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Anne Brown

Effect of Class Act NG Adjuvant on Glyphosate Efficacy in Corn

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Abstract

There is little information on the effect of the co-application of glyphosate with Class Act NG adjuvant on weed control efficacy and corn yield under Ontario environmental conditions. This study consisted of 6 field experiments that were conducted in Ontario during 2021 and 2022 to determine if the addition of Class Act NG (2.5% v/v) to glyphosate at 450, 900 and 1350 g ae ha⁻¹ would improve weed control and result in a concomitant increase in corn yield. The co-application of glyphosate with Class Act NG resulted in no visible corn injury at 1 and 4 weeks after herbicide application (WAA). The addition of Class Act NG to glyphosate at 450 g ae ha⁻¹ improved control of common lambsquarters, velvetleaf, Powell amaranth, common ragweed, and barnyardgrass as much as 20, 14, 9, 8, and 7%, respectively but there was no improvement in control of giant foxtail, or green foxtail and there was no increase in corn yield. The addition of Class Act NG to glyphosate at 900 g ae ha⁻¹ improved common lambsquarters control 6 and 5% at 4 and 8 WAA, respectively and improved barnyardgrass control 4% at 4 WAA. The addition of Class Act NG to glyphosate at 1350 g ae ha⁻¹ provided no improvement in control of velvetleaf, Powell amaranth, common ragweed, common lambsquarters, barnyardgrass, giant foxtail, or green foxtail and there was no increase in corn yield. Based on this data the co-application of glyphosate with Class Act NG results in improved control of some annual broadleaf and grass weeds (common lambsquarters, velvetleaf, Powell amaranth, common ragweed and barnyardgrass) when glyphosate is applied at 450 or 900 g ae ha⁻¹; however, when glyphosate is applied at 1350 g ae ha⁻¹ there was no improvement in weed control. The addition of Class Act NG to glyphosate at 450, 900 and 1350 g ae ha⁻¹ did not result in an increase in corn yield.

Keywords: barnyardgrass, common lambsquarters, common ragweed, giant foxtail, green foxtails, Powell amaranth, velvetleaf

1. Introduction

Corn (*Zea mays* L.) is one of the most valuable and dominant grain crops in the world that is grown for human and animal food, biofuel, and other industrial uses (Baker, 2018). Ontario is the main corn-producing province in Canada. Most of the corn produced in Ontario is used for animal feed (60%) and the remainder is used for various industrial uses (40%) (OMAFRA, 2023a).

Ontario farmers seed approximately 820,000 hectares of grain corn with a farm-gate value of approximately \$1.6 billion annually (OMAFRA, 2023b; Soltani et al., 2022). Weed interference can reduce corn yield dramatically; consequently, effective weed management programs are essential for profitable corn production. A meta-analysis by the Weed Science Society of America (WSSA) determined that corn producers in North America would lose 50% of their production (148 million tonnes) with a value of US\$26.7 billion if no weed management tactics are implemented (Soltani et al., 2016a). Despite the rapid evolution of glyphosate-resistant (GR) weeds from the repeated use of glyphosate in GR crops, many crop producers continue to depend on glyphosate as a major component of their weed management programs due to its excellent weed control efficacy, wide margin of crop safety, no residues affecting future crops in the rotation, low environmental impact, and low cost (Beckie et al., 2014; Sikkema & Soltani, 2007). Currently, more than 95% of the corn in Ontario is seeded GR hybrids (Beckie et al., 2014).

Glyphosate provides effective, broad-spectrum control of annual, biennial, and perennial grass and broadleaf weeds. However, there has been variable weed control; one reason for the variable control is attributed to the quality of water used for the herbicide carrier (Pratt et al., 2003; Thelen et al., 1995). Some studies have concluded that the inclusion of additives such as ammonium sulfate (AMS) could help improve the weed control

efficacy with glyphosate for the control of some weed species especially when glyphosate is applied at low rates (Nurse et al., 2008; Soltani et al., 2011, 2016b). It has been suggested that the negatively charged sulfate ion (SO_4^{--}) from water conditioners that include AMS can bind to the positively charged cations in water including calcium, iron, magnesium, potassium, and sodium present in water thus preventing them from binding to the negatively charged glyphosate which results in increased herbicide absorption and subsequent greater weed control efficacy (Thelen et al., 1995; Winfield United, 2023). Hall et al. (2000) reported that the addition of AMS to glyphosate is necessary to adequately control weeds such as velvetleaf regardless of water hardness.

Class Act NG is a new adjuvant marketed by Winfield Solutions, LLC (Winfield Solutions, LLC, St. Paul, MN, USA) as a water conditioner and surfactant spreader sticker for use with some herbicides including glyphosate (Winfield United, 2023). According to the label, Class Act NG contains 50.5% ammonium sulfate, corn syrup, alkyl polyglucoside, and 45.5% constituents that are ineffective as spray adjuvants (Winfield Solutions, 2023). In addition to AMS, Class Act NG includes a nonionic surfactant (CornSorb[®] Technology, Winfield Solutions, LLC, St. Paul, MN, USA) and antifoaming agent in a liquid premix (Winfield Solutions, 2023). Class Act NG as a hard water conditioner is designed to help the movement of the herbicide across the non-living leaf cuticle into living plant cells (Winfield Solutions, 2023). There is little information on the effect of Class Act NG on weed control efficacy with glyphosate for the control of common, wide-spread, annual grass and broadleaf weeds in corn under Ontario environmental conditions. This data is imperative so Ontario corn producers can make science-based decisions to maximize weed control efficacy and corn yield while minimizing weed management costs.

The objective of this study was to ascertain if the addition of Class Act NG to glyphosate at 450, 900, and 1350 g ae ha⁻¹ would improve the control of common weeds in Ontario including velvetleaf, Powell amaranth, common ragweed, common lambsquarters, barnyardgrass, giant foxtail, and green foxtail.

2. Materials and Methods

Six field experiments (3 in 2021 and 3 in 2022) were conducted at Huron Research Station, Exeter, ON, and the University of Guelph, Ridgetown Campus, Ridgetown, Ontario. Detailed information including soil characteristics, corn seeding and emergence dates, herbicide application dates, and weather conditions at herbicide application for each site are listed in Table 1. Seedbed preparation included fall mouldboard plowing followed by two passes with a field cultivator with rolling basket harrows in the spring.

Table 1. Year, location, soil characteristics, corn seeding and emergence dates, herbicide application dates, and weather conditions at application for six experiments conducted in Ontario, Canada in 2021 and 2022

Year	Location	Texture	Soil characteristics ^a						Seeding date	Emergence date	Application date	Application weather conditions		
			Sand	Silt	Clay	Organic matter	pH	Air temperature				Relative humidity	Wind speed	
			----- % -----											
E1	2021	Ridgetown A	Loam	33	31	36	4.1	7.3	May 14	May 21	June 11	24.7	69.7	4.3
E2	2021	Ridgetown B	Clay loam	27	38	35	4.2	7.4	May 18	May 23	June 16	24.0	43.1	1.2
E3	2021	Exeter	Clay loam	29	44	27	4.4	7.9	April 27	May 17	June 2	20.4	59.1	1.5
E4	2022	Ridgetown A	Clay loam	30	31	39	4.7	7.2	May 12	May 19	June 15	26.6	81.0	7.8
E5	2022	Ridgetown B	Loam	26	36	38	4.1	7.3	May 13	May 20	June 17	24.2	57.1	6.9
E6	2022	Ridgetown C	Sandy loam	74	15	11	3.1	6.4	May 12	May 19	June 15	29.5	78.1	2.9

Note. ^a Soil cores were extracted to a depth of 15 cm and analyzed by A&L Canada Laboratories Inc. (2136 Jetstream Road, London, ON) to determine soil characteristics.

Experiments were established as a randomized complete block design with four replications. Treatments included a weedy control and glyphosate at 450, 900, and 1350 g ae ha⁻¹ applied alone or with Class Act NG adjuvant at 2.5% v/v (Tables 2-4). Each plot was 3 m wide and 8 or 10 m long and consisted of four rows spaced 0.75 m apart of GR 'DKC39-97 RIB'/'DKC 42-04RIB' corn. Corn was planted at a rate of approximately 85,000 seeds ha⁻¹.

Herbicide treatments were applied postemergence when weeds were approximately 10 cm in height (V2-4 corn growth stage) using a CO₂-pressurized backpack sprayer calibrated to deliver 200 L ha⁻¹ aqueous solution at 240

kPa. The boom was 1.5 m wide with four ULD120-02 nozzles (Hypro, New Brighton, MN, USA) spaced 0.5 m apart producing a spray width of 2.0 m.

Corn injury was visually evaluated 1 and 4 weeks after herbicide application (WAA) and weed control was visually evaluated 4 and 8 WAA on a scale of 0 (no injury/control) to 100% (complete necrosis/control). Corn was combined at harvest maturity with a small plot combine; weight and seed moisture content were recorded. Corn yield was adjusted to 15.5% seed moisture content prior to analysis.

Data analyses were conducted using the GLIMMIX procedure in SAS Studio v9.4, OnDemand for Academics (SAS Institute, Cary, NC) with a significance level of 0.05. Data were pooled across environments. The fixed effect of herbicide treatment and the random effects of environment (location-year combinations), block nested within environment, and the environment-by-treatment interaction accounted for model variance. Treatments with zero variance were excluded from analyses. Visual estimates of weed control at 4 and 8 WAA were analyzed for each species using a Gaussian (identity link), beta (logit link), or binomial (complementary log-link or probit link) distribution. When required, data were arcsine square-root transformed prior to analysis. Corn yield was analyzed using a Gaussian distribution (identity link). Pearson chi-square/degrees of freedom ratio and Shapiro-wilk statistic were used to evaluate model fitness and identify potential overdispersion of residuals, respectively. Plots of normal probability and studentized residuals were generated to verify assumptions of normality and homogeneity of variance, respectively. Non-orthogonal contrasts were used to evaluate the effect of Class Act NG on glyphosate efficacy. All treatments were independently compared to zero using a t-test. Tukey-Kramer multiple range test was used to separate least square means. Data transformed using the arcsine square root function were back-transformed post-analysis.

3. Results and Discussion

Data were pooled and averaged over years and locations when there was no statistically significant interaction between year, location, and treatments (Tables 2-4). Weeds were included when present in 2 or more sites.

3.1 Corn Injury

At 1 and 4 WAA, there was no visible corn injury with any of the herbicide treatments evaluated (data not presented). This is consistent with other studies showing no/minimal corn injury from glyphosate applied alone or co-applied with AMS (Nurse et al., 2008; Soltani et al., 2011, 2016b).

3.2 Velvetleaf (*ABUTH*)

Glyphosate (450 g ae ha⁻¹) controlled velvetleaf 78% at 4 WAA and 81% at 8 WAA; the addition of Class Act NG improved control 14% at 8 WAA (Table 2). Glyphosate (900 g ae ha⁻¹) controlled velvetleaf 91% and 4 WAA and 97% at 8 WAA; the addition of Class Act NG did not significantly improve control (Table 2). Glyphosate (1350 g ae ha⁻¹) controlled velvetleaf 97% at 4 WAA and 99% at 8 WAA; the addition of Class Act NG adjuvant did not significantly improve control (Table 2). Based on non-orthogonal contrasts, averaged across all glyphosate rates, the addition of Class Act NG to glyphosate improved velvetleaf control 7% at 4 WAA and 3% at 8 WAA (Table 2). In other studies, the addition of AMS to glyphosate applied at 450, 675, or 900 g ae ha⁻¹ provided little or no improvement in velvetleaf control (Soltani et al., 2016b). Soltani et al. (2011) observed no benefit from the addition of AMS to glyphosate (900 g ae ha⁻¹) for the control of velvetleaf in corn. However, Pratt et al. (2003) reported that velvetleaf control can be improved up to 70% when added to 280 g ae ha⁻¹ of glyphosate; however, this is 31% of the lowest registered rate in Ontario. Nurse et al. (2008) and Young et al. (2003) also reported that the addition of AMS to glyphosate, at doses of < 450 g ae ha⁻¹ (half the label rate) can improve velvetleaf control. Hall et al. (2000) reported that the addition of AMS (2.5 L ha⁻¹) to glyphosate at 125, 250, 500 or 1000 g ae ha⁻¹ can improve velvetleaf control regardless of water hardness.

Table 2. Visible control and non-orthogonal contrasts for velvetleaf (ABUTH), Powell amaranth (AMAPO), and common ragweed (AMBEL) 4 and 8 weeks after application (WAA) of glyphosate and glyphosate plus Class Act NG adjuvant applied postemergence in corn in field trials across Ontario, Canada in 2021 and 2022

Herbicide treatment	Rate	Control					
		ABUTH		AMAPO		AMBEL	
		4 WAA	8 WAA	4 WAA	8 WAA	4 WAA	8 WAA
	g ae ha ⁻¹	%					
Untreated control	-	0 c	0 c	0 c	0 c	0 d	0 c
Glyphosate	450	78 b	81 b	89 b	91 b	81 c	80 b
Glyphosate + Class Act NG ^b	450	90 ab	95 a	98 a	98 a	88 bc	88 ab
Glyphosate	900	91 ab	97 a	99 a	98 a	90 ab	90 a
Glyphosate + Class Act NG	900	98 a	99 a	99 a	99 a	93 ab	93 a
Glyphosate	1350	97 ab	99 a	99 a	99 a	95 ab	95 a
Glyphosate + Class Act NG	1350	98 a	99 a	99 a	99 a	95 a	94 a
<i>Contrasts</i>							
Glyphosate vs. glyphosate + Class Act NG	450	78 vs. 90 NS	81 vs. 95**	89 vs. 98**	91 vs. 98 **	81 vs. 88*	80 vs. 88*
Glyphosate vs. glyphosate + Class Act NG	900	91 vs. 98 NS	97 vs. 99 NS	99 vs. 99 NS	98 vs. 99 NS	90 vs. 93 NS	90 vs. 93 NS
Glyphosate vs. glyphosate + Class Act NG	1350	97 vs. 98 NS	99 vs. 99 NS	99 vs. 99 NS	99 vs. 99 NS	95 vs. 95 NS	95 vs. 94 NS
Glyphosate vs. glyphosate + Class Act NG ^d		90 vs. 97*	95 vs. 98**	97 vs. 99*	97 vs. 99*	89 vs. 93 *	89 vs. 92 NS

Note. Abbreviations: WAA; weeks after application.

^a The nontreated control was excluded from visible control analyses; herbicide tank-mixtures were compared to the nontreated control using p-values generated from Least Square Means comparisons.

^b Class Act NG was included at 2.5% v/v (Winfield Solutions, LLC, St. Paul, MN, USA).

^c Means followed by the same letter within a column are not significantly different according to Tukey-Kramer multiple range test ($P > 0.05$).

^d Averaged across all treatment levels.

* significant at $P < 0.05$, ** significant at $P < 0.01$.

3.3 Powell Amaranth (AMPO)

Glyphosate (450 g ae ha⁻¹) controlled Powell amaranth 89% at 4 WAA and 91% at 8 WAA; the addition of Class Act NG improved control 9% at 4 WAA and 7% at 8 WAA (Table 2). Glyphosate at 900 g ae ha⁻¹ or 1350 g ae ha⁻¹ controlled Powell amaranth 98-99% at 4 and 8 WAA; non-orthogonal contrasts indicated that the addition of Class Act NG to glyphosate at 900 g ae ha⁻¹ or 1350 g ae ha⁻¹ provided no improvement of Powell amaranth at 4 or 8 WAA (Table 2). Averaged across all glyphosate rates, the addition of Class Act NG to glyphosate improved Powell amaranth control 2% at 4 and 8 WAA (Table 2). These results are similar to other studies in which redroot pigweed was controlled 95-100% when glyphosate was applied with or without 2% AMS at rates of at least 450 g ae ha⁻¹ (Guza et al., 2002; Krausz et al., 1996). In other studies, the addition of AMS to glyphosate applied at 450, 675, or 900 g ae ha⁻¹ did not improve redroot pigweed control in corn (Soltani et al., 2016b). Another study also found no benefit from the addition AMS (2.5 L ha⁻¹) to glyphosate (900 g ae ha⁻¹) for the control of pigweed in corn (Soltani et al., 2011). Mahoney et al. (2014) reported that the addition of AMS to glyphosate applied at 900 g ae ha⁻¹ regardless of the carrier water hardness provided negligible effects on pigweed control in corn. Nurse et al. (2008) reported improvement in the control of redroot pigweed at 2 WAA when AMS was added to glyphosate at 225 g ae ha⁻¹ (25% of the lowest label rate in Ontario).

3.4 Common Ragweed (AMBEL)

Glyphosate (450 g ae ha⁻¹) controlled common ragweed 81% at 4 WAA and 80% at 8 WAA; based on non-orthogonal contrasts the addition of Class Act NG improved common ragweed control 7 and 8% at 4 and 8 WAA, respectively (Table 2). Glyphosate at 900 and 1350 g ae ha⁻¹ controlled common ragweed 90 and 95%, respectively at 4 and 8 WAA; the addition of Class Act NG provided no improvement in common ragweed control at 4 or 8 WAA (Table 2). Based on non-orthogonal contrasts, averaged across all glyphosate rates, the addition of Class Act NG to glyphosate improved common ragweed control 4% at 4 WAA but provided no improvement of common ragweed control at 8 WAA (Table 2). Results are similar to other studies where glyphosate applied at 450, 675, and 900 g ae ha⁻¹ controlled common ragweed 80-97%, 85-99%, and 86-99%, respectively; the addition of AMS (2.5 L ha⁻¹) provided little to no added benefit for the control of common

ragweed in corn (Soltani et al., 2016b). Another study also found no benefit from the addition of AMS (2.5 L ha⁻¹) to glyphosate (900 g ae ha⁻¹) for the control of common ragweed in corn (Soltani et al., 2011).

3.5 Common Lambsquarters (CHEAL)

Glyphosate (450 g ae ha⁻¹) controlled common lambsquarters 77% at 4 WAA and 70% at 8 WAA; the addition of Class Act NG improved control 14% at 4 WAA and 20% at 8 WAA (Table 3). Non-orthogonal contrasts indicated that the addition of Class Act NG to glyphosate (900 g ae ha⁻¹) improved common lambsquarters control 6% at 4 WAA and 5% at 8 WAA (Table 3). The addition of Class Act NG to glyphosate (1350 g ae ha⁻¹) did not improve common lambsquarters control at 4 or 8 WAA (Table 3). Averaged across all glyphosate rates, the addition of Class Act NG to glyphosate improved common lambsquarters control 6% at 4 WAA and 7% at 8 WAA (Table 3). Results are similar to other studies where glyphosate applied at 450, 675, and 900 g ae ha⁻¹ controlled common lambsquarters 91-99%, 93-100%, and 94-100%, respectively and the addition of AMS (2.5 L ha⁻¹) did not result in improved control of common lambsquarters with glyphosate at high rates in corn (Soltani et al., 2016b). Another study has also shown no benefit with the addition AMS (2.5 L ha⁻¹) to glyphosate at 900 g ae ha⁻¹ (label rate) for the control of common lambsquarters in corn (Soltani et al., 2011). Nurse et al. (2008) reported that the addition of 2% AMS to glyphosate at rates below 450 g ae ha⁻¹ can improve common lambsquarters control but there is no improvement in the control when glyphosate is applied at rates above 450 g ae ha⁻¹.

Table 3. Visible control and non-orthogonal contrasts for common lambsquarters (CHEAL), barnyardgrass (ECHCG), and giant foxtail (SETFA) 4 and 8 weeks after application (WAA) of glyphosate and glyphosate plus class act NG applied postemergence in corn in field trials across Ontario, Canada in 2021 and 2022

Herbicide treatment	Rate	Control					
		CHEAL		ECHCG		SETFA	
		4 WAA	8 WAA	4 WAA	8 WAA	4 WAA	8 WAA
	g ae ha ⁻¹	----- % -----					
Untreated control	-	0 d	0 d	0 c	0 b	0 b	0 b
Glyphosate	450	77 c	70 c	85 b	82 a	85 a	98 a
Glyphosate + Class Act NG ^b	450	91 b	90 b	92 ab	90 a	99 a	99 a
Glyphosate	900	91 b	91 ab	91 ab	89 a	100 a	99 a
Glyphosate + Class Act NG	900	97 ab	96 ab	95 a	93 a	100 a	100 a
Glyphosate	1350	97 ab	96 ab	95 a	93 a	100 a	100 a
Glyphosate + Class Act NG	1350	98 a	97 a	95 a	93 a	100 a	100 a
<i>Contrasts</i>							
Glyphosate vs. glyphosate + Class Act NG	450	77 vs. 91**	70 vs. 90**	85 vs. 92*	82 vs. 90 NS	85 vs. 99 NS	98 vs. 99 NS
Glyphosate vs. glyphosate + Class Act NG	900	91 vs. 97*	91 vs. 96*	91 vs. 95*	89 vs. 93 NS	100 vs. 100 NS	99 vs. 100 NS
Glyphosate vs. glyphosate + Class Act NG	1350	97 vs. 98 NS	96 vs. 97 NS	95 vs. 95 NS	93 vs. 93 NS	100 vs. 100 NS	100 vs. 100 NS
Glyphosate vs. glyphosate + Class Act NG ^d		90 vs. 96**	88 vs. 95**	91 vs. 94**	88 vs. 92 NS	95 vs. 99 NS	99 vs. 100 NS

Note. Abbreviations: WAA; weeks after application.

^a The nontreated control was excluded from visible control analyses; herbicide tank-mixtures were compared to the nontreated control using p-values generated from Least Square Means comparisons.

^b Class Act NG was included at 2.5% v/v (Winfield Solutions, LLC, St. Paul, MN, USA).

^c Means followed by the same letter within a column are not significantly different according to Tukey-Kramer multiple range test ($P > 0.05$).

^d Averaged across all treatment levels.

* significant at $P < 0.05$, ** significant at $P < 0.01$.

3.6 Barnyardgrass (ECHCG)

Glyphosate (450 g ae ha⁻¹) controlled barnyardgrass 85% at 4 WAA and 82% at 8 WAA; based on non-orthogonal contrasts the addition of Class Act NG adjuvant improved barnyardgrass control 7% at 4 WAA (Table 3). Glyphosate (900 g ae ha⁻¹) controlled barnyardgrass 91% at 4 WAA and 89% at 8 WAA; based on non-orthogonal contrasts the addition of Class Act NG adjuvant improved barnyardgrass control 4% at 4 WAA (Table 3). Glyphosate at 1350 g ae ha⁻¹ controlled barnyardgrass 95 and 93% at 4 and 8 WAA, respectively; the addition of Class Act NG adjuvant provided no improvement in control of barnyardgrass at 4 or 8 WAA (Table

3). Non-orthogonal contrasts indicated that the addition of Class Act NG adjuvant to glyphosate (averaged across all rates) improved barnyardgrass control 3% at 4 WAA but provided improved control at 8 WAA (Table 3). Results are similar to another study where glyphosate applied at 450, 675, and 900 g ae ha⁻¹ controlled barnyardgrass 90-98%, 95-100%, and 97-100%, respectively (Soltani et al., 2016b); the addition of AMS (2.5 L ha⁻¹) to glyphosate did not improve barnyardgrass control in corn (Soltani et al., 2016b). Another study also found no benefit in the control of annual grasses including barnyardgrass with the addition of AMS (2.5 L ha⁻¹) to glyphosate applied at 900 g ae ha⁻¹ in corn (Soltani et al., 2011).

3.7 Giant Foxtail (*SETFA*)

Glyphosate at 450, 900, and 1350 g ae ha⁻¹ controlled giant foxtail 85, 100, and 100%, respectively at 4 WAA; the addition of Class Act NG did not improve giant foxtail control (Table 3). Glyphosate at 450, 900, and 1350 g ae ha⁻¹ controlled giant foxtail 98, 99, and 100%, respectively at 8 WAA; the addition of Class Act NG did not improve giant foxtail control (Table 3). Based on non-orthogonal contrasts, averaged across all glyphosate rates, the addition of Class Act NG to glyphosate provided no improvement in giant foxtail control at 4 and 8 WAA (Table 3). Nurse et al. (2008) reported that the addition of 2% AMS to glyphosate at rates above 225 g ae ha⁻¹ provides no improvement in the control of annual grasses (including foxtails) in corn. In another study, orthogonal contrasts indicated that there was no improvement in the percent control of annual grasses such as foxtails when AMS (2.5 L ha⁻¹) was added to glyphosate (900 g ae ha⁻¹) using water sources with various hardness 2, 4, and 8 WAA (Soltani et al., 2011).

3.8 Green Foxtail (*SETVI*)

Glyphosate at 450, 900, and 1350 g ae ha⁻¹ controlled green foxtail 88, 96, and 98%, respectively at 4 WAA; the addition of Class Act NG did not improve green foxtail control (Table 4). Glyphosate at 450, 900, and 1350 g ae ha⁻¹ controlled green foxtail 92, 96, and 97%, respectively at 8 WAA; the addition of Class Act NG did not improve green foxtail control (Table 4). Based on non-orthogonal contrasts, averaged across all glyphosate rates, the addition of Class Act NG to glyphosate provided no improvement in green foxtail control at 4 and 8 WAA (Table 4). Results are similar to other studies where glyphosate applied at 450, 675, and 900 g ae ha⁻¹ controlled green foxtail 91-100%, 96-100%, and 97-100%, respectively and the addition of AMS (2.5 L ha⁻¹) provided no improvement in the control of green foxtail in corn (Soltani et al., 2016b). Another study also found no benefit in the control of green foxtail in corn with the addition of AMS (2.5 L ha⁻¹) to glyphosate (900 g ae ha⁻¹) (Soltani et al., 2011).

Table 4. Visible control of green foxtail (SINAR) 4 and 8 weeks after application (WAA), corn grain yield, and non-orthogonal contrasts from glyphosate and glyphosate plus Class Act NG applied postemergence in corn in field trials across Ontario, Canada in 2021 and 2022

Herbicide treatment	Rate	Control		Yield
		SETVI		
		4 WAA	8 WAA	
	g ae ha ⁻¹	----- % -----		kg ha ⁻¹
Untreated control	-	0 b	0 b	3,550 b
Glyphosate	450	88 a	92 a	9,290 a
Glyphosate + Class Act NG ^b	450	96 a	96 a	10,210 a
Glyphosate	900	96 a	96 a	10,490 a
Glyphosate + Class Act NG	900	97 a	97 a	10,440 a
Glyphosate	1350	98 a	97 a	10,480 a
Glyphosate + Class Act NG	1350	98 a	97 a	10,320 a
<i>Contrasts</i>				
Glyphosate vs. glyphosate + Class Act NG	450	88 vs. 96 NS	92 vs. 96 NS	9,290 vs. 10,210 NS
Glyphosate vs. glyphosate + Class Act NG	900	96 vs. 97 NS	96 vs. 97 NS	10,490 vs. 10,440 NS
Glyphosate vs. glyphosate + Class Act NG	1350	98 vs. 98 NS	97 vs. 97 NS	10,480 vs. 10,320 NS
Glyphosate vs. glyphosate + Class Act NG ^d		94 vs. 97 NS	95 vs. 97 NS	10,080 vs. 10,330 NS

Note. Abbreviations: WAA; weeks after application.

^a The nontreated control was excluded from visible control analyses; herbicide tank-mixtures were compared to the nontreated control using p-values generated from Least Square Means comparisons.

^b Class Act NG was included at 2.5% v/v (Winfield Solutions, LLC, St. Paul, MN, USA).

^c Means followed by the same letter within a column are not significantly different according to Tukey-Kramer multiple range test ($P > 0.05$).

^d Averaged across all treatment levels.

* significant at $P < 0.05$, ** significant at $P < 0.01$.

3.9 Corn Yield

Weed inference reduced corn yield up to 66% (highest yielding treatment compared to the weedy control) in this study. Reduced weed interference with glyphosate increased corn yield 162 to 195% compared to the untreated (weedy) control. There was no improvement in corn yield from the addition of Class Act NG to glyphosate at 450, 900, and 1350 g ae ha⁻¹ (Table 4).

Results are similar to other studies where glyphosate at 450, 675, or 900 g ae ha⁻¹ applied with and without AMS (2.5 L ha⁻¹) resulted in similar corn yield (Soltani et al., 2016b). Another study also found no yield benefit when AMS (2.5 L ha⁻¹) was added to glyphosate at 900 g ae ha⁻¹ in corn (Soltani et al., 2011). Nurse et al. (2008) also reported that the addition of AMS (2.5 L ha⁻¹) to glyphosate at 225, 450, 675, and 900 g ae ha⁻¹ provided no improvement in con yield.

