	32.4 (1.5)
8: SR ^f MESZ	3911 (117)	30.7 (1.6)	11.6 (0.3)	38.0 (2.1)	13.1 (0.6)	104.3 (5.7)	38.5 (1.7)
9: SR MESZ + Fol(+Zn) ^g	3564 (83)	38.4 (1.1)	11.0 (0.3)	28.6 (1.1)	11.9 (0.5)	129.6 (6.4)	36.3 (1.7)
10: SR MESZ + Fol(+Zn,+K)	3626 (92)	40.6 (1.4)	11.0 (0.3)	27.0 (1.1)	12.2 (0.6)	139.2 (7.8)	36.6 (1.8)
11: SR MESZ + Fol(+Zn,+bio ^h)	3665 (74)	40.6 (1.1)	11.5 (0.3)	28.0 (1.0)	11.9 (0.6)	133.6 (8.0)	36.6 (2.1)
12: SR MESZ + Fol(+Zn,+bio,+K)	3944 (102)	36.8 (1.0)	11.5 (0.3)	31.0 (1.1)	13.0 (0.7)	123.2 (7.8)	37.3 (2.2)
Site-Year							
Carman_2023	4211 (71)	40.5 (1.1)	13.0 (0.5)	31.4 (1.3)	9.0 (0.5)	89.5 (4.6)	26.4 (1.9)
Sperling_2023	3167 (44)	36.8 (0.9)	10.3 (0.3)	27.6 (1.0)	8.0 (0.4)	91.3 (3.9)	25.7 (1.7)
Carman_2024	4239 (55)	29.7 (0.7)	12.2 (0.4)	40.6 (1.5)	14.9 (0.7)	104.1 (4.4)	42.7 (2.8)
Sperling_2024	3631 (209)	33.0 (2.6)	9.9 (0.6)	31.2 (3.1)	20.0 (2.3)	188.5 (24.9)	55.4 (6.5)
ANCOVA							
Effect				P value			
Relative elevation	0.6586	0.2137	0.0628	0.5836	0.0008	0.0059	0.0002
Rel. elev. x Site-Year	0.2311	0.2579	0.0009	0.0286	0.0067	0.0005	0.2838
Site-Year	<.0001	0.0049	0.0008	0.0250	0.0041	0.0966	0.0132
Treatment	0.0049	<.0001	0.0377	<.0001	0.0383	<.0001	0.0003
Site-Year x Treatment	0.3183	<.0001	0.2970	<.0001	0.2134	0.0091	0.2608
Contrast Site-Year							
2023 vs. 2024	0.0296	<.0001	0.2109	0.0023	<.0001		<.0001
2023: Carman vs. Sperling	<.0001	0.0208	0.0001	0.0301	0.1640		0.8025
2024: Carman vs. Sperling	0.0109	0.2127	0.0041	0.0126	0.0240		0.0649

^a The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. ^b Side-banded at 5 cm beside and below the seed furrow. ^c Monoammonium phosphate. ^d MicroEssentials[®] SZ[®]. ^e Broadcast-incorporation. ^f Seed-row. ^g Foliar application at wheat Zadok’s growing stages Z12 and Z65. ^h Biostimulants blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. ⁱ Means and standard error (Treatment, n=16; Site-Year, n=48) have been calculated using the LSMEANS function in PROC GLIMMIX in SAS. Contrasts for treatment effects are presented in Figure 2.5 & Figure 2.6, and contrast for interaction of site-year by treatment in Figure 2.7 – Figure 2.9.

Treatment contrasts (Figure 2.5 and Figure 2.6) showed that foliar Zn application significantly increased grain Zn concentration (27.2%) and uptake (25.9%). Adding K to the Zn+biostimulant foliar significantly reduced grain Zn concentration by 9.3% and numerically lowered uptake by 7.8%. Using SR MESZ + foliar Zn as the baseline, adding biostimulant had no effect, whereas adding K (without biostimulant) produced small, non-significant increases in concentration (5.8%) and uptake (7.4%). Apparent advantages of MESZ banding over broadcast and seed-row placement over side-band were confounded because foliar Zn was applied only with seed-row MESZ. Compared with MAP+Zn, MESZ resulted in lower grain Zn concentration and uptake by 11.0–12.1% (. Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6). For MESZ, banding versus broadcast had little impact on grain Zn concentration but increased uptake by 12.4%. Compared to broadcast-incorporation, side-banding MAP+Zn

increased grain Zn concentration and uptake by 8.1% and 14.7% respectively. MAP alone did not differ from the control for grain Zn concentration or uptake.

Among site-years, Sperling_2023 and Carman_2024 showed the strongest grain Zn responses, specifically in SR MESZ + foliar Zn treatments (T9–T12) at Z12 and Z65 (Figure 2.7 and Figure 2.8). At Carman_2024, foliar Zn to SR MESZ significantly increased grain Zn concentration by 15.0 mg kg⁻¹ (+60%) to 40.0 mg kg⁻¹; adding biostimulant or K raised concentrations further to 41.9 and 41.1 mg kg⁻¹ but were not significantly different. At Sperling_2023, foliar Zn to SR MESZ increased grain Zn concentration by 8.8 mg kg⁻¹ (+27.4%) to 40.9 mg kg⁻¹ according to foliar treatment average. Taken individually, Zn concentration increased as follow: biostimulant < K < foliar Zn alone with the respective concentration 43.0, 43.1, and 44.7 mg kg⁻¹. Carman_2023 and Sperling_2024 showed similar numerical trends, despite being statistically insignificant. Uptake mirrored these effects, with foliar Zn application significantly increasing Zn uptake by 28.7% at Sperling_2023 and 57.0% at Carman_2024.

Placement and product contrasts showed mixed results across site-years. For MESZ, compared to seed-row placement, side-banding increased grain Zn concentration by 3.8% at Carman_2023 and 6.7% at Carman_2024, while decreasing it by 11.0% at Sperling_2024 and had no impact at Sperling_2023. In contrast, side-banding increased Zn uptake by 6.5% at Carman_2024, decreased it by 4.9% at Sperling_2023, and by 12.1% at Sperling_2024, while showing no impact at Carman_2023. Compared to broadcast-incorporation, side-banding MESZ decreased concentration by 7.1% at Carman_2023 and 20.1% at Sperling_2024 and showed no effect at other site-years. However, due to contrasting yield influences, grain Zn uptake at Sperling_2024 was 14.3% lower for side-banding, while that at other site-years was 5.8% to 35.3% higher for side-banding, compared with broadcast-incorporation. For MAP+Zn and MAP alone, side-banding

(43.71 and 45.4 mg kg⁻¹ respectively) significantly increased Zn concentration and uptake at Sperling_2023 by approximately 34% over broadcast-incorporation. Compared to the control, MAP resulted in significantly higher Zn concentration and uptake at only Sperling_2023 (~23%), but not at other site-years. Overall, Carman_2024 was the only site with consistent, statistically significant increases in both grain Zn concentration (22.9%) and uptake (30.7%) when Zn was added, reflecting its low background Zn levels (Table 2.1).

For grain P, concentrations were consistently higher at Carman than Sperling, while greater yields in 2024 led to 1.7 times and 2.5 times higher P uptake at Carman_2024 and Sperling_2024, compared to 2023 (. Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6). Contrast analysis showed that, relative to broadcast-incorporation, side-banded MAP lowered P concentration by 7.2% but did not affect uptake (. Grain Zn concentration was

significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6). For MESZ, banding resulted in P concentration and uptake similar to broadcast-incorporation, whereas within banded placements, side-banding increased uptake by 15.3% over seed-row placement. MAP did not differ from the control. For MAP+Zn, compared with broadcast-incorporation, side-banding decreased P concentration by 9.3% but did not affect uptake. Foliar Zn in combination with seed-row MESZ significantly lowered grain P concentration by 5.4% but did not affect uptake relative to the corresponding treatment without foliar Zn. Adding biostimulants and K to the foliar Zn increased grain P concentration by 7.6% and uptake by 9.2% compared with foliar Zn alone.

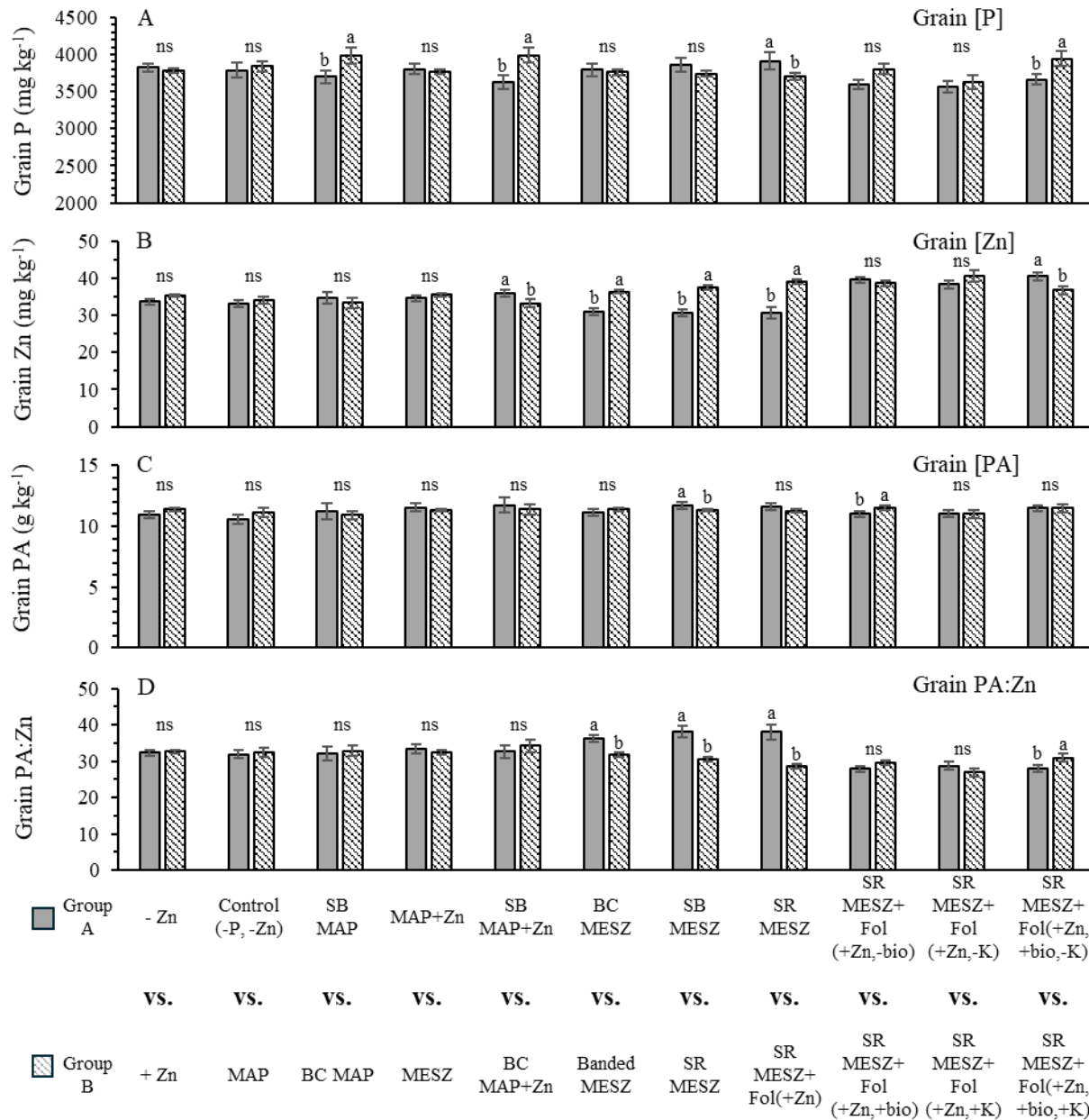


Figure 2.5 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain concentrations of P (A), Zn (B), phytic acid (PA) (C), and grain phytic acid to Zn molar ratio (PA:Zn) (D) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials® SZ®. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

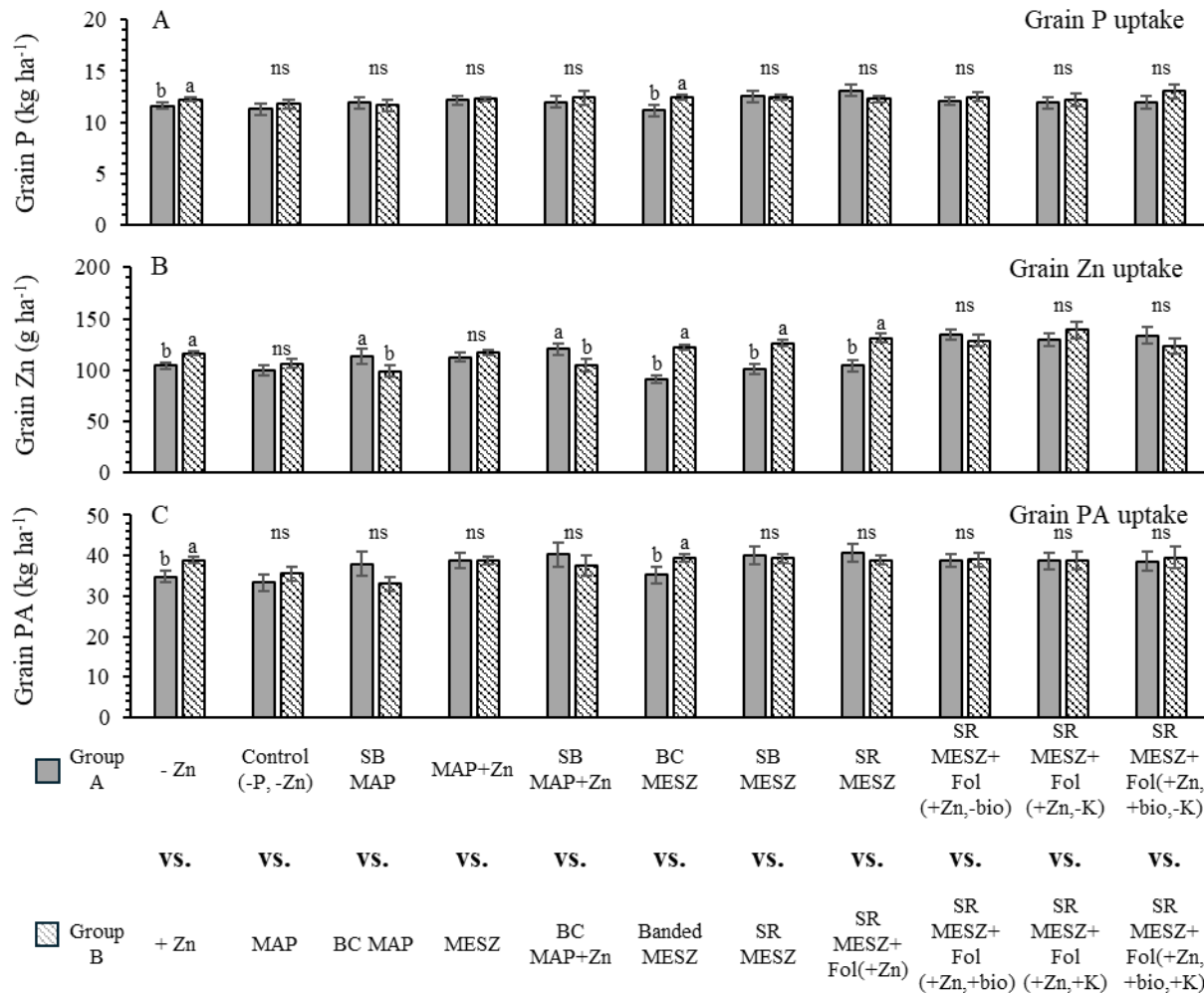


Figure 2.6 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain uptake of P (A), Zn (B), and phytic acid (PA) (C) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials® SZ®. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