4. Conclusions

This research shows that the addition of Class Act NG (2.5% v/v) to glyphosate at 450 g ae ha⁻¹ can provide improved control of common lambsquarters, velvetleaf, Powell amaranth, common ragweed and barnyardgrass but does not provide any improvement in control of giant foxtail or green foxtail or corn yield. The addition of Class Act NG (2.5% v/v) to glyphosate at 900 g ae ha⁻¹ provides improved control of common lambsquarters and barnyardgrass but does not provide improved control of velvetleaf, Powell amaranth, common ragweed, giant foxtail, and green foxtail or corn yield. The addition of Class Act NG (2.5% v/v) to glyphosate at 1350 g ae ha⁻¹ does not provide any improvement in control of velvetleaf, Powell amaranth, common ragweed, common lambsquarters, barnyardgrass, giant foxtail, and green foxtail or corn yield. Based on this data the effect of Class Act NG (2.5% v/v) on weed control efficacy with glyphosate in corn is most frequent at the low rate of 450 g ae ha⁻¹ and is weed species-specific.

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Authors Contributions

Drs. Peter Sikkema and Nader Soltani were responsible for the study design and writing of this manuscript. Christian Willemsse conducted the statistical analysis of the data collected.

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Evaluation of Mungbean Varieties for Adaptation to Rice-Based Cropping Systems and Profitability in North-West Cambodia

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Abstract

Two varieties of mungbean (*Vigna radiata* (L.) Wilczek) are most commonly grown in Battambang Province North-West Cambodia: KPS-2 (Thailand); and DX-208 (Vietnam). From the buyer's point of view, the ideal variety would have large shiny seeds and from the farmer's point of view, resistance to pod-shattering for single-pick or mechanical harvesting is a priority. KPS-2 has resistance to pod-shattering but small seeds and DX-208 has large seeds but has pods that shatter readily. The ideal variety would have both traits. This study evaluated 17 released mungbean varieties from Cambodia, Thailand, Vietnam and Australia for grain yield, seed weight and resistance to pod-shattering in a series of experiments from 2019 to 2021. In 2019, the four Cambodian varieties were evaluated alongside 11 Australian public varieties. Five of the Australian varieties were rejected because of low seed weight, dull seed coat and unacceptable color. Cambodian variety CMB-1 was rejected because of dull seed coat and indeterminate maturity. In 2021, the six remaining Australian varieties were re-evaluated together with locally grown varieties (DX-208, KPS-2) and Cambodian varieties (CARDI Chey, CMB-2 and CMB-3). The seed weight for Emerald was very similar to that for DX-208. Seed weights for CARDI Chey, CMB-3, King and Shantung were not significantly different to KPS-2. In laboratory testing for resistance to pod shattering, Delta, Emerald and KPS-2 were the most resistant and DX-208 was the most susceptible to pod shattering. The Australian varieties Delta and Emerald are recommended for further testing across other mungbean growing regions of Cambodia before submission for registration and commercial release.

Keywords: mungbean, varieties, pod-shattering, seed weight, seed lustre

1. Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), with a short duration of 60-90 days, is a low-cost diversification option for rice-rice and rice-wheat cropping systems in South and South-East Asia (Shanmugasundaram et al., 2009). After cassava, maize and soybean, mungbean was the fourth most important non-rice crop in Cambodia in 2019 with an area of 34,577 ha (Anonymous, 2020). Mungbean is grown in 22 out of 25 Provinces and the largest mungbean growing regions are in the Tonle Sap active flood plain and on recently cleared upland areas such as in Preah Vihear and Monduliri Provinces (Figure 1). In 2019, the Tonle Sap flood plain accounted for 13,451 ha (39%) of the mungbean area with a mean yield of 1,079 kg ha⁻¹. There were 13,405 ha (39%) of mungbean in Monduliri, Preah Vihear and Stung Treng Provinces in 2019 with a mean yield of 1,174 kg ha⁻¹. This study was carried out in Battambang Province where the mean yield of mungbean was below average at 769 kg ha⁻¹ (Anonymous, 2020).

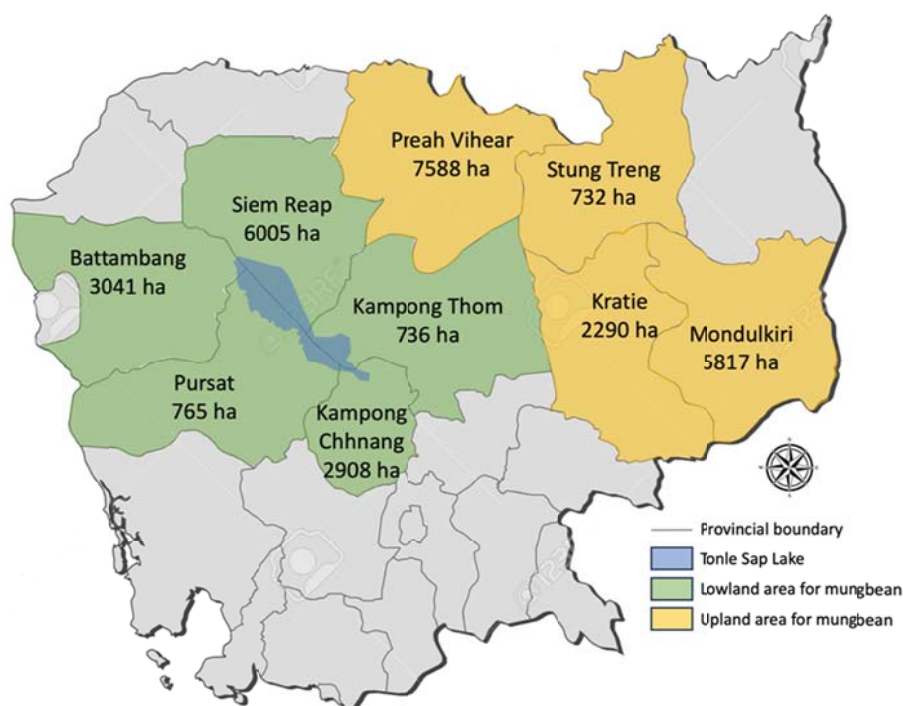


Figure 1. The main mungbean production regions in Cambodia (the area shaded green is mungbean grown after receding flood waters in the Tonle Sap floodplain and the area shaded yellow is mungbean grown in the upland after forest clearance)

There are many benefits of rice-mungbean rotations in rice cropping systems. For example, nitrogen fixed by mungbean ranged from 26-36 kg ha⁻¹ with residual nitrogen of up to 26 kg ha⁻¹ left for the following rice crop (Ahmad et al., 2001). Rice paddy yields were 0.6-1.1 t ha⁻¹ higher in mungbean-rice rotations compared with rice monocultures (Ahmad et al., 2001). In Cambodia, incorporation of mungbean (with 10 kg P ha⁻¹ applied) into the rotation with rice added up to 21 kg N ha⁻¹ and increased rice yield to over 4 t ha⁻¹ compared with around 3 t ha⁻¹ in the control (Ro et al., 2016). Rice-mungbean rotations also provided higher farmer incomes and soil organic carbon than rice monocultures in India (Nath et al., 2019; Malik et al., 2021).

In the past, attempts have been made to include mungbean rotations in Cambodian lowland rice systems with limited success as mungbean establishment is often poor with poor crop growth and low yield (Chan et al., 2004; Ouk et al., 2007). A major limitation for growing mungbean after rice into the dry season is the shallow root system of mungbean in lowlands where hard pans limit rooting depth to less than 20 cm in most soil types (Som et al., 2011). This shallow rooting zone limits the supply of water and nutrients throughout crop growth. If non-rice crops planted after rice in the lowlands are to become feasible, it would be necessary to deep-rip hard pans or select soils where soil strength does not increase with depth. Rhizobial inoculation of mungbean has been shown to eliminate the need for application of nitrogenous fertilisers in upland cropping systems in Cambodia (Pin et al., 2009). Mungbean yields were increased by 7% with application of rhizobial inoculant and this was greater than the yield increase from 40 kg ha⁻¹ of N applied as urea fertiliser. An additional benefit, not measured, is the increased levels of N available to crops following mungbean.

Mungbean is a low-input, high-value diversification crop option in lowland rice systems with an established value chain in North-West Cambodia (Campbell-Ross et al., 2019; Rootsey et al., 2017). Current farmer practice for mungbean in the Battambang Tonle Sap flood plain is to commence land preparation after flood waters recede in late October to early November after which the fields are ploughed one or two times and harrowed. Seed is hand-broadcast at 30 kg ha⁻¹ in mid-November (Martin et al., 2020). The fields are then harrowed again to incorporate the seed. In the absence of irrigation, the crop relies on residual soil water after the wet season flood recedes. In Siem Reap Province, mungbean is planted after the wet season flood waters recede without tillage and at seeding rates of 200-300 kg ha⁻¹. In Kampong Chhnang Province, mungbean is hand broadcast into

rice crop residues after rice harvest at the end of the wet season also at 200-300 kg ha⁻¹. In both Siem Reap and Kampong Chhnang, mungbean seed is broadcast when surface water has not completely drained from the field.

In Battambang, once the crop is established, the average total rainfall during the 3-month growing season is 29 mm. No fertiliser is applied at crop establishment but foliar applications of trace elements and growth regulators are applied to promote flowering. In-crop herbicides are not usually applied because weeds tend to be less competitive with mungbean under the dry seedbed conditions. Until recently, mungbean in North-West Cambodia have been picked by hand with the first pick at around 60 days after sowing (DAS) and the second pick at around 90 DAS. Mungbean growers are now looking for varieties with pods that do not shatter and which can be harvested once at 90 DAS, either by hand or machine. Two varieties are most commonly grown in Battambang Province, KPS-2 from Thailand and DX-208 from Vietnam. From the buyer's point of view, the ideal variety would have large shiny seeds, whereas the farmers' priority is resistance to pod-shattering. KPS-2 has resistance to pod-shattering but small seeds and DX-208 has large seeds but has pods that shatter readily. The ideal variety would have both traits.

The Cambodian Agricultural Research and Development Institute (CARDI) released CARDI Chey mungbean (VC 1973A) in 2002 (CARDI, 2005). CARDI went on to evaluate 25 mungbean breeding lines and varieties between 2003 and 2005. The lines were obtained from the Asian Vegetable Research and Development Center (AVRDC), Australia and Thailand (Ouk et al., 2009). Three Cambodian Mungbean (CMB) varieties were released from this program: CMB-1 (VC 4152); CMB-2 (VC 3541B); and CMB-3 (HL33-6) from the Australian collection (James, 2020). Although officially released, none of these Cambodian mungbean varieties are commercially available.

Hand-picking requires labor which is becoming scarce, so mungbean varieties that mature synchronously and can be hand-picked or machine-harvested at one time are the key for keeping mungbean production attractive to farmers (Schafleitner & Nair, 2020). This is especially important in Cambodia, where the growing shortage and increasing cost of rural labor has already forced farmers to reduce the number of picks. Pod-shattering is the natural process of pod dehiscence that facilitates dispersal of seeds of wild plants at physiological maturity. Pod-shattering, as an evolved seed dispersal mechanism, is an important cause of yield loss in crops such as mungbean, which are still in the process of domestication (Nirmalbharati & Sumangala, 2016). Although mungbean germplasm is subject to various studies such as molecular diversity analysis (Nirmalbharati & Sumangala, 2016), and screening for pod shattering in mutant populations (Vairam et al., 2017), no useful published comparisons can be found on pod-shattering in commercial mungbean varieties. In 2018, three Thai and four Vietnamese varieties were evaluated at two sites in Battambang (Campbell-Ross et al., 2019) but rejected for evaluation in this study because they had lower seed weights compared to DX-208. However, the Australian National Mungbean Improvement Program includes resistance to pod-shattering as a selection criterion (Douglas, 2007). Since all mungbean are harvested by combine machine in Australia, it is reasonable to expect that Australian varieties might have genetic resistance to pod-shattering and were considered for evaluation in this study. In 2018, Cambodian government approval was obtained to import a collection of 11 public mungbean varieties from the Australian Mungbean Collection. These varieties were evaluated in comparison with the four Cambodian varieties and the locally grown DX-208 and KPS-2 varieties in 2019 and 2021.

The objectives of this study were to (1) evaluate commercial mungbean varieties from Thailand, Vietnam and Australia for grain yield, seed size and resistance to pod-shattering compared to locally grown and registered varieties and (2) assess the economic benefit to farmers of adopting alternative varieties compared to the currently-grown varieties DX-208 and KPS-2.

2. Method

Experiments were carried out in the dry season from January to April in 2019 and between December and March in 2021 at Don Bosco Agro Technical School (DBAS) (13°04'37"N 103°10'24"E) in Battambang Province. The experiment in the wet season 2021 (August to November) was located in Ta Haen Muoy (TAHM) village in Battambang Province (13°4'42"N, 103°15'51"E). Records of monthly rainfall (mm) and mean monthly maximum and minimum temperature (°C) were obtained from the Department of Water Resources and Meteorology weather station at Veal Bek Chan, Battambang (13°5'25"N; 103°12'51"E) (Table 1).

The soil in the experimental areas is an alfisol, known in Cambodia as Toul Samroung (White et al., 1997). The soil has a brown or gray clayey or loamy topsoil that develops moderate to large cracks on drying. The cracks extend deeper than 3-4 cm into the profile. The topsoil has a blocky structure and is very hard when dry. Internal drainage is slow. A plough pan is common. Rice is grown in the wet season and mungbean can be grown as an

opportunity crop at the end of the wet season. Typically, the land is prepared with two passes of a rotavator following rice harvest.

Table 1. Monthly rainfall (mm), mean monthly maximum temperature (°C) and mean monthly minimum temperature (°C) for experimental growing periods for 2019 and 2021

Dry season experiments	January	February	March	April
<i>2019</i>				
Rainfall (mm)	41	0	6	32
Maximum temperature (°C)	34.9	37.8	38.3	36.1
Minimum temperature (°C)	22.6	24.7	25.4	26.3

<i>2021</i>				
Rainfall (mm)	0	1	1	81
Maximum temperature (°C)	35	38.5	41	40.3
Minimum temperature (°C)	15	20.2	23	24.5
Wet season experiment	August	September	October	November
<i>2021</i>				
Rainfall (mm)	137	296	230	64
Maximum temperature (°C)	34.4	32.1	31.4	31.6
Minimum temperature (°C)	26.1	25.3	25	24.4

In all experiments, weeds were removed by hand-weeding. Powdery mildew (*Erysiphe polygoni* D.C.) occurred in mungbean at DBAS but did not cause economic yield loss. Virus symptoms similar to Mungbean Yellow Mosaic Virus (MYMV) was present in the wet season crop in 2021 but did not cause economic yield loss. An Integrated Pest Management (IPM) strategy was used to manage insect pests (Martin et al., 2021). A liquid rhizobial inoculant containing *Bradyrhizobium* spp. was used for inoculation of the mungbean seed (Tittabutr et al., 2007). The application rate was 100 mL of inoculant per 5 kg of mungbean seed. A total of 17 mungbean varieties were evaluated in this study (Table 2).

Table 2. Mungbean varieties included in this study

Variety	Accession No.	Reference
Berken	Unknown	
CARDI Chey	VC 1973A	CARDI, 2005
Celera	Unknown	
CMB-1	VC 4512	Ouk et al., 2009
CMB-2	VC 3541B	Ouk et al., 2009
CMB-3	HL33-6	James, 2020
Delta	Unknown	
DX-208	Unknown	
Emerald	ARGL 94-5	Imrie, 1995
Green Diamond	Unknown	
King		Cook, 1982
KPS-2	VC 2768A	Shanmugasundaram et al., 2009
Putland		Yeates et al., 1992
Satin	ARGL 88-3	Lawn & Kommol, 1989
Shantung	ARGL 88-4	Imrie, 1989
White Gold	Unknown	
Yellow Sun	Unknown	

The mungbean growth stage identification method from Pookpakdi et al. (1992) was used (Table 3). Three plants at three locations in each plot (nine plants) in each plot were tagged and the number of flowers, green and brown

Pods were recorded from these plants throughout the crop growth period. Counts were made of open flowers (R_1), pods 1-5 cm (R_2), pods > 5 cm (R_3), full seed R_4 and brown pods (R_5). At harvest (picks), measurements were made of pods per plant, seeds per pod and 100 seed weight on tagged plants.

Table 3. Reproductive stages for mungbean (Pookpakdi et al., 1992)

Stage	Abbrev. title	Description
R_1	Beginning flower	One open flower at any node on the main stem
R_2	Beginning pod	One pod of 1.0 cm length between nodes 4-6 of the main stem
R_3	Beginning seed	One pod of 5.0 cm length found on any of the top three nodes on the main stem
R_4	Full seed	One pod on any of the top three nodes has constriction between seed
R_5	Start maturity	One pod on the main stem turns to brown, dark brown or black
R_6	First harvest	Fifty percent of pods on the plant are mature
R_7	Second harvest	R_7 is reached after the remaining pods on the plant mature

2.1 Experiment in 2019

A Cambodian government import certificate was obtained for eleven Australian public mungbean varieties. The small quantity of seed available ruled out the possibility of replication of the Australian varieties. A field experiment was designed to include the locally grown varieties (DX-208, KPS-2) and the Cambodian varieties (CARDI Chey, CMB-1, CMB-2, CMB-3). The local varieties were replicated in the experiment. The experiment was located at DBAS. The seed bed was prepared by rotavator and beds formed by hand labor on 1.4 m centers. Seeds were planted by hand on 14th January 2019 in three rows, 30 cm apart. Single seeds were planted at 10 cm spacing within rows. The mungbean beds were separated by two rows of sweet corn with row-spacing of 70 cm. Data were recorded according to the schedule in Table 4. Establishment counts were taken from three 1.0 × 0.6 m quadrats per plot. Three sets of three plants (nine) were tagged in each plot for recording of phenological data.

2.2 Experiment in the Dry Season 2020-21

Seed samples collected from the 2019 experiment were dried to 10% moisture content and stored in sealed containers at 5°C until germination testing in November 2020. The experiment was located at DBAS. The previous crop was rice which was combine-harvested at the end of October 2020. The field was rotavated twice on 8th November 2020 and free water drained from the field. Once the seedbed was sufficiently dry, the field was rotavated again and hand-planting commenced on 11th December 2020. The experiment was laid out in a randomized complete block design with four replicates. Plots were 1.5 × 5.0 m with four rows at 30 cm spacing. Single seeds were planted at 15 cm spacing within rows (22 seeds m⁻²). Establishment counts were made at 15 DAS from three quadrats (2 rows × 1 m: 0.6 m²) from each plot. At 60 and 73 DAS, data were collected from three tagged plants per plot for reproductive stage of development (Pookpakdi et al., 1992) open flowers (R_1); pods 1-5 cm long (R_2); pods > 5 cm (R_3); pods showing seed definition (R_4); brown pods (R_5). The number of shattered pods per plant was recorded from tagged plants at 60 and 85 DAS. The crop was harvested at one time at 90 DAS. At 15 DAS, four blue sticky traps (Anonymous, 2021) were installed 10 m apart in a square in the experimental area for monitoring and control of bean flower thrips (*Megalurothrips usitatus* Bagnall) (Tang et al., 2016). Sticky traps were replaced with fresh traps after 10 days and replacement repeated to give three times of sticky trap deployment up to 60 DAS. A bio-insecticide containing *Beauveria bassiana* Balsamo-Crivelli, was applied as a 2% solution in water at a rate of 500 g ha⁻¹ at 15 and 25 DAS. The *B. bassiana* strain used was IPL/BB/M1/01 (Mascarin & Jaronski, 2016) containing 1 × 10⁹ colony forming units (CFU) g⁻¹ as a wettable powder. Virtako 40 WG (chlorantraniliprole 20% + thiamethoxam 20 %) was applied at 100 g ha⁻¹ at 32 DAS to control leaf folders (*Omiodes diemenalis* Guenée and *O. indicata* Fabricius).

2.3 Experiment in the Wet Season 2021

The experiment was located in a non-rice area on Toul Samroung soil. The previous crop was maize. Glyphosate 480 g L⁻¹ was applied at 2 L ha⁻¹ on 17th August, the field was rotavated on 19th August and the experiment was planted by hand on the 24th August 2021. The experiment was laid out in a randomized complete block design with four replicates. Plots were 2.0 × 6.0 m with five rows at 40 cm spacing and within-row plant spacing of 10 cm. (25 seeds m⁻²). At planting, blue and yellow sticky traps were placed at each of the four corners of the experiment and replaced weekly until 63 DAS. Beauveria applications were the same as in the previous experiment. No chemical insecticides were applied. Establishment counts were taken from three 1.0 × 1.2 m

quadrats per plot 14 DAS. Flower and pod counts commenced on 28th September (35 DAS). At 60 and 73 DAS, data were collected from three tagged plants per plot for reproductive stage of development (Pookpakdi et al., 1992): open flowers (R_1); pods 1-5 cm long (R_2); pods > 5 cm (R_3); pods showing seed definition (R_4); brown pods (R_5). Four samples per plot were taken from the first pick to record 100 seed weight for each variety. The number of shattered pods per plant was recorded from tagged plants at 60 and 85 DAS. The crop was hand-picked at 63 and 84 DAS. Testing for resistance to pod shattering was done using an oven drying method in the laboratory (Antwi-Boasiako, 2017; AVRDC, 1997). Twenty-five pods were taken at random from the first pod harvest (63 DAS) for each of the 40 plots. Samples were kept in paper bags at room temperature for 60 days to allow moisture content to equilibrate. The pods were then oven dried at 80°C for 12 hours. Pods that opened to release the seeds or opened but did not release seeds were considered shattered. The number of shattered pods were expressed as the ratio of shattered pods to total pods.

2.4 Statistical Analysis

In all experiments, analysis of variance (ANOVA) was performed on numerical measurements using IBM® SPSS® version 22. The means were compared using Duncan's Multiple Range post-hoc analysis (Duncan, 1955). Three samples per plot were included in the analysis for plant establishment and plant height. Nine samples per plot were analysed for numbers of flowers and pods and one whole-plot sample was analyzed for whole-plot measurements.

3. Results

3.1 Experiment in 2019

There were no significant differences between varieties for plant establishment, the mean of which was 15.4 plants m⁻². Celera had significantly more open flowers than all other varieties at 42 DAS and Green Diamond had significantly more brown pods at 63 DAS than all other varieties and White Gold and CARDI Chey had significantly more brown pods than Putland and Delta (Table 4). The mean plant height at maturity was 71 cm (Table 4) and CMB-2 was significantly taller than DX-208. There were no significant differences for seeds per pod between varieties with a mean of 12 seeds per pod. White Gold had significantly higher 100 seed weights than DX-208, whereas Satin, Celera, Green Diamond and Putland had significantly lower 100 seed weights than KPS-2. The 100 seed weight of CMB-1 was not significantly different from KPS-2. 100 seed weights for Emerald, CARDI Chey, Yellow Sun, Berken, CMB-2, Satin, King and Delta were not significantly different from DX-208 (Table 4). The mean total yield was 1,820 kg ha⁻¹ and there were no significant differences between varieties. However, there were significant differences at pick 3 where Putland showed later maturity than other varieties.

Celera, CMB-1, Green Diamond, Putland, and Satin were rejected for further evaluation because seeds were too small to be acceptable to the Cambodian market. Berken and Emerald were retained but likely to be rejected because of small seed size. Satin was also unacceptable to the market because of dull seed coat. Yellow Sun was also rejected because of yellow seed coat colour. Six Australian varieties (Berken, Delta, Emerald, King, Shantung, White Gold) were retained for further evaluation in comparison with Cambodian varieties (CARDI Chey, CMB-2) and locally grown varieties (DX-208, KPS-2). Insufficient data were obtained to reliably determine the degree of pod-shattering and all 10 varieties require further evaluation of the extent of pod-shattering.

Table 4. Differences between varieties for plant establishment, reproductive development, plant height and yield components in 2019

Variety	Open flowers	Brown pods	100 seed weight (g)	Height (cm)	Pick 3 yield (kg ha ⁻¹)
	42 DAS	63 DAS	83 DAS	83 DAS	83 DAS
Berken	2.3	1.2	8.17	64	136
CARDI Chey	1.6	2.9	7.97	73	132
Celera	6.1	2.2	4.35	73	205
CMB-1	1.4	2.5	7.53	60	130
CMB-2	2.1	2	8.35	78	98
CMB-3	2.4	1.6	7.71	73	203
Delta	1.6	0.8	8.58	65	296
DX-208	2.8	1.5	8.03	61	249
Emerald	2.4	1.5	7.92	73	95
Green Diamond	1.8	5.6	3.89	67	141
King	1.7	1.2	8.49	77	226
KPS-2	1.3	2	7.01	65	205
Putland	1.3	0	3.75	70	694
Satin	2.1	1.5	6.37	76	156
Shantung	1.4	1.4	8.48	74	127
White Gold	2	2.8	8.81	76	62
Yellow Sun	4.3	2.2	8.04	73	187
S.E.	0.725	0.843	0.222	4.4	63
P value	<0.001	0.004	< 0.001	< 0.001	0.002

3.2 Experiment in the Dry Season 2021

Plant establishment, 13 plants m⁻², was low due to dry seedbed conditions and there were no significant differences between varieties (Table 5). At 68 DAS, there were no significant differences between varieties for open flowers. DX-208 had significantly more pods 1-5 cm than all other varieties except Emerald, CARDI Chey and KPS-2. DX-208 also had significantly more brown pods at 68 DAS than all other varieties except Delta. DX-208 had significantly more shattering than all other varieties. At 83 DAS, the total yield of whole pods and grain yield for Delta was significantly greater than for King and CARDI Chey. There were no differences for shelling ratio. The 100 seed weight of Berken was significantly less than for DX-208 and was therefore eliminated from further evaluation and replaced by CMB-3.

Table 5. Differences between varieties for plant establishment, reproductive development, plant height and yield components in the dry season, 2021

Variety	Estab.	Pods 1-5 cm	Brown pods	Shattered pods	Pod yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	100 seed weight (g)
	14 DAS	68 DAS	68 DAS	68 DAS	83 DAS	83 DAS	83 DAS
Berken	16	6.8	13.6	0.00	739	521	5.8
CARDI Chey	9	10.0	8.3	0.17	298	172	7.0
CMB-2	13	6.6	14.3	0.08	909	642	7.4
Delta	20	4.0	25.7	0.00	1401	914	7.3
DX-208	17	12.8	20.0	1.25	1055	687	7.8
Emerald	12	9.3	15.5	0.00	943	618	7.3
King	9	5.3	8.3	0	295	195	7.5
KPS-2	13	8.2	12.2	0	685	492	6.6
Shantung	14	2.8	17.2	0	636	416	7.3
White Gold	12	5.5	10.3	0	986	689	7.2
S.E.	1.92	1.69	2.85	0.13	221.46	151.14	0.23
P value	0.002	0.002	0.001	< 0.001	0.037	0.042	0.001

3.3 Experiment in the Wet Season 2021

The mean plant establishment at 14 DAS was 46 plants m⁻² and there were no significant differences between varieties. At 35 DAS there were significant differences for open flowers with Delta having more open flowers than KPS-2 and DX-208 (Table 6). Delta had significantly more pods 1-5 cm than all other varieties. At 50 DAS, King had significantly more open flowers and pods 1-5 than all other varieties. Delta had more brown pods but not significantly more than KPS-2, CMB-2 or CARDI Chey. At 56 DAS, CMB-2 and CMB-3 had significantly more open flowers than White Gold and King had more pods > 5 cm than all other varieties. Delta had significantly more brown pods than all other varieties. The grain yield of Emerald was significantly greater than for all other varieties at the first pick (63 DAS) and for total yield at 84 DAS. The yield at the second pick was only 33 kg ha⁻¹ with no significant differences between varieties.

Locally grown variety, DX-208 had the highest 100 seed weight and two varieties King and KPS-2 had significantly lower seed weight compared to DX-208. The seed weight for Emerald was very similar to that for DX-208. Seed weights for CARDI Chey, CMB-3, King and Shantung were not significantly different to KPS-2 (Figure 2). In the laboratory pod shattering test, DX-208 (72%) had significantly more shattered pods than all other varieties (Figure 3). The low incidence of pod-shattering for Emerald and Delta was equivalent to that of KPS-2. Pod-shattering could be considered as marginally acceptable for King, Shantung and White Gold (Figure 3). However, King and Shantung might not be acceptable to the market because of small seed size. Delta and Emerald best satisfy the dual criteria of large seed size and resistance to pod-shattering.

Table 6. Differences between varieties for plant establishment, reproductive development, plant height and yield components in the wet season, 2021

Variety	Open flowers	Open flowers	Brown Pods	Shattered pods (%)	100 seed weight (g)	Grain yield	Grain yield	Total grain yield
	35 DAS	56 DAS	56 DAS	63 DAS ¹	63 DAS	63 DAS	84 DAS	(kg ha ⁻¹)
CARDI Chey	3.1	2	15.3	42	5.11	869	45	1266
CMB-2	3.8	2.3	16.3	42	5.48	584	55	973
CMB-3	4	2.3	17	34	5.20	680	39	1020
Delta	4.2	0.5	23.9	10	5.42	797	47	1218
DX-208	1.8	1.9	12.5	72	5.74	742	46	1119
Emerald	3.2	0.4	13.2	3	5.57	1167	32	1671
King	0.3	0.8	7.1	23	4.86	518	32	766
KPS-2	1.8	0.8	12.1	6	4.78	753	39	1134
Shantung	1.8	1	10.8	12	5.16	677	25	994
White Gold	2	0.2	8.5	27	5.38	765	27	1137
S.E.	0.67	0.57	2.04	5.9	0.118	86.9	11.3	123.3
P value	0.001	0.048	< 0.001	< 0.001	< 0.001	0.002	NS	0.003

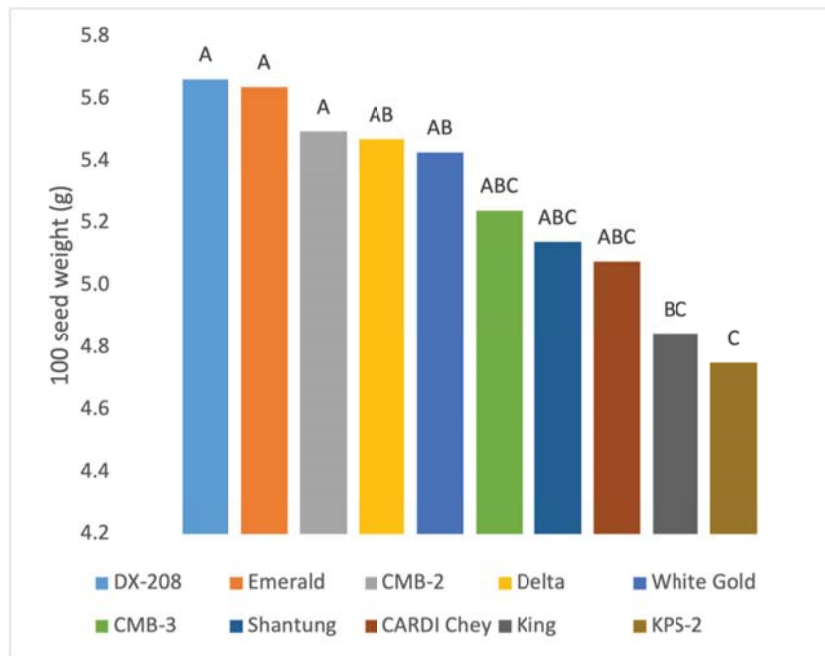


Figure 2. Differences between varieties for 100 seed weight (g)

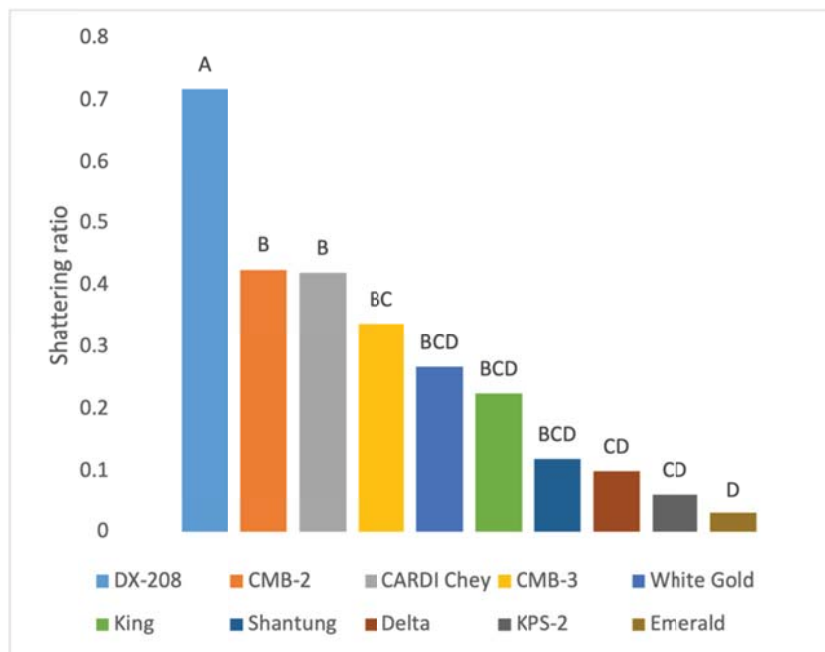


Figure 3. Differences between varieties for pod shattering ratio

4. Discussion

Mungbean is grown in two main agro-ecosystems in Cambodia. Approximately 39% of the crop is planted after flood waters recede in the Tonle Sap active flood plain in November each year. Another 39% of the mungbean crop is planted during the early wet season (February-July) in land recently cleared of forest in the uplands of northern Cambodia (Anonymous, 2020). In the active flood plain in Battambang Province, the market demand is for mungbean with large shiny seeds and this trait is met by the Vietnamese variety DX-208. However, DX-208 is prone to pod-shattering and some farmers also grow the Thai variety KPS-2 which is resistant to pod-shattering. However, KPS-2 has smaller seeds and receives a lower price compared to DX-208. Mungbean growers in North-West Cambodia are therefore looking for a variety that combines both traits.

CARDI evaluated 25 breeding lines from AVRDC, Australia and Thailand between 2003 and 2005 (Ouk et al., 2009). This program resulted in the release of CMB-1 (VC 4512), CMB-2 (VC 3541B) and CMB-3 (HL33-6). However, no data were presented on seed weight or pod-shattering. Our study included a selection of 17 commercial mungbean varieties from Australia, Thailand and Vietnam and included the Cambodian varieties (CARDI Chey, CMB-1, CMB-2, CMB-3) (Table 2).

Campbell-Ross et al. (2019) evaluated commercial Thai and Vietnamese mungbean varieties at two sites in Battambang in 2018. All of these varieties had a significantly lower seed weight compared to DX-208. Although there is ongoing research with mungbean germplasm focussed on the non-shattering trait (Nirmalbharati et al., 2016; Vairam, 2017), there appears to be no published research on pod-shattering characteristics of commercialized mungbean varieties. The Australian mungbean improvement program selects for the non-shattering trait (Douglas, 2017). Therefore, a request was made to the Australian mungbean improvement program to access commercial Australian mungbean varieties for evaluation in North-West Cambodia. Eleven public Australian mungbean varieties were obtained in 2018 for evaluation in 2019. Small quantities of seed were available and no conclusive data on pod-shattering were obtained in 2019. However, five of the Australian varieties could be eliminated on the basis of small seed size (Celera, Green Diamond, Putland and Satin); non-shiny seed coat (Satin) and yellow seed coat (Yellow Sun). CMB-1 was rejected because of indeterminate maturity and having a dull seed coat.

Six Australian mungbean varieties were re-evaluated in both dry and wet seasons in 2021 together with the locally grown varieties (DX-208 and KPS-2) and registered Cambodian varieties, CARDI Chey, CMB-2 and CMB-3. A laboratory pod shattering test (Antwi-Boasiako, 2017; AVRDC, 2017) was carried out on pods harvested at 63 DAS in the 2021 wet season experiment. Delta, Emerald and KPS-2 were resistant to pod shattering (1-10%). Shantung and King were moderately resistant (11-25%). White Gold, CARDI Chey, CMB-2 and CMB-3 were moderately susceptible (26-50%) and DX-208 was susceptible to pod-shattering (>50%). This is an important consideration because combine harvesting of mungbean is already being carried out on several thousand hectares at around 90 DAS in Siem Reap Province without earlier hand-picking. This study has identified suitable varieties for these conditions.

An assessment was made of the economic benefit to farmers of adopting varieties Delta and Emerald compared to existing varieties DX-208 and KPS-2. The mean of grain yields obtained in 2019 and 2021 were used together with the farm-gate price obtained in 2021 (Table 7). The increased total revenue compared to DX-208 was \$144 ha⁻¹ (11%) for Delta and \$315 ha⁻¹ (24%) for Emerald.

Table 7. Economic advantage of Delta and Emerald compared to DX-208 and KPS-2 (USD)

Variety	Delta	Emerald	DX-208	KPS-2
Yield	1,360	1,546	1,294	1,475
Price kg	\$1.09	\$1.07	\$1.04	\$0.94
Total Revenue (USD kg ⁻¹)	\$1,482	\$1,654	\$1,339	\$1,379

The importance of improved varieties can be over-emphasised in systems constrained by abiotic and biotic environmental factors. The grain yield potential of mungbean is around 3,000 kg ha⁻¹ (Martin et al., 2020) and yields of up to 2,000 kg ha⁻¹ have been achieved in our study. In comparison, the mean grain yield of mungbean in Cambodia is around 1,000 kg ha⁻¹ but only about 750 kg ha⁻¹ in Battambang Province where this work was conducted (Anonymous, 2020). These figures suggest that the yield potential of the available mungbean varieties is not an important factor limiting yields for mungbean planted after flood in North West Cambodia.

Mungbean production in this system relies on residual wet season water stored in the soil. Local farmers generally plough twice, broadcast seed and harrow to incorporate the seed. This soil disturbance is not necessary as the crop can be planted after flood with a machine drill seeder without any other soil disturbance. Mungbean are direct-seeded in Siem Reap Province where the mean yield is 1,000 kg ha⁻¹ (Anonymous, 2020). Drill seeder services are available in Battambang Province, so availability of machine planter service providers is not a constraint to adoption.

The potential economic benefits of adopting reduced tillage and machine planting of mungbean are substantial. A partial budget can be used to compare the costs and benefits of alternative crop establishment methods (CIMMYT, 1988). Here we consider that farmers are faced with a decision between their current practice of hand broadcasting and the alternative of reduced tillage and machine planting. It is assumed that hand

broadcasting results in mean yields of 750 kg ha⁻¹ while machine planting gives a mean yield of 1,000 kg ha⁻¹ primarily through the benefit of saved soil water (Table 8). Reduced tillage and machine planting has the potential to increase yields, to reduce input costs and to increase income of smallholder mungbean growers by at least \$320 ha⁻¹. On-farm demonstrations are required to increase awareness of this benefit. In addition to the economic benefits of machine planting, machine harvesting of the new varieties with reduced shattering provide a solution to the acute labor shortage due to migration.

Table 8. Partial budget for hand broadcasting vs machine planting of mungbean (USD)

	Hand broadcasting	Machine planting
Expected yield (kg ha ⁻¹)	750	1,000
Assumed price (\$ kg ⁻¹)	1	1
Total Revenue (\$ ha ⁻¹)	750	1,000
Ploughing (2 × \$30 ha ⁻¹)	60	0
Harrowing (1 × \$15 ha ⁻¹)	15	0
Seed (30, 22 kg ha ⁻¹ × \$1.95 kg ⁻¹)	58	43
Cost of planting (\$ ha ⁻¹)	10	30
Total costs that vary (\$ ha ⁻¹)	143	73
Return over specified costs (\$ ha ⁻¹)	607	927

This study has shown that, with improved varieties, mungbean yields of up to 1,500 kg ha⁻¹ can be reliably achieved and that varieties resistant to pod shattering such as Delta and Emerald enable one pick and the possibility of machine harvesting. In recent years there has been considerable outmigration from rural areas in Cambodia. This has had significant impact on farming practices across the country, especially meaning that farmers can no longer depend on a ready supply of laborers, which in turn, has resulted in greater investment in agricultural machinery to maintain production (Chhim et al., 2015). Traditionally, rice fields were harvested manually and required a large number of people over a short period of time. With increased labor cost and migration to the cities and internationally, labor is now in short supply and rice is increasingly harvested by machine, particularly by combine harvester and the number of combines in Cambodia reached 5,500 units in 2015 (Som et al., 2019). This research on non-shattering mungbean is globally significant as many developing countries including India, Bangladesh and Vietnam are facing similar labor shortages and are moving towards mechanical harvesting of mungbean in a single pick. Mungbean are harvested at one time by combine machine in Siem Reap Province. However, adoption of combine harvesting of mungbean is limited because of lack of varieties suitable for a single pick.

In conclusion, this study evaluated 17 public mungbean varieties from Cambodia (4), Thailand (1), Vietnam (1) and Australia (11) for grain yield, seed size, resistance to pod-shattering, determinate flowering and suitability for machine harvesting. The Australian varieties, Delta and Emerald, best satisfied the criteria of having both large seeds as well as resistance to pod-shattering. These varieties should be suitable for single-pick harvesting or machine harvesting and are recommended for further testing across other mungbean growing regions of Cambodia before submission for registration and commercial release. Future research should also focus on environmental yield determinants such as improved water-use efficiency together with improved varieties.

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Bat Guano Application Rate in Horticulture in Cambodia: An Experiment With Tomato

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Abstract

Bat guano is rich in carbon, nitrogen, essential minerals and beneficial microbes. It is considered as a potential organic fertilizer for plant growth and productivity. The aim of the study was to test the effect of varying amounts of bat guano on plant growth and productivity of tomato. The results showed that the growth and productivity increased significantly with the amounts of bat guano applied. With the amount of 0.5 or 1 t/ha was found to have the greatest impact compared to other treatment and control groups. The amount of 0.5 t/ha (or 35 g/plant) is an appreciate amount and recommended for tomato production.

Keywords: Chemical fertilizer, Food safety, Organic farm, Soil health, Vegetable

1. Introduction

For healthy and safe food, long-term sustainability and concerns regarding the environmental pollution related to the unregulated utilization of chemical fertilizers, organic farming has become an essential priority worldwide (Al-Erwy et al., 2016). Organic fertilizer has been promoted to avoid or reduce the negative effects attributed to the use of chemical (or synthetic) fertilizer. Vegetables and fruits grown on overfertilized soils are more susceptible to attacks by insects and disease (Karungi et al., 2006). Underfertilization can lead to the deterioration of soil characteristics and fertility, and as well it can lead to a reduction in fruit nutrition values and edible qualities (Shimbo et al., 2001). Organic fertilizers havelongterm benefits including enhanced soil quality, improve biodiversity, as well as the reduction of production cost and environmental pollution.

In Cambodia, although chemical fertilizers are most commonly applied to deal with nutrient deficiencies, livestock manure, compost and bat guano are also extensively used throughout the country as organic forms of fertilizer (Sothearen et al., 2014). Bat guano is collected from bat caves and commonly traded and used as fertilizer in the country (Furey et al., 2016). Bat guano serves as a source of organic manure for improvement of growth and photosynthetic response in crop plants (Palita et al., 2021). It is rich in carbon, nitrogen, essential minerals and beneficial microbes, and mineralises very quickly (Dimande et al., 2023a). Previous studies have documented with high amount of bat guano applied to tomato plants in Nigeria (Karimou et al., 2020), and a combination of bat guato and fermented seaweedd application for cherry tomato production in Cambodia (Anuada et al., 2021) and other studies on other crops (e.g., Sothearen et al., 2014; Dimande et al., 2023a; Dimande et al., 2023b). Providing an appropriate amount and option for a specific crop is an entry point of promoting organic farming.

Consequently, the aim of the study was to test varying rates of bat guano application to determine appropriate application rates for tomato (*Solanum lycopersicum*) production in Cambodia. The findings of this study will provide better understanding of the appropriate amount of bat guano needed for tomato production and to avoid over or under fertilization. This could lead to a more efficient use of natural sources of organic fertilizer. In a context of the rise in input costs, natural sources of fertilization are in demand (Arndt et al., 2023). Tomato is a good source of nutritive value, containing high vitamins specifically vitamin C and other minerals like phosphorus, iron, and calcium (Bhowmik et al., 2012) and is an increasingly important vegetable crop in rural Northwest Cambodia (Hav et al., 2021) In this region, the limestone mountains are habitat for thousands of bats

and there is a continuous supply of bat guano from the caves there. Applications rates, based on experiments, will help to effectively manage this valuable natural resource.

2. Method

2.1 Experimental Design

The experiment was conducted in a shade net-house (size—length: 10 m, wide: 8 m, high: 3 m) of the National University of Battambang (NUBB), in Northwest Cambodia (13°5'6.95"N, 103°13'15.25"E). Average (mean±sd)—air temperature: 30.4±4.7 °C, relative humidity: 57.8±6.6 %, and light density: 11,602.6±357 lux, during growing condition from October 25, 2020 to March 15, 2021.

A completely randomized design was used with four different treatments, with 10 replications (single plant per pot) for each treatment and the control. Four amounts of bat guano were included in the experiment, i.e., 0 t/ha for control, 0.25 t/ha (17 g/plant), 0.50 t/ha (35 g/plant), and 1.0 t/ha (70 g/plant) were applied into each treatment when preparing soil for each plastic pot. The plastic pots with a size of 20 L of volume, with 30 cm of diameter and 36 cm of height, were used as containers for the substrate.

The soil was brought from Sangkae river bank, approximately 2 km away from the experimental site. The soil was classified as Brown hydromorphic soil group (Crocker, 1962), a loam texture containing clay: 25.10%, silt: 36.52%, sand: 37.39%, carbon (C): 2.12%, total N: 0.19%, organic matter: 3.64%, total phosphorus (P₂O₅): 0.07%, available P: 39.0 ppm, cation exchange capacity: 20.50 m.e/100 g soil, calcium (Ca): 17.0 m.e/100 g soil, magnesium (Mg): 4.60 m.e/100 g soil, sodium (Na): 3.46 m.e/100 g soil, potassium (K): 0.82 m.e/100 g soil, total exchange base: 25.88 m.e/100 g soil, exchange acidity: 1.5 m.e/100 g soil, exchange aluminium (Al): 0.01, electrode conductivity: 198.9 µS/cm, and pH_{KCl} (1:5): 6.62. The bat guano was brought from Romsaysak bat cave (lat.: 13.0234949, lon.: 102.9991074) in Battambang, containing 9.01% total nitrogen (N), 3.78% total phosphorus (P₂O₅), and 1.40% total potassium (K), with 5.34% moisture content using oven dry at 105 °C for 24 hours.

Makis F1 hybrid tomato variety was used for this experiment; the seeds were sown on trays containing mixed soil and composted cow manure (2:1) then kept under the net house and watered twice a day. Similar size seedlings with 2-3 leaves per plant with height of 18 to 20 cm, were transplanted in the container substrates when they were at 3 weeks old, one plant per each container of substrate. The plants were watered twice a day (at 8 AM and 4 PM).

2.2 Data Collection and Statistical Analysis

To compare plant growth and productivity among the treatments and control group, plant height, branch number per plant, fruit number per plant, and total fruit yield per plant parameters were measured at the end of the experiment (110 days after transplanting).

To test the significance of difference in means of each variable for multiple groups, one-way analysis of variance or the Kruskal-Wallis test (Kruskal & Wallis, 1952) was used due to the fact that the data have an unnormal distribution. Tukey's test (Tukey, 1949) was applied to compare all possible pairs of mean for each variable. All pairwise comparisons using least significant difference at $\alpha = 0.05$. All statistical analyses were performed with the R statistical software, version 3.6.3 (R Core Team, 2020); and the plots were performed using 'ggplot2' R package (Wickham, 2011). To visualize correlations between all pairwise combinations of the variables included plant height, branch number per plant, fruit number per plant, and total fruit yield per plant, and amount of guano applied, bivariate relationships were analysed using Pearson's correlation coefficient for numerical variables by performing correlation matrix using scatter plots in the 'Performance Analytics' R package (Carl & Peterson, 2010).

3. Results

The growth and productivities of the tomato plants were found to be significantly affected by different amounts of bat guano application (Figure 1; $P < 0.001$). The greatest plant height, branch number, fruit number and total fruit yield per plant were found in the treatment with the amount of 0.5 or 1.0 t/ha guano fertilizer, whereas the least plant growth and productivity in the control group. Between the amounts of 0.5 and 1.0 t/ha guano fertilizers were not significantly different for the plant growth and productivity, indicated that 0.5 t/ha (35 g/plant) was an optimal rate for tomato plant production in clay loam soil condition. Applying 35 g/plant of bat guano fertilizer could increase the fruit yield by double than those without guano fertilizer application.

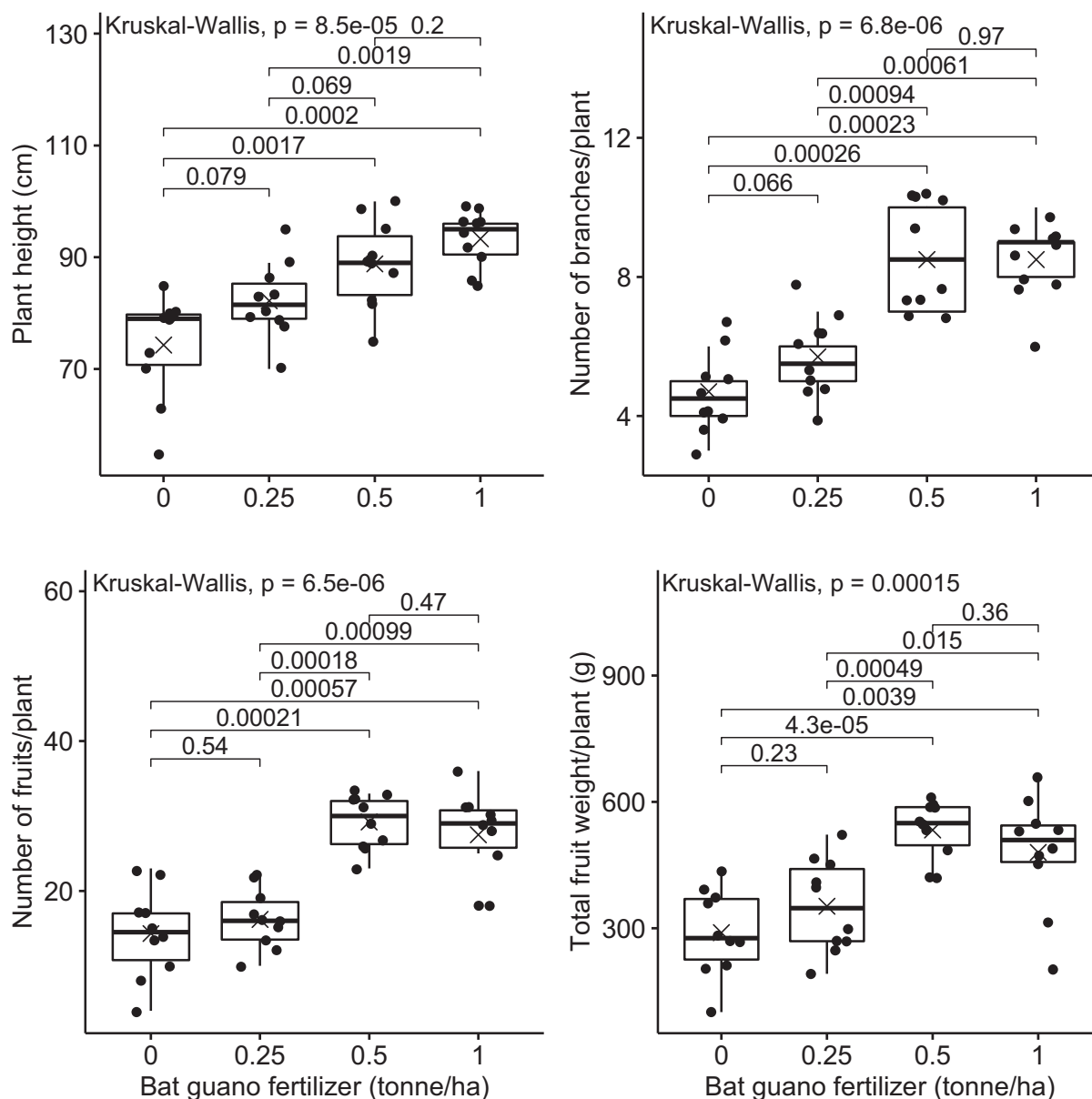


Figure 1. Effect of different rates (0; 0.25; 0.5 and 1 tonne/ha) of bat guano application on cherry tomato plant growth and productivity; plant height (top left:), branch number (top right), fruit number (bottom left), and fruit weight (bottom right)

The Pearson's correlation matrix plot (Figure 2) showed that the amounts of bat guano application (from 0 to 1 t/ha) were significantly positively correlated to the plant height, branch number, fruit number, and total fruit weight per plant.

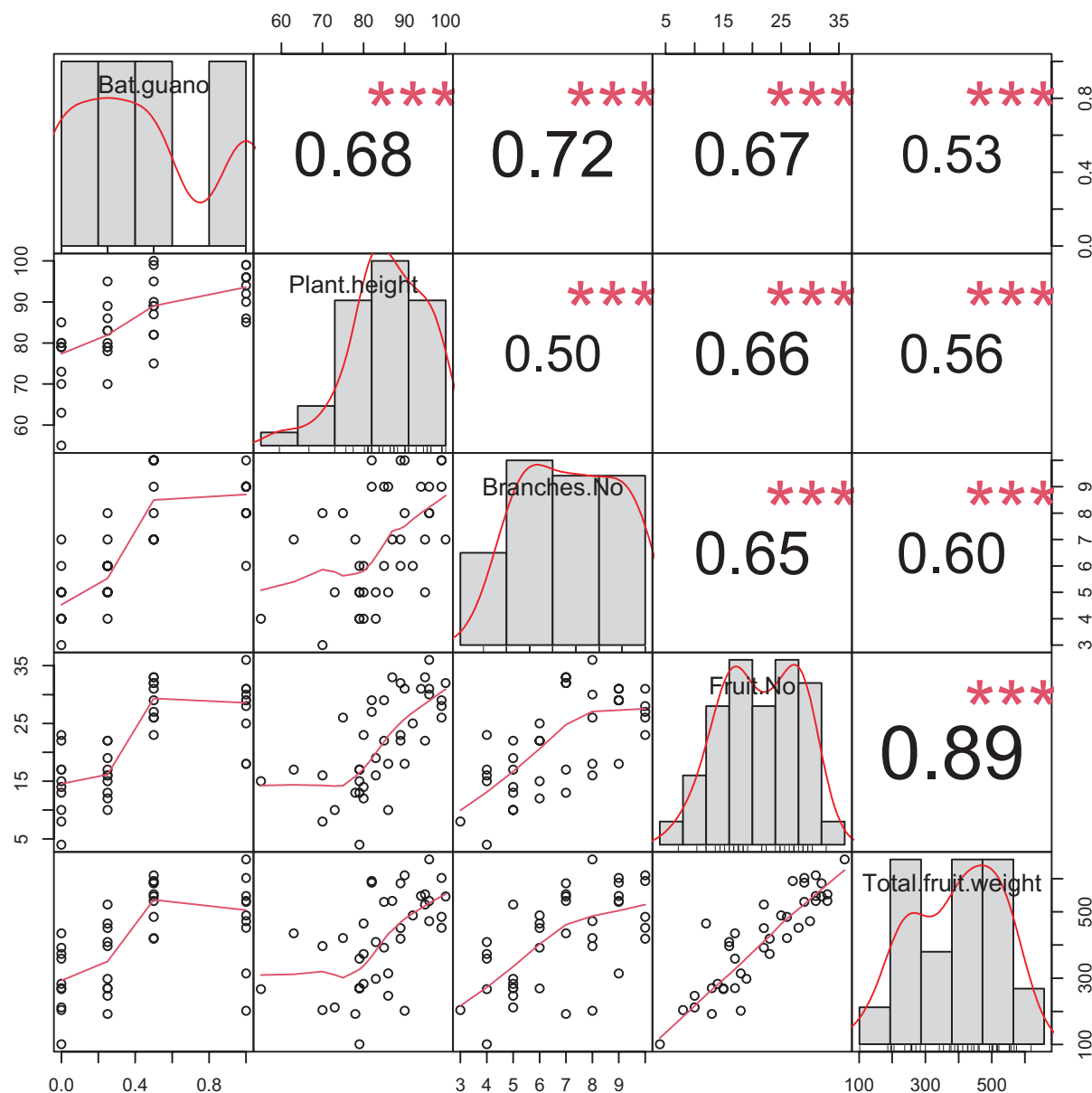


Figure 2. The Pearson’s correlation matrix of the plant growth (plant height and branch number) and productivity (fruit number and total fruit weight per plant) variables and different amounts of bat guano application. Note: Bat guano: 0; 0.25; 0.5 and 1 tonne/ha, Plant.height: maximum plant height (cm), Branches. No: branch number per plant, Fruit No: fruit number per plant, and Total.fruit.weight: total fruit weight per plant (g/plant); *** $P < 0.001$

4. Discussion

The results of this investigation demonstrated that bat guano could be used as an organic fertilizer for tomato production as the plant growth and production increased with the amount of bat guano applied. However, the results show the amount of 0.5 t/ha (35 g/plant) would be an optimal rate for tomato production, applying more than this optimal amount would not increase plant growth and productivity, unless for other reasons such as soil quality improvement. The results are similar to previous studies on other tomato variety (Karimou et al., 2020), however, the present findings provided additional proofs to indicate less than 0.25 t/ha of guano is not enough amount for tomato growth and production, as well as more than 0.5 t/ha does not achieve better growth and yields for the soil conditions of the present study. Previous study (Anuada et al., 2021) recommended a combination between 0.5 t/ha of bat guano and 500 L/ha of fermented seaweed for cherry tomato production in Cambodia. Bat guano is rich in carbon, nitrogen, essential minerals and beneficial microbes; excess amount of

the guano does not cause negative plant growth and productivity, but may increase production cost. Bat guano mineralises very quickly (Dimande et al., 2023a), the application of bat guano to soils could also improve physical properties of soils and microbial population and the environment (Ghasem et al., 2014; Bhambe et al. 2017; Dimande et al., 2023b; Onunwa et al., 2023). Bat guano has been considered as important biowastes of the cave ecosystems containing high amounts of minerals (Giurgiu et al., 2013). The minerals of bat guano include phosphates and sulphates of potassium, ammonium, sodium, magnesium, and calcium (Shahack-Gross et al., 2004).