Similar to grain P, grain concentrations and uptake of PA were affected by fertilizer treatments and site-years, but not their interaction (. Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6). PA concentrations were consistently higher at Carman than at Sperling in both years. Greater yields in 2024 led to 1.6- and 2.2-fold higher PA uptake at Carman_2024 and Sperling_2024, respectively, compared to 2023. Compared to control, MAP alone did not significantly change PA concentration and uptake, while adding Zn had little effect on PA concentration but increased uptake significantly by 11.7%, consistent with yield gains. PA concentration and uptake did not differ between MAP+Zn and MESZ. For MESZ, side-banding increased PA concentration by 3.3% and uptake by 1.9%, compared to seedrow placement. Foliar applications generally did not affect PA, except that foliar Zn without biostimulants (T9 and T10) slightly decreased PA concentration by 4.1%.

Grain K concentration was affected only by site-year, whereas grain Fe concentration was affected only by fertilizer treatment. In contrast, K and Fe uptake were influenced by both treatment and site-year, with no interaction (Appendix Table A-2). Grain K concentrations were highest at Sperling_2024 and lowest at Sperling_2023. Higher yields in 2024 led to 1.6–4.5 times higher K and Fe uptake than in 2023. Contrast analysis showed that foliar Zn reduced grain Fe concentration by 28.6% and uptake by 28.0%, respectively (Appendix Figure A-10). Adding K to the foliar Zn + biostimulant treatment significantly increased grain Fe concentration by 29.3% and numerically increased uptake by 30.5%. Compared to control, MAP did not affect grain Fe. The placement effect was only evident for MAP+Zn, where side-banding decreased grain Fe concentration by 38.0% and uptake by 34.0% compared to broadcast-incorporation. For grain K, adding Zn increased uptake by 7.5% compared to the treatment without Zn. Compared to broadcast-incorporation, banding MESZ significantly increased grain K uptake by 18.4%. MAP+Zn and MESZ did not differ significantly.

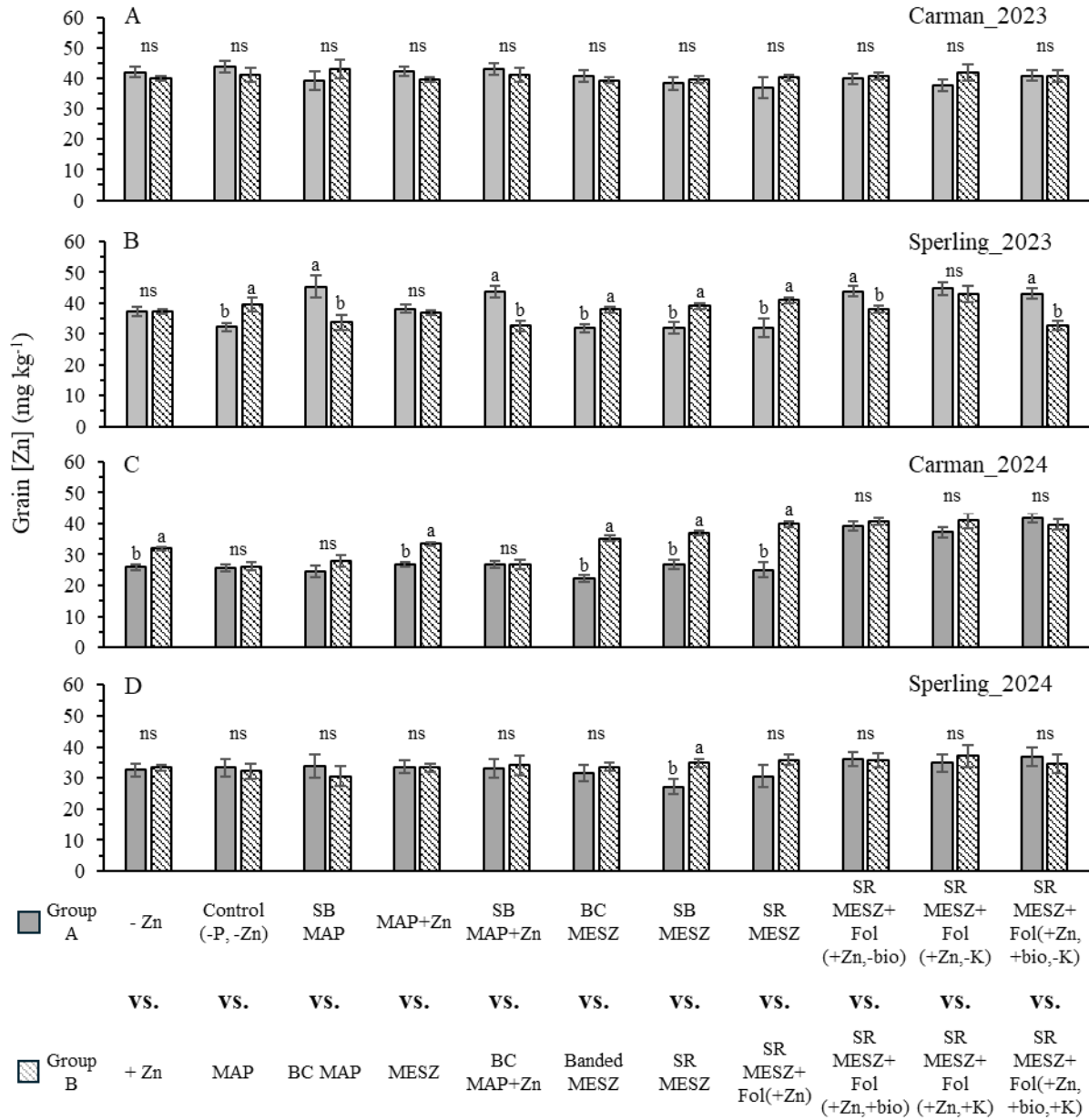


Figure 2.7 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain Zn concentration for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The '+' or '-' refers to "added" or "not added", respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below the seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials® SZ®. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok's growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

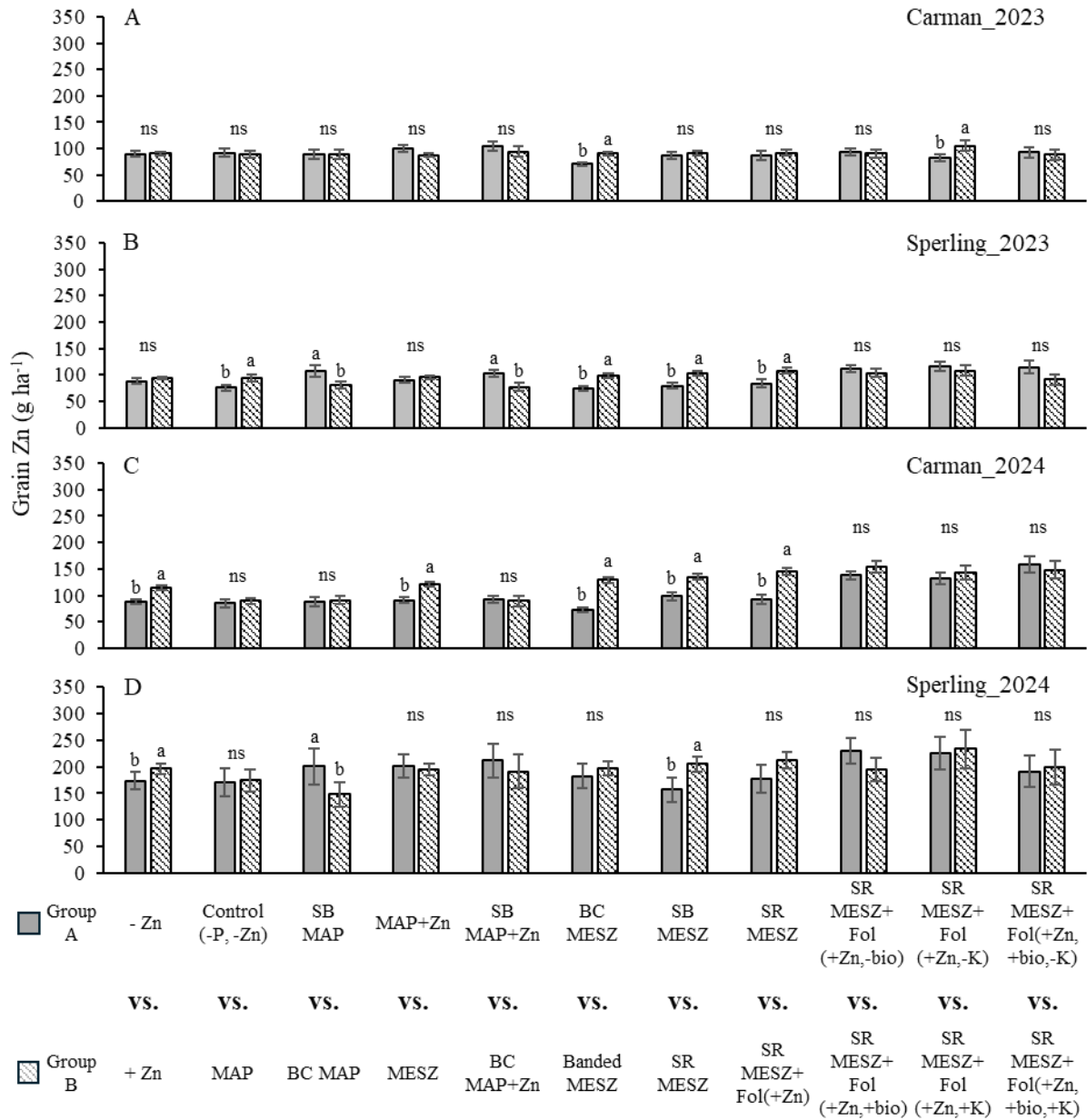


Figure 2.8 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain Zn uptake for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The '+' or '-' refers to "added" or "not added", respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below the seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials® SZ®. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok's growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

2.4.5 Grain Zn Bioavailability

Grain Zn bioavailability, expressed as the phytic acid to Zn (PA:Zn) molar ratio, was significantly affected by the relative elevation \times site-year interaction, even though grain Zn and P concentrations were unaffected (. Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions.

Table 2.6). In both years, PA:Zn increased with elevation at Carman but decreased at Sperling. Among site-years, PA:Zn were highest at Carman_2024 (40.6), followed by Carman_2023 (31.4) and Sperling_2024 (31.2), with the lowest value at Sperling_2023 (27.6).

Across treatments, PA:Zn ratios ranged between 27.0 and 38.0. Adding foliar Zn to SR MESZ reduced PA:Zn by 24.6% relative to SR MESZ alone, improving Zn bioavailability (Figure 2.5). In contrast, adding K to the foliar solution increased the molar ratio by 10.5%, reducing bioavailability. Although contrast analysis suggested improved Zn bioavailability with MESZ over

MAP + Zn, banding over broadcast incorporation, and seed-row over side-band, these effects were confounded by the treatment structure because foliar Zn was applied only with seed-row MESZ. Consistent with treatment means Table 2.1, MESZ increased PA:Zn by 12.0% versus MAP + Zn, lowering bioavailability (. Grain Zn concentration was significantly affected by fertilizer treatment, site-year and their interactions (**Error! Not a valid bookmark self-reference.**). The interaction likely reflects contrasts in baseline Zn fertility, and weather on Zn fertilizer uptake. Concentrations were highest at Carman_2023 and lowest at Carman_2024. At Carman_2023, severe drought likely limited fertilizer dissolution and root uptake; even foliar Zn did not raise grain Zn, and the elevated concentrations are best explained by yield reduction. By contrast, Sperling_2023, also dry, showed clearer fertilizer responses than Carman_2023, plausibly because greater soil moisture. Within the Sperling sites, more treatments responded in 2023 than in the wetter 2024 season; we speculate that preceding canola at Sperling_2023 reduced arbuscular mycorrhizal fungi, shifting the crop's reliance toward applied fertilizer. Despite lower concentrations in 2024, higher yields increased total Zn removal: grain Zn uptake was 1.2 times higher at Carman_2024 and 2.1 times higher at Sperling_2024 compared to 2023, indicating that the applied Zn rate was insufficient to sustain biofortification targets in high yielding conditions. Table 2.6). For MESZ, banding slightly lowered PA:Zn by 4.7% compared to broadcast-incorporation, whereas there was no difference between side-band and seed-row placements. Placement effects were negligible for MAP and MAP+Zn, and MAP alone did not differ from the control.

At all site-years, on the trial average, Zn application did not affect PA:Zn when compared to control treatments without Zn. However, when looking into more specific treatment, compared to soil application alone, soil plus foliar Zn decreased PA:Zn at three site-years, being 39.6% at

Carman_2024, 24.8% at Sperling_2023, and 21.2% at Sperling_2024. At Sperling_2023 only, combining SR MESZ with foliar applications of Zn, biostimulants, and K increased PA:Zn by 34.9%, indicating reduced Zn bioavailability. Surprisingly, MAP alone increased PA:Zn at Carman_2023 (20.3%), decreased it at Carman_2024 (20.0%), whereas had no impact at Sperling in both years.

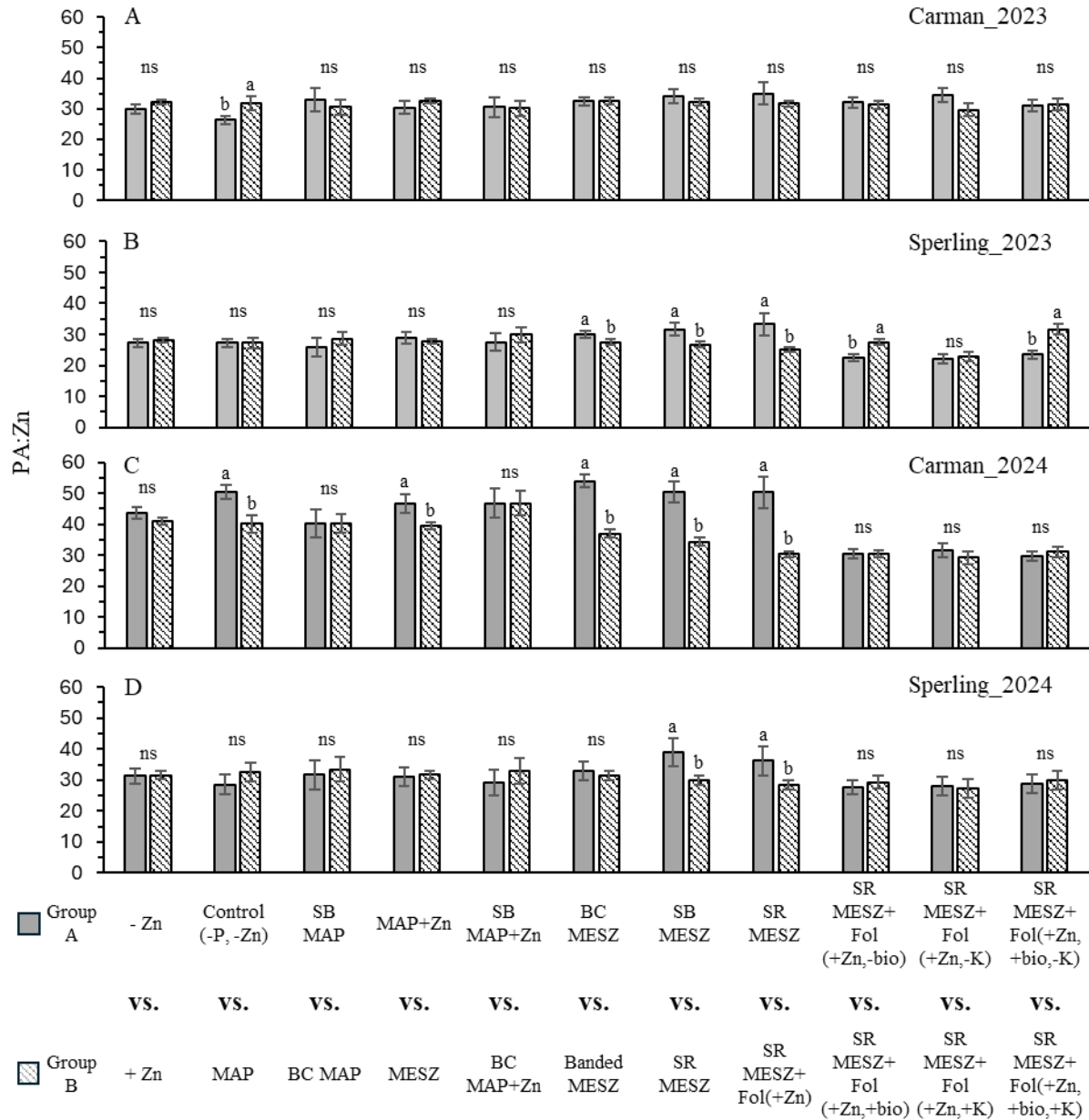


Figure 2.9 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat grain phytic acid to Zn molar ratio (PA:Zn) for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials® SZ®. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

2.4.6 Relationships of Grain Zn and P Concentrations with Grain Yield, Protein, and Other Nutrients

Across the four site-years, grain Zn concentration was associated negatively ($P < 0.001$) with yield ($r = -0.338$) and protein ($r = -0.244$), while positively ($P = 0.0132$) with grain K ($r = 0.179$) (Figure 2.10). There was no significant relationship between grain Zn and grain P, Fe, or PA concentrations. Grain P correlated ($P < 0.0001$) positively with grain PA ($r = 0.480$), Fe ($r = 0.357$), and K ($r = 0.861$) concentrations and showed no relationship with yield (Figure 2.11).

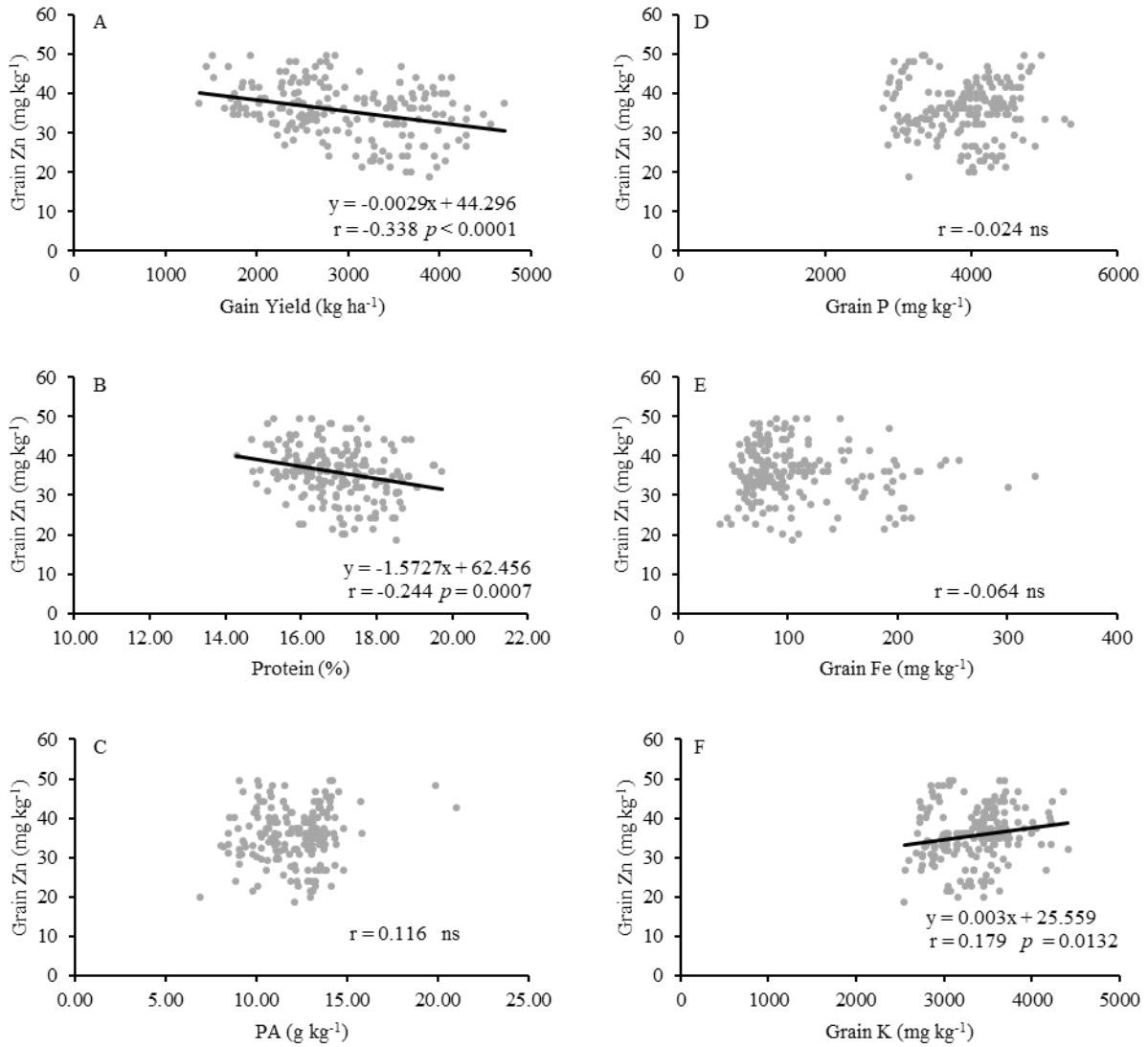


Figure 2.10 Linear relationships of grain Zn concentration with grain yield (A), protein (B), phytic acid (PA) (C), P (D), Fe (E), and K (F) concentrations.

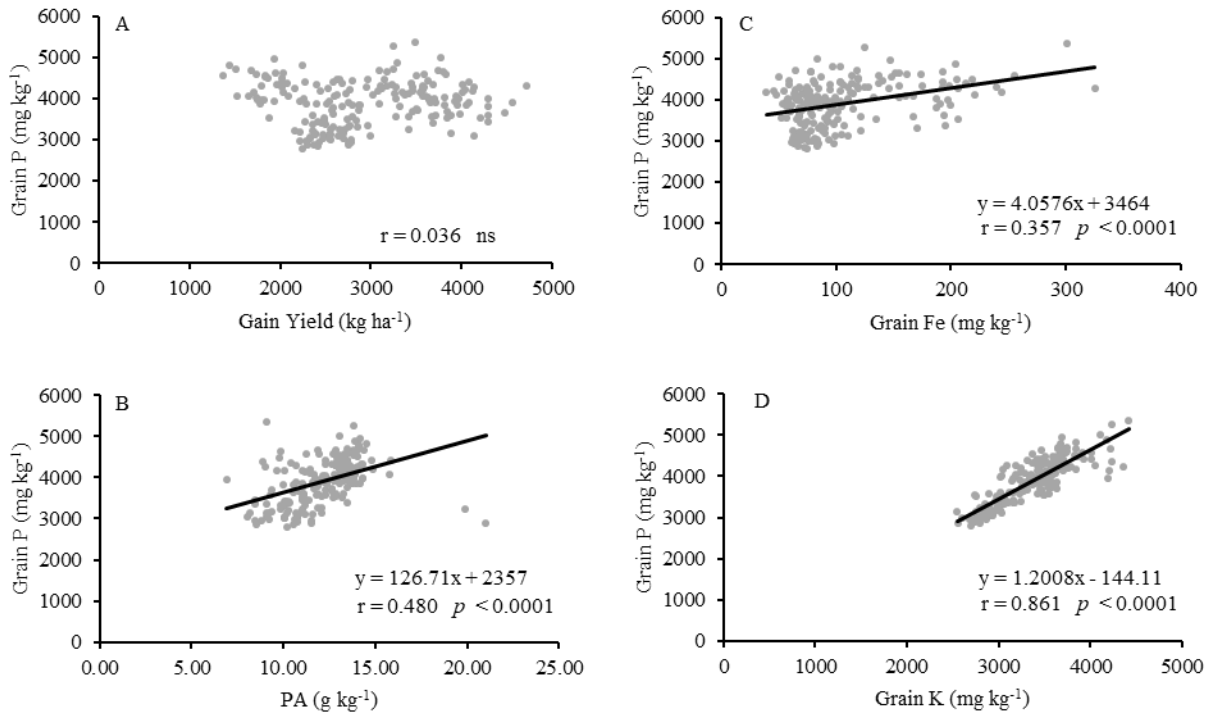


Figure 2.11 Linear relationships of grain P concentration with grain yield (A), phytic acid (PA) (B), Fe (C), and K (D) concentrations.

2.5 Discussion

2.5.1 Effects of P and Zn Fertilization Strategy on Spring Wheat Establishment, Development, and Yield

Early plant densities were used to detect a potential salt injury from concentrated fertilizer near the germinating seed. In this study, we did not observe apparent salt injury from any treatment, and differences in counts mainly reflected seedbed and moisture variability in early stage. This aligns with observations that wheat has relatively high salt tolerance (Grant and Flaten, 2019) and that seed-placed P rarely reduces wheat stands (Karamanos et al., 2017), since later plant densities did not differ significantly between treatments. However, in soil with less buffering capacity a chelated-Zn (e.g. EDTA) could be preferable with its low salt index.

Early-season biomass was greater with banded fertilizer, underscoring the importance of P placement under cold prairie spring conditions (Grant and Bailey, 1998; Hopkins and Ellsworth, 2005; Grant and Flaten, 2019). This effect was accentuated when P was combined with Zn, which can improve seedling vigor. However, the early advantage of banding diminished as crops approached reproduction, leaving Zn addition as the main factor supporting greater biomass, likely attributable to improved moisture conditions (Ghanem et al., 2025), which improve yield potential, and lower background Zn fertility, particularly at Carman. Band placement increased late-season biomass more for MAP than for MESZ, consistent with Mooleki et al. (2010). Also, the combination of the seedrow MESZ with foliar Zn with K showed an important biomass gain at 10 DAF when compared to foliar treatment with Zn only. This result may be attributable to a greater number of tillers for this treatment, finding supported by a study of Zafar et al. (2016). However, the exact mechanism contributing to the biomass increase remains to be determined.

Yield response and ultimately economic return, drive the adoption of new agronomic practices. In this study, Zn supply increased wheat yield, contrasting with results from the P-deficient sites from Saskatchewan (Rahman et al., 2022), suggesting that soil P fertility is one of the factors that determines yield response to Zn. Supporting evidence from a pot study on alkaline soil showed a stronger yield response to Zn under optimal P conditions than under low P conditions (Naeem et al., 2018). In this study, yields with MAP application were similar to those of the control, indicating that soil P was not limiting and crop Zn response was evident where P fertility was sufficient. Compared with soil application only, soil plus foliar Zn did not significantly increase yield, unlike results from several Middle Eastern studies (Cakmak, 2010; Zou et al., 2012; Zarea, 2025). These lack of response in this studies suggest that Zn was not the most limiting factor for yield improvement. Grain yield also did not differ between MAP+Zn (granular ZnSO₄) and MESZ, supporting that uniform distribution of low Zn doses can be as effective as higher, uneven applications. By contrast, banded MAP, MAP+Zn, and MESZ improved yield over broadcast incorporation, in line with Canadian prairie P studies (Strong and Soper, 1974; Grant and Bailey, 1998; Grant et al., 2001; Grant and Flaten, 2019). Since there was no difference between seed-row and side-band placement for MESZ, considering equipment costs, seed-row MESZ may be economically attractive, pending budget analysis.