The use of organic fertilizer can avoid or reduce the negative effects attributed to the use of chemical (or synthetic) fertilizer. Applying chemical fertilizer leads to the deterioration of soil characteristics and fertility, and as well it leads to a reduction in fruit nutrition values and edible qualities (Shimbo et al., 2001), including the protein content of crops, and the carbohydrate quality (Marzouk & Kassem, 2011). Other issues caused by chemical fertilizer, excess potassium content on chemically overfertilized soil decreases Vitamin C, carotene content and antioxidant compounds in vegetables (Toor et al., 2006).

5. Conclusions

Bat guano application on tomato enhances plant growth and productivity of the tomato plant. Application rates of 500 kg/ha or 35 g/plant is an appropriate amount for tomato production, with less not providing sufficient nutrient requirements, and more not being efficient with this limited natural resource. Bat guano is recommended for tomato production as an alternative organic source of nutrients for sustainable soil fertility management.

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Authors Contributions

Dr. Pao Srean and Dr. David R. Ader were responsible for study design and revising. Longdy Korn and Channaty Ngang were responsible for data collection. Dr. Pao Srean drafted the manuscript and Dr. David R. Ader revised it. All authors read and approved the final manuscript.

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Analysis of Labor Productivity in Single and Multi-household Grassland Management Patterns: A Case Study in Maqu County, Qinghai-Tibetan Plateau

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Abstract

This study investigated labor productivity in meat and milk/dairy production within single and multi-household management patterns, based on primary data collected from 156 randomly selected herder households in Maqu County, Tibetan Plateau. The results showed that in the rotational grazing system, herder households in both single and multi-household management patterns achieved higher labor productivity for meat production (70.36 Kg/man-day and 51.21 Kg/man-day, respectively) compared to the overall study households (40.89 Kg/man-day). In contrast, within the continuous grazing system, the single-household management pattern recorded lower labor productivity for meat production (23.04 Kg/man-day). Significantly, regional variations in the distance between pastures and market centers led herder households in the single-household management pattern within the continuous grazing system to achieve superior labor productivity for milk and dairy production (19.74 \$/man-day) compared to the overall study households (15.44 \$/man-day). In the rotational grazing system, labor productivity for milk and dairy production stood at 12.63 \$/man-day for the single-household management pattern and 8.30 \$/man-day for the multi-household management pattern. These findings underscore the complexities associated with achieving high labor productivity simultaneously in both meat and milk/dairy production within the same grassland management pattern. While the multi-household management pattern shows promise in reducing labor inputs, it also grapples with challenges in achieving substantial production levels for meat and milk/dairy products. To address these challenges, policymakers should consider follow-up measures that prioritize the simultaneous enhancement of meat and milk/dairy production within the multi-household management pattern. Special attention should be given to reducing the distance between herder households and market centers to facilitate the sale of milk/dairy products. Simply advocating for the broader adoption of the multi-household management pattern may fall short without addressing these production-related hurdles.

Keywords: labor productivity, meat production, milk and dairy production, grassland management patterns, Tibetan Plateau

1. Introduction

China's expansive grasslands, covering a substantial 400 million hectares, represent approximately 42% of the world's total land area. Among these, the Qinghai-Tibetan Plateau (QTP) stands as the largest alpine grassland globally, serving as a crucial habitat for nearly 41 million Tibetan sheep, 13 million yaks (Long et al., 2009), and more than 1.3 million cattle and cattle-yak hybrids. This unique ecosystem supports a human population exceeding 9.8 million individuals (Long, 2003). However, ecological challenges, including reduced biodiversity, soil erosion, and the emergence of sandstorms resulting from inadequate grassland management practices (Akiyama & Kawamura, 2007), combined with the impact of climate change (Hao et al., 2014), have placed constraints on the sustainable development of animal husbandry and posed substantial threats to the fundamental ecological and societal stability of the QTP (Pulido et al., 2018).

The privatization of grasslands has garnered support in various countries and regions, including China, Central Asia, and South Africa, as a strategy to address ecological challenges stemming from overgrazing. This approach is also intended to incentivize herders to embrace sustainable grassland management practices (Ybarra, 2009;

Mwangi, 2016). In China, the Household Responsibility System (HRS) was instituted in the 1980s, leading to the privatization of grasslands and livestock through the implementation of fixed fencing (Yang et al., 2020). This marked a shift away from the collective model characteristic of the communist revolution, which had spurred labor motivation and yielded a period of heightened agricultural production in the 1990s (Li et al., 2018). Nevertheless, the rigid property ownership, defined pasture boundaries, and independent livestock management inherent in the single-household management pattern (SHMP) have constrained the flexibility of grassland management and the mobility of grazing (Hobbs et al., 2008). Over time, this has contributed to the progressive degradation of grasslands, resulting in diminished pastoral productivity and escalating production costs. Consequently, a segment of herders has been compelled to discontinue livestock rearing (Zhou & Dong, 2013).

In response to the challenges stemming from the HRS, the central government of China introduced the Grassland Ecological Protection Award Policy in pastoral areas across the country, a measure initiated in 2011. This policy involves stringent controls on the number of livestock that herder households are permitted to raise per unit area (Zhang et al., 2019). While numerous ecological studies have affirmed that grassland degradation in China has been mitigated and improved (Zhao et al., 2021; Wang et al., 2022), herder households have incurred substantial economic losses as the policy subsidies have not adequately compensated for the significant reduction in livestock income (Xiao et al., 2022). Furthermore, the strict stocking rate regulations have compelled herder households to transition from exclusive grassland grazing to a combined approach of grazing and shed feeding (Wang et al., 2019). This shift, accompanied by rising forage and labor costs, has led to a considerable exodus of herders from pastoral areas (Liu, 2016).

Within the context outlined above, ecological research rooted in grazing experiments has suggested that the spontaneously emerging multi-household management pattern (MHMP) may constitute a more efficacious approach. In the MHMP, two or more herder households unite contiguous pastures, a practice that not only augments grazing mobility, thereby fostering grassland restoration (Cao et al., 2018; Li et al., 2018; Zhang et al., 2020), but also facilitates labor savings through collaborative efforts among the households within the MHMP (Cao et al., 2011). In 2015, the Chinese central government initiated a policy aimed at promoting grassland circulation, entailing the separation of land ownership, rights consolidation, and land use rights (Yang et al., 2021). Furthermore, in 2019, it advocated for the transition from the SHMP to the MHMP, encouraging MHMP members to collectively procure and share production resources (General Office of the State Council, 2019). Despite these concerted efforts, however, the SHMP still predominates in grassland management across China, encompassing 75% of the total contracted grassland area, whereas the MHMP accounts for only 23% (Wang et al., 2020). In practical terms, widespread adoption of the MHMP has yet to materialize (Yang et al., 2021).

Given the labor-intensive nature of current livestock production methods in alpine pastoral areas (Conte & Tilt, 2014), coupled with the primary objective of laborers engaged in agricultural and pastoral practices to obtain tradable agricultural and pastoral products (Fang et al., 2010), it is pertinent to recognize that while the mutual cooperation advocated within the MHMP may yield labor savings, the relationship between labor inputs and outputs warrants careful consideration. In essence, a reduction in labor input contributes positively to production only if, under *ceteris paribus* conditions (assuming other production factors remain constant), the marginal product of labor—*i.e.*, the additional output produced per unit of labor—either remains steady or increases as a consequence of the reduced labor input (Mankiw, 2014). In simpler terms, a decrease in labor input benefits production only if the quantity of products produced per unit of time does not decrease by the same proportion as the reduction in labor input. However, it is worth noting that much of the existing research has predominantly centered on assessing the ecological aspects of grasslands in the context of the SHMP and the MHMP. These studies, often conducted through grazing experiments, have demonstrated that the MHMP exhibits a higher degree of resilience in terms of vegetation and soil characteristics (Cao et al., 2018; Li et al., 2018; Zhang et al., 2020). Additionally, some research efforts have explored the factors contributing to the formation of the MHMP, the associated property rights systems, and the composition of household income. Through a combination of qualitative and quantitative analyses, these studies have revealed that the spontaneous emergence of large-scale MHMP among herder households in the context of land transfer has effectively augmented herders' non-farm income (Cao et al., 2017; Yang et al., 2020; Zhou et al., 2021). Nevertheless, there is a notable absence of analysis concerning the labor productivity of herder households in different grassland management patterns. Therefore, this study aims to analyze labor productivity in both single (SHMP) and multi-household management patterns (MHMP), with the goal of providing new insights for policymakers to further enhance measures related to the expansion of the multi-household management pattern from the perspective of labor inputs and outputs.

2. Materials and Methods

2.1 Study Site

Maqu County, situated in Gansu Province in northwest China (coordinates 35°58'N, 101°53'E, at an elevation of 3500 m above sea level), constitutes a significant pastoral region within the area. It serves as a pivotal hub for livestock production in the region, characterized by an annual rainfall ranging from 450 to 780 mm and an average annual temperature of 1.8 °C. The expansive grassland terrain encompasses approximately 87×10^4 ha, with alpine meadows occupying the majority at 59%. As of the year 2020, Maqu County boasted a total population of approximately 57,000 inhabitants, of which herders accounted for a substantial 75%. The primary source of income for these herders is derived from livestock trading, constituting approximately 89% of their total income (Du et al., 2022). The remaining income is generated through the sale of livestock by-products (Li et al., 2022).

2.2 Data Collection

In this study, surveys were conducted using telephone and face-to-face questionnaires between November 2021 and March 2022. These surveys were administered in randomly selected villages around Maqu County. Respondent selection was carried out in a randomized manner, ensuring representation from each chosen village. Prior to commencing the survey, a preliminary assessment was undertaken to ensure the clarity and uniformity of the questionnaire. This questionnaire included fundamental inquiries about the herders' households, their management of animal husbandry, and their household gross income.

To ensure the precision of responses and mitigate potential language barriers, we enlisted the assistance of six local Tibetans to administer the questionnaires. This team consisted of five college students and one civil servant, all proficient in the local dialect and customs. Additionally, all enumerators underwent standardized training to minimize potential biases resulting from variations in the interpretation of questionnaire questions. Prior to commencing each questionnaire, we provided a comprehensive explanation of the study's objectives to each participant and obtained their consent. With the invaluable assistance of the local enumerators, we successfully collected a total of 165 questionnaires, resulting in an effective response rate of 94.55%. Within this dataset, 121 questionnaires were obtained from single-household management patterns (SHMP), including 83 from continuous grazing systems (CGS) and 38 from rotational grazing systems (RGS). The remaining 35 questionnaires were associated with multi-household management patterns (MHMP), all of which belonged to RGS. It's worth noting that nine questionnaires were excluded from the analysis due to invalid or missing data.

2.3 Research Methods

The choice of productivity measurement methods depends on the specific objectives of the measurement and the accessibility of relevant data. Labor productivity is a commonly assessed metric, typically quantified by the ratio of output to labor input, with either gross output or value added used as a surrogate for output (OECD, 2001). Output can be quantified in either monetary or in-kind units, while labor input can be represented by various metrics, including the number of hours worked, the number of days worked, the number of shifts worked, or the average size of the labor force (Kubálková, 2009).

As livestock production encompasses both meat and milk production (Food and Agriculture Organization of the United Nations, 2018), the labor productivity assessment in this study was divided into two components: meat production and milk and dairy production. The selection of measurement units was determined based on prevailing herder practices within the study area and data availability. Experienced herders typically possess a keen understanding of livestock weights at various stages of growth. Consequently, this study quantified meat production in terms of live animal weights. In addition, milk and dairy production were evaluated using monetary units since herders typically did not record the weights of milk and dairy products but rather tracked their income from livestock by-products. Labor inputs were measured in terms of the number of days worked. To standardize labor input, a full day of labor (comprising 24 hours daily and 365 days annually) was converted into working days, accounting for 10 hours daily and 364 days yearly. The conversion rate for monetary units was pegged to the exchange rate set by the People's Bank of China for December 2021, with 6.5 RMB equating to 1 USD. The labor productivity models for meat production (LP_m) and milk and dairy production (LP_d) were therefore defined as follows:

$$LP_m = \frac{\sum_{i=1}^n E_i - B_i}{\sum_{i=1}^n T_i} \quad (1)$$

$$LP_d = \frac{\sum_{i=1}^n C_i}{\sum_{i=1}^n D_i} \quad (2)$$

where, E_i represents the total weight of livestock at the end of the year (including livestock sold, stocked, and self-consumed) for the i^{th} household, B_i represents the total weight of livestock at the beginning of the year for the i^{th} household, T_i represents the total input of working days corresponding to meat production for the i^{th} household; C_i represents the cash income from milk and dairy production obtained by the i^{th} household in selling units, D_i represents the total input of working days corresponding to milk and dairy production for the i^{th} household, and n represents the total number of households corresponding to each management pattern.

Given the potential influence of continuous grazing systems (CGS) and rotational grazing systems (RGS) on labor inputs (Windh et al., 2019), this study conducted separate analyses for the grazing systems under both the single-household management pattern (SHMP) and the multi-household management pattern (MHMP).

2.4 Input and Output Variables Description

Meat production involves various key activities, including livestock release and gathering, vaccinations, fence repairs, forage sowing and harvesting, pasture transfers, and livestock sales, as delineated in Table 1. The average full-year labor input for meat production varied among herder households within different management patterns and grazing systems. In SHMP-RGS, herder households worked an average of 113.87 days per year for meat production, while in SHMP-CGS, they worked 103.09 days. Both of these exceeded the overall study household average of 100.79 working days. In contrast, herder households within MHMP-RGS expended an average of 81.12 working days, which was lower than the overall study household average. Specifically, herder households in MHMP-RGS devoted 77.31 working days throughout the year to livestock management, which was lower than the 95.3 working days spent by the overall study households. Conversely, herder households in SHMP-CGS and SHMP-RGS invested an average of 98.9 and 104.02 working days throughout the year, respectively, exceeding the overall study household average. For livestock vaccinations, which took place between June and July each year, the average full-year labor input of herder households in MHMP-RGS was less than the 0.49 working days of the overall study households. Conversely, herder households in SHMP-CGS and SHMP-RGS expended 0.52 and 0.61 working days throughout the year, respectively, exceeding the overall study household average. Regarding fence repairs, herder households in both SHMP-CGS and MHMP-RGS contributed a lower average full-year labor input than the 3.12 working days of the overall study households. However, herder households in SHMP-RGS allocated an average of 4.60 working days throughout the year, surpassing the overall study household average. For forage sowing and harvesting, activities occurring between June and July and September and October each year, herder households in both SHMP-CGS and MHMP-RGS devoted an average full-year labor input lower than the 0.56 working days of the overall study households. Conversely, the average full-year labor input for herder households in SHMP-RGS exceeded the overall study household average. Regarding pasture transfers, a practice undertaken by herders in both SHMP-RGS and MHMP-RGS from winter to summer pastures at the end of April or the beginning of May each year and again to winter pastures at the end of September or the beginning of October, the average full-year labor input of herder households in SHMP-RGS was higher than the 1.08 working days of the overall study households. In contrast, herder households in MHMP-RGS spent 0.65 working days throughout the year, which was lower than the overall study household average. Lastly, herders transported their available livestock to the local sole trade center at the end of the production cycle in October for trading based on market prices and livestock weights. Herder households in SHMP-RGS expended an average full-year labor input exceeding the 0.82 working days of the overall study households. Conversely, herder households in both SHMP-CGS and MHMP-RGS allocated 0.76 and 0.48 working days throughout the year, respectively, which were less than the overall study household average.

Milk and dairy production activities encompass milking, as well as the production of ghee and cheese, which typically occur between May and September. In terms of labor input, herder households in both SHMP-CGS and MHMP-RGS dedicated fewer working days compared to the overall study households, which averaged 64.92 working days for these activities. However, herder households in SHMP-RGS allocated more time to milk and dairy production, averaging 78.26 working days. Specifically, the average labor inputs for milking, ghee making, and cheese making in SHMP-RGS were 42.80, 26.14, and 9.32 working days, respectively, surpassing the corresponding figures for the overall study households. In the case of herder households in SHMP-CGS, they expended 42.20 working days on milking, which exceeded the overall average. However, their average labor

input for ghee and cheese making was 21.39 and 0 working days, respectively, lower than the overall study household averages. Within MHMP-RGS, the average labor input for milking and ghee making was lower than the figures for the overall study households. Nonetheless, the average labor input for cheese making was 3.2 working days, which exceeded the corresponding value for the overall study households.

Table 2 displays meat production by herder households from yaks and Tibetan sheep. In SHMP-RGS, herder households exhibited higher average initial weights, end weights, and growth weights for the total number of yaks they raised, recording 26,204.89 kg, 34,015.13 kg, and 7,810.24 kg, respectively. This contrasts with the overall study households, which reported 13,461.78 kg, 17,509.71 kg, and 4,047.93 kg, respectively. Conversely, the average initial weights, end weights, and growth weights of the total number of Tibetan sheep raised in SHMP-RGS were lower than those observed in the overall study households. For SHMP-CGS and MHMP-RGS, the average initial and end weights of the total number of yaks raised by herder households were lower than those reported for the overall study households. However, the average initial and end weights of the total number of Tibetan sheep raised were higher than those of the overall study households. In SHMP-CGS, the growth weight of the total number of yaks raised by herder households was lower than that recorded for the overall study households. In contrast, the growth weight of the total number of Tibetan sheep raised was higher in SHMP-CGS. Within MHMP-RGS, the growth weight of the total number of yaks raised by herder households exceeded that observed in the overall study households, whereas the growth weight of the total number of Tibetan sheep raised was lower in MHMP-RGS compared to the overall study households.

Table 2 also presents the cash income generated by herder households through milk, ghee, and cheese production. In SHMP-CGS, the average cash income derived from milk and ghee exceeded that of the overall study households. Specifically, herder households garnered an average of \$825.61 from milk and \$463.08 from ghee, in contrast to \$684.31 and \$346.76 for the overall study households, respectively. However, the average cash income from cheese was lower in SHMP-CGS, with herder households earning an average of less than \$16.06, compared to the overall study households. In SHMP-RGS, the average cash income from milk and cheese surpassed that of the overall study households, with herder households earning an average of \$716.45 and \$33.60, respectively. Nonetheless, the average cash income from ghee was lower, with herder households earning \$293.47, compared to the overall study households. Within MHMP-RGS, the average cash income from milk and ghee was inferior to that of the overall study households, while the average cash income from cheese was higher.

Table 1. Labor inputs of herder households in livestock production in each management pattern

Item	SHMP-CGS ¹ (n = 83)		SHMP-RGS ² (n = 38)		MHMP-RGS ³ (n = 35)		Overall (N = 156)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Meat production (man-day⁴)</i>								
Release of livestock	40.35*	9.87	43.11	20.48	21.51*	7.52	36.79	15.27
Gathering of livestock	58.55	9.87	60.91	20.75	55.80	10.35	58.51	13.45
Livestock vaccinations	0.52	0.40	0.61*	0.14	0.29*	0.20	0.49	0.33
Fence repairs	2.86	1.95	4.60*	3.04	2.12*	0.90	3.12	2.28
Forage sowing	0.01*	0.08	0.62*	0.40	0.10*	0.05	0.18	0.32
Forage harvesting	0.04*	0.19	1.31*	0.76	0.18*	0.06	0.38	0.67
Pasture transfer ⁵	-	-	1.47*	0.79	0.65*	0.18	1.08	0.71
Livestock sales	0.76*	0.18	1.25*	0.62	0.48*	0.23	0.82	0.44
Total	103.09	21.45	113.87	40.94	81.12*	17.47	100.79	29.03
<i>Milk and dairy production (man-day)</i>								
Milking	42.20	12.05	42.80	15.79	33.00*	11.69	40.28	13.48
Ghee making	21.39	5.87	26.14*	6.86	17.40*	5.73	21.65	6.76
Cheese making	0.00	0.00	9.32*	15.45	3.20	0.98	2.99	8.47
Total	63.59	14.04	78.26*	24.93	53.60*	15.67	64.92	19.49

Note. * denotes the significance level of 0.05 for the difference in means of the corresponding indicator between herder households in each grassland management pattern and the overall study households. SD denotes the standard deviation of the mean. ¹ SHMP-CGS = single household management pattern-continuous grazing system. ² SHMP-RGS = single household management pattern-rotational grazing system. ³ MHMP-RGS = multi household management pattern-rotational grazing system. ⁴ Man-day denotes the amount of work done by one person in one day (10 hours daily × 364 days yearly). ⁵ Pasture transfer denotes the transfer of livestock between summer and winter pastures.

Source: Authors' calculations from field study data.

Table 2. Output of herder households in livestock production in each management pattern

Item	SHMP-CGS (n = 83)		SHMP-RGS (n = 38)		MHMP-RGS (n = 35)		Overall (N = 156)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Meat production</i>								
Kg	Yak	Sheep	Yak	Sheep	Yak	Sheep	Yak	Sheep
Initial weight	8855.34*	378.84 (5606.29)	26204.89*	135.26* (611.45)	10550.23*	493.49 (1435.47)	13461.78 (11763.76)	345.23 (822.44)
End weight	11093.31*	467.54 (556.70)	34015.13*	151.55* (680.15)	14805.54 (9908.85)	532.94 (1529.76)	17509.71 (14383.42)	405.24 (899.58)
Growth weight	2237.98*	88.70* (1421.13)	7810.24* (3057.96)	16.29* (70.31)	4255.31 (2429.24)	39.46 (133.41)	4047.93 (3135.17)	60.01 (110.28)
<i>Milk and dairy production</i>								
\$ ¹	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Milk	825.61*	472.07	716.45	451.45	314.35*	273.70	684.31	474.16
Ghee	463.08*	264.78	293.47	184.92	128.76*	112.11	346.76	258.23
Cheese	00.00	00.00	33.60	117.21	64.18*	90.29	16.06	81.96
Total	1288.69*	736.85	1043.52	642.33	507.29*	472.46	1053.65	729.18

Note. * denotes the significance level of 0.05 for the difference in means of the corresponding indicator between herder households in each grassland management pattern and the overall study households. Values denote the average with the standard deviation in parenthesis. SD denotes the standard deviation of the mean. ¹ Based on the exchange rate of the People's Bank of China in December 2021, 1\$ = 6.5 RMB.

Source: Authors' calculations from field study data.

3. Results and Discussion

3.1 Descriptive Analysis of the Sample Households

Table 3 illustrates that family members constituted the primary labor force in the research area, with young adults predominantly comprising the available workforce across all management patterns. The average available labor force within herder households in SHMP-CGS and SHMP-RGS consisted of two individuals, which was consistent with the overall study household figures. However, in MHMP-RGS, the average available labor force was three individuals. Despite having an average of over 22 years of engagement in production, the available labor force within herder households had limited educational backgrounds, typically receiving only 2-3 years of formal education across different management patterns.

The principal livestock reared by herder households were yaks and Tibetan sheep. The average pasture area owned increased progressively from SHMP-CGS (58.20 ha) to MHMP-RGS (103.81 ha) and SHMP-RGS (186.24 ha). The average numbers of yaks and Tibetan sheep reared in the respective management patterns were as follows: 36 and 9, 45 and 10, and 80 and 3. Specifically, 31, 31, and 36 households exclusively reared yaks in the three respective management patterns, while mixed rearing was conducted by 52, 4, and 2 households, respectively. Production practices also varied among the management patterns. Herders in both SHMP-RGS (33.74 km) and MHMP-RGS (17.34 km) engaged in livestock transfers between summer and winter pastures according to seasonal shifts, adapted grazing routes to accommodate livestock foraging, and utilized superior animals for breeding purposes. Forage seeding was performed by all households in SHMP-RGS and MHMP-RGS, but only by three households in SHMP-CGS. Notably, across all three management patterns, herder households were situated at varying distances from the local market center, spanning from 9.01 to 25.69 km. Consequently, ownership of motorbikes was a common occurrence.

Table 3. Descriptive statistics of each management pattern

Variable	SHMP-CGS (n = 83)	SHMP-RGS (n = 38)	MHMP-RGS (n = 35)	Overall (N = 156)
<i>Demographic characteristics</i>				
Age of the labor force per household (years)	37.42 (6.26)	38.87 (7.08)	42.06 (6.96)	38.81 (6.84)
Time in education of the labor force per household (years)	1.76 (2.66)	2.98 (4.00)	2.26 (2.28)	2.17 (2.99)
Production experience of the labor force per household (years)	22.77 (6.09)	24.36 (8.48)	25.82 (8.89)	23.84 (7.46)
Available labor force per household ¹ (people)	2.37 (0.91)	2.29 (0.93)	2.80 (0.87)	2.45 (0.92)
<i>Management characteristics</i>				
Yak rearing (households)	31.00	36.00	31.00	98.00
Mixed rearing ² (households)	52.00	2.00	4.00	58.00
Sowing of forage seed (households)	3.00	38.00	35.00	76.00
Ownership of motor vehicle (households)	62.00	27.00	20.00	109.00
No. of yaks reared (households)	36.00	80.00	45.00	49.00
No. of Tibetan sheep reared (households)	9.00	3.00	10.00	7.00
Area of pasture per household (ha) ³	58.20 (31.66)	186.24 (119.38)	103.81 (52.47)	99.62 (85.43)
Distance between pastures ⁴ (km)	-	33.74 (29.46)	17.34 (7.03)	25.88 (23.18)
Distance of households to market (km)	9.01 (6.25)	25.19 (15.04)	25.69 (13.42)	16.92 (13.62)

Note. Values denote the average with the standard deviation in parenthesis. ¹ Available labor denotes labor force that normally participates in production all year round. ² Mixed rearing includes yaks and Tibetan sheep. ³ 1ha = 15mu. ⁴ Distance between pastures denotes the distance between summer and winter pastures.

Source: Authors' calculations from field study data.

3.2 Labor Productivity for Livestock Production in Each Management Pattern

3.2.1 Labor Productivity in SHMP-CGS

Table 4 displays the labor productivity for meat production among herder households in SHMP-CGS. The range of labor productivity in this pattern varied from 4.71 Kg/man-day to 70.73 Kg/man-day, with a mean of 23.04 Kg/man-day, which was lower than that of the overall study households (40.89 Kg/man-day). Notably, the majority of herder households (63.85%) demonstrated labor productivity between 10 Kg/man-day and 30

Kg/man-day. It is important to note that in SHMP-CGS, livestock are confined to a single pasture throughout the year, limiting their mobility. Studies have shown that limited mobility can increase pasture trampling (Yeh et al., 2014), resulting in higher soil bulk density and lower vegetation cover and aboveground biomass (Klimeš et al., 2013). Consequently, this may restrict livestock growth by limiting their access to fresh and adequate native grasses (Kerven et al., 2016).

In Table 5, the labor productivity for milk and dairy production among herder households is shown. The range of labor productivity varied from \$5.92/man-day to \$39.74/man-day, with a mean of \$19.74/man-day, which was higher than that of the overall study households (\$15.44/man-day). Furthermore, the majority of herder households (68.68%) demonstrated labor productivity between \$10/man-day and \$30/man-day. Herder households in SHMP-CGS had an advantage in terms of market access, with a distance of only 9.01 km to the market center, enabling same-day delivery of milk and dairy products via motorcycle. Consequently, the average labor time spent by herder households on milking and dairy production activities between May and September was approximately 63.59 working days, resulting in an average annual income from the sale of milk and dairy products of \$1,288.69. This finding aligns with previous observations that the milk sales of herder households are influenced by market distance, as reported by Hussen (2007).

3.2.2 Labor Productivity in SHMP-RGS

In SHMP-RGS (Table 4), the labor productivity for meat production among herder households ranged from 29.13 Kg/man-day to 125.68 Kg/man-day, with a mean of 70.36 Kg/man-day, surpassing that of the overall study households (40.89 Kg/man-day). Additionally, the majority of herder households (50.00%) achieved labor productivity levels between 60.00 Kg/man-day and 80.00 Kg/man-day. Previous research has underscored the significance of rotational grazing in improving livestock production. The practice of transferring livestock between summer and winter pastures enhances pasture recovery periods, native grass utilization efficiency, and livestock mobility, thereby benefiting livestock growth (Zhang et al., 2020; Kerven et al., 2016).

The labor productivity for milk and dairy production among herder households in SHMP-RGS ranged from \$3.76/man-day to \$26.95/man-day, with a mean of \$12.63/man-day. This was slightly lower than the labor productivity of the overall study households, which averaged \$15.44/man-day (Table 5). The majority of herder households in SHMP-RGS (84.21%) demonstrated labor productivity levels between \$0/man-day and \$20/man-day. The larger livestock scale owned by herder households in SHMP-RGS resulted in increased labor time spent on milking and dairy production activities (80 yaks/household; 78.26 man-days/household). However, due to the distance from the market center (25.19 km) and the requirement for fresh high-quality milk at the collection point, herders couldn't deliver milk collected at dusk on the same day. Consequently, the average annual income earned by herder households, excluding milk and dairy products used for self-consumption and donations to monasteries, amounted to approximately \$1043.52.

3.2.3 Labor Productivity in MHMP-RGS

In MHMP-RGS (Table 4), the labor productivity for meat production among herder households ranged from 18.28 Kg/man-day to 108.62 Kg/man-day, with a mean of 51.21 Kg/man-day. This was higher than the labor productivity of the overall study households, which averaged 40.89 Kg/man-day. Additionally, the majority of herder households in MHMP-RGS (51.43%) demonstrated labor productivity levels between 30.00 Kg/man-day and 60.00 Kg/man-day. The MHMP-RGS pattern allows member households to combine adjacent pastures, providing better opportunities for livestock to access high-quality, abundant native grasses and drinkable water (Klimeš et al., 2013). Furthermore, member households monitor and control the number of livestock reared by each other, enabling livestock to choose more palatable plants. This results in prolonged rumination time and a greater nutrient supply to the rumen, facilitating livestock weight gain (Askar et al., 2013).

The labor productivity for milk and dairy production among herder households ranged from \$2.37/man-day to \$22.56/man-day, with a mean of \$8.30/man-day. This labor productivity was concentrated between \$0/man-day and \$30/man-day, which was lower than that of the overall study households (\$15.44/man-day) (Table 5). Furthermore, the majority of herder households (77.14%) exhibited labor productivity levels between \$0/man-day and \$10/man-day. In the multi-household management pattern, the number of livestock reared by each member household was strictly controlled, leading to a reduction in the average labor time spent by herder households on milking and dairy production activities (45 yak/household; 53.60 man-day/household). Additionally, due to the relative distance of these households from the market center (25.69 km) and their collective living arrangement, opportunities for bartering between members of the multi-household management pattern with livestock by-products were available. Ultimately, the average annual income obtained by member households from the sale of milk and dairy products was \$507.29.

Table 4. Labor productivity for meat production in each management pattern

Productivity (Kg/man-day)	SHMP-CGS		SHMP-RGS		MHMP-RGS		Overall	
	Freq	%	Freq	%	Freq	%	Freq	%
(1.00, 10.00]	11	13.25	0	0	0	0	11	7.05
(10.00, 20.00]	34	40.96	0	0	1	2.86	35	22.44
(20.00, 30.00]	19	22.89	1	2.63	6	17.14	26	16.67
(30.00, 40.00]	9	10.84	1	2.63	5	14.29	15	9.62
(40.00, 50.00]	4	4.82	1	2.63	6	17.14	11	7.05
(50.00, 60.00]	2	2.41	7	18.42	7	20.00	16	10.26
(60.00, 70.00]	3	3.61	10	26.32	5	14.29	18	11.54
(70.00, 80.00]	1	1.20	9	23.68	2	5.71	12	7.69
(80.00, 90.00]	0	0	5	13.16	1	2.86	6	3.85
(90.00, 100.00]	0	0	1	2.63	0	0	1	0.64
(100.00, 110.00]	0	0	1	2.63	2	5.71	3	1.92
(110.00, 120.00]	0	0	0	0	0	0	0	0
(120.00, 130.00]	0	0	2	5.26	0	0	2	1.28
Total	83	100	38	100	35	100	156	100
Min	4.71		29.13		18.28		4.71	
Max	70.73		125.68		108.62		125.68	
Mean	23.04* (14.55)		70.36* (19.42)		51.21* (21.25)		40.89 (26.62)	

Note. * denotes the significance level of 0.05 for the difference in means of the corresponding indicator between herder households in each grassland management pattern and the overall study households. Freq is the abbreviation for frequency.

Source: Authors' estimations from field study data.

Table 5. Labor productivity for milk and dairy production in each management pattern

Productivity (\$/man-day)	SHMP-CGS		SHMP-RGS		MHMP-RGS		Overall	
	Freq	%	Freq	%	Freq	%	Freq	%
(00.00, 10.00]	11	13.25	13	34.21	27	77.14	51	32.69
(10.00, 20.00]	35	42.17	19	50.00	7	20.00	61	39.10
(20.00, 30.00]	22	26.51	6	15.79	1	2.86	29	18.59
(30.00, 40.00]	15	18.07	0	0	0	0	15	9.62
Total	83	100	38	100	35	100	156	100
Min	5.92		3.76		2.37		2.37	
Max	39.74		26.95		22.56		39.74	
Mean	19.74* (8.88)		12.63* (5.89)		8.30* (5.12)		15.44 (8.89)	

Note. * denotes the significance level of 0.05 for the difference in means of the corresponding indicator between herder households in each grassland management pattern and the overall study households. Freq is the abbreviation for frequency.

Source: Authors' estimations from field study data.

4. Conclusions

Based on data collected from a survey of 156 herder households in Maqu County on the Qinghai-Tibet Plateau, this study analyzed labor productivity in meat and milk/dairy production within both single and multi-household management patterns. The results revealed noteworthy differences among these patterns. In the rotational grazing system, herder households in both single and multi-household management patterns achieved higher labor productivity in meat production compared to the overall study households (40.89 Kg/man-day). They performed at 70.36 Kg/man-day and 51.21 Kg/man-day, respectively. However, herder households in the single-household management pattern within the continuous grazing system exhibited lower labor productivity for meat production, measuring only 23.04 Kg/man-day. Conversely, due to varying distances from pastures to

market centers in different management patterns, herder households in the single-household management pattern within the continuous grazing system achieved higher labor productivity in milk and dairy production compared to the overall study households (15.44 \$/man-day). Their productivity reached 19.74 \$/man-day. For herder households in the single and multi-household management patterns within the rotational grazing system, their labor productivity in milk and dairy production was 12.63 \$/man-day and 8.30 \$/man-day, respectively. These findings underscore the challenges associated with achieving high labor productivity simultaneously in both meat production and milk and dairy production within the same grassland management pattern.

The findings of this study hold significant implications for policymakers, providing valuable insights into the multi-household management pattern in terms of labor inputs and outputs. While the multi-household management pattern demonstrates potential in reducing labor inputs within herder households, it also faces challenges in achieving substantial levels of meat and milk/dairy production. Therefore, policymakers should consider implementing follow-up measures that prioritize the simultaneous increase in meat and milk/dairy production within the multi-household management pattern. This necessitates specific efforts to reduce the distance between herder households and market centers, thus facilitating the sale of milk/dairy products. Merely advocating for the broader adoption of the multi-household management pattern may not suffice without addressing these production-related challenges.

It's essential to acknowledge the limitations of this study. The ecological and socio-economic contexts in other grassland regions may differ significantly from those in the Gannan grassland. Consequently, caution should be exercised when attempting to generalize the results to other areas. To validate these findings and offer a more comprehensive understanding of grassland management practices, future research could explore similar studies in diverse pastoral regions characterized by varying environmental and socio-economic conditions.

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Cost-Benefit Analysis of Herders' Household Business Scale in the Multi Household Grassland Management Patterns: A Case Study of Maqu County in Qinghai-Tibetan Plateau

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Abstract

This study conducted an analysis of total production costs, gross production values, and net margins across varying scales (small, medium, and large) within herder households operating under the multi-household management pattern. Data was sourced from a random sample of 35 herder households representing six multi-household management patterns in Maqu County, Qinghai-Tibet Plateau. The results revealed that average total production costs per sheep unit were \$168.43, \$107.36, and \$92.89 for small, medium, and large-scale operations, respectively. Gross production values in these scales were \$243.50/SSU, \$245.23/SSU, and \$239.53/SSU. Significantly, large and medium-scale herder households achieved higher net margins, at \$146.64/SSU and \$137.87/SSU, while small-scale households obtained \$75.06/SSU. An intriguing revelation is that net margins for large and medium-scale households predominantly fall within the range of \$100.01/SSU to \$200.00/SSU, signifying that while scaling may curtail total production costs per sheep unit, it does not assure enduring increases in net margins. These findings hold paramount implications for policymakers as they reassess the feasibility of upscaling multi-household management pattern operations for grassland ecological restoration on the Qinghai-Tibet Plateau. While scaling up can yield cost efficiencies, it does not inherently translate into sustained net profit growth. Hence, astute consideration of these insights is imperative in evaluating the potential of scaling up multi-household management patterns for grassland ecological restoration initiatives.

Keywords: cost-benefit analysis, grassland animal husbandry, multi-household management pattern, business scale, Tibet Plateau

1. Introduction

China possesses the second-largest grassland area globally, comprising approximately 41% of its territory (Feng et al., 2021; Lan et al., 2021). The Qinghai-Tibetan Plateau (QTP), with its total grassland (steppe and meadow) area of 8.7 million ha, accounts for 51.3% of the plateau's total area (Piao et al., 2006). The grassland systems on the QTP play a critical role not only in global carbon sequestration and biodiversity conservation but also as an indicator of climate and ecological changes worldwide (Chen & Zhu, 2015; Mu et al., 2017; Zhang et al., 2015). These systems are predominantly characterized by animal husbandry and are integral to supporting the natural environment, sustaining local livelihoods, and preserving the unique cultural heritage of the QTP (Miller, 1999; Jianlin et al., 2002). However, in recent years, the grassland ecological environment has faced significant threats and challenges resulting from inappropriate grassland management practices and climate change. These challenges include a reduction in grassland area, an increase in land desertification, and a decrease in vegetation coverage (Akiyama & Kawamura, 2007; Castellani et al., 2017; Li et al., 2013). Official estimates suggest that approximately 90% of Chinese grasslands exhibit some degree of degradation, with degradation increasing at a rate of 200 km² per year (General Office of the State Council [GOSC], 2002). Consequently, it is of utmost significance to implement scientific and effective grassland management strategies to ensure the sustainable development of grassland resources.

The role of the land ownership system in mitigating grassland degradation has been widely debated. In the early 1980s, the Chinese central government introduced the household responsibility system (HRS) in pastoral areas, following its success in agricultural regions (Yang et al., 2020). Grassland privatization has been considered a

necessary strategy to address the “tragedy of the commons” and combat grassland ecological degradation (Wang et al., 2010). Under this system, individual families were allocated grasslands and livestock, replacing the collective pattern prevalent from the 1950s to the 1970s based on the production team system (Yang et al., 2021). This transition contributed to significant growth in grain production in China during the 1990s (Li et al., 2018). However, the single-household management pattern (SHMP) resulting from grassland fragmentation has its drawbacks. Under this pattern, individual families bear the risks alone, with fixed pasture locations and boundaries, limiting flexibility in grassland management and mobility of grazing. Moreover, the SHMP has led to a breakdown of collective cooperation, loss of migratory grazing knowledge (Huang et al., 2017; McGinlay et al., 2017; Hobbs et al., 2008; Wang, 2013). Traditional grazing systems are characterized by the spatial and temporal heterogeneity of native grass and water resources, as well as the collective nature of life and production. Overgrazing resulting from grassland privatization has led to decreased grassland productivity, reduced ecosystem services, increased environmental disasters, and higher levels of poverty among herders. As a result, many scholars and policymakers have advocated for the rebuilding of collective actions (Chen & Zhu, 2015; Li et al., 2007) to address these challenges.

The multi-household management pattern (MHMP), characterized by voluntary combination of pastures by adjacent herder families, has been advocated as a more suitable approach to grassland management compared to the single-household management pattern (SHMP). Researchers have highlighted its ecological benefits, including increased grazing mobility, reduced pasture trampling frequency, and improved resilience to overgrazing, resulting in decreased grassland degradation (Cao et al., 2013; Fernández-Giménez et al., 2015). Additionally, given the labor-intensive nature of grassland animal husbandry in alpine rangelands, the MHMP has potential to generate economies of scale by expanding the business scale and pooling resources to save fencing expenditures and labor inputs (Cao et al., 2009; Cao et al., 2011; Wei & Lu, 2010). In 2015, the Chinese central government introduced a policy aimed at redistributing land ownership, contracting rights, and usage rights to encourage smallholder herders to consolidate their operations with larger herders (GOSC, 2019; Yang et al., 2021). However, MHMP still face challenges related to small business scale (Tian et al., 2009; Wang, 2016; Zhou et al., 2021).

The expansion of herder households’ business scale as production operators is aimed at increasing economic benefits, with the reduction of production costs per unit of product just serving as a means to achieve higher revenue (Liu & Huang, 2010). Xu et al. (2011) argued that farmers are willing to expand their business scale even if the unit cost of agricultural products does not decrease or even increases, as long as the net margin increases. This highlights the potential disconnect between the initial intentions of government or researchers in promoting business scale among herder households within the MHMP and the actual motivations of herders when engaging in production activities. It’s crucial to emphasize that much of the existing research has predominantly focused on assessing the ecological dimensions of grasslands within the context of both the SHMP and the MHMP. These studies, often conducted through grazing experiments, consistently illustrate that the MHMP displays a higher degree of resilience concerning vegetation and soil characteristics (Cao et al., 2018; Li et al., 2018; Zhang et al., 2020). Moreover, certain research endeavors have delved into the factors contributing to the formation of the MHMP, the associated property rights systems, and the composition of household income. These investigations, employing a combination of qualitative and quantitative analyses, have unveiled that the spontaneous emergence of large-scale MHMP among herder households, facilitated by land transfers, has effectively augmented herders’ non-farm income (Cao et al., 2017; Tian et al., 2009; Wei & Guo, 2014; Yang et al., 2020; Zhou et al., 2021). Nevertheless, it’s important to acknowledge that limited research has quantified labor inputs for activities such as shearing and cost expenditures on items like dog food and fencing within both the SHMP and the MHMP, and certain cost items remain unexplored (Cao et al., 2011). While Gillespie et al. (2008) argued that other costs, such as veterinary expenses, minerals and supplements, transportation, utilities, and taxes, are contingent on the number of animals or the extent of land, herders typically possess the adaptability to make decisions and adjust their production practices to align with the dynamic socio-ecological system within which their ranch operates (Lubell et al., 2013). Therefore, the specific objective of this study is to analyze the total production costs, gross product value, and net margins of herder households involved in small, medium, and large-scale operations within the MHMP. This objective is pursued with the aim of providing a more enriched understanding to policymakers regarding the expansion of operational scale among herder households in the multi-household management pattern, viewed through the prism of cost-benefit analysis.

2. Materials and Method

2.1 Study Area

Gansu Province, located in northwest China (32°31'-42°57'N, 92°13'-108°46'E), has the country's fifth largest pastoral area. Maqu County, located on the northeastern edge of the Qinghai-Tibet Plateau (35°58'N, 101°53'E), is vital to Gansu Province's livestock output. The county is located at an elevation of 3,500 meters above sea level and receives 450 to 780 mm of rain per year. The region's average yearly temperature is 1.8 °C, with January having the lowest monthly temperature of -10.7 °C and July having the highest at 11.7 °C. The area experiences 270 frost days on average each year. The entire grassland area is around 87×10^4 ha, with alpine meadows accounting for 59% of that total. Maqu County's expected resident population in 2020 was around 57,000, with herders accounting for 75% of the overall population. Currently, herder households rely primarily on livestock trading, which accounts for around 89% of their income (Du et al., 2022).

2.2 Grassland Management Pattern

The two main livestock species raised on the Qinghai-Tibet Plateau are yaks and Tibetan sheep. Herders move their livestock from winter pastures at the foot of the mountains to higher summer pastures where they camp out toward the end of April or the beginning of May. Yaks normally mate between August and September when they are around 2 years old, giving birth to young between May and June the following year (Cui et al., 2016). Similar to this, Tibetan sheep begin breeding around August or September when they are between one and two years old, and lambs are born in January or February of the following year (Li & Guo, 2016). Yaks are milked from May to September, and any extra milk is used for ghee production, cheesemaking, sales, and self-consumption. To supply forage for the winter, forage seeds are seeded between June and July, and the harvest takes place between September and October. Herders also shear sheep and vaccinate both yaks and Tibetan sheep in the months of June and July. The movement of animals from summer pastures to winter pastures occurs once more around the end of September or the beginning of October. At the conclusion of the production cycle in October, herders transport all livestock older than two or three years old (excluding breeding animals) to the only nearby trading markets for trading based on market price and livestock weight.

2.3 Data Collection

This study employed a combination of telephone and in-person interviews to collect data from randomly selected MHMP in the study area during the period between February and March 2022. Prior to the official survey, an informal test was conducted in villages around Maqu County to ensure the logical coherence of the questionnaire design. The questionnaire encompassed a range of inquiries regarding the demographic profile of herder households. These inquiries included gender, household size, age, education level, and the prior work experience of the labor force. Additionally, the questionnaire gathered information related to animal husbandry management. This information encompassed details about the type and size of pastures, production activities, working hours and days, livestock types, numbers, weights, and breeding techniques. Furthermore, the questionnaire included inquiries about the types, quantities, and amounts of production materials utilized. Lastly, the questionnaire sought information about the sources of household income, encompassing livestock, byproducts, and non-livestock revenue.

To overcome language barriers, this study enlisted the assistance of six local individuals, comprising five college students and one government servant. These individuals were tasked with filling out questionnaires on behalf of respondents who primarily spoke Tibetan. Additionally, prior to conducting the formal survey, all enumerators underwent comprehensive training to guarantee uniformity and minimize the potential for response bias. Before administering each questionnaire, enumerators provided an explanation of the survey's objectives and obtained voluntary agreement from the participants. In total, 35 herder households, representing six MHMP, were interviewed with the assistance of the six local Tibetan enumerators.

2.4 Data Analysis

Based on the actual number of livestock owned by the herder households surveyed (Table 1), the minimum number of standard sheep units was 45.43, the maximum number was 303.18 SSU, and the average number was 132.06 SSU. Examining the distribution of sheep units, 37.14% of households had livestock numbers below 100 SSU, while another 37.14% had numbers ranging between 100.01 and 200 SSU. The remaining 25.71% of households had livestock numbers exceeding 200.01 SSU. Consequently, the number of sheep units falls into three categories: small, medium, and large business scale, based on these ranges.

The total production cost of the MHMP was calculated using multiple input items such as labor, fodder, fence maintenance, dog food, transportation, immunization, livestock insurance, and veterinarian services. The

evaluation of these inputs took into account the actual quantity used as well as the costs paid by each herder household among MHMP. The pasture area was established by the actual pasture area possessed by each herder household among MHMP to ensure consistency in measurement. After that, the area was converted to hectares using a conversion ratio of 15 mu per hectare. In order to standardize the measurement, the labor input was changed from natural days (24 hours per day, 365 days per year) to working days (10 hours per day, 364 days per year). The monetary results were initially expressed in Chinese Renminbi (RMB), and they were then converted to US dollars using the average exchange rate used by the People's Bank of China in 2021. The various kinds of animals were converted using livestock calculation units. For instance, one sheep unit was equal to a 50-kg adult ewe with a lamb and a yearly intake of 1.8 kg of hay (Ministry of Agriculture and Rural Affairs [MARA], 2020). The total production cost was calculated using the following formula:

$$C_{total\ production(\$ / SSU)} = C_{labor} + C_{fodder} + C_{fence} + C_{dog\ food} + C_{transportation} + C_{vaccination} + C_{insurance} + C_{veterinarian} \quad (1)$$

Depreciation of fences and motorbikes was calculated using the straight-line method, which evenly distributes the cost over the estimated useful life of the assets. To account for the leasing of pastures by herders to address grass shortages (Yan et al., 2011), the market rental price of pastures in rotational grazing systems (RGS) was used as a proxy for the value of native grass in this study. Specifically, the market rental prices provided by the local pasture management office were utilized. For summer pasture in RGS, the rental price was \$184.62/ha/year, while for winter pasture in RGS, it was \$246.15/ha/year. In the context of this study, the cost of using the native grass ($C_{native\ grass}$) was represented by the pasture rental cost ($C_{pasture\ rental}$).

Additionally, the gross production value and net margin were calculated following the guidelines provided by the MARA (2019).

$$V_{gross\ production(\$ / SSU)} = V_{income\ from\ live\ livestock\ sales} + V_{cash\ value\ of\ remaining\ live\ livestock} + V_{income\ from\ livestock\ by-products\ sales} \quad (2)$$

$$Net\ Margin(\$ / SSU) = V_{gross\ production} - C_{total\ production} \quad (3)$$

Where, $V_{income\ from\ livestock\ by-products}$ denotes the actual income from the sale of milk, ghee, cheese, wool and hides throughout the year for each herder household among MHMP.

The data collected on inputs, outputs, and explanatory variables were initially entered into Excel software. Descriptive statistics were then computed to summarize and analyze the data.

Table 1. Number of livestock reared by herder households in different business scales

Interval (SSU ¹ /household)	N	%	Min	Max	Mean	Std. Dev.
≤100.00	13	37.14	45.43	94.58	71.49	17.78
100.01~200	13	37.14	102.79	182.63	120.78	22.57
≥200.01	9	25.71	207.52	303.18	235.84	33.75
Total	35	100	45.43	303.18	132.06	69.73

Note. ¹ SSU is an abbreviation for Standard Sheep Unit based on the agricultural industry standard of the People's Republic of China.

Source: Authors' calculations from field study data.

3. Results and Discussion

3.1 Descriptive Analysis of Herder Households in Different Scales

According to various business scales, herder households are shown in Table 2 to have the following managerial and demographic characteristics. In the small, medium, and big large-sales, there were, correspondingly, 13, 13, and 9 households on average. In addition, these households had an average of about 3 workers available. There were mainly young and middle-aged people present, and the average length of time spent engaging in production activities ranged from 16.92 to 30.85 years. The labor force's average time in school, which ranged from 1 to 4 years, was found to be relatively short.

Yaks were the main livestock that herder households raised in the MHMP, with Tibetan sheep coming in as the second most frequent animal. With 78.90 ha for small-scale, 87.55 ha for medium-scale, and 163.27 ha for large-scale, the average pasture area showed an upward trend. A similar increase was seen in the average amount of livestock reared per hectare, with the small-scale category recording 0.94 SSU per hectare, the medium-scale 1.46 SSU per hectare, and the large-scale 1.86 SSU per hectare. In the MHMP, moving livestock from summer to

winter pastures is an essential production activity, with the distances required increasing from small to medium to large-scales. Motorcycles are a practical and often used means of transportation in the neighborhood as a result of the decreasing distances from the local market center on a medium, small, and large-scales.

Table 2. Descriptive statistics of herder households in different business scales

Variable	Small	Medium	Large	Overall
<i>Demographic characteristics</i>				
Number of households per scale	13	13	9	35
Age of the labor force per household (years)	44.69 (4.26)	43.55 (8.33)	36.11 (4.44)	42.06 (6.96)
Time in education of the labor force per household (years)	2.09 (1.25)	1.44 (2.16)	3.69 (3.04)	2.26 (2.28)
Production experience of the labor force per household (years)	30.85 (4.02)	26.94 (8.97)	16.92 (7.64)	25.82 (8.89)
Available labor force per household (people)	3.08 (0.64)	2.69 (1.03)	2.56 (0.88)	2.80 (0.87)
<i>Management characteristics</i>				
Shared labor ¹ (households)	13	13	9	35
Yak rearing (households)	13	13	9	35
Mixed rearing ² (households)	0	2	2	4
Ownership of motor vehicle (%)	100	100	100	100
Area of summer pasture per household ³ (ha)	35.71 (10.80)	41.64 (13.30)	84.57 (39.85)	50.48 (29.97)
Area of winter pasture per household (ha ⁴)	43.19 (6.32)	45.91 (9.65)	78.70 (32.03)	53.33 (22.79)
Stocking rate (SSU/ha)	0.94 (0.30)	1.46 (0.48)	1.86 (1.09)	1.37 (0.73)
Distance between pastures ⁵ (km)	21.54 (2.40)	19.84 (3.18)	7.67 (6.49)	17.34 (7.03)
Distance of patterns to market (km)	27.31 (2.59)	31.54 (8.51)	14.89 (21.34)	25.69 (13.42)

Note. Figures in parentheses indicate the standard deviation of the means. Small scale, 100 SSU or fewer; medium scale, 100.01 to 200 SSU; large scale, more than 200.01 SSU. ¹ Shared labor represents the mutual production of labor among households. ² Mixed rearing includes rearing of yaks and Tibetan sheep. ³ Pasture area denotes the actual pasture area owned by each herder households. ⁴ 1 ha = 15 mu. ⁵ Distance between pastures denotes the distance between summer and winter pastures.

Source: Authors' calculations from field study data.

3.2 Production Costs of Herder Households in Different Scales

Fodder expenses constitute a substantial cost component in livestock rearing (Table 3). Due to the scarcity of native grass, herders commonly lease pastures as an alternative to buying commercial forage. The rental price for summer pasture in the study area was \$184.62/ha per year. However, during winter, when native grass is insufficient and commercial forage prices rise, the rental price for winter pasture increases to \$246.15/ha per year. Herder households in the small and medium business scale, with limited pasture area, opt to lease more winter pasture than summer pasture to minimize reliance on expensive commercial feed during winter. This leads to an average pasture lease expenditure of \$109.35/ha and \$108.64/ha, respectively. In contrast, herder households in the large-scale have larger areas of both summer and winter pasture, with an average expenditure of \$107.50/ha. Furthermore, herders cultivate and harvest their own forage between June and October, providing hay as a source of feed during the winter months from January to April. However, given that herder households in the large-scale reared an average of 1.86 sheep units per hectare, while those in the medium and small-scales reared 1.46 and 0.94 SSU per hectare, respectively, additional fodder such as hay, corn, and silage is necessary during winter. As a result, the average expenditure on livestock forage per sheep unit for the large and medium-scale amounted to \$1.23 and \$1.17, respectively, exceeding the overall average of \$0.96 across all study households. In comparison, the average livestock forage expenditure per sheep unit for the small-scale was \$0.57.