2.5.2 Nutrient Uptake and PA Concentration

Soil-applied Zn rates in this study were based on practical recommendations to maximize yield potential rather than to meet biofortification targets. Further studies should test alternative Zn sources and combinations, rates, and banding locations, with and without additives, to identify strategies that supply Zn for the whole growing season and deliver both yield and improved grain Zn concentration and bioavailability. The limited time for optimal soil-Zn supplies helps explain

why combining soil-applied and foliar Zn clearly increased grain and straw Zn concentrations. Treatment effects on Zn concentration were generally similar for straw, but differences were more pronounced in treatment that received foliar applications, suggesting greater Zn immobilization in stem tissues. MESZ treatments tended to result in lower Zn concentrations, likely due to lower Zn rates or slower Zn release from MESZ than from ZnSO₄ granules. Additionally, the presence of ammonium sulfate in non-MESZ treatments may have acidified the rhizosphere and thus increased Zn solubility.

Grain and straw Zn concentrations and uptake were generally higher with side-band fertilizer placement. This contrasts with Montalvo et al. (2016), who reported that co-banding Zn with P fertilizers in alkaline soils resulted in greater Zn precipitation than broadcasted application. In our study, the highest grain Zn concentrations were observed with side-banded MAP+Zn, although a significant effect was detected only at Sperling_2023. This response was likely due to the 2023 drought, together with the high water-holding capacity of clay soil and relatively cold conditions, which slowed fertilizer dissolution in the band, limiting immediate Zn–P precipitation following application but enabling later uptake as roots developed (Caldwell et al., 1998). A second, site-specific factor may also have contributed: Sperling_2023 followed canola, a non-mycorrhizal Brassicaceae crop that can suppress arbuscular mycorrhizal fungi, potentially altering nutrient uptake from the soil reserve. Banded MESZ did not show the same effect, likely due to the lower Zn rate supplied with this product. MAP alone also increased Zn uptake at the Sperling sites, which had the highest DTPA-Zn, highlighting the role of early P supply in stimulating root growth and exploration (Grant and Bailey, 1998), thereby enhancing Zn acquisition (Havlin et al., 2014). These findings support our first hypothesis that banding Zn fertilizer, particularly MAP+Zn, is the most effective soil-applied strategy to increase grain Zn uptake, especially in fine-textured soils where

ZnSO₄ is less prone to leaching. By contrast, MESZ may be more suitable for coarse-textured soils under wet conditions. These observations are based on limited number of site-year, additional multi-site, multi-year trials are needed to validate and refine these recommendations for the Canadian Prairies.

For P, both straw and grain concentrations responded similarly across fertilizer treatments. While grain P uptake was higher with side-banded placement, P concentrations were reduced, likely due to yield dilution. Foliar Zn combined with soil MESZ application reduced grain and straw P concentration, whereas adding biostimulants and K to this treatment maintained higher P levels. These results suggest that Zn application had a greater impact on root P uptake than on within plant translocation, consistent with Zn-mediated down-regulation of P uptake in roots, where adequate Zn control P transporter protein at the root surface (Marschner, 2012; Ding et al., 2021). Soil-applied Zn at seeding had no such effect, highlighting lower Zn availability later in the season. We also observed that MAP did not increase grain yield or P concentrations in grain over control, suggesting the background soil P fertility was sufficient and that P fertilizer primarily replenishes soil reserves rather than directly raising crop P uptake. Consequently, PA concentration in grain was more influenced by site-year than by fertilizer treatments. For example, even with relatively high application rates, MAP did not increase grain PA concentration and production relative to the control, and foliar Zn did not affect PA significantly. These results contrast with our hypothesis that P fertilization would increase grain PA concentration and with earlier reports by Yang et al. (2011a), Bharti et al. (2013), and Saha et al. (2020), potentially due to the higher Zn rates used in those studies.

For other nutrients, high background soil K at both sites resulted in similar grain and straw concentrations across fertilizer treatments. Fe concentrations mostly increased when P and Zn

fertilizers were broadcast-incorporated, likely due to broader root exploration under less concentrated nutrient zones (Rahman et al., 2022). In contrast, foliar Zn application suppressed Fe uptake in straw and grain, suggesting that Zn could compete with Fe for transport and protein-binding sites in plants (Stanton et al., 2022). The impacts of foliar Zn on other nutrient availability warrant further investigation, especially for subsequent crops such as soybean that are more susceptible to Fe deficiency. Nevertheless, grain Fe concentrations in this study remained well above the 59 mg kg⁻¹ threshold, indicating adequate levels for plant growth and human nutrition (Roy et al., 2022).

2.5.3 Wheat Zn Biofortification with Foliar Application

Across the four site-years, combining foliar Zn with soil-applied Zn was essential to increase grain Zn concentration while countering the dilution effect induced by yield gain. Still, grain Zn correlated negatively with yield in this study. Similar yield-Zn trade-offs have been reported by studies across the world (Cakmak et al., 2010; Zou et al., 2012; Cakmak and Kutman, 2018; Zhao et al., 2018; Tran et al., 2021; Rahman et al., 2022; Hou et al., 2025), emphasizing the importance of Zn fertilization in achieving both grain yield and Zn enrichment. Consistent with earlier studies and with the exception of Carman_2023, soil-applied Zn alone, ranging from 2.5 kg Zn ha⁻¹ in this study to 11.4 kg Zn ha⁻¹ in Zou et al. (2012) was insufficient to raise grain Zn concentration above the 40 mg kg⁻¹ threshold target for dietary intake (Cakmak and Kutman, 2018; HarvestPlus, 2025), especially under high-yield conditions. For Carman_2023, yield potential and root uptake were extremely limited that the higher Zn concentration is not attributable to the fertilization strategies. It is worth noting that a different source of Zn, such as EDTA, could have potentially increased the Zn concentration even further. However, this approach included an effect from a different variable and would have increased the Zn foliar product cost by at least one-fold.

Despite lower application rates and different application timings than in previous studies, foliar Zn application showed notable enrichment in grain Zn in this study. Across sites, foliar Zn consistently raised grain Zn concentration, but was only significant at two sites, supporting our hypothesis that combining soil-applied and foliar Zn is more effective than soil application alone. Adding foliar K produced modest further increase, but not significant, suggesting that K supply could improve Zn uptake (Naeem et al., 2018). The biostimulant mix (salicylic acid + betaine) produced comparable Zn concentrations to foliar K, likely through different mechanisms. K supply improves plant water status, stomatal conductance, enzyme activation, and osmoregulation, thereby enhancing P and Zn uptake and translocation to grain, especially under drought conditions (Naeem et al., 2018; Safar-Noori et al., 2018). In contrast, salicylic acid and betaine act primarily as antioxidants and osmoprotectants (Dubey et al., 2021), mitigating oxidative stress and improving cell turgor to maintain active nutrient uptake and translocation in the plant (Shemi et al., 2021; Nieves-Cordones et al., 2019). Taken together, in a context where Zn is not a limiting yield factor, increasing foliar Zn rate, combined with optimized timing and consideration of biostimulant mix, might be the most effective strategy for Zn biofortification, consistent with the meta-analysis of Hui et al. (2025). However, the lack of a strong yield increase with the addition of foliar products and their related cost ($\approx 50\text{--}70$ \$ ha⁻¹ for foliar Zn and biostimulant or K applied twice), made this strategy not sustainable economically.

2.5.4 Impact of P and Zn Fertilization Strategy on Phytic Acid to Zn Molar Ratio

The phytic acid to Zn (PA:Zn) molar ratio is a widely used indicator of Zn bioavailability in cereal grains. According to WHO (1996), a diet with a PA:Zn ratio below 15 is necessary to positively impact human Zn nutrition. Lowering the PA:Zn ratio in wheat is therefore critical in regions where wheat is a staple food. . In this study, the lowest PA concentration was observed at Sperling_2024

(9.9 g kg⁻¹). To achieve PA:Zn \leq 15 at this PA level, grain Zn needs to exceed 65 mg kg⁻¹, a level reported in some trials (Cakmak et al., 2010; Zou et al., 2012) but unlikely to be agronomically or economically feasible under Prairie conditions.

In this study, PA:Zn responses to P fertilization varied across site-years. While P application generally increased PA:Zn ratios, a few exceptions were observed, such as at Carman 2024 (Figure 2.9). These inconsistencies lacked a clear site-specific explanation. Granular Zn additions to MAP generally did not improve bioavailability, and MESZ treatments often increased PA:Zn due to lower grain Zn concentrations. Fertilizer placement did not significantly impact Zn bioavailability, contrasting with Zhao et al. (2018), likely due to the lower Zn application rates used in this study.

The most notable improvements in Zn bioavailability were achieved when foliar Zn applications were combined with seed-row MESZ. However, when looking at treatment average, the data suggests that the response would have probably been stronger if the foliar Zn was applied to the MAP+Zn treatment. This supports our hypothesis that foliar Zn application would reduce the PA:Zn ratio and thus improve Zn bioavailability. These observations are consistent with findings in rice (Saha et al., 2020), winter wheat (Yang et al., 2011a; Wang et al., 2020), and spring wheat (Cakmak, 2008; Esfandiari et al., 2016). Adding K to foliar Zn did not consistently improve PA:Zn, aligning with findings in wheat (Gu et al., 2023). The biostimulant mix did not affect the molar ratio when combined with foliar Zn, though at Sperling_2023 PA:Zn increased, likely due to strong yield gains and attendant dilution of grain Zn.

In this study, no fertilizer treatment reduced PA:Zn below 15. However, the PA:Zn metric of unprocessed wheat whole grain likely overestimates values in typical end-use products, since milling, fermentation, and cooking reduces phytic acid in flour, bread, and other wheat-based products (Sathe and Venkatachalam, 2001; Gibson et al., 2018). Future work should set whole-

grain targets that account for phytate reductions during milling/fermentation/cooking of end-use products. From a practical standpoint, foliar Zn applications improved PA:Zn but may be difficult to justify economically in the Prairies, where yield responses are generally small and premiums for nutrient density are not offered, unlike occasional protein premiums. Additionally, we did not observe a positive relationship between grain Zn and protein, as reported in other studies (Hou et al., 2025). Adoption of foliar Zn will depend on its integration into existing spray passes at minimal additional cost or on the emergence of market or policy incentives.

2.6 Conclusion

Through plot-based field trials across multiple years and sites, this study demonstrated how different fertilization strategies affect spring wheat productivity and grain nutritional quality under Prairie conditions. Foliar Zn applications were the most effective strategies at increasing grain Zn concentration and were the only treatments to increase Zn concentration over the lower limit of the 40–50 mg Zn kg⁻¹ dietary target. Contrary to expectations, P fertilization did not reduce Zn bioavailability. Instead, Zn fertilization emerged as the key driver of improved bioavailability, especially in offsetting the yield-associated dilution of Zn concentration. Fertilizer placement significantly influenced yield, with side-banding consistently outperforming broadcast-incorporation across formulations. Responses varied across site-years, making difficult to promote any recommendations at this point. Despite these gains, no treatment reduced the PA:Zn molar ratio below the nutritional threshold of 15. Higher foliar Zn rates combined with seed-row applied MESZ should be explored for the potential to achieve such a target, though substantially greater Zn inputs may be required. Nevertheless, for Prairie wheat production systems where yield responses to Zn applications are generally small, Canadians are generally not Zn deficient, and there is no price premium for nutrient density of marketed grains, meanings there is currently no

return on investment for farmers. This would need to change before there is large-scale adoption of biofortification strategies. In addition, the distribution system is not adapted for a small and progressive adoption and may not be economically viable for smaller marketers to adopt a bagging strategy like the edible bean distributor did. Further research should integrate Zn biofortification into diverse farming systems and optimize foliar Zn rates, potentially with biostimulants, to balance nutritional quality, agronomic performance, and profitability. Overall, the findings provide practical insights for enhancing Zn bioavailability in commercial spring wheat production, while highlighting the need for both agronomic and economic feasibility.

2.7 References

- Aldesuquy, H.S., Abbas, M.A., Abo-Hamed, S.A., Elhakem, A.H., and Alsokari, S.S. 2012. Glycine betaine and salicylic acid induced modification in productivity of two different cultivars of wheat grown under water stress. *J. Stress Physiol. Biochem.* **8**: 72–89. [Online] Available: <https://www.researchgate.net/publication/233916194> [Accessed 6 Aug. 2025].
- AgriMaps 2025. Manitoba AgriMaps. Manitoba Government. [Online] Available: <https://experience.arcgis.com/experience/90713dfec03b47d6a754628191cbeb4a/page/Page> [Accessed 7 Sep. 2025].
- Alessi, J., and Power, J.F. 1980. Effects of banded and residual fertilizer phosphorus on dryland spring wheat yield in the Northern Plains [USA]. *SSSA J.* **44**: 792–796. doi:10.2136/sssaj1980.03615995004400040027x.

Alloway, B.J. 2008. Zinc in Soils and Crop Nutrition. 2nd edition. IZA and IFA, Brussels, Belgium and Paris, France. 139pp. [Online] Available: <https://www.fertilizer.org/resource/zinc-in-soils-and-crop-nutrition/> [Accessed 30 Aug. 2025].

Bharti, K., Pandey, N., Shankhdhar, D., Srivastava, P.C., and Shankhdhar, S.C. 2013. Improving nutritional quality of wheat through soil and foliar zinc application. *Plant Soil Environ.* **59**: 348–352. doi:10.17221/158/2013-PSE.

Bertrand, I., Holloway, R.E., Armstrong, R.D., and McLaughlin, M.J. 2003. Chemical characteristics of phosphorus in alkaline soils from southern Australia. *Aust. J. Soil Res.* **41**: 61–76. doi:10.1071/SR02021.

Borrill, P., Connorton, J.M., Balk, J., Miller, A.J., Sanders, D., and Uauy, C. 2014. Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. *Front. Plant Sci.* **5**: 53. doi:10.3389/fpls.2014.00053.

Bouis, H.E. 2003. Micronutrient fortification of plants through plant breeding: Can it improve nutrition in man at low cost? *Proc. Nutr. Soc.* **62**: 403–411. doi:10.1079/PNS2003262.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil.* **302**: 1–17. doi:10.1007/s11104-007-9466-3.