In the MHMP, member families play a crucial role as the primary labor force in livestock production activities. The average total labor cost for herder households in the small-scale was \$27.00/SSU for the year, surpassing the average of \$19.27/SSU across all study households. Conversely, the medium and large-scale had lower average total labor costs of \$16.87/SSU and \$11.54/SSU, respectively. A daily recurring task for herders involves releasing livestock at dawn for free grazing and gathering them at dusk for housing. Due to the limited area of winter pastures owned by herder families in the small-scale, they strive to minimize expenses on commercial forage during the winter season by maximizing the use of winter pastures for grazing. As a result, the labor cost

for livestock management in the small-scale was \$16.97/SSU, while it was \$9.34/SSU and \$6.27/SSU in the medium and large-scales, respectively. Furthermore, herder households in the small, medium, and large-scales implement forage sowing and harvesting within the barns to avoid grassland destruction through cultivation and to optimize the use of livestock manure as fertilizer. This approach results in a limited area for forage planting, and the labor cost for forage sowing and harvesting was \$0.03/SSU, \$0.04/SSU, and \$0.03/SSU, respectively. Routine fence maintenance is carried out irregularly. In the small-scale, herders can inspect the fences regularly due to the small pasture area. However, herder households in the medium and large-scales repair and replace the fences only when they notice any damage, resulting in labor costs for routine fence maintenance of \$0.41/SSU in the small scale and \$0.29/SSU and \$0.17/SSU in the medium and large scales, respectively. Moreover, herder households in the medium and large-scales practice mixed rearing of yaks and Tibetan sheep, which requires labor for shearing, incurring a cost of \$0.05/SSU and \$0.07/SSU, respectively. Livestock transfer between summer and winter pastures takes place in early October and late April. As the distance between pastures decreases from small (21.54 km) to medium (19.84 km) and large-scale (7.67 km), the labor cost for this activity decreases accordingly, amounting to \$0.18/SSU, \$0.10/SSU, and \$0.03/SSU, respectively. Regarding livestock vaccination, the average labor cost was consistent across the small, medium, and large-scales, approximately \$0.03/SSU or \$0.04/SSU. This is because vaccination activities are typically conducted within the respective barns of herder households, minimizing livestock escape and reducing labor and time spent on capturing livestock. As the number of livestock reared per unit area gradually increases in small, medium, and large-scale farms, herder households adopt different strategies to maximize milk and dairy product production. Herders in the small-scale can only increase their time investment on individual yaks, resulting in an average labor cost of \$9.25/SSU, while herders in the medium and large-scales can obtain more milk and dairy products from the overall yak herd, with average labor costs of \$7.01/SSU and \$4.97/SSU, respectively.

The boundaries that separate adjacent family pastures within the grassland grazing area are demarcated by fences. Specifically, the average cost of fence maintenance was \$4.79/ha in the small-scale, \$5.21/ha in the medium-scale, and \$4.48/ha in the large-scale. It is noteworthy that the cost expenditure in both the small and large-scales surpasses the overall average of \$4.86/ha. The national construction cost of grassland fencing is estimated at \$38.08/ha (MARA, 2003), with an anticipated lifespan of approximately 10 years (Cao et al., 2011). Consequently, the annual depreciation cost amounts to \$3.81/ha. In addition to depreciation, the chances of fencing damage from livestock collisions may be affected by the area of the pasture and the number of livestock. These costs were \$0.98/ha and \$1.40/ha for the small and medium-scales, respectively, and \$0.67/ha for the large-scale.

Motorcycles serve as the primary mode of transportation for herders in the study area. The average transportation cost for the small-scale was \$4.24/SSU, which is higher than the overall average of \$3.07/SSU. This difference can be attributed to the specific requirement of transporting fresh milk from June to September, where the milk must reach the collection point on the same day. Due to the small-scale's average distance of 27.31 km from the market center and the limited total amount of milk to be transported in a single day, the transportation costs on motorcycles amount to \$2.82/SSU. On the other hand, herder households in the medium-scale can transport a larger amount of milk in a single day, despite being further away from the market centers, resulting in an average cost of \$1.66/SSU for motorcycle transportation. The large-scale, being the closest to the market center among all study households, incurs an average transportation cost of \$1.10/SSU for motorcycles. Additionally, during the period when livestock is transported to the market in late September or early October, the cost of renting a truck varies based on the number of livestock to be sold. Herder households in the large-scale, with relatively large pasture areas and the ability to winterize their livestock, can sell them when they reach adulthood. Conversely, the small-scale typically minimizes the number of wintering animals to reduce the purchase of commercial fodder during winter. For the large-scale, the average transportation cost for truck rental was \$0.35/SSU, while it amounted to \$1.37/SSU for the medium-scale and \$1.43/SSU for the small-scale.

Livestock insurance plays a crucial role in mitigating losses caused by disease outbreaks, extreme weather conditions, and wildlife attacks, all of which can lead to livestock mortality. In the pastoral area, the cost of livestock insurance is standardized, and herders are responsible for only 10% of the cost per animal, with the government subsidizing the remaining amount. On average, the cost of livestock insurance for all study households amounts to \$0.82/SSU. Disease epidemics pose a significant risk to livestock mortality. Therefore, herders prioritize annual livestock vaccinations to minimize losses. In the medium and large-scales, the average cost of livestock vaccination was \$0.20/SSU, which exceeds the overall study household average of \$0.18/SSU. This difference can be attributed to the mixed rearing of yaks and Tibetan sheep in the medium-scale, which

requires different types of livestock vaccinations. For the small-scale, the average cost of livestock vaccination was \$0.17/SSU.

Herders rely on dogs to safeguard their livestock against theft or attacks from wild animals. The main source of dog food is typically leftovers, although households with a larger number of dogs may need to purchase additional commercial food to supplement their dietary needs. However, it is important to note that the total number of dogs owned per household in the overall pastoral area typically does not exceed three. In the small and medium-scales, the average cost of dog food amounts to \$0.36/SSU and \$0.44/SSU, respectively, which surpasses the overall study household average of \$0.34/SSU. Conversely, the average cost of dog food in the large-scale was \$0.16/SSU. The relatively lower cost of dog food in the larger-scale may be attributed to the increased number of livestock reared per unit area, which corresponds to a higher number of dogs owned. Additionally, the proximity of pastures in the large-scale to urban areas may reduce the likelihood of livestock theft and wildlife attacks relative to more distant pastures, leading to a decrease in the number of dogs owned by herder households.

Veterinary costs in livestock production encompass expenses related to medicines and veterinary visits. In the small-scale, the average cost of veterinarians amounted to \$0.28/SSU, exceeding the overall study household average of \$0.19/SSU. The relatively higher veterinary costs in the small-scale can be attributed to the fact that herder households in this category have fewer livestock, which allows for easier identification of diseased livestock, enabling timely treatment. They may administer readily available medicines based on their own experience or seek consultation with a veterinarian. In contrast, the medium-scale incurred an average cost of \$0.18/SSU for veterinarians, while the large-scale had a lower average cost of \$0.07/SSU for veterinary services.

Based on the above observed input items, the average total production of herder households in the small-scale was \$168.43/SSU. The majority of herder households (76.93%) had average total production costs concentrated between \$100.01/SSU and \$200/SSU, as shown in Table 4. This average total production cost in the small-scale was higher than the overall study households' average of \$126.32/SSU. For the medium and large-scales, the average total production cost was \$107.36/SSU and \$92.89/SSU, respectively. In both medium and large-scales, the majority of herder households (92.30% and 88.88%, respectively) had average total production costs concentrated between \$50.01/SSU and \$150/SSU.

Table 3. Input costs for herder households in different business scales

Item	Small	Medium	Large	Overall
Pasture rental ¹ (\$/ha)	109.35 (1.60)	108.64 (1.48)	107.50* (1.28)	108.61 (1.61)
Livestock fodder (\$/SSU)	0.57* (0.15)	1.17 (1.76)	1.23 (0.79)	0.96 (1.16)
Hay	0 (0.00)	0.12 (0.44)	0.10 (0.20)	0.07 (0.28)
Corn	0 (0.00)	0.46 (1.34)	0.48 (0.87)	0.29 (0.93)
Silage	0 (0.00)	0 (0.00)	0.13* (0.15)	0.03 (0.09)
Forage seed	0.57 (0.15)	0.59 (0.16)	0.54 (0.05)	0.53 (0.07)
Labor force ² (\$/SSU)	27.00* (7.25)	16.87* (2.85)	11.54* (2.55)	19.27 (7.99)
Release of livestock	4.24* (1.18)	2.41* (0.36)	2.06* (0.63)	3.00 (1.26)
Gathering of livestock	12.73* (3.55)	6.93* (1.55)	4.21* (1.10)	8.38 (4.27)
Livestock vaccinations	0.04 (0.02)	0.03* (0.00)	0.04* (0.00)	0.03 (0.01)
Sheep shearing	0 (0.00)	0.05 (0.13)	0.07 (0.14)	0.04 (0.11)
Fence repairs	0.41* (0.18)	0.29 (0.14)	0.17* (0.05)	0.30 (0.17)
Forage sowing	0.01* (0.00)	0.01* (0.00)	0.01 (0.00)	0.01 (0.00)
Forage harvesting	0.02* (0.01)	0.03* (0.01)	0.02* (0.00)	0.02 (0.01)
Pasture transfer	0.18* (0.05)	0.10* (0.02)	0.03* (0.01)	0.11 (0.07)
Livestock sales	0.11* (0.06)	0.07 (0.02)	0.02* (0.02)	0.08 (0.05)
Milking	4.77 (1.07)	4.60 (0.72)	3.19* (0.49)	4.30 (1.04)
Ghee making	3.86* (1.52)	2.02* (0.75)	1.49* (0.48)	2.57 (1.46)
Cheese making	0.62* (0.15)	0.39* (0.10)	0.29* (0.06)	0.45 (0.18)
Fence maintenance ³ (\$/ha)	4.79 (0.61)	5.21 (0.78)	4.48* (0.22)	4.86 (0.67)
Fence replacement (\$/ha)	0.98 (0.61)	1.40 (0.78)	0.67* (0.22)	1.05 (0.67)
Fence depreciation (\$/ha)	3.81 (0.00)	3.81 (0.00)	3.81 (0.00)	3.81 (0.00)
Dog food ⁴ (\$/SSU)	0.36 (0.89)	0.44 (0.68)	0.16 (0.32)	0.34 (0.69)
Livestock vaccination (\$/SSU)	0.17* (0.01)	0.20 (0.08)	0.20 (0.09)	0.18 (0.06)
Veterinarian ⁵ (\$/SSU)	0.28* (0.16)	0.18 (0.04)	0.07* (0.04)	0.19 (0.13)
Transportation (\$/SSU)	4.24 (3.19)	3.03 (2.01)	1.45* (1.41)	3.07 (2.59)
Truck rental	1.43 (1.80)	1.37 (1.87)	0.35* (0.20)	1.13 (1.61)
Motorcycle ⁶	2.82 (2.56)	1.66 (1.77)	1.10* (1.33)	1.95 (2.09)
Livestock insurance (\$/SSU)	0.83 (0.06)	0.83 (0.05)	0.79 (0.05)	0.82 (0.05)
Total ⁷ (\$/SSU)	168.43* (53.71)	107.36* (25.73)	92.89* (35.06)	126.32 (51.50)

Note. * denotes the significance level of 0.05 for the difference in means of the corresponding indicator between herder households in each grassland management pattern and the overall study households. Figures in parentheses indicate the standard deviation of the means. Small scale, 100 SSU or fewer; medium scale, 100.01 to 200 SSU; large scale, more than 200.01 SSU. ¹ Cost of renting summer and winter pastures for 6 months in RGS was \$92.31/ha and \$123.08/ha, respectively. ² Labor includes all herders engaged in production activities; Daily wage based on the actual expenses paid to herders, and working hour represents the sum of the time spent by herders in all activities over the year. A full year is 70 hours per week × 52 work weeks. ³ Fence maintenance includes the cost to repair pasture fencing, and livestock enclosures as well as depreciation costs. ⁴ Dog food includes the commercial nutritional food purchased for dogs. ⁵ Veterinarian includes the cost of medicine and veterinary consultation. ⁶ Motorbike includes the cost of fuel, maintenance and depreciation costs. ⁷ Under total production costs, the cost of pasture rental and fence maintenance based on unit pasture area will be calculated on the basis of standard sheep unit.

Source: Authors' estimations from field study data.

Table 4. Distribution of total production costs for herder households in different business scales

Interval (\$/SSU)	Small		Medium		Large		Overall	
	Freq	%	Freq	%	Freq	%	Freq	%
[0, 50.00)	0	0	0	0	1	11.11	1	2.86
[50.01, 100.00)	0	0	6	46.15	4	44.44	10	28.57
[100.01, 150.00)	7	53.85	6	46.15	4	44.44	17	48.57
[150.01, 200.00)	3	23.08	1	7.69	0	0	4	11.43
[200.01, 250.00)	2	15.38	0	0	0	0	2	5.71
[250.01, 300.00)	1	7.69	0	0	0	0	1	2.86
Total	13	100	13	100	9	100	35	100

Note. Small scale, 100 SSU or fewer; medium scale, 100.01 to 200 SSU; large scale, more than 200.01 SSU.

Source: Authors' estimations from field study data.

3.3 Gross Production Values and the Net Margin of the Multi-household Pattern in Different Scales

Livestock and livestock by-products contribute to the gross production value of herder households, as shown in Table 5. In the small and medium-scales, the gross production value reached \$243.50/SSU and \$245.23/SSU, respectively, which surpasses the overall study household average of \$243.20/SSU, and the large-scale generated \$239.53/SSU in gross production value. The gross production value of herder households in the small-scale (76.93%) was mainly concentrated between \$230/SSU and \$240/SSU, while the gross production value of herder households in both the medium (100%) and large-scales (88.89%) were concentrated between \$240.01/SSU and \$250/SSU (Table 6). During October each year, livestock trading takes place based on local market prices and the weight of each animal. The cash income from live animals amounted to \$240.91/SSU in the small-scale, \$241.46/SSU in the medium-scale, and \$234.41/SSU in the large-scale. Additionally, the proximity of the large-scale to market centers within the overall study households facilitates greater commercialization of livestock by-products (Lubungu et al., 2012), resulting in an average income of \$5.11/SSU from livestock by-products. The average income from livestock by-products in the medium and small-scales were \$3.77/SSU and \$2.59/SSU, respectively. The welfare of producers in terms of net margins can be evaluated by considering the total production costs of input items. With decreasing total production costs from small to large-scales, the average net margin from livestock production in the large and medium-scales were \$146.64/SSU and \$137.87/SSU, respectively, after deducting production costs. These net margins surpass the overall study household average of \$116.80/SSU. The average net margin obtained by the small-scale was \$75.06/SSU. The net margins obtained by herder households in the large (100%) and medium-scales (92.31%) were mainly concentrated between \$100.01/SSU and \$200/SSU, while those obtained by herder households in the small-scale (76.93%) were mainly concentrated between \$50.01/SSU and \$150/SSU.

Table 5. Returns to herder households in different business scales

Item	Small	Medium	Large	Overall
Livestock ¹ (\$/SSU)	240.91 (4.88)	241.46 (5.37)	234.41* (3.21)	239.44 (5.48)
Livestock by-products (\$/SSU)	2.59* (0.59)	3.77 (0.37)	5.11* (1.29)	3.68 (1.25)
Milk	1.46* (0.38)	2.20 (0.31)	2.88* (0.84)	2.10 (0.76)
Ghee	0.60* (0.15)	0.90 (0.13)	1.18* (0.34)	0.86 (0.31)
Cheese	0.24* (0.09)	0.28* (0.16)	0.71* (0.45)	0.38 (0.31)
Wool	0 (0.00)	1.01 (2.47)	1.43 (2.83)	0.74 (2.10)
Hide	0.16* (0.04)	0.10* (0.02)	0.05* (0.01)	0.11 (0.05)
Gross product value (\$/SSU)	243.50 (4.75)	245.23 (5.36)	239.53* (4.11)	243.12 (5.22)
Total production cost (\$/SSU)	168.43* (53.71)	107.36* (25.73)	92.89* (35.06)	126.32 (51.50)
Net margin (\$/SSU)	75.06* (50.88)	137.87* (21.75)	146.64* (32.72)	116.80 (49.03)

Note. * denotes the significance level of 0.05 for the difference in means of the corresponding indicator between herder households in each grassland management pattern and the overall study households. Figures in parentheses indicate the standard deviation of the means. Small scale, 100 SSU or fewer; medium scale, 100.01 to 200 SSU; large scale, more than 200.01 SSU. ¹ Livestock includes live sales of yaks and Tibetan sheep.

Source: Authors' estimations from field study data.

Table 6. Distribution of gross production value and net margin of herder households in different business scales

	Small		Medium		Large		Overall	
	Freq	%	Freq	%	Freq	%	Freq	%
<i>Production value (\$/SSU)</i>								
[230.00, 240.00)	10	76.92	0	0	0	0	10	28.57
[240.01, 250.00)	3	23.08	13	100	8	88.89	24	68.57
[250.01, 260.00)	0	0	0	0	1	11.11	1	2.86
Total	13	100	13	100	9	100	35	100
<i>Net margin (\$/SSU)</i>								
< 0	1	7.69	0	0	0	0	1	2.86
[0, 50.00)	2	15.38	0	0	0	0	2	5.71
[50.01, 100.00)	3	23.08	1	7.69	0	0	4	11.43
[100.01, 150.00)	7	53.85	7	53.85	5	55.56	19	54.29
[150.01, 200.00)	0	0	5	38.46	4	44.44	9	25.71
Total	13	100	13	100	9	100	35	100

Note. Small scale, 100 SSU or fewer; medium scale, 100.01 to 200 SSU; large scale, more than 200.01 SSU.

Source: Authors' estimations from field study data.

4. Conclusions

Based on data collected from a random sample of 35 herder households in six multi-household management patterns in Maqu County on the Qinghai-Tibet Plateau, this study examined the total production cost, gross production value, and net margin of herder households at different business scales: small, medium, and large. The key findings revealed that the average total production costs per sheep unit in the small, medium, and large-scale operations were \$168.43, \$107.36, and \$92.89, respectively. Likewise, the gross production values in these respective scales were \$243.50/SSU, \$245.23/SSU, and \$239.53/SSU. Notably, herder households in the large and medium-scale operations achieved higher net margins of \$146.64/SSU and \$137.87/SSU, while those in the small-scale operation received a net margin of \$75.06/SSU. Interestingly, the net margins of herder households in the large and medium-scale operations were primarily concentrated between \$100.01/SSU and \$200.00/SSU. This implies that, although there is potential for decreasing the total production cost per sheep unit as the business scale increases, expanding the business scale of herder households in multi-household management patterns may not necessarily result in sustainable increases in net margins. These findings offer valuable insights for policymakers when considering the feasibility of scaling up multi-household management pattern operations. While scaling up can potentially lead to cost savings, it does not necessarily translate into a sustained increase in net profit. Therefore, careful consideration of these insights is essential when evaluating the potential of scaling up multi-household management patterns for grassland ecological restoration.

Despite the significant findings, it is important to acknowledge several limitations of this study. Firstly, the study area was limited to the Gannan grassland in the Gansu pasture area, which may restrict the generalizability of the findings to other regions. Future research should consider expanding the study area to enhance the broader applicability of the results. Secondly, due to constraints in terms of time and financial resources, this study only analyzed one production cycle and focused on a relatively small sample of 6 multi-household patterns comprising 35 herder households. It is crucial to conduct further research with a larger sample size and examine the long-term changes in cost-benefit relationships across different scales of herder households in multi-household patterns.

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Antixenosis in Constitutive Resistance in Maize Genotypes to the Stink Bug *Diceraeus melacanthus*

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Abstract

Corn is one of the most important agricultural crops in the world, however, it can be affected by numerous phytophagous insects that causing economic losses in production. *Diceraeus melacanthus* belongs to the complex of pests that attack maize. The objective of this work was to evaluate 17 maize genotypes regarding the effects of antixenosis resistance to the stink bug *D. melacanthus*. The experiments were carried out in a greenhouse with maize plants in stage V2 and adult stink bugs. Bioassays of attractiveness and food preference were carried out, in addition to evaluating physical and morphological factors of the plants, such as tissue hardness, number of punctures and colorimetric factors. Genotypes 30A37, IAC 8390, Defender, NS 77 and Supremo Tg were the ones that expressed the greatest antixenotic effects in tests free choice and no-choice, among these Defender and Supremo Tg due to possible morphological causes such as plant tissue hardness. Although the IAC 8390 genotype also presents great potential for resistance, it was not possible to attribute its causes, so more studies should be conducted to evaluate the possible chemical constituents that give them this characteristic.

Keywords: injuries, green-belly stink bug, corn pests, plant resistance

1. Introduction

Injuries caused by insects are frequently observed in cultivated plants, mainly species of economic importance such as corn (Aguirre et al., 2016; Fernandes, Ávila, Silva, & Zulin, 2020). Naturally, some plants may have mechanisms and/or structures that allow them to be less damaged by herbivorous insects than a susceptible plant (Smith & Clement, 2012), being considered resistant the one that presents this capacity.

The term resistance by antixenosis is designated to plants that present physical, morphological or chemical factors that affect the behavior of the insect, making it less preferred by the insect as a host, when compared to a susceptible plant (Smith & Clement, 2012; Boiça Junior et al., 2018). In other words, it is the negative behavioral response of the insect to plants that do not serve as hosts.

Morphological characteristics of plants, such as tissue hardness, leaf serosity, trichomes and architecture, can interfere with insects' preference for feeding, oviposition or shelter, functioning as a barrier to insect attack (Rao & Panwar, 2000; Schoonhoven, Van Loon, & Dicke, 2005; Boiça Junior et al., 2018).

When it comes to sucking insects such as stink bugs, an important factor to consider is the hardness of the plant tissue. Plants with rigid tissues are less preferred as hosts, as they limit the feeding capacity of insects (Peeters, 2002). The deposition of lignin, cellulose, suberin and other macromolecules in the cell wall of the plant gives greater hardness to the tissues, causing some resistance to the entry of the styles of sucking insects (Schoonhoven et al., 2005; Boiça Junior et al., 2018).

The amount of lignin responsible for the stiffness of the stem of maize plants is a constitutive characteristic of the plant, and normally does not change in response to insect feeding (Meihls, Kaur, & Jander, 2012). Plants that have this characteristic may have an advantage regarding the injury caused by the stink bug *Diceraeus melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae).

Color is a physical factor that can also influence the attraction of insects (Vaishampayan, Waldbauer, & Kogan, 1975). Genetic changes that alter the coloration of host plants can be implemented as a type of antixenosis resistance (Baldin, Pannuti, & Bentivenha, 2019). Although there are not many works in the literature that show the effects of color on insect preference, some authors report effects of this characteristic on the attractiveness of some agricultural pests (Schelick-Souza, Baldin, Morando, & Lourenção, 2011; Diaz-Montano, Fail, Noul, & Shelton, 2012).

Therefore, the objective of this study was to evaluate the attractiveness and food preference of the stink bug *D. melacanthus* among 17 corn genotypes, evaluated by morphological and physical characteristics.

2. Material and Methods

2.1 Creation of Insects

The initial population of *D. melacanthus* used in study was collected in the field, at the UNESP/FCAV farm in the municipality of Jaboticabal-São Paulo, in an area of grain cultivation. Another part of the initial population of stink bugs was also collected in the field, in the municipality of Formosa do Oeste-Paraná. Still, part of the insects came from the acquisition of the company PROMIP. The intention of mixing populations was to increase genetic variability and prevent population depletion. All insects were identified based on their morphological characteristics (Personal identification).

The rearing was maintained at the Plant Resistance to Insects laboratory at UNESP/FCAV, under controlled conditions of temperature (25 ± 3 °C), relative humidity ($65\pm 10\%$ RH) and photoperiod (14 L). The stink bugs were fed “ad libitum” with a natural diet consisting of bean pods (*Phaseolus vulgaris* L.), privet fruits (*Ligustrum lucidum* Aiton) and okra (*Abelmoschus esculentus* L.), dried soybean seeds (*Glycine max* Merrill), peanut (*Arachis hypogaea* L.) and sunflower (*Helianthus annuus* L.) randomly arranged in the rearing arena, according to the methodology adapted from Canassa, Baldin, Bentivenha, Pannuti, and Lourenção (2017).

Adults of *D. melacanthus* were reared in acrylic arenas (30 cm × 30 cm × 40 cm) sealed at the front with voile fabric for aeration and lined internally with filter paper. Furthermore, inside the breeding arenas, food and dry cotton pads were arranged, used as substrate for laying.

Eggs were collected daily and placed in Petri dishes (9 cm in diameter) until the nymphs hatched. Second-instar nymphs were placed in plastic boxes (10 cm × 15 cm × 30 cm) until the fifth instar, and fed the same diet as adults, subsequently transferred to acrylic cages. From the second generation, 15-day-old adult stink bugs were used in the bioassays.

2.2 Conducting the Plants

In this study, 17 maize genotypes were selected, containing different technologies and also conventional genotypes (Table 1). The genotypes were seeded in 500 ml plastic cups filled with a soil mixture (Dystrophic Red Latosol) (Andrioli & Centurion, 1999), sand and bovine organic compost in the proportions of 3:1:1, at a depth of one centimeter. In each container a single plant was conducted, which is considered a repetition. In addition, during the entire period of the experiment, the plants were kept in a greenhouse protected by an anti-aphid screen and irrigated according to the plant's water needs.

2.3 Antixenosis Bioassay

The influence of corn genotypes on the expression of antixenosis to the stink bug *D. melacanthus* was evaluated by free-choice and free-choice food preference tests. The experimental design used was randomized blocks (DBC), with ten replications, and the bioassays were conducted in a greenhouse.

For the no-choice test, each repetition composed of a plant at the V2 stage was covered with a “voile” fabric arena and released a couple of the stink bug *D. melacanthus* per repetition. The infestation lasted 72 hours, during which time the preference of insects was evaluated at the following times: 15 and 30 minutes, 1, 3, 6, 12, 24, 48 and 72 hours. The preference evaluation was carried out by evaluating the presence of the insect feeding on the plant or not.

For the free-choice test, plants of the same age as the previous test were used, however, the genotypes were divided into two groups of nine, repeating only the standard resistant genotype IAC 8390 (Bueno et al., 2020). Group 1 was composed by genotypes 22M12, 10A40, Feroz, Defender, CD 3612, AG 8700, 2B533, MG 580, IAC 8390 and group 2 Supremo Tg, Supremo vip, K9960, NS 77, 2B512, 2B610, 30A37, IAC 8046, IAC8390. The division was necessary to reduce the influence of one genotype over the other.

The genotypes of each group were arranged equidistant within the arena, covered by a filo type fabric. In the center of the arenas, nine couples of stink bugs were released, referring to one couple per genotype. The insects

remained in contact with the plants for 72 hours and the evaluations of food preference were carried out at the same times described for the previous test, recording the number of insects in each genotype. Furthermore, in both bioassays the insects were left for 12 hours without food before infestation.

Based on preference tests for groups 1 and 2, the genotypes from which group 3 originated were selected, comprising genotypes 10A40, CD 3612, MG 580, Supremo Tg, K9960, NS 77, 30A37, Defender, 2B610, IAC8390. The attractiveness of group 3 was evaluated following the same pattern as the other groups.

2.4 Test of Punctures

For the puncture test, corn plants were grown in a greenhouse following the methodology described for the antixenosis bioassay. Plants at the V2 stage were used, as this is the critical phase of the crop for attack by *D. melacanthus* (Fernandes et al., 2020). An unsexed adult bug was attached to the first fully expanded leaf of the plants, with the aid of a mini plastic arena measuring three centimeters in diameter. The insects were attached to the plants for 72 hours and after this period the leaves were removed from the plant and stained to account for the punctures. The bioassay was conducted in a randomized block design (DBC) with 8 replications.

The methodology for coloring the leaves was adapted from Rossetto and Lourenção (1981), in which a solution of water and acid fuchsin was prepared (0.5 L of distilled water and 0.5 g of fuchsin), and the leaves were submerged in this solution for 15 minutes. Then they were washed in distilled water to remove excess dye, dried on filter paper and evaluated under a stereoscope magnifying glass to count the number of punctures.