Cakmak, I., Kalayci, M., Kaya, Y., Torun, A.A., Aydin, N., Wang, Y., Arisoy, Z., Erdem, H., Yazici, A., Gokmen, O., Ozturk, L., and Horst, W.J. 2010. Biofortification and localization of zinc in wheat grain. *J. Agric. Food Chem.* **58**: 9092–9102. doi:10.1021/jf101197h.

Cakmak, I., and Kutman, U.B. 2018. Agronomic biofortification of cereals with zinc: A review. *Eur. J. Soil Sci.* **69**: 172–180. doi:10.1111/ejss.12437.

Caldwell, M.M., Dawson, T.E., and Richards, J.H. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia*. **113**: 151–161. doi:10.1007/s004420050363.

Cereals Canada 2024. Canada's 2023 new wheat crop report. [Online] Available: <https://cerealscanada.ca/2023-wheat-crop> [Accessed 27 Mar. 2024].

Clark, R.B., and Baligar, V.C. 2000. Acidic and alkaline soil constraints on plant mineral nutrition. Pages 133–177. in R.E. Wilkinson (Ed.). *Plant-Environment Interactions*, 2nd edition. Marcel Dekker, New York, NY. U.S.A. doi:10.1201/9780824746568.

Ding, J., Liu, L., Wang, C., Shi, L., Xu, F., and Cai, H. 2021. High level of zinc triggers phosphorus starvation by inhibiting root-to-shoot translocation and preferential distribution of phosphorus in rice plants. *Environ. Pollut.* **277**: 116778. doi:10.1016/j.envpol.2021.116778.

Dubey, A., Kumar, A., Malla, M.A., Chowdhary, K., Singh, G., Ravikanth, G., Harish, Sharma, S., Saati-Santamaria, Z., Menéndez, E., and Dames, J.F. 2021. Approaches for the amelioration of adverse effects of drought stress on crop plants. *Front. Biosci.-Landmark*. **26**: 928–947. doi:10.52586/4998.

Edwards, M. 2017. Back transformation of data from log. [Online] Available: https://sassyfridays.ca/wp-content/uploads/2017/10/log_back_transformations2.pdf [Accessed 26 Nov. 2025].

Esfandiari, E., Abdoli, M., Mousavi, S.-B., and Sadeghzadeh, B. 2016. Impact of foliar zinc application on agronomic traits and grain quality parameters of wheat grown in zinc-deficient soil. *Indian J. Plant Physiol.* **21**: 263–270. doi:10.1007/s40502-016-0225-4.

FAOSTAT 2022. Crop and livestock products. [Online] Available: <https://www.fao.org/faostat/en/#data/QCL> [Accessed 6 Feb. 2024].

Gao, X., Mohr, R.M., McLaren, D.L., and Grant, C.A. 2011. Grain cadmium and zinc concentrations in wheat as affected by genotypic variation and potassium chloride fertilization. *Field Crops Res.* **122**: 95-103. doi:10.1016/j.fcr.2011.03.005.

Gawalko, E.J., Garrett, R.G., and Nowicki, T.W. 2002. Cadmium, copper, iron, manganese, selenium, and zinc in Canadian spring wheat. *Commun. Soil Sci. Plant Anal.* **33**: 3121–3133. doi:10.1081/CSS-120014510.

Ghanem, H.E., Hamza, D.A., Zain El-Abdeen, A.A., Elbatrawy, W.S., and El-Habashy, H.M. 2025. Influence of zinc foliar spray on growth, some important physiological processes, yield and yield attributes of bread wheat under water stress. *Sci. Rep.* **15**: 14943. doi:10.1038/s41598-025-94728-1.

Gibson, R.S., Raboy, V., and King, J.C. 2018. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutr. Rev.* **76**: 793–804. doi:10.1093/nutrit/nuy028.

Goh, T.B., Karamanos, R.E., and Lee, J. 2013. Effects of phosphorus form on short-term solubility and availability in soils. *Commun. Soil Sci. Plant Anal.* **44**: 136–144. doi:10.1080/00103624.2013.736162.

Goldberg, S., and Suarez, D. L. 2014. A new soil test for quantitative measurement of available and adsorbed boron. *SSSA J.* **78**: 480–485. doi:10.2136/sssaj2013.09.0404.

Grant, C.A., and Bailey, L.D. 1998. Nitrogen, phosphorus and zinc management effects on grain yield and cadmium concentration in two cultivars of durum wheat. *Can. J. Plant Sci.* **78**: 63–70. doi:10.4141/P96-189.

Grant, C.A., and Flaten, D.N. 2019. 4R management of phosphorus fertilizer in the northern Great Plains. *J. Environ. Qual.* **48**: 1356–1369. doi:10.2134/jeq2019.02.0061.

Grant, C.A., Flaten, D.N., Tomaszewicz, D.J., and Sheppard, S.C. 2001. The importance of early season phosphorus nutrition. *Can. J. Plant Sci.* **81**: 211–224. doi:10.4141/P00-093.

Gu, X.Y., Liu, Y., Liu, Y.H., Paliwal, J., and Wen, X.X. 2023. Effects of foliar spraying of potassium fertilizer on the contents of microelement, phytic acid and HMW-GS in wheat flour. *J. Cereal Sci.* **110**: 103621. doi:10.1016/j.jcs.2022.103621.

HarvestPlus 2025. Zinc wheat - HarvestPlus. [Online] Available: <https://www.harvestplus.org/crop/zinc-wheat/> [Accessed 7 Aug. 2025].

Haug, W., and Lantzsch, H.-J. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.* **34**: 1423–1426. doi:10.1002/jsfa.2740341217.

Havlin, J.L., Beaton, J.D., Tisdale, S.L., and Nelson, W.L. 2014. *Soil fertility and fertilizers; An introduction to nutrient management*. 8th edition, Pearson, Upper Saddle River, NJ, U.S.A. 516 pp. [Online] Available: <https://www.pearson.com/en-ca/subject-catalog/p/9780137593392> [Accessed 30 Aug. 2025].

Hopkins, B., and Ellsworth, J. 2005. Phosphorus availability with alkaline/calcareous soil. *Western Nutrient Management Conf.* **6**: 88–93. Salt Lake City, UT. U.S.A. [Online] Available: <https://www.researchgate.net/publication/237734366>.

Hou, J., Gao, X., and Entz, M.H. 2025. Enhancing zinc and iron bioavailability through crop rotation and organic farming: insights from a long-term study. *Field Crops Res.* **322**: 109710. doi:10.1016/j.fcr.2024.109710.

Hui, X., Luo, L., Chen, Y., Palta, J.A., and Wang, Z. 2025. Zinc agronomic biofortification in wheat and its drivers: a global meta-analysis. *Nat. Commun.* **16**: 3913. doi:10.1038/s41467-025-58397-y.

Ivanović, D., Dodig, D., Đurić, N., Kandić, V., Tamindžić, G., Nikolić, N., and Savić, J. 2021. Zinc biofortification of bread winter wheat grain by single zinc foliar application. *Cereal Res. Commun.* **49**: 673–679. doi:10.1007/s42976-021-00144-2.

Karamanos, R., Flore, N., and Harapiak, J. 2017. The impact of phosphorus fertilizer placement on crop production. *Agric. Res. Technol.* **11**: 555817. doi:10.19080/ARTOAJ.2017.11.555817.

Marschner, P. (Ed.). 2012. *Marschner's Mineral Nutrition of Higher Plants*. 3rd edition. Elsevier. San Diego, CA. U.S.A. 651 pp. doi:10.1016/C2009-0-63043-9.

Miller, R.O., Jacobsen, J.S., and Skogley, E.O. 1993. Aerial accumulation and partitioning of nutrients by hard red spring wheat. *Commun. Soil Sci. Plant Anal.* **24**: 2389–2407. doi:10.1080/00103629309368963.

Montalvo, D., Degryse, F., da Silva, R.C., Baird, R., and McLaughlin, M.J. 2016. Agronomic effectiveness of zinc sources as micronutrient fertilizer. Pages 215–267. in D.L. Sparks (Ed.). *Advances in Agronomy*. **139**. Elsevier. San Diego, CA. U.S.A. doi:10.1016/bs.agron.2016.05.004.

Mooleki, S.P., Malhi, S.S., Lemke, R.L., Schoenau, J.J., Lafond, G., Brandt, S., Hultgreen, G.E., Wang, H., and May, W.E. 2010. Effect of form, placement and rate of N fertilizer, and placement of P fertilizer on wheat in Saskatchewan. *Can. J. Plant Sci.* **90**: 319–337. doi:10.4141/CJPS09075.

Naeem, A., Aslam, M., and Lodhi, A. 2018. Improved potassium nutrition retrieves phosphorus-induced decrease in zinc uptake and grain zinc concentration of wheat. *J. Sci. Food Agric.* **98**: 4351–4356. doi:10.1002/jsfa.8961.

NCERA-13. 2015. Recommended Chemical Soil Test Procedures for the North Central Region. Nathan, M., and Gelderman, R. (Ed.). Revised 2012 edition. Extension University of Missouri and NCERA-13. Columbia, MO. U.S.A. Publ. SB1001/NCR221. 72 pp. [Online] Available: <https://extension.missouri.edu/publications/sb1001> [Accessed 6 Aug. 2025].

Nieves-Cordones, M., López-Delacalle, M., Ródenas, R., Martínez, V., Rubio, F., and Rivero, R.M. 2019. Critical responses to nutrient deprivation: a comprehensive review on the role of ROS and RNS. *Environ. Exp. Bot.* **161**: 74–85. doi:10.1016/j.envexpbot.2018.10.039.

Rahman, N., Peak, D., and Schoenau, J. 2022. Antagonistic effect of copper and zinc in fertilization of spring wheat under low soil phosphorus conditions. *Can. J. Soil Sci.* **102**: 797–809. doi:10.1139/cjss-2021-0189.

Raza, M.A.S., Saleem, M.F., and Khan, I.H. 2015. Combined application of glycinebetaine and potassium on the nutrient uptake performance of wheat under drought stress. *Pak. J. Agric. Sci.* **52**: 19–26. [Online] Available: <http://www.pakjas.com.pk>.

Raza, M.A.S., Saleem, M.F., Shah, G.M., Khan, I.H., and Raza, A. 2014. Exogenous application of glycinebetaine and potassium for improving water relations and grain yield of wheat under drought. *J. Soil Sci. Plant Nutr.* **14**: 348–364. doi:10.4067/S0718-95162014005000028.

Roy, C., Kumar, S., Ranjan, R.D., Kumhar, S.R., and Govindan, V. 2022. Genomic approaches for improving grain zinc and iron content in wheat. *Front. Genet.* **13**: 1045955. doi:10.3389/fgene.2022.1045955.

Safar-Noori, M., Assaha, D.V.M., and Saneoka, H. 2018. Effect of salicylic acid and potassium application on yield and grain nutritional quality of wheat under drought stress condition. *Cereal Res. Commun.* **46**: 558–568. doi:10.1556/0806.46.2018.026.

Saha, B.N., Saha, S., Saha, S., Roy, P.D., Bhowmik, A., and Hazra, G.C. 2020. Zinc (Zn) application methods influences Zn and iron (Fe) bioavailability in brown rice. *Cereal Res. Commun.* **48**: 293–299. doi:10.1007/s42976-020-00038-9.

Sathe, S.K., and Venkatachalam, M. 2001. Influence of processing technologies on phytate and its removal. Pages 157–188. in N.R. Reddy and S.K. Sathe (Eds.). *Food Phytates*. 1st edition. CRC Press, Boca Raton, FL. U.S.A. [Online] Available: <https://www.taylorfrancis.com/chapters/edit/10.1201/9781420014419-12/> [Accessed 6 Aug. 2025].

Shemi, R., Wang, R., Gheith, E.-S. M.S., Hussain, H.A., Hussain, S., Irfan, M., Cholidah, L., Zhang, K., Zhang, S., and Wang, L. 2021. Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. *Sci. Rep.* **11**: 3195. doi:10.1038/s41598-021-82264-7.

Silva, V.M., Putti, F.F., White, P.J., and Reis, A.R. dos. 2021. Phytic Acid accumulation in plants: Biosynthesis pathway regulation and role in human diet. *Plant Physiol. Biochem.* **164**: 132–146. doi:10.1016/j.plaphy.2021.04.035.

Stanton, C., Sanders, D., Krämer, U., and Podar, D. 2022. Zinc in plants: integrating homeostasis and biofortification. *Mol. Plant.* **15**: 65–85. doi:10.1016/j.molp.2021.12.008.

Stein, A.J., Nestel, P., Meenakshi, J., Qaim, M., Sachdev, H., and Bhutta, Z.A. 2007. Plant breeding to control zinc deficiency in india: How cost-effective is biofortification? *Public Health Nutr.* **10**: 492–501. doi:10.1017/S1368980007223857.

Strong, W.M., and Soper, R.J. 1974. Phosphorus utilization by flax, wheat, rape, and buckwheat from a band or pellet-like application. I. reaction zone root proliferation 1. *Agron. J.* **66**: 597–601. doi:10.2134/agronj1974.00021962006600050001x.

Thitisaksakul, M., Jiménez, R.C., Arias, M.C., and Beckles, D.M. 2012. Effects of environmental factors on cereal starch biosynthesis and composition. *J. Cereal Sci.* **56**: 67–80. doi:10.1016/j.jcs.2012.04.002.

Tran, B.T.T., Cavagnaro, T.R., Able, J.A., and Watts-Williams, S.J. 2021. Bioavailability of zinc and iron in durum wheat: A trade-off between grain weight and nutrition? *Plants, People, Planet.* **3**: 627–639. doi:10.1002/ppp3.10151.

Wang, S., Tian, X., and Liu, Q. 2020. The effectiveness of foliar applications of zinc and biostimulants to increase zinc concentration and bioavailability of wheat grain. *Agronomy.* **10**: 178. doi:10.3390/agronomy10020178.

Westerman, R.L. 1990. *Soil Testing and Plant Analysis*. 3rd edition. Soil Science Society of America, Madison, WI, U.S.A. 784 pp. [Online] Available: <https://acsess.onlinelibrary.wiley.com/doi/pdf/10.2136/sssabookser3.3ed.frontmatter> [Accessed 6 Aug. 2025].

WHO. 1996. Trace elements in human nutrition and health. 1st edition. World Health Organization, Geneva, Switzerland. 343 pp. [Online] Available: <https://www.who.int/publications/i/item/9241561734> [Accessed 26 Aug. 2025].

Xu, M., Liu, M., Si, L., Ma, Q., Sun, T., Wang, J., Chen, K., Wang, X., and Wu, L. 2022. Spraying high concentrations of chelated zinc enhances zinc biofortification in wheat grain. *J. Sci. Food Agric.* **102**: 3590–3598. doi:10.1002/jsfa.11705.

Yang, X.W., Tian, X.H., Gale W.J., Cao, Y.X., Lu, X.C., and Zhao, A.Q. 2011a. Effect of soil and foliar zinc application on zinc concentration and bioavailability in wheat grain grown on potentially zinc-deficient soil. *Cereal Res. Commun.* **39**: 535–543. doi:10.1556/CRC.39.2011.4.8.

Yang, X., Tian, X., Lu, X., Cao, Y., and Chen, Z. 2011b. Impacts of phosphorus and zinc levels on phosphorus and zinc nutrition and phytic acid concentration in wheat (*Triticum aestivum* L.). *J. Sci. Food Agric.* **91**: 2322–2328. doi:10.1002/jsfa.4459.

Zafar, S., Ashraf, M.Y., Anwar, S., Ali, Q. and Noman, A. 2016. Yield enhancement in wheat by soil and foliar fertilization of K and Zn under saline environment. *Soil Environ.* 35: 46-55. [Online] Available: <https://www.se.org.pk/Papers.aspx?issueid=74> [Accessed 3 Dec. 2025].

Zarea, M.J. 2025. Foliar application of *Azospirillum brasilense*, salicylic acid and zinc on wheat performance under rain-fed condition. *Cereal Res. Commun.* **53**: 1073–1090. doi:10.1007/s42976-024-00570-y.

Zhang, X., Guo, D., Blennow, A., and Zörb, C. 2021. Mineral nutrients and crop starch quality. *Trends Food Sci. Technol.* **114**: 148–157. doi:10.1016/j.tifs.2021.05.016.

Zhao, A., Yang, S., Wang, B., Tian, X., and Zhang, Y. 2018. Effects of ZnSO₄ and Zn-EDTA broadcast or banded to soil on Zn bioavailability in wheat (*Triticum aestivum* L.) and Zn fractions in soil. *Chemosphere*. **205**: 350–360. doi:10.1016/j.chemosphere.2018.04.115.

Zou, C.Q., Zhang, Y.Q., Rashid, A., Ram, H., Savasli, E., Arisoy, R.Z., Ortiz-Monasterio, I., Simunji, S., Wang, Z.H., Sohu, V., Hassan, M., Kaya, Y., Onder, O., Lungu, O., Mujahid, M.Y., Joshi, A.K., Zelenskiy, Y., Zhang, F.S., and Cakmak, I. 2012. Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant Soil*. **361**: 119–130. doi:10.1007/s11104-012-1369-2.

3. SYNTHESIS

This chapter summarizes the findings of the field trials and situates the thesis within the broader goal of improving wheat nutritional quality. The core premise of biofortification is to ensure that foods consumed by vulnerable populations support healthy development and reduce illness associated with micronutrient deficiencies. Modern farming excels at calorie production, but often at the expense of micronutrient density. While assigning responsibility for nutrient-dense end food products is beyond the scope of this study, our results point to practical directions if Canadian producers are asked, or choose, to deliver nutrient-dense grain. The sections below synthesize key findings and accomplishments, limitations, and recommendations for future research and practice.

3.1 Key Findings

Enhancing nutrient density at scale is difficult when consumers are distant from production, so agronomic biofortification must align with productivity goals as producers or their families are not directly affected by Zn deficiency in their diet. In our trials, soil-applied Zn increased yield but did not substantially raise grain Zn concentration. In contrast, foliar Zn reliably increased grain Zn but delivered limited or inconsistent additional yield gains, making adoption sensitive to added input costs, consistent with findings from other studies (Cakmak and Kutman, 2018; Cakmak et al., 2010; Zou et al., 2012; Hui et al., 2025). Across treatments, P and Zn soil placement had little effect on grain Zn concentration, contrasting with Montalvo et al. (2016). This may reflect spring banding that shortens contact time compared to broadcast incorporation and reduces Zn–P co-precipitation in calcareous soils. Grain Zn concentration was the most effective factor to improve

Zn bioavailability, indexed by the phytic acid:Zn molar ratio. P fertilization had limited direct impact on bioavailability. In contrast, background soil fertility set the baseline: Carman_2024, with Zn fertility lowest among sites and P similar to others, exhibited the poorest bioavailability on average. Foliar Zn combined with soil-applied Zn was the most effective fertilization strategy to improve Zn bioavailability in wheat grain when compared to soil-applied Zn alone.

3.2 Project Accomplishments

This work shows that Zn biofortification strategies can be integrated into common Prairie wheat operations and spray windows: seed-row MESZ or side-band MAP + ZnSO₄ at seeding, with well-timed foliar Zn (\pm K, \pm biostimulants) at Z12 and Z65. These approaches are operationally feasible and compatible with existing equipment and passes. While some tuning is needed to stabilize responses across contrasting soils and seasons, the study demonstrates a practical pathway to raise grain Zn concentration and to do so within commercial production constraints.

3.3 Limitation and Analytical Gaps

Two design choices limit the extent to which these findings can be generalized. First, background fertility: using MESZ effectively supplied more P than was optimal for already adequate soils (Manitoba Soil Fertility Advisory Committee, 2007). Then, adding treatments with different P rates and even one with Zn only could have helped clarifying the P role in Zn biofortification. Nitrogen rates were also set above guideline levels to target high yield potential (\sim 5–5.5 t ha⁻¹), as reflected in consistently high grain protein (>15%). Together, these findings suggest that responses to foliar Zn could differ under the Manitoba Soil Fertility Guide P and N rates recommendation guidelines. Second, genetics: only one hard red spring cultivar was tested, despite known genotypic differences in foliar Zn uptake and remobilization, warranting a multi-cultivar screen. Several

additional measurements would have strengthened both mechanism and end-use relevance: serial tissue sampling to track seasonal Zn uptake and remobilization (especially after the early foliar pass); partitioning grain Zn into soluble vs. insoluble pools and quantifying inorganic P to better index nutritional status; milling fractions or flour extraction to match human consumption (since bran is often removed); dough functionality (gluten/protein fractions) to test potential quality co-benefits of Zn or betaine; and quantifying betaine in grain, given its potential human-health co-benefits (Arumugam et al., 2021). The previous crop residue effect was also overlooked in the field selection process, to confirm our suspicion of reduced arbuscular mycorrhizal fungi colonization on the canola stubble, an environmental DNA study could be done on archived plot soil sample to potentially confirm the difference between sites.

3.4 Future Research

Future work on Zn biofortification should prioritize foliar strategies, optimizing timing, rate, and formulation, to enhance leaf absorption and whole-plant translocation. Industry trials and personal communications with foliar product developers suggest that adding a modest N source to biostimulant-Zn sprays can improve uptake and movement of Zn within wheat. Two linked questions follow: (i) does crop N status (e.g., predicted grain protein 13% vs. 15%) modulate the Zn response when N is included in the tank? and (ii) does added N up-regulate synthesis of Zn ligands/transporters (peptides, nicotianamine, deoxymugineic acid), irrespective of the crop's overall N status? Field factorials that combine soil-applied N (low/moderate/high) with foliar Zn vs. Zn+N at early milk stages could test these mechanisms directly. For instance, Wang et al. (2015) found the greatest grain Zn when moderate soil N was paired with foliar Zn+N at early milk stage in wheat grown under semi-arid conditions in Northwestern China.

Because our trials separated nutrition from crop protection for safety/logistics, the compatibility remains an open question. Follow-up experiments should co-apply foliar Zn with common herbicide passes (Z12–Z15) and fungicide passes (Z65) to quantify any combined effects on grain Zn and yield. Newer fungicide formulations (e.g., strobilurin-containing or multi-mode of action mixes) can extend photosynthetic duration and “stay-green,” potentially enlarging the sink for Zn remobilization (Carucci et al., 2020; Woore and Holland, 2020). Testing Zn responses with and without these chemistries would clarify whether plant-health fungicides amplify biofortification gains.

Raising foliar Zn rates and adding N increases the risk of leaf burn and yield loss (Xu et al., 2022). Formulation science should therefore accompany agronomy: evaluate chelators/complexing agents (e.g., fulvic acids) that improve droplet spread, reduce surface crystallization/osmotic injury (Ducatti and Tironi, 2023), and have shown promise for elevating grain Zn versus ZnSO₄ alone (Wang et al., 2020). Dose–response trials should report both efficacy (grain Zn, PA:Zn) and safety (visible injury, flag-leaf function, yield) across carrier volumes typical of Prairie operations.

Finally, research should address late-season constraints. Identifying the primary yield-limiting factors during grain filling (water, N remobilization, disease pressure, heat, nutrient deficiencies) may justify a second foliar Zn window post-anthesis when the grain sink is strongest. Coupling foliar Zn with interventions that sustain canopy function could maximize grain Zn and yield potential, strengthening the economic case for biofortification.

3.5 Recommendations

3.5.1 For Farmers

Our results, together with the meta-analysis by Hui et al. (2025), indicate that fields already producing grain Zn > 30 mg kg⁻¹ can often reach biofortification targets with foliar Zn alone, provided applications are well-timed. In this study, soils with DTPA-Zn ≥ 1.18 mg kg⁻¹ could be suitable for a foliar-only strategy. Where DTPA-Zn < 1.18 mg kg⁻¹, especially under high-yield potential, both soil Zn supplementation and foliar Zn are needed to meet targets. When soil Zn is required, banded ZnSO₄ plus foliar Zn delivered the strongest biofortification response in our conditions, particularly where the agronomic P requirement is low (i.e., when MESZ would over-supply P or under-supply Zn). However, soil-applied Zn in a band seems required to achieve maximum yield potential regardless of soil DTPA-Zn test results.

3.5.2 For Researchers

This study and the random-forest analysis in Hui et al. (2025) identify foliar Zn rate and spray timing as the most influential factors to raise grain Zn. As foliar Zn rate and solution strength increase, incorporate chelating agents and/or spreader adjuvants that improve leaf coverage and absorption while minimizing phytotoxicity (chlorosis/leaf burn) that can depress yield. The key challenge is to identify effective combinations of nutrients, biostimulants, and chelators that increase both grain Zn and yield. Progress will require field-specific diagnostics (soil tests, tissue tests, yield potential) rather than generic Zn-only recommendations. Ultimately, profitability will determine adoption, and biofortification practices must fit existing spray passes and pencil out economically, otherwise they will remain uncommon among Canadian growers.

3.6 References

- Arumugam, M.K., Paal, M.C., Donohue, T.M., Ganesan, M., Osna, N.A., and Kharbanda, K.K. 2021. Beneficial effects of betaine: A comprehensive review. *Biology*. **10**: 456. doi:10.3390/biology10060456.
- Cakmak, I., and Kutman, U.B. 2018. Agronomic biofortification of cereals with zinc: A review. *Eur. J. Soil Sci.* **69**: 172–180. doi:10.1111/ejss.12437.
- Cakmak, I., Pfeiffer, W.H., and McClafferty, B. 2010. Review: Biofortification of durum wheat with zinc and iron. *Cereal Chem.* **87**: 10–20. doi:10.1094/CCHEM-87-1-0010.
- Carucci, F., Gatta, G., Gagliardi, A., Vita, P.D., and Giuliani, M.M. 2020. Strobilurin effects on nitrogen use efficiency for the yield and protein in durum wheat grown under rainfed mediterranean conditions. *Agronomy*. **10**: 1508. doi:10.3390/agronomy10101508.
- Ducatti, R.D.B., and Tironi, S.P. 2023. Enhancing the efficiency and sustainability of foliar fertilization in agriculture. *Agron. Sci. Biotechnol.* **10**: 1–21. doi:10.33158/ASB.r200.v10.2024.
- Hui, X., Luo, L., Chen, Y., Palta, J.A., and Wang, Z. 2025. Zinc agronomic biofortification in wheat and its drivers: A global meta-analysis. *Nat. Commun.* **16**: 3913. doi:10.1038/s41467-025-58397-y.
- Manitoba Soil Fertility Advisory Committee. 2007. Manitoba Soil Fertility Guide. Revised March 2007 edition. Manitoba Agriculture. Winnipeg, MB, Canada. Publi. MG-5662. 83 pp. [Online] Available: <https://www.gov.mb.ca/agriculture/crops/soil-fertility/soil-fertility-guide/> [Accessed 26 Sept. 2025].

Montalvo, D., Degryse, F., da Silva, R.C., Baird, R., and McLaughlin, M.J. 2016. Agronomic effectiveness of zinc sources as micronutrient fertilizer. Pages 215–267. in D.L. Sparks (Ed.). *Advances in Agronomy*. **139**. Elsevier. San Diego, CA. U.S.A. doi:10.1016/bs.agron.2016.05.004.

Wang, S., Li, M., Tian, X., Li, J., Li, H., Ni, Y., Zhao, J., Chen, Y., Guo, C., and Zhao, A. 2015. Foliar zinc, nitrogen, and phosphorus application effects on micronutrient concentrations in winter wheat. *Agron. J.* **107**: 61–70. doi:10.2134/agronj14.0414.

Wang, S., Tian, X., and Liu, Q. 2020. The effectiveness of foliar applications of zinc and biostimulants to increase zinc concentration and bioavailability of wheat grain. *Agronomy*. **10**: 178. doi:10.3390/agronomy10020178.

Woore, M.S., and Holland, J.B. 2020. Genetic variation for response to mixed triazole and strobilurin application in diverse maize. *Agrosyst. Geosci. Environ.* **3**: e20054. doi:10.1002/agg2.20054.

Xu, M., Liu, M., Si, L., Ma, Q., Sun, T., Wang, J., Chen, K., Wang, X., and Wu, L. 2022. Spraying high concentrations of chelated zinc enhances zinc biofortification in wheat grain. *J. Sci. Food Agric.* **102**: 3590–3598. doi:10.1002/jsfa.11705.