Table 1. Maize genotypes evaluated in the experiment with respective companies and employed technologies

Genotypes	Company	Technology
Sempre 22M12	Sempre [®]	Viptera
Sempre 10A40	Sempre [®]	Top
Feroz	Syngenta [®]	Viptera 3
Defender	Syngenta [®]	Viptera
Supremo	Syngenta [®]	Tg
Supremo	Syngenta [®]	Viptera 3
CD 3612	Coodetec	Power Core
K9960	KWS	Viptera 3
AG 8700	Agrocere [®]	VT PRO 3
NS 77	Nidera Sementes [®]	PRO 2
2B533	Forseed [®]	Power Core
2B512	Forseed [®]	Power Core
2B610	Forseed [®]	Power Core
MG 580	Morgan [®]	Power Core
30A37	Morgan [®]	Power Core
IAC 8046	Instituto Agronômico	Convencional
IAC 8390	Instituto Agronômico	Convencional

2.5 Hardness Test and Colorimetry

The plants used for the evaluation of stem hardness and colorimetry were conducted in the same way as previously described for the antixenosis bioassay. Both evaluations were carried out at the Integrated Pest Management laboratory of the Instituto Federal Goiano, Campus de Urutaí-GO.

In the hardness test, five plants were used for each genotype, at the V2 stage. In each plant, two points on the stem were measured, located one cm below the first fully expanded leaf on opposite sides, totaling 10 repetitions per genotype. To carry out the test, an ENGCO model Penetrometer (Piracicaba, São Paulo, Brazil) with a P 1500 tip was used to simulate the insect's stylus. The device was adjusted to perform a displacement of 2 mm at a speed of 7 mm/s towards the interior of the culm, and during this movement, measure the force used to perforate the plant tissue. The results were expressed in kilogram-force per centimeter (kgf/cm²).

The plants used in the hardness test were reused for colorimetric evaluations. Color was measured on the first and second fully expanded leaves of each plant, totaling 10 replicates per genotype. Konica Minolta model colorimeter (CR 10 Plus, Osaka, Japan) was used for these evaluations. The coloring was given by the indices L*,

a^* and b^* , with the Hue angle equivalent to $[\text{tangent arc } (b^*/a^*)]$ and the Chroma to $[(a^{*2} + b^{*2})^{1/2}]$ (Minolta, 1998). Hue angle values were transformed according to McGuire (1992).

2.6 Statistical Analysis

In the antixenosis bioassays the attractiveness data were submitted to analysis of variance (ANOVA). As the assumptions of normality and homoscedasticity were not met by the Kolmogorov-Smirnov and Levene tests, respectively, in no test was the data ranked for this variable. Means were calculated using the Scott-Knott test at 5% probability.

The preference index (PI) for *D. melacanthus* adults was calculated using the formula proposed by Kogan and Goeden (1970) for all genotypes studied and using the resistant genotype IAC 8390 as standard (Bueno et al., 2020), in addition to the genotypes designated as susceptible. The equation used was:

$$IP = 2G/G + P \quad (1)$$

In which, the number of insects in the evaluated genotype (G) and number of insects in the standard genotype (P) (resistant/susceptible) were used. By this calculation, preference indexes equal to 1 indicate similar attraction between the evaluated genotype and the susceptible standard (neutral), $IP < 1$ indicates less attraction in the evaluated genotype (deterrence) and $IP > 1$ indicates greater attraction in relation to the standard (stimulation).

For data on the number of punctures, stem hardness and colorimetric components, normality and homogeneity were analyzed using the Kolmogorov-Smirnoff and Levene tests, respectively, and the data were transformed, if necessary. Means were submitted to analysis of variance (ANOVA) and compared using the Scott Knott test at 5% probability.

The parameters were correlated to analyze the interactions between them. All analyzes were performed in the R 3.6.1 software (R Core Time, 2019).

3. Results

For attractiveness in the no-choice test, all genotypes were evaluated separately for the presence or absence of the bug in the plant at the times studied, and no significant difference was observed between genotypes at any of the evaluated times. However, when observing the average of all times, a lower average of insects attracted to genotypes 30A37, Defender, IAC 8046, AG 8700, 2B610, Supremo Vip3 and IAC 8390 was observed (Table 2).

The same pattern verified for the average attractiveness of the genotypes was also observed when evaluating the preference index of the stink bugs (Figure 1). The genotypes Supremo Vip3, IAC 8390, 2B610, AG 8700, IAC 8046, Defender and 30A37 were considered deterrents in relation to *D. melacanthus* feeding.

For the free-choice test, the genotypes present in group 1 showed statistical differences for attractiveness only in the 3 h evaluation, with genotypes 22M12, 10A40, CD 3612 and MG 580 being the least preferred by stink bugs. In the other periods, no significant difference was observed between the genotypes (Table 3).

Tabela 2. Attractiveness at different sampling times and average (\pm SEM) of adults of *Diceraeus melacanthus* in maize genotypes in a no-choice test

Genotypes	15	30	1h	3h	6h
22M12	1.0 \pm 0.26	1.0 \pm 0.15	0.9 \pm 0.23	0.4 \pm 0.22	0.5 \pm 0.22
10A40	0.8 \pm 0.20	0.6 \pm 0.22	0.7 \pm 0.21	0.7 \pm 0.26	0.9 \pm 0.28
Feroz	0.8 \pm 0.20	1.1 \pm 0.23	0.9 \pm 0.23	0.6 \pm 0.22	0.6 \pm 0.16
Defender	0.7 \pm 0.26	0.6 \pm 0.16	0.7 \pm 0.15	0.4 \pm 0.16	0.1 \pm 0.10
Supremo Tg	0.7 \pm 0.21	0.9 \pm 0.28	0.8 \pm 0.20	0.9 \pm 0.31	0.8 \pm 0.20
Supremo Vip	0.8 \pm 0.20	0.7 \pm 0.15	0.5 \pm 0.22	0.3 \pm 0.15	0.5 \pm 0.22
CD 3612	1.2 \pm 0.25	1.1 \pm 0.23	1.0 \pm 0.21	0.4 \pm 0.16	0.6 \pm 0.16
K9960	0.6 \pm 0.27	1.0 \pm 0.30	0.9 \pm 0.28	0.6 \pm 0.27	0.5 \pm 0.27
AG 8700	0.7 \pm 0.15	0.5 \pm 0.17	0.7 \pm 0.21	0.3 \pm 0.15	0.4 \pm 0.22
NS 77	1.0 \pm 0.26	1.0 \pm 0.26	1.0 \pm 0.21	0.4 \pm 0.16	0.2 \pm 0.13
2B533	1.1 \pm 0.28	1.1 \pm 0.23	1.0 \pm 0.21	0.9 \pm 0.28	0.5 \pm 0.27
2B512	1.0 \pm 0.26	1.0 \pm 0.26	0.6 \pm 0.27	0.5 \pm 0.22	0.6 \pm 0.27
2B610	0.7 \pm 0.21	0.7 \pm 0.21	0.5 \pm 0.22	0.5 \pm 0.22	0.3 \pm 0.21
MG 580	1.1 \pm 0.23	1.0 \pm 0.15	0.8 \pm 0.13	0.5 \pm 0.17	0.3 \pm 0.21
30A37	0.6 \pm 0.22	0.3 \pm 0.15	0.3 \pm 0.15	0.2 \pm 0.13	0.3 \pm 0.15
IAC 8046	0.8 \pm 0.29	0.9 \pm 0.23	0.5 \pm 0.22	0.3 \pm 0.15	0.1 \pm 0.10
IAC 8390	0.6 \pm 0.27	0.8 \pm 0.25	0.5 \pm 0.17	0.9 \pm 0.28	0.2 \pm 0.13
F	0.6437	1.1523	1.0776	0.762	1.4269
<i>p</i> -valor	0.844 ^{ns}	0.3129 ^{ns}	0.381 ^{ns}	0.726 ^{ns}	0.1358 ^{ns}
Genotypes	12 h	24 h	48 h	72 h	Mean
22M12	0.3 \pm 0.21	0.6 \pm 0.27	0.9 \pm 0.18	0.6 \pm 0.27	0.69 \pm 0.07 a
10A40	0.0 \pm 0.0	0.3 \pm 0.15	0.5 \pm 0.22	0.3 \pm 0.15	0.53 \pm 0.07 a
Feroz	0.0 \pm 0.0	0.4 \pm 0.16	0.4 \pm 0.22	0.2 \pm 0.13	0.56 \pm 0.06 a
Defender	0.3 \pm 0.15	0.2 \pm 0.20	0.3 \pm 0.15	0.0 \pm 0.0	0.37 \pm 0.05 b
Supremo Tg	0.3 \pm 0.15	0.4 \pm 0.16	0.5 \pm 0.17	0.4 \pm 0.16	0.63 \pm 0.07 a
Supremo Vip	0.3 \pm 0.15	0.3 \pm 0.15	0.5 \pm 0.17	0.4 \pm 0.22	0.48 \pm 0.06 b
CD 3612	0.1 \pm 0.10	0.3 \pm 0.21	0.3 \pm 0.15	0.6 \pm 0.16	0.62 \pm 0.07 a
K9960	0.7 \pm 0.30	0.6 \pm 0.27	0.4 \pm 0.16	0.8 \pm 0.25	0.68 \pm 0.08 a
AG 8700	0.2 \pm 0.13	0.6 \pm 0.22	0.4 \pm 0.16	0.2 \pm 0.13	0.44 \pm 0.05 b
NS 77	0.3 \pm 0.21	0.2 \pm 0.13	0.3 \pm 0.15	0.5 \pm 0.17	0.54 \pm 0.07 a
2B533	0.3 \pm 0.15	0.2 \pm 0.13	0.6 \pm 0.22	0.4 \pm 0.16	0.68 \pm 0.07 a
2B512	0.4 \pm 0.22	0.3 \pm 0.15	0.2 \pm 0.13	0.3 \pm 0.15	0.54 \pm 0.07 a
2B610	0.2 \pm 0.20	0.2 \pm 0.13	0.5 \pm 0.22	0.4 \pm 0.16	0.44 \pm 0.06 b
MG 580	0.4 \pm 0.22	0.3 \pm 0.21	0.6 \pm 0.27	0.3 \pm 0.15	0.59 \pm 0.07 a
30A37	0.2 \pm 0.13	0.2 \pm 0.13	0.2 \pm 0.13	0.5 \pm 0.17	0.31 \pm 0.05 b
IAC 8046	0.3 \pm 0.15	0.3 \pm 0.15	0.2 \pm 0.13	0.1 \pm 0.10	0.39 \pm 0.06 b
IAC 8390	0.3 \pm 0.15	0.2 \pm 0.13	0.6 \pm 0.27	0.2 \pm 0.13	0.48 \pm 0.07 b
F	0.7547	0.5574	0.9183	1.3092	2.6577
<i>p</i> -valor	0.7339 ^{ns}	0.911 ^{ns}	0.5497 ^{ns}	0.198 ^{ns}	0.0003*

Note. *Means followed by the same letter in the columns did not differ statistically, using the Scott-Knott test at 5% probability, SEM = Standard Error of the Mean.

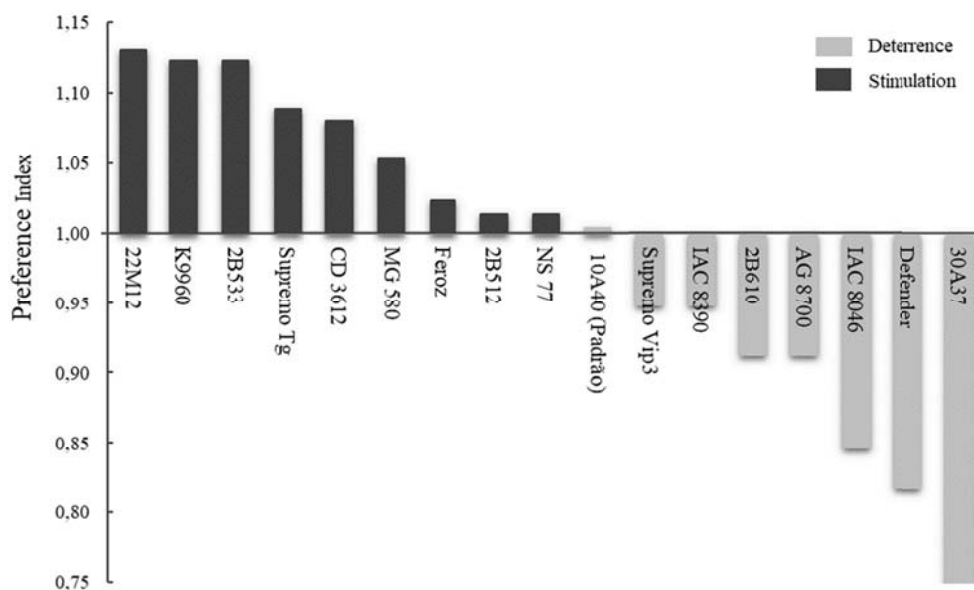


Figure 1. Preference index of *Diceræus melacanthus* to maize genotypes for the no-choice test

Tabela 3. Attractiveness at different sampling times and mean (\pm SEM) of *Diceræus melacanthus* adults in test maize genotypes with free choice for group 1

Genotypes	15	30	1h	3h	6h
22M12	0.5 \pm 0.22	0.4 \pm 0.22	0.5 \pm 0.27	0 \pm 0.0 b	0.7 \pm 0.26
10A40	0.0 \pm 0.0	0.2 \pm 0.13	0.2 \pm 0.13	0.2 \pm 0.20 b	0.3 \pm 0.21
Feroz	0.6 \pm 0.22	0.7 \pm 0.21	0.8 \pm 0.25	0.6 \pm 0.22 a	0.4 \pm 0.0
Defender	0.8 \pm 0.29	0.6 \pm 0.27	1.0 \pm 0.33	1.7 \pm 0.96 a	1.4 \pm 0.62
CD 3612	0.6 \pm 0.22	0.7 \pm 0.26	0.5 \pm 0.22	0.3 \pm 0.21 b	0.7 \pm 0.40
AG 8700	0.4 \pm 0.22	0.9 \pm 0.31	0.7 \pm 0.30	0.7 \pm 0.30 a	0.6 \pm 0.27
2B533	0.7 \pm 0.30	0.9 \pm 0.28	0.7 \pm 0.26	0.6 \pm 0.16 a	0.9 \pm 0.31
MG 580	0.1 \pm 0.10	0.2 \pm 0.13	0.3 \pm 0.15	0.4 \pm 0.22 b	0.2 \pm 0.13
IAC 8390	0.4 \pm 0.16	0.4 \pm 0.16	0.3 \pm 0.15	0.5 \pm 0.17 a	0.6 \pm 0.22

F	1.6432	1.3825	1.0595	2.1277	1.2993
<i>p</i> -valor	0.1255 ^{ns}	0.2167 ^{ns}	0.3997 ^{ns}	0.0422*	0.2557 ^{ns}

Genotypes	12 h	24 h	48 h	72 h	Mean
22M12	0.7 \pm 0.26	0.6 \pm 0.27	0.8 \pm 0.33	0.2 \pm 0.13	0.43 \pm 0.07 b
10A40	0.3 \pm 0.21	0.4 \pm 0.22	0.7 \pm 0.21	0.4 \pm 0.22	0.32 \pm 0.06 b
Feroz	0.4 \pm 0.0	0.5 \pm 0.22	0.4 \pm 0.22	0.4 \pm 0.16	0.53 \pm 0.07 b
Defender	1.4 \pm 0.62	0.7 \pm 0.33	0.7 \pm 0.26	1.0 \pm 0.52	0.99 \pm 0.15 a
CD 3612	0.7 \pm 0.40	0.7 \pm 0.33	0.3 \pm 0.15	0.2 \pm 0.13	0.50 \pm 0.08 b
AG 8700	0.6 \pm 0.27	0.7 \pm 0.42	0.9 \pm 0.23	0.5 \pm 0.31	0.76 \pm 0.11 a
2B533	0.9 \pm 0.31	1.2 \pm 0.51	1.3 \pm 0.47	0.8 \pm 0.44	0.89 \pm 0.11 a
MG 580	0.2 \pm 0.13	0.6 \pm 0.22	0.4 \pm 0.22	1.1 \pm 0.41	0.41 \pm 0.07 b
IAC 8390	0.6 \pm 0.22	0.2 \pm 0.13	0.5 \pm 0.22	0.8 \pm 0.36	0.50 \pm 0.06 b

F	1.0362	0.445	0.9776	0.792	3.8839
<i>p</i> -valor	0.4161 ^{ns}	0.8904 ^{ns}	0.4595 ^{ns}	0.6112 ^{ns}	<0.001*

Note. *Means followed by the same letter in the columns did not differ statistically from each other, using the Scott-Knott test at 5% probability, SEM = Standard Error of the Mean.

When observing the average attractiveness (Table 3) of the genotypes in relation to the evaluation periods, Defender, 2B533 and AG 8700 were more attractive, and the other genotypes presented averages equal to the resistance standard IAC 8390 (Bueno et al., 2020).

As for group 2, a significant difference was observed at 6 and 48 hours, with the genotypes Supremo Tg, IAC 8390, NS 77 and K9960 being the least attractive, maintaining this trend in the aforementioned time interval. These same genotypes maintained the lowest attractiveness pattern when compared in terms of average attractiveness between all evaluation periods, adding the 30A37 genotype to the group (Table 4).

Table 4. Attractiveness at different sampling times and mean (\pm EPM) of adults of *Diceraeus melacanthus* in maize genotypes in test with chance of choice for group 2

Genotypes	15	30	1h	3h	6h
Supremo Tg	0.3 \pm 0.21	0.4 \pm 0.22	0.4 \pm 0.22	0.3 \pm 0.15	0.0 \pm 0.00 b
Supremo vip	0.8 \pm 0.13	0.5 \pm 0.17	0.4 \pm 0.16	0.3 \pm 0.15	1.0 \pm 0.33 a
K9960	0.5 \pm 0.22	0.9 \pm 0.35	0.4 \pm 0.16	0.5 \pm 0.31	0.7 \pm 0.60 b
NS 77	0.4 \pm 0.16	0.3 \pm 0.15	0.3 \pm 0.15	0.0 \pm 0.00	0.4 \pm 0.22 b
2B512	0.4 \pm 0.16	0.8 \pm 0.20	0.7 \pm 0.15	0.4 \pm 0.22	0.7 \pm 0.21 a
2B610	0.5 \pm 0.27	0.8 \pm 0.47	0.8 \pm 0.47	0.5 \pm 0.22	1.2 \pm 0.47 a
30A37	0.5 \pm 0.22	0.4 \pm 0.16	0.1 \pm 0.10	0.1 \pm 0.10	0.6 \pm 0.22 a
IAC8046	0.7 \pm 0.40	0.8 \pm 0.42	0.9 \pm 0.41	0.8 \pm 0.33	1.1 \pm 0.28 a
IAC8390	0.2 \pm 0.13	0.3 \pm 0.15	0.4 \pm 0.22	0.4 \pm 0.22	0.1 \pm 0.10 b
F	1.0017	0.6984	1.0387	1.1611	2.9269
<i>p</i> -valor	0.4414 ^{ns}	0.6919 ^{ns}	0.4144 ^{ns}	0.3328 ^{ns}	0.0063*
Genotypes	12 h	24 h	48 h	72 h	Mean
22M12	0.6 \pm 0.40	0.4 \pm 0.22	0.1 \pm 0.10 b	0.2 \pm 0.13	0.30 \pm 0.06 c
10A40	0.8 \pm 0.39	0.9 \pm 0.31	0.9 \pm 0.41 a	0.7 \pm 0.60	0.70 \pm 0.10 b
Feroz	0.1 \pm 0.10	0.2 \pm 0.20	0.2 \pm 0.13 b	0.1 \pm 0.10	0.40 \pm 0.09 c
Defender	0.3 \pm 0.15	0.4 \pm 0.31	0.6 \pm 0.40 b	0.6 \pm 0.27	0.37 \pm 0.07 c
CD 3612	1.0 \pm 0.54	1.4 \pm 0.40	0.6 \pm 0.22 a	0.4 \pm 0.16	0.71 \pm 0.09 b
AG 8700	2.0 \pm 0.60	1.7 \pm 0.60	1.8 \pm 0.47 a	0.6 \pm 0.40	1.10 \pm 0.15 a
2B533	0.5 \pm 0.27	0.7 \pm 0.30	0.7 \pm 0.21 a	1.0 \pm 0.33	0.51 \pm 0.07 c
MG 580	0.9 \pm 0.43	0.8 \pm 0.29	0.8 \pm 0.25 a	0.4 \pm 0.16	0.80 \pm 0.10 b
IAC 8390	0.4 \pm 0.22	0.5 \pm 0.31	0.3 \pm 0.21 b	0.4 \pm 0.27	0.33 \pm 0.06 c
F	2.0073	1.9405	2.9134	1.1199	6.9222
<i>p</i> -valor	0.0557 ^{ns}	0.0649 ^{ns}	0.0065*	0.3589 ^{ns}	<0.001*

Note. *Means followed by the same letter in the columns did not differ statistically from each other, using the Scott-Knott test at 5% probability, SEM = Standard Error of the Mean.

For the preference index, the Feroz and 30A37 genotypes were determined as susceptible standards, respectively for groups 1 and 2. For group 1, the genotypes CD 3612, IAC 8390, 22M12, MG580 and 10A40 were considered feed deterrents. And, while for group 2 the genotypes that showed deterrence were K9960, NS 77, IAC 8390 and Supremo Tg (Figure 2).

For group 3, the 30 min, 6 and 12h evaluations showed significant differences, with the greatest attractiveness being for genotypes 10A40, MG580 and 2B610 at 30 minutes. In the 6h evaluation, the most attractive genotypes were IAC 8390, 10A40, Defender and 2B610, and at 12h 2B610 and MG (Table 5).

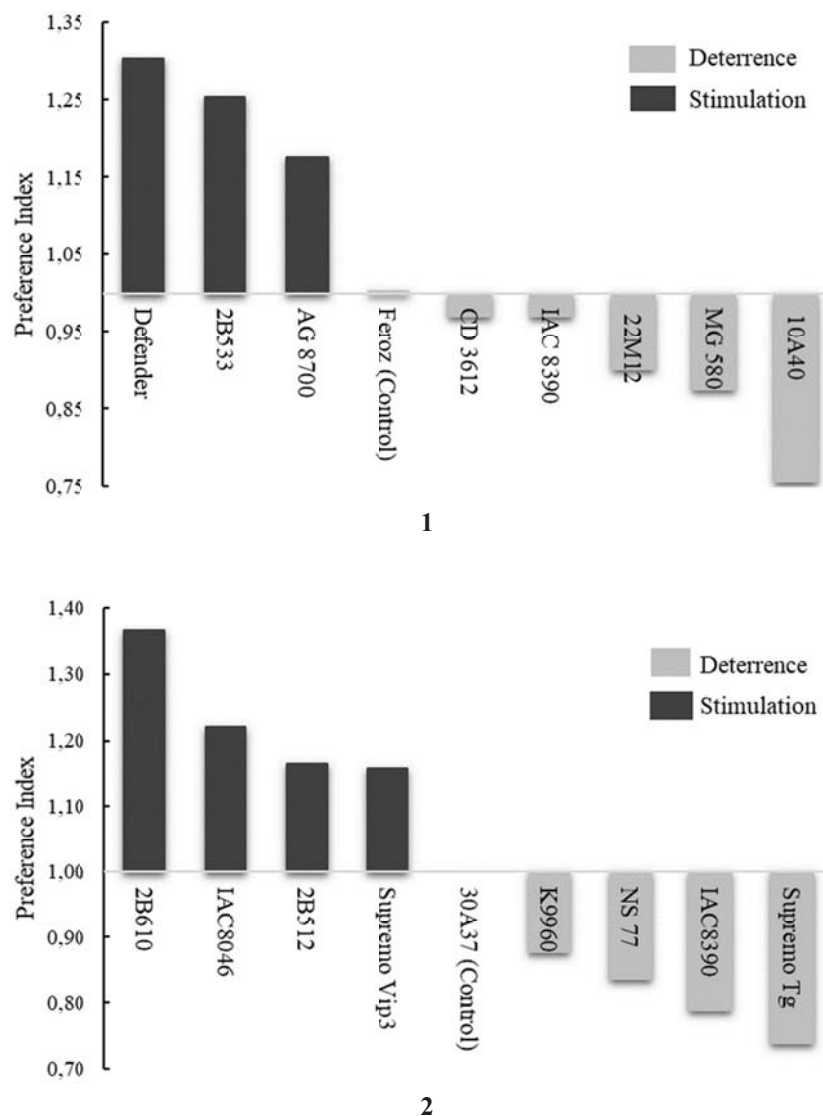


Figure 2. Preference index of groups 1 and 2 of the stink bug *Diceraeus melacanthus* in maize genotypes in free-choice test

As for average attractiveness, 30A37, K9960, IAC 8390, Supremo Tg and NS 77 stood out with the lowest average number of attracted insects (Table 5). These same genotypes were considered deterrents for food when evaluated by the preference index (Figure 3).

The number of punctures factor showed differentiation between the genotypes, with 2B512, AG 8700 and IAC 8046 being those that had the highest number of punctures, while Defender, Supremo Tg, CD 3612 and NS 77 had the lowest average number of punctures, although they did not have statistically different from the others (Table 6).

Another morphological factor studied was stem hardness and, among all genotypes, 2B512, 2B610, Defender, 22M12, Supremo Vip and Supremo Tg were those with the highest hardness (Table 6).

Table 5. Attractiveness at different sampling times and mean (\pm EPM) of *Diceraeus melacanthus* adults in test maize genotypes with free choice for group 3

Genotypes	15	30	1h	3h	6h
10A40	0.6 \pm 0.27	0.6 \pm 0.27 a	0.3 \pm 0.15	0.1 \pm 0.10	0.5 \pm 0.22 a
Defender	0.5 \pm 0.31	0.2 \pm 0.20 b	0.2 \pm 0.20	0.1 \pm 0.10	0.5 \pm 0.22 a
Supremo Tg	0.2 \pm 0.13	0.1 \pm 0.10 b	0.4 \pm 0.16	0.1 \pm 0.10	0.0 \pm 0.00 b
CD 3612	0.4 \pm 0.22	0.4 \pm 0.31 b	0.5 \pm 0.40	0.1 \pm 0.10	0.0 \pm 0.00 b
K9960	0.3 \pm 0.15	0.3 \pm 0.15 b	0.4 \pm 0.16	0.0 \pm 0.00	0.1 \pm 0.10 b
NS 77	0.0 \pm 0.00	0.2 \pm 0.20 b	0.2 \pm 0.20	0.1 \pm 0.10	0.1 \pm 0.10 b
2B610	0.8 \pm 0.39	0.9 \pm 0.31 a	0.7 \pm 0.30	0.3 \pm 0.15	0.5 \pm 0.17 a
MG 580	0.5 \pm 0.17	0.6 \pm 0.16 a	0.4 \pm 0.16	0.0 \pm 0.00	0.1 \pm 0.10 b
30A37	0.0 \pm 0.00	0.1 \pm 0.10 b	0.0 \pm 0.00	0.0 \pm 0.00	0.1 \pm 0.10 b
IAC8390	0.2 \pm 0.13	0.2 \pm 0.13 b	0.4 \pm 0.22	0.0 \pm 0.00	0.4 \pm 0.22 a
F	1.6655 ^{ns}	2.0639	1.0403	1.1515	2.3133
<i>p</i> -valor	0.109	0.041*	0.4148 ^{ns}	0.3358 ^{ns}	0.02172*
Genotypes	12 h	24 h	48 h	72 h	Mean
10A40	0.3 \pm 0.15	0.9 \pm 0.31	0.3 \pm 0.21	0.43 \pm 0.07 b	0.3 \pm 0.15 b
Defender	0.5 \pm 0.27	0.6 \pm 0.31	0.2 \pm 0.13	0.35 \pm 0.07 b	0.4 \pm 0.16 b
Supremo Tg	0.3 \pm 0.21	0.1 \pm 0.10	0.2 \pm 0.13	0.18 \pm 0.04 c	0.3 \pm 0.15 b
CD 3612	0.2 \pm 0.13	0.3 \pm 0.15	0.3 \pm 0.21	0.28 \pm 0.07 c	0.4 \pm 0.22 b
K9960	0.1 \pm 0.10	0.1 \pm 0.10	0.0 \pm 0.00	0.15 \pm 0.04 c	0.1 \pm 0.10 b
NS 77	0.2 \pm 0.13	0.1 \pm 0.10	0.6 \pm 0.34	0.18 \pm 0.06 c	0.2 \pm 0.13 b
2B610	0.1 \pm 0.10	0.7 \pm 0.40	0.4 \pm 0.22	0.56 \pm 0.09 a	0.7 \pm 0.15 a
MG 580	0.3 \pm .15	0.3 \pm 0.15	0.2 \pm 0.13	0.34 \pm 0.05 b	0.7 \pm 0.21 a
30A37	0.0 \pm 0.00	0.3 \pm 0.15	0.4 \pm 0.22	0.12 \pm 0.04 c	0.2 \pm 0.13 b
IAC8390	0.0 \pm 0.00	0.1 \pm 0.10	0.1 \pm 0.10	0.17 \pm 0.04 c	0.1 \pm 0.10 b
F	0.9632	1.5885	0.5758	5.890	1.9606
<i>p</i> -valor	0.4757 ^{ns}	0.1306 ^{ns}	0.8137 ^{ns}	<0.001*	0.05321*

Note. * Means followed by the same letter in the columns did not differ statistically from each other, using the Scott-Knott test at 5% probability, SEM = Standard Error of the Mean.