Zou, C.Q., Zhang, Y.Q., Rashid, A., Ram, H., Savasli, E., Arisoy, R.Z., Ortiz-Monasterio, I., Simunji, S., Wang, Z.H., Sohu, V., Hassan, M., Kaya, Y., Onder, O., Lungu, O., Mujahid, M.Y., Joshi, A.K., Zelenskiy, Y., Zhang, F.S., and Cakmak, I. 2012. Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant Soil*. **361**: 119–130. doi:10.1007/s11104-012-1369-2.

4. APPENDICES

Appendix A: Supplementary Material for Chapter 2

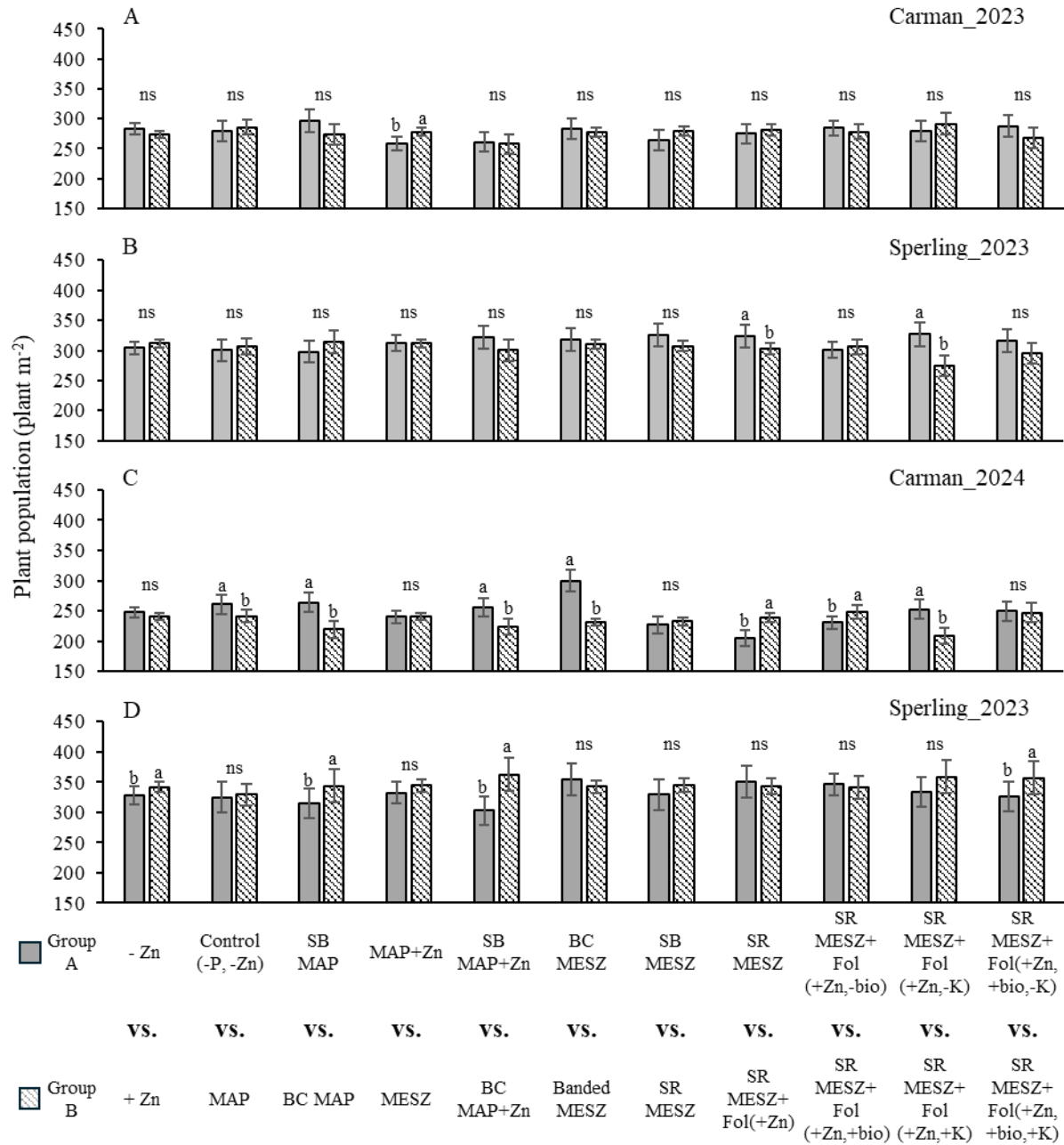


Figure A-6 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat plant densities at Zadok's stage Z11 for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The '+' or '-' refers to "added" or "not added", respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok's growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

Table A-1 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on straw nutrient concentrations and uptake of iron (Fe), P, potassium (K) and Zn at Carman and Sperling from 2023 to 2024.

Treatment	Straw concentration				Straw uptake			
	K	P	Fe	Zn	K	P	Fe	Zn
	mg kg ⁻¹				g ha ⁻¹			
	Means (SEM) ⁱ							
1: Control (-P,-Zn) ^a	13684 (407)	369 (23)	115 (12)	9.0 (0.9)	34257 (1858)	930 (67)	312 (33)	23.2 (2.2)
2: SB ^b MAP ^c	13354 (595)	356 (20)	112 (12)	9.2 (0.7)	32871 (2185)	883 (46)	302 (39)	23.8 (2.0)
3: SB MAP+ Zn granular	13386 (463)	342 (28)	103 (13)	8.9 (0.6)	36004 (2649)	924 (67)	301 (40)	25.0 (2.1)
4: SB MESZ ^d	13601 (506)	383 (16)	96 (13)	7.6 (0.5)	34697 (2970)	982 (76)	264 (39)	19.9 (2.0)
5: BC ^e MAP	13917 (428)	415 (18)	110 (11)	8.0 (0.6)	37093 (2594)	1113 (88)	318 (37)	22.1 (2.2)
6: BC MAP+ Zn granular	13672 (527)	354 (24)	104 (12)	7.7 (0.5)	33426 (2144)	870 (70)	276 (34)	19.5 (1.7)
7: BC MESZ	13300 (424)	364 (22)	111 (10)	6.7 (0.4)	31590 (2287)	873 (78)	284 (34)	16.5 (1.3)
8: SR ^f MESZ	13583 (386)	380 (22)	122 (12)	7.4 (0.5)	37064 (2140)	1045 (84)	358 (42)	20.9 (1.8)
9: SR MESZ + Fol(+Zn) ^g	13539 (405)	340 (15)	83 (8)	11.7 (1.0)	35473 (2092)	897 (50)	233 (23)	31.6 (3.3)
10: SR MESZ + Fol(+Zn,+K)	13567 (464)	344 (21)	91 (9)	12.2 (0.8)	36594 (2096)	934 (57)	264 (33)	34.3 (2.8)
11: SR MESZ + Fol(+Zn,+bio ^h)	14110 (589)	339 (14)	74 (8)	12.3 (1.0)	37955 (2677)	918 (55)	217 (26)	34.7 (3.5)
12: SR MESZ + Fol(+Zn,+bio,+K)	13531 (441)	359 (18)	103 (10)	9.7 (0.6)	35562 (3150)	949 (72)	294 (34)	26.8 (2.8)
Site-Year								
Carman_2023	13086 (588)	358 (13)	103 (10)	10.4 (0.7)	14748 (1207)	397 (18)	119 (15)	12.7 (0.9)
Sperling_2023	12004 (480)	194 (6)	103 (8)	7.1 (0.4)	17581 (1272)	285 (11)	154 (16)	10.6 (0.7)
Carman_2024	13945 (566)	729 (21)	97 (7)	10.4 (0.6)	66521 (4873)	3485 (130)	460 (47)	49.0 (2.9)
Sperling_2024	15679 (1261)	338 (38)	104 (28)	8.7 (1.5)	89689 (14151)	2000 (320)	791 (237)	52.9 (12)
ANCOVA								
Effect					P value			
Relative elevation	0.5875	0.8513	0.1120	0.9455	0.0005	0.0009	0.0004	0.0757
Rel. elev. x Site-Year	0.0275	0.2541	0.1007	0.0203	0.0440	0.0163	0.0947	0.0348
Site-Year	<.0001	<.0001	0.1692	0.0004	<.0001	<.0001	<.0001	<.0001
Treatment	0.8490	0.0245	0.0118	<.0001	0.1842	0.2366	0.0178	<.0001
Site-Year x Treatment	0.1027	0.2963	0.0479	0.0003	0.1006	0.0642	0.0610	0.0123
Contrast Site-Year								
2023 vs. 2024	0.0046	<.0001		0.34823	<.0001	<.0001	<.0001	<.0001
2023: Carman vs. Sperling	0.20753	<.0001		0.30519	0.1000	0.0011	0.1113	0.8225
2024: Carman vs. Sperling	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001

^a The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. ^b Side-banded at 5 cm beside and below the seed furrow. ^c Monoammonium phosphate. ^d MicroEssentials[®] SZ[®]. ^e Broadcast-incorporation. ^f Seed-row. ^g Foliar application at wheat Zadok’s growing stages Z12 and Z65. ^h Biostimulants blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. ⁱ Means and standard error (Treatment, n=16; Site-Year, n=48) were calculated with the LSMEANS function, and contrast p-values with the LSMESTIMATE function, both in PROC GLIMMIX in SAS. Contrasts for treatment effects are presented in Figure A-7, Figure A-8 and interaction in Figure A-9.

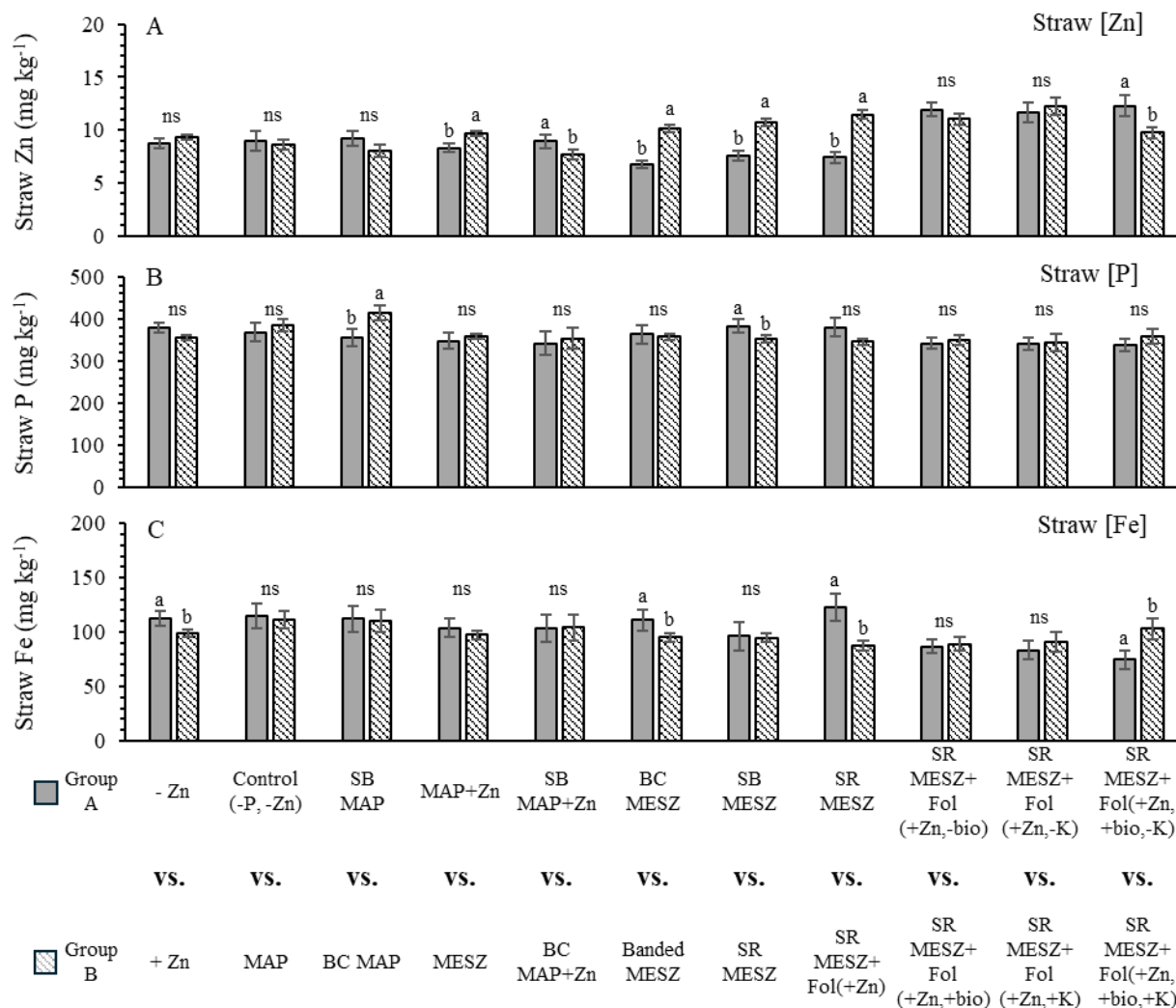


Figure A-7 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw concentrations of Zn (A), P (B), and iron (Fe) (C) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=16$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

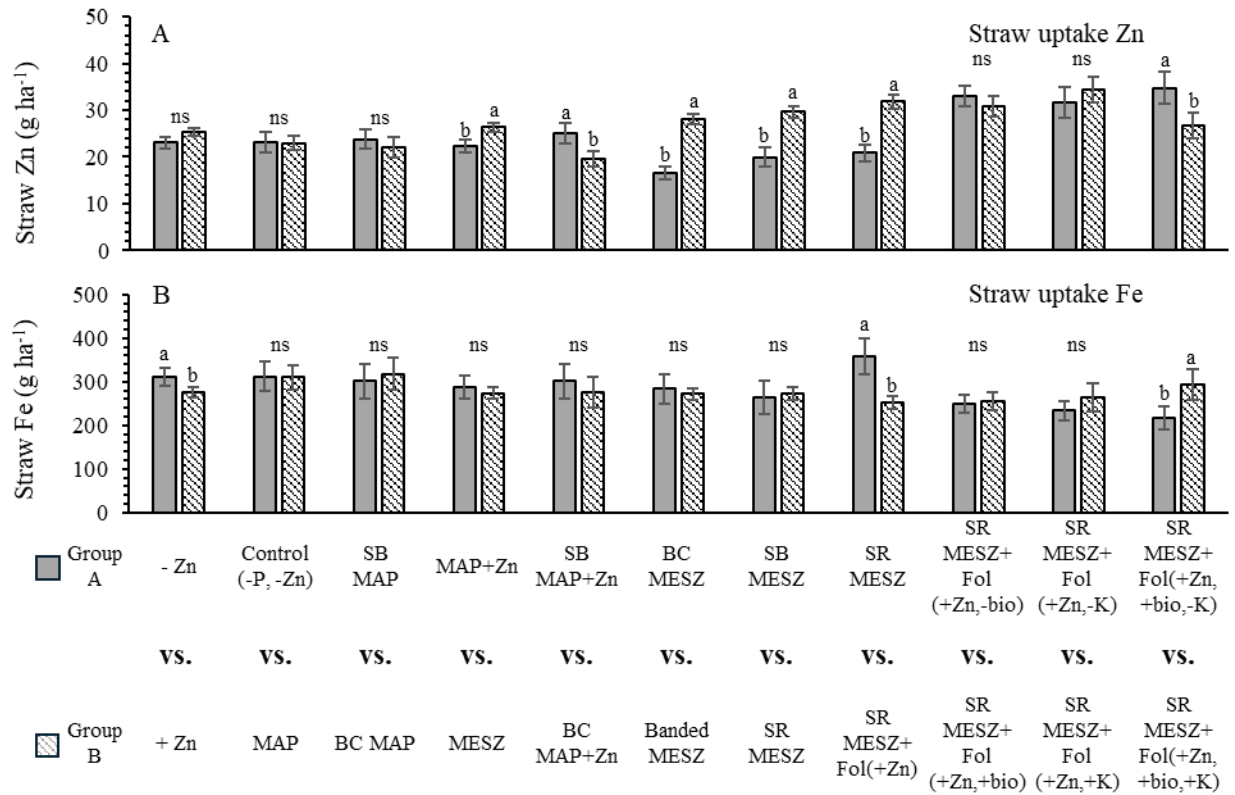


Figure A-8 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw uptake of Zn (A), and iron (Fe) (B) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=16$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. SR = seed-row. Banded = SB and SR placements. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

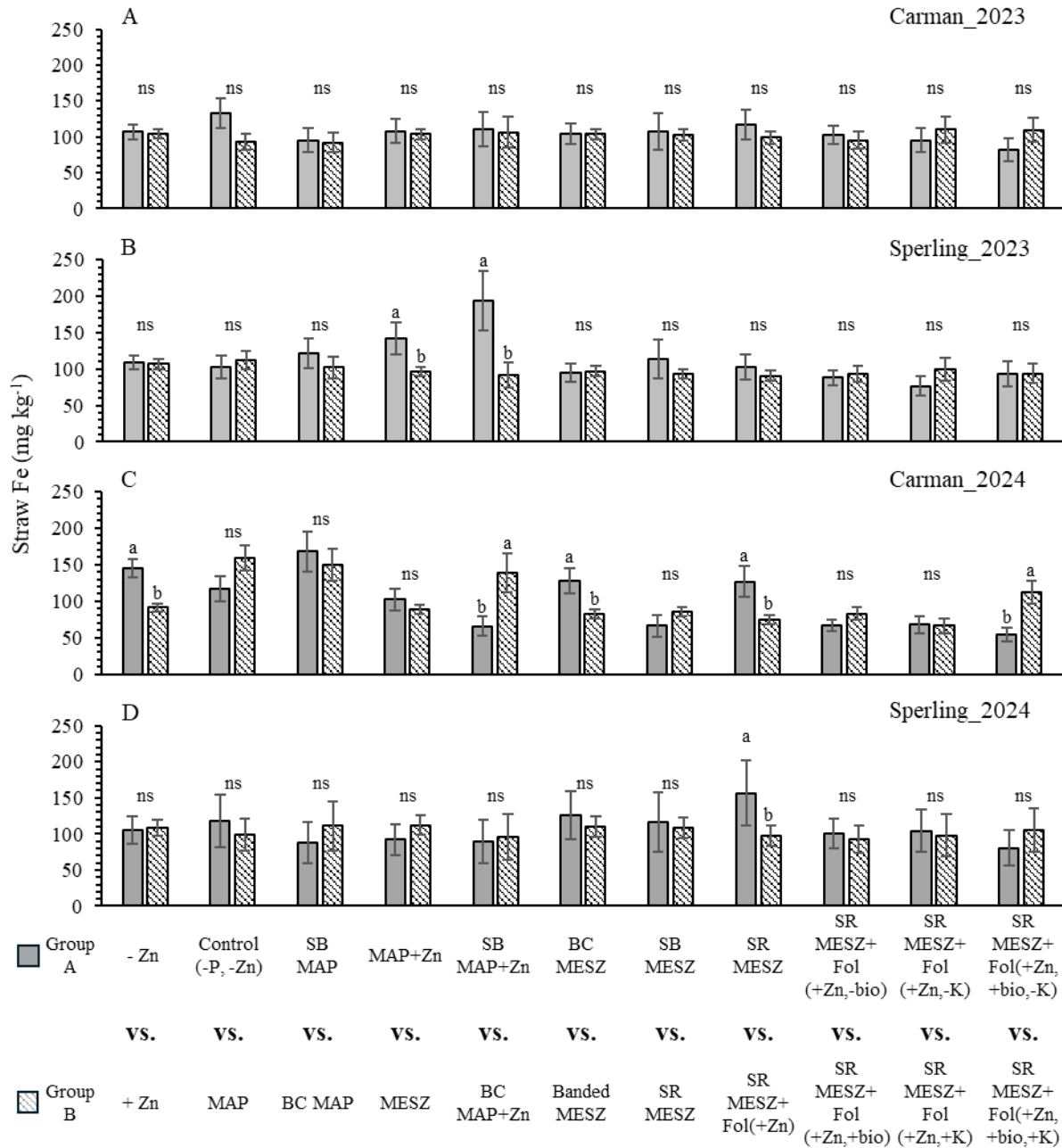


Figure A-9 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer effects on wheat straw iron (Fe) concentrations for Carman_2023 (A), Sperling_2023 (B), Carman_2024 (C), and Sperling_2024 (D). Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The '+' or '-' refers to "added" or "not added", respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. Banded = SB and SR placements. SR = seed-row. Fol = foliar application at Zadok's growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

Table A-2 Effects of phosphorus (P) and zinc (Zn) fertilizer treatments on grain nutrient concentrations and uptake of iron (Fe), and potassium (K) at Carman and Sperling from 2023 to 2024.

Treatment	Grain concentration		Grain uptake	
	K	Fe	K	Fe
	mg kg ⁻¹		g ha ⁻¹	
	Means (SEM) ⁱ			
1: Control (-P,-Zn) ^a	3325 (96)	115.4 (12.7)	9629 (511)	355.6 (38.4)
2: SB ^b MAP ^c	3405 (90)	91.5 (11.4)	10707 (579)	306.0 (32.6)
3: SB MAP+ Zn granular	3374 (76)	80.2 (3.6)	10877 (524)	273.3 (23.5)
4: SB MESZ ^d	3358 (98)	107.1 (9.2)	10651 (448)	361.4 (39.3)
5: BC ^e MAP	3426 (109)	106.1 (18.4)	9757 (504)	320.6 (56.3)
6: BC MAP+ Zn granular	3540 (95)	129.2 (13.1)	10724 (604)	413.9 (48.9)
7: BC MESZ	3250 (78)	109.3 (11.5)	9314 (405)	334.0 (36.5)
8: SR ^f MESZ	3339 (102)	130.1 (12.8)	10931 (433)	449.6 (44.4)
9: SR MESZ + Fol(+Zn) ^g	3347 (83)	81.7 (6.2)	10950 (500)	288.6 (31.0)
10: SR MESZ + Fol(+Zn,+K)	3419 (101)	93.5 (7.7)	11298 (616)	329.2 (39.1)
11: SR MESZ + Fol(+Zn,+bio ^h)	3467 (74)	85.6 (4.1)	11079 (662)	293.8 (27.1)
12: SR MESZ + Fol(+Zn,+bio,+K)	3470 (70)	110.7 (12.4)	11241 (657)	383.3 (51.2)
Site-Year				
Carman_2023	3560 (65)	100.2 (8.4)	7887 (389)	216.1 (16.0)
Sperling_2023	2923 (45)	78.7 (5.9)	7361 (299)	198.9 (12.3)
Carman_2024	3490 (50)	92.0 (7.0)	12307 (499)	344.7 (20.0)
Sperling_2024	3651 (220)	149.4 (22.3)	17603 (2104)	899.6 (227.3)
ANCOVA				
Effect			P value	
Relative elevation	0.5263	0.0354	0.023	0.0387
Rel. elev. x Site-Year	0.7033	0.4624	0.0042	0.0022
Site-Year	0.0258	0.4184	0.0188	0.0011
Treatment	0.2419	<.0001	0.0118	0.0061
Site-Year x Treatment	0.4203	0.1000	0.1545	0.3478
Contrast Site-Year				
2023 vs. 2024	0.0050		<.0001	<.0001
2023: Carman vs. Sperling	<.0001		0.31144	0.3944
2024: Carman vs. Sperling	0.4891		0.00751	0.0005

^a The '+' or '-' refers to "added" or "not added", respectively. ^b Side-banded at 5 cm beside and below the seed furrow. ^c Monoammonium phosphate. ^d MicroEssentials[®] SZ[®]. ^e Broadcast-incorporation. ^f Seed-row. ^g Foliar application at wheat Zadok's growing stages Z12 and Z65. ^h Biostimulants blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. ⁱ Means and standard error (Treatment, n=16; Site-Year, n=48) were calculated with the LSMEANS function, and contrast p-values with the LSMESTIMATE function, both in PROC GLIMMIX in SAS. Contrasts for treatment effects are presented in Figure A-10.

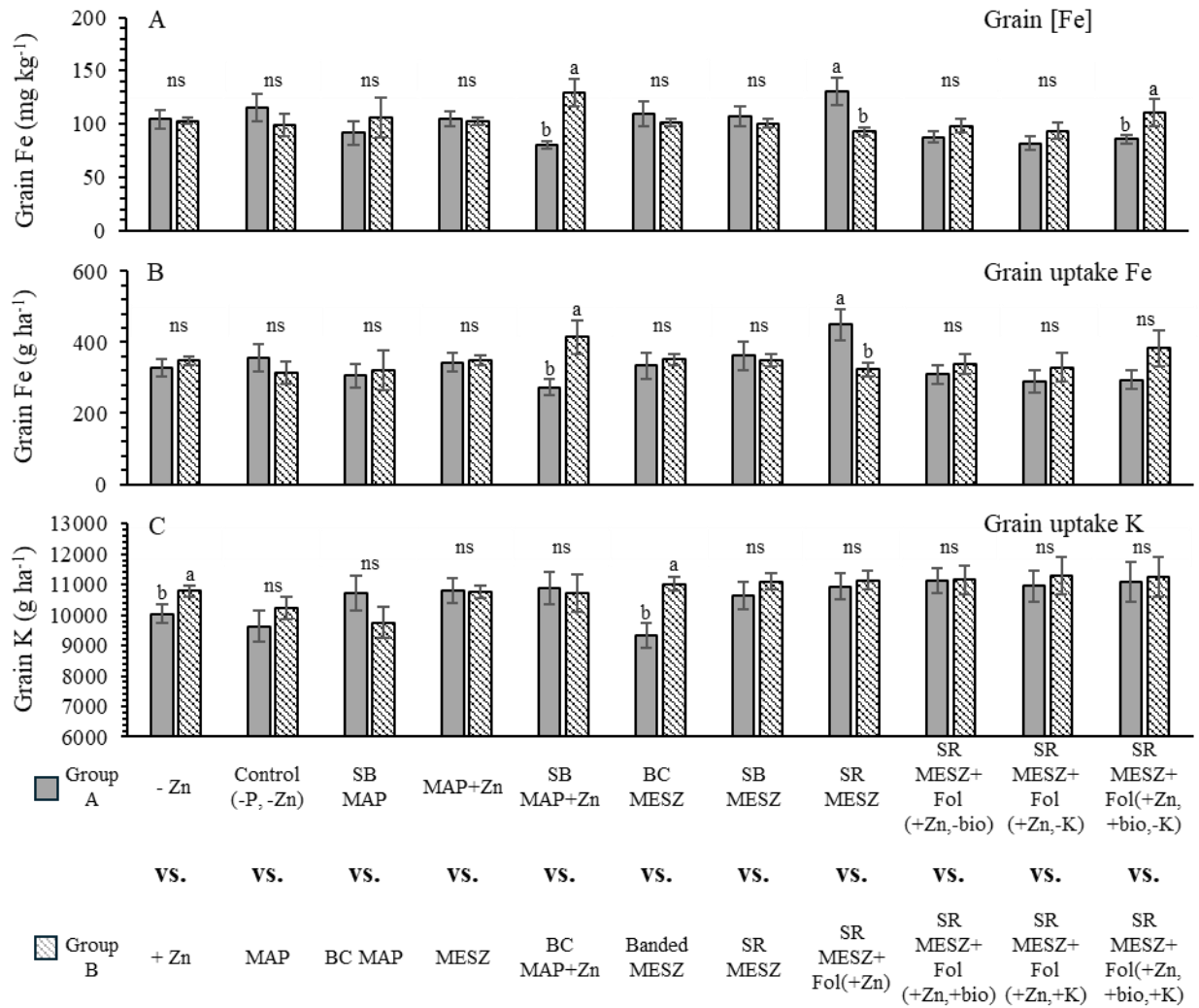


Figure A-10 Contrast analysis of phosphorus (P) and zinc (Zn) fertilizer treatment effects on wheat grain concentration of iron (Fe) (A), and grain uptake of Fe (B), and potassium (K) (C) at Carman and Sperling, 2023–2024. Different letters within a group pair indicate significant differences at $p < 0.05$; ns indicates non-significant. Error bars represent standard errors of the means ($n=4$). The ‘+’ or ‘-’ refers to “added” or “not added”, respectively. MAP = monoammonium phosphate. SB = side-banded at 5 cm beside and below seed furrow. BC = Broadcast-incorporation. MESZ = MicroEssentials[®] SZ[®]. Banded = SB and SR placements. SR = seed-row. Fol = foliar application at Zadok’s growing stages Z12 and Z65. Bio = Biostimulant blend of Ez-Gro Betaine and EZ-GRO SA 10%-L. K = potassium.