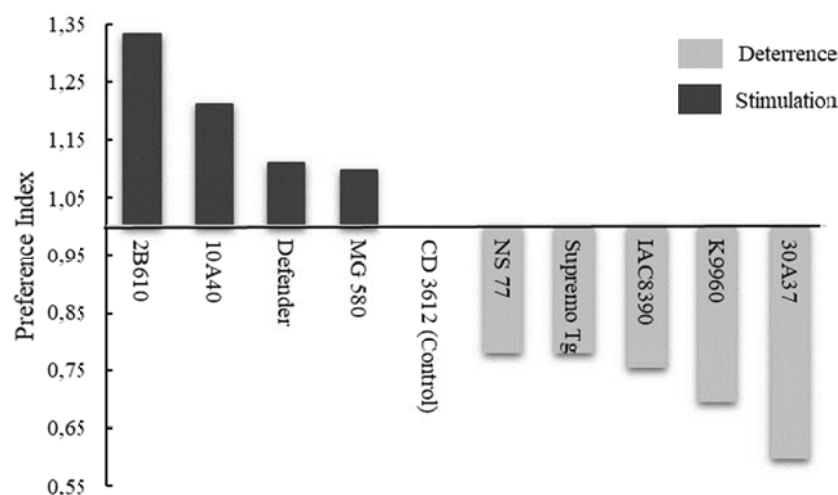


Figure 3. Group 3 preference index of the stink bug *Diceraeus melacanthus* on maize genotypes in a free-choice test

For the physical factor color, among the three components evaluated, the Hue angle did not present a significant difference between the genotypes, considering that this component is related to the green color. For luminosity

(L*) the genotypes K9960, 30A37, CD 3612, Feroz, AG8700, 10A40, IAC 8046 and 22M12 presented the highest values, and statistically differed from the others. These same genotypes correlated with chroma values, also showing the highest values for this component (Table 6).

There was a significant and negative correlation between stem hardness and the colorimetric parameters L* and chroma. In general, genotypes with greater hardness had lower L* and chroma values, indicating that harder plants are darker (Table 7). Another factor that showed a negative correlation with hardness was the number of punctures, however, it did not show significance.

Table 6. Number of punctures, stem hardness and factors related to the color of maize plants exposed to the stink bug *Diceraeus melacanthus*

Genotypes	Number of punctures ¹	Stem hardness (Kgf/cm ²) ¹	Color		
			L*	Hue	Chroma ²
22M12	7.00±4.00 b	10.21±0.75 a	39.01±1.20 a	178.78±0.02a	23.74±1.68 a
10A40	6.00±2.07 b	9.05±0.78 a	37.55±0.59 a	178.80±0.02 a	21.98±0.89 a
Feroz	8.00±1.76 b	7.10±0.53 b	37.12±0.65 a	178.79±0.02 a	22.54±1.16 a
Defender	4.00±0.82 b	10.39±0.71 a	35.37±0.80 b	178.83±0.02 a	20.81±1.43 b
Supremo Tg	4.50±1.61 b	9.52±0.70 a	35.14±0.70 b	178.83±0.02 a	20.39±1.23b
Supremo Vip	6.88±1.54 b	9.53±0.38 a	34.91±0.75 b	178.91±0.08 a	19.66±1.80 b
CD 3612	5.50±1.61 b	7.43±0.70 b	37.08±0.92 a	178.91±0.10 a	18.38±1.44 a
K9960	5.88±1.38 b	7.49±0.61 b	36.32±0.71 a	178.81±0.01 a	20.96±0.97 a
AG 8700	14.12±2.84 a	6.73±0.52 b	37.27±0.86 a	178.79±0.01 a	23.27±1.20 a
NS 77	5.00±1.29 b	6.93±0.65 b	36.91±1.35 b	178.82±0.02 a	22.09±1.37 a
2B533	7.00±2.29 b	7.31±0.71b	33.88±0.87b	178.84±0.02 a	18.31±1.11 b
2B512	9.62±2.44 a	10.56±0.56 a	35.13±0.98 b	178.87±0.02 a	17.40±1.28 b
2B610	8.00±1.03 b	10.50±0.81 a	34.14±0.65 b	178.86±0.01 a	17.51±1.05 b
MG 580	5.75±0.75 b	9.03±0.41 a	34.41±0.79 b	178.88±0.02 a	15.30±1.36 b
30A37	7.88±2.53 b	7.51±0.63 b	36.89±0.59 a	178.85±0.02 a	18.23±1.28 a
IAC 8046	5.75±1.08 b	5.57±0.90 b	38.78±1.46 a	178.77±0.02 a	23.72±1.01 a
IAC 8390	14.12±3.24 a	6.33±1.00 b	34.82±0.74 b	178.83±0.01 a	20.04±1.12 b
F	1.9384	5.7196	3.0554	1.4778	3.6974
<i>p</i> -valor	0.0231*	<0.0001*	<0.0001*	0.1145 ^{ns}	<0.0001*

Note. * Means followed by the same letter in the columns did not differ statistically, using the Scott-Knott test at 5% probability; ^{ns} = not significant; ¹ transformed into square root + 1; ² transformed into log x + 0.5; L* = luminosity; Hue = Hue angle.

All colorimetric parameters showed a significant correlation with each other, with a positive trend between Chroma and L* and a negative trend between both and the Hue angle, that is, genotypes with higher values of L* and Chroma were correlated, increasing concomitantly. As for the Hue angle, the trend was inversely proportional.

Attractiveness did not present a significant correlation with any of studied factors.

Table 7. Correlation between attractiveness, luminosity (L*), Hue angle (Hue), Chroma, stem hardness and number of punctures (NPT) factors

	Attractiveness	L*	Hue	Chroma	NPT
Dureza	0.1181793 0.1248	-0.2085334 0.0063	0.1483285 0.0535	-0.1898057 0.0131	-0.1353424 0.0784
NPT	-0.04805352 0.5338	0.02489809 0.7472	0.07598796 0.3251	-0.05914185 0.4436	
Chroma	-0.0464491 0.5475	0.6621006 <0.0001	-0.7268138 <0.0001		
Hue	0.1029532 0.1816	-0.2840046 0.0001			
L*	-0.0489053 0.5265				

4. Discussion

Studies are constantly carried out with the intention of seeking maize genotypes that present characteristics of resistance to herbivorous insects. Resistant plants stand out as an important strategy to be used in pest management, as they are non-polluting, cause less interference in the ecosystem and have a persistent effect (Lara, 1991; Boiça Junior et al., 2018).

Antixenosis resistance may occur due to chemical and/or biophysical plant factors that affect insect behavior (Smith & Clement, 2012). Among the maize genotypes studied, some stood out for being less attractive and preferred for feeding in the free-choice test, namely 30A37, K9960, IAC 8390, NS 77 and supreme Tg. For the no-choice attractiveness and food preference tests, the genotypes that stood out for being less preferred by *D. melacanthus* were 30A37, IAC 8390, IAC 8046 and Defender.

Morphological characteristics of plants, such as plant tissue hardness, may be responsible for promoting antixenosis resistance (Santiago, Barros-Rios, & Malvar, 2013). Although this characteristic is observed especially for chewing insects, it is also suggested that it may influence the feeding capacity of sucking insects, since they are linked to the insertion capacity in the insect's mouthparts in the plant. The present study suggests that this factor may have influenced the stink bugs' non-preference for the Defender and Supremo Tg genotypes, as they are among those with the highest values for stem hardness (Table 6).

The level of hardness of plant tissue implies a thick cell wall or with greater amounts of fibers. Cellulose, hemicellulose and lignin are the components of the fiber and confer mechanical resistance to the cutting or chewing action of the insect jaws, in addition to reducing the nutritional quality of the food (Santiago et al., 2013). It can be inferred that the hardness of the plant tissue can also affect the penetration of the stylet of sucking insects. Complementing the hardness results found for the Defender and Supremo Tg genotypes, which showed around 70% fewer punctures caused by the stink bug than the most fed genotype (Table 6), a fact that contributes to the hypothesis of antixenosis resistance for these genotypes.

Based on the values attributed from the correlation analysis, a tendency was observed for genotypes with greater stem hardness to present smaller amounts of punctures (Table 7), confirming the hypothesis that stem hardness influences the bug's feeding.

Some studies evaluated the effect of maize plant tissue hardness in reducing injuries caused by insects. Bergvinson, Hamilton, and Arnason (1994) quantified the tenacity of corn leaves and observed a negative correlation between this factor and damage caused by the European stem borer (*Ostrinia nubilalis* (Hübner)). Studies by Hedin, Daves, Willims, and Salin (1984) demonstrated that maize lines resistant to the corn borer (*Diatraea grandiosella* Dyar) have higher amounts of hemicellulose and crude fiber, being negatively correlated with food damage caused by the pest.

The stink bug *D. melacanthus* prefers to feed at the base of the stem of plants, and in general, the regions farther from the apex tend to be more lignified, as observed by Hoffman and McEvoy (1985) in plants of *Anaphalis margaritacea* L., a fact that, together with genotypes that already have high resistance, further limits the insect's feeding.

The IAC 8390 genotype, which also showed an antixenotic effect on the bug *D. melacanthus*, did not stand out in terms of morphological characteristics of tissue hardness, so that it could attribute resistance to this factor. It

can be inferred that, probably factors related to chemical constituents of this genotype have a greater effect on the deterrence for feeding to the stink bug.

Studies conducted by Bueno et al. (2020) with *D. melacanthus* concluded that the corn genotype IAC 8390 also showed expression of antixenosis to this bug, results that corroborate those found in this work. In addition, other studies prove the resistance of the IAC 8390 genotype to other organisms such as the nematode *Pratylenchus brachyurus* (Godfrey) Goodey (Nikuma et al., 2012) and the fungus *Puccinia polysora* Underw (Dudienas, Duarte, Gallo, & Dias de Sá, 2017). However, in both works the causes of resistance were not elucidated either.

The IAC 8390 genotype also expressed antixenosis, in addition to antibiosis for *Dalbulus maidis* (DeLong & Wolcott), reducing insect oviposition and larval viability (Faria, 2020). All these findings indicate great potential for the IAC 8390 genotype in pest management, however, more studies must be conducted to find the main resistance characteristic of this genotype.

The colorimetric characteristic of the plants is also an important factor for the selection of host plants, however, this topic is rarely studied (Cunningham & Zalucki, 2014), mainly regarding sucking insects. The parameters related to the color of the maize plants studied showed statistical differences between the genotypes ($F = 3.697$; $p \leq 0.0001$), and the genotypes less attracted to insects were correlated with lower L^* and Chroma values, indicating that the stink bug was attracted to plants with a darker color.

As already mentioned, studies relating to plant color and insect feeding preference are rare, however some studies have been conducted regarding oviposition preference. Mercader and Scriber (2007) observed greater oviposition of *Papilio glaucus* L. on dark green plants compared to lighter ones. In contrast Schlick-Souza et al. (2018) observed lower oviposition rates for genotypes with darker colors for *Chrysodeixis includens* (Walker) in soybean.

As loci mapping studies with quantitative traits and transgenic lines related to sucking insect resistance in maize are rare, one should take full advantage of the use of natural resistance variation in maize plant breeding (Meihls et al. 2012), demonstrating the relevance of the study in question.

5. Conclusion

It was concluded that the 30A37, IAC 8390, Defender, NS 77 and Supremo Tg genotypes had a greater antixenotic effect on the stink bug *D. melacanthus*. It is suggested that Defender and Supremo Tg had this effect due to possible morphological characteristics, such as plant tissue hardness. Although the IAC 8390 genotype also presents great resistance potential, it was not possible to assign its causes, among the characteristics studied, inferred that other factors are responsible for the resistance. Thus, complementary studies should be carried out to evaluate other possible causes of resistance, such as chemical ones, which may be influencing these genotypes.

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Authors Contributions

Prof. Dr. Arlindo was responsible for study design. Mrs. Francieli helped for data collection. Dr. Thaise drafted the manuscript and Prof. Julio revised it. All authors read and approved the final manuscript. Every author contributed equally to the study.

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Essential Oils in the Control of *Fusarium solani*

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Abstract

Fusarium solani is a soil pathogen and causes disease in several economically important crops. The development of research with essential oils is important. These present a diversity of active substances, with fungicidal properties. This study aimed to evaluate the effect of essential oils from *Copaifera reticulata*, *Cymbopogon nardus*, *Origanum majorana*, *Allium sativum*, *Rosmarinus officinalis*, *Piper nigrum* and *Cymbopogon citratus* on *Fusarium solani*. The oils were extracted using the technique of steam dragging, in a Clevenger apparatus. In Petri dishes containing essential oils and PDA medium, a fungal mycelium disc was added. After seven days, the mycelial growth, germination and sporulation of the fungus were evaluated. The essential oils of *C. nardus*, *C. citratus* and *A. sativum* were more efficient in controlling *F. solani*.

Keywords: biological control, soil-dwelling pathogen, sustainability

1. Introduction

The passion fruit is a plant of tropical climate, of the Passifloraceae family. The largest and most important genus of this family is *Passiflora* to which passion fruit (*Passiflora* spp.) belongs (Ramaiya et al., 2020). The genus *Passiflora* has more than 500 species, most of them producing fruits for fresh consumption (Faleiro et al., 2019). No Brasil a produção chega a cerca de 602.651 toneladas (IBGE, 2019), sendo considerado o maior produtor mundial de maracujá (FAO, 2019).

However, the productive potential of the culture is much greater. In addition, this low production is usually associated with inadequate management of the orchard, low-quality seedlings or seeds, lack of adequate technologies, and mainly phytosanitary problems (Moreira et al., 2017). Among these phytosanitary problems are soil-dwelling fungi. *Fusarium solani* (*Nectria haematococca*), considered the main causal agent of collar rot in passion fruit (Preisigke et al., 2015).

F. solani produces cylindrical microconidia and spindle-shaped macroconidia, in addition to numerous resistance structures, namely chlamydospores, which are difficult to eradicate from infested areas (Gupta & Meenu, 2019). The infection starts at the taproot and progresses to the neck (Ambrósio et al., 2018) as the rot progresses, the bark lesion darkens, and then the tissue at the bark site crumbles (Buritcá et al., 2017).

Tissues below the shell rupture at various points lengthwise; meanwhile, the canker advances laterally, encircling the root and neck and, more deeply, destroys the vessels, which causes the reflex symptoms of wilting, yellowing, and drying of the foliage (Freitas et al., 2016; Preisigke et al., 2015).

Some soil-dwelling fungi management measures have been proposed (Cruz et al., 2015; Sameza et al., 2016). Due to difficulties related to its control and increasing pressure from society to use techniques that harm the environment less, many types of research have been carried out with alternative means of control (Sampaio et al., 2016; Araújo et al., 2017). Among these measures, the use of essential oils has been gaining ground in research within universities and showing promising results (Silva et al., 2019; Habbadi et al., 2018).

Essential oils are substances from the secondary metabolism of plants, and these are liquid, hydrophobic, highly concentrated volatiles (Sharma et al., 2017). Essential oils are known to have fungicidal (Avanço et al., 2017; Kumar et al., 2016), antimicrobial (Bagy and Abo-Elyousr, 2019; Najafgholi et al., 2017), nematicidal (Jardim et al., 2017), and insecticides (Polatoglu et al., 2017; Oliveira et al., 2018).

The use of essential oils as fungicides has numerous advantages: they may contain compounds that fungi cannot inactivate (Ghalem, 2016), they are less aggressive to the environment, they can be biodegraded, and have multiple modes of action, which expands the spectrum of action over time while acting selectively (Nazzaro et al., 2017). To understand the fungicidal activity of essential oils on *F. solani*, this study extracted essential oils from seven different plant species and evaluated their action on mycelial growth, sporulation, and germination of *F. solani*.

2. Method

The experiments were set up at the State University of Montes Claros (Unimontes) in the Plant Physiology and Phytopathology laboratories.

2.1 Obtaining Essential Oils

Seven different plant species were have used *Copaifera reticulata*, *Cymbopogon nardus*, *Origanum majorana*, *Allium sativum*, *Rosmarinus officinalis*, *Piper nigrum*, and *Cymbopogon citratus*. For the extraction of essential oils, we used the technique of dragging by water vapor, in a Clevenger apparatus. The fresh leaves of the seven plant species were have added to distilled water, and they were crushed in a blender.

Then transferred to round-bottomed balloons. The extraction process took about four hours. The extracted oil was transferred to glass containers with the aid of a Pasteur pipette. To remove excess moisture, anhydrous sodium sulfate (Na_2SO_4) was added to the glass vessels. The oils were stored in a freezer at $-18\text{ }^\circ\text{C}$.

2.2 Obtaining the Fungal Isolate

The MR-35 isolate of *F. solani* was obtained from the fungal collection of the Laboratory of Phytopathology of Unimontes. This was cultivated in Petri dishes with a diameter of nine centimeters containing 20 mL of PDA (Potato-Dextrose-Agar) medium, in the continuous dark at $25\text{ }^\circ\text{C}$ for seven days. After that, 5 mm disks were removed from the edge of the colony to be used in the antibiosis test.

2.3 Effect of Essential Oils on the *in vitro* Mycelial Growth of *F. solani*

We added 200 μL of each essential oil to nine-centimeter Petri dishes containing 20 mL of BDA at a temperature of $45\text{ }^\circ\text{C}$. Next, 5 mm discs containing *F. solani* mycelium were placed in the center of the Petri dishes, which were sealed and conditioned in BOD in the continuous dark for seven days at $25\text{ }^\circ\text{C}$. The treatment contained only BDA. After seven days, the mycelial growth of *F. solani* was evaluated with the aid of a millimeter ruler.

2.4 Effect of Essential Oils on *F. solani* Germination

To estimate the germination of *F. solani*, sterile distilled water plus tween 80 were added to the colonies with seven days of growth and, with the aid of a glass slide, the conidia were disaggregated. The suspension was filtered through sterile gauze. A 100 μL aliquot of this suspension was added to the center of a seven-centimeter-diameter Petri dish with agar medium, which were incubated at room temperature for 12 hours. After this period, 100 germinated and non-germinated conidia were quantified, and the germinated ones were those with a germ tube greater than or equal to the length of the conidia.

2.5 Effect of Essential Oils on Sporulation of *F. solani*

To evaluate the sporulation of *F. solani*, the suspension described above was used. About 500 μL of this suspension was taken to a Neubauer chamber under an optical microscope for spore quantification. The trial was set up in a completely randomized design, with eight treatments (control and seven oils) and four replications. The variables were submitted to analysis of variance and the means were compared by the Scott Knott test at 5% significance. The percentage of reduction of variables was calculated using the methodology proposed by Mourão et al. (2003). Statistical analyzes were performed using R version 5.3 software.

3. Results and Discussion

In the Results There was a significant effect of different essential oils on mycelial growth, germination and sporulation of *F. solani* ($p < 0.05$). By means of the Scott-Knott test, it was possible to verify that the essential oils of *C. nardus*, *A. sativum*, *C. citratus*, *O. majorana*, *C. reticulata* and *P. nigrum*, were statistically different from the control ($p < 0.05$) for the response variable colony diameter (Table 1). As for the variables twinning conidia and sporulation, the best results were found in *C. nardus*, *A. sativum*, *C. citratus* and *O. majorana* oils (Table 1).

Table 1. Diameter, germinated conidia and sporulation of *Fusarium solani* as a function of different essential oils in PDA culture medium

Treatment	Colony Diameter (cm)	Germinated Spores	Sporulation ($\times 10^5$)
<i>Cymbopogon nardus</i>	0a	0a	0a
<i>Allium sativum</i>	0a	0a	0a
<i>Cymbopogon citratus</i>	0a	0a	0a
<i>Origanum majorana</i>	0.90a	10.33a	11.67a
<i>Copaifera reticulata</i>	6.30b	62.67b	87.33b
<i>Piper nigrum</i>	6.60b	81.67c	73.67b
<i>Rosmarinus officinalis</i>	7.43c	78.33c	75.67b
Control	8.26c	100d	124.67c
Coefficient of variation (%)	16.43	22.77	25.19

Note. Means followed by the same letter in the column do not differ from each other by the Scott Knott test at 5% probability.

The diameter of the *F. solani* colony reduced by up to 100% when it was in the presence of the essential oils of *C. nardus*, *A. sativum*, *C. citratus* (Figures 1 and 2). The essential oil of *O. majorana* reduced the colony diameter by 89.1%. The smallest reductions were verified by the oils *C. reticulata*, *P. nigrum*, *R. officinalis* (Figure 1). The effectiveness of essential oils against the diameter of *F. solani* was on the order of that of *C. nardus* > *A. sativum* > *C. citratus* > *O. Majorana* > *C. reticulata* > *P. nigrum* > *R. officinalis* (Figure 2).

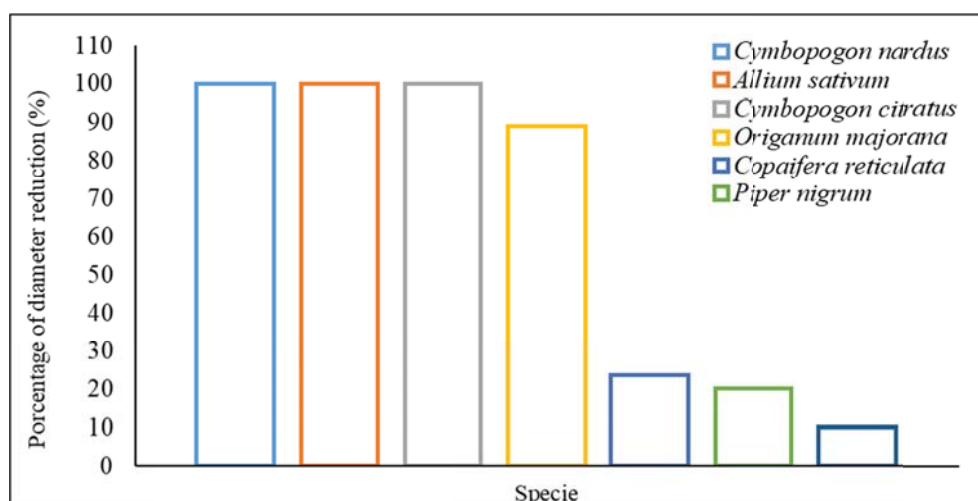


Figure 1. Percentage of reduction in colony diameter of *F. solani* as a function of different essential oils

The fat-soluble nature of essential oils enables interaction with cellular structures that have a lipid constitution, resulting in increased membrane permeability and consequent electrolyte imbalance and cell death (Steffen et al., 2019). Damage to the cytoplasmic membrane of lipoproteins causes cytoplasmic leakage, as well as the death of hyphae (Santos et al., 2013), thus reducing the mycelial growth of the fungal colony (Figure 2).

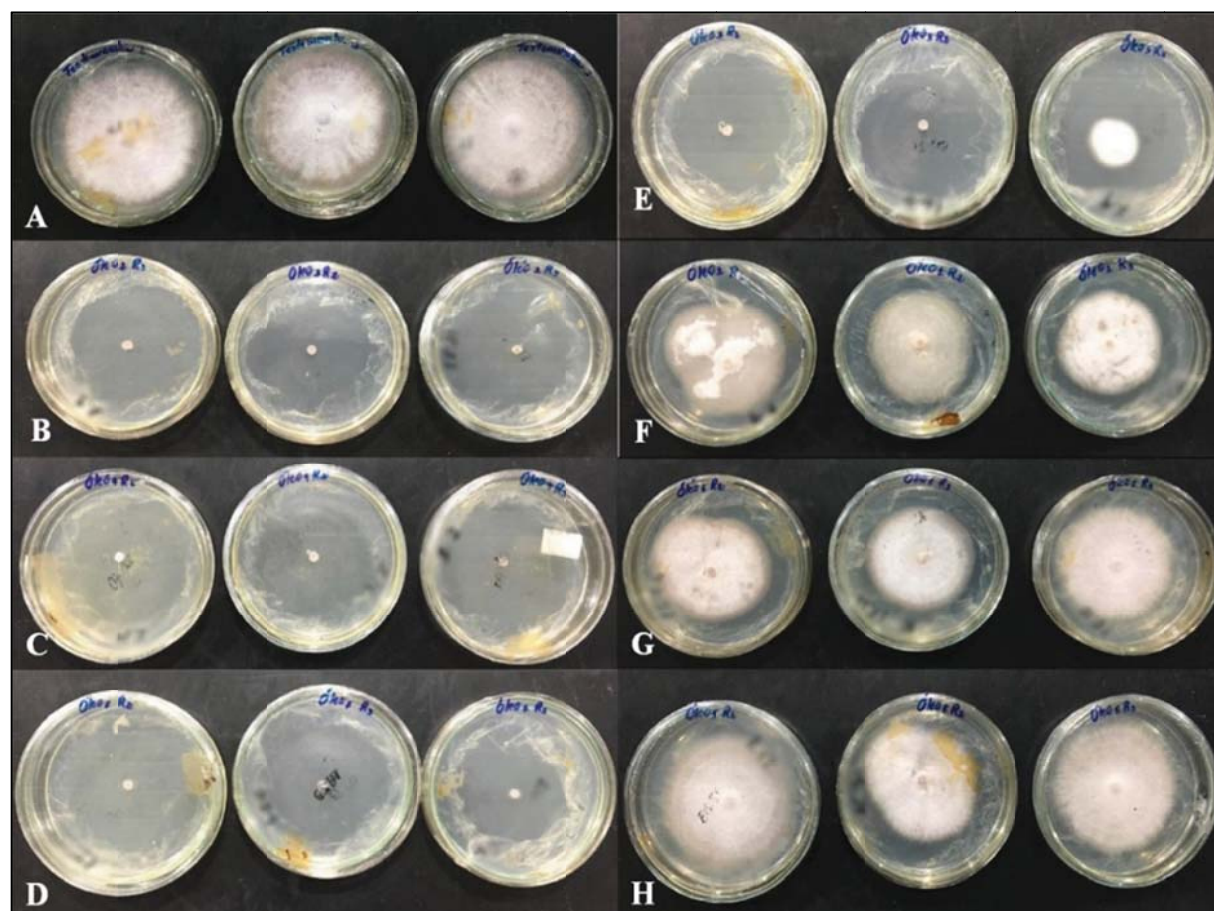


Figure 2. Diameter of the colony of *F. solani*. Control (A), *C. nardus* (B), *A. sativum* (C), *C. citratus* (D), *O. majorana* (E), *C. reticulata* (F), *P. nigrum* (G), *R. officinalis* (H)

The reductions in conidia germination reached up to 100% for the essential oils *C. nardus*, *A. sativum* and *C. citratus* (Figure 3). The smallest reduction was observed with *P. nigrum* and *R. officinalis* oils. Several studies have proven the antifungal effects of essential oils from the *Cymbopogon* genus (Mohajeri et al., 2018). The chemical constituents of essential oils have a variety of targets, including the membrane and cytoplasm, and in certain situations, they completely change the morphology of cells (Salas et al., 2016). Products derived from secondary metabolism plants are potentially useful for the development of new products capable of circumventing the problems caused by *F. solani* (Araújo et al., 2017).

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Authors Contributions

RAA, SPAMC, IOS, MJM, MNM, TJPS, IPSS, SGVC and GGL were responsible for study design and revising. ICCB, TCM, RCFR, AAX, VACC, LGAS, DFO, PDSS, MIAS, RVS and SNMB was responsible for data collection. CARM, RMA, NCN, MPG, PNCS, SSS, MJL, WGSL, HSNS, NLF and MRJ drafted the manuscript revised it. All authors read and approved the final manuscript.

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