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Residual effect of cadmium applications in different crop rotations and environments on durum wheat cadmium accumulation

J. Hirzel, J. Retamal-Salgado, I. Walter, and I. Matus

Abstract: Cadmium (Cd) is one of the heavy metals commonly present in soils that is toxic for living organisms and carcinogenic in humans. The uptake of Cd by a crop depends on various factors such as soil properties, plant-soil interaction, fertilizer management, and previous crop. The aim of the present study was to evaluate the residual effect of three Cd rates applied in three preceding crops (durum wheat [*Triticum turgidum* L. var durum], white lupine [*Lupinus albus* L.], and narrow-leafed lupine [*Lupineus angustifolius* L.]) on total dry matter, grain yield, and Cd concentration and uptake in three durum wheat cultivars in four environments. The residual effect of a cadmium chloride ($CdCl_2$) dose applied in the preceding crop to durum wheat was affected by location and previous crop, with the greatest effect in La Serena with narrow-leafed lupines as the preceding crop. Finally, in soils where a critical level of Cd is present, narrow-leafed lupines should not be used as a previous crop to durum wheat.

Key words: cadmium—crop rotations—residual effects

Cadmium (Cd) is one of the heavy metals commonly present in soils and is toxic for living organisms and carcinogenic in humans (Grant et al. 1998). The World Health Organization (WHO) considers toxic for humans a daily intake of 1 μg kg^{-1} body weight, or 70 μg $person^{-1}$ (Walker and Herman 2000; Grant et al. 1998). The ingestion of Cd in minor quantities in food, water, or air accumulates in the human body and remains for a long time, causing health problems, particularly in the kidneys (Grant et al. 1998). The European Union identified maximal Cd concentrations of 50, 100, 200, and 200 μg kg^{-1} in fruits, root vegetables, wheat (*Triticum aestivum* L.), and lettuce (*Lactuca sativa*), respectively (Berg and Licht 2002).

The amount and bioavailability of Cd in soils depend on soil factors, fertilization management, exposure to pollution sources, crop rotation, and management practices (Oliver et al. 1993; Grant et al. 1998; Baize et al. 2003; Chen et al. 2008; Grant et al. 2010; Gao and Grant 2012; McDowell et al. 2013). According to Lehoczky and Kiss (2006), a soil total Cd concentration of 1 mg kg^{-1} was considered high risk in Hungarian soils. A similar value is indicated for Chilean soils by González et al. (1997) and Villanueva (2003).

Although Cd is not an essential plant nutrient, its uptake can be in higher quantities than other trace elements without any adverse effects on plant growth (Grant et al. 1998). There are differences in how much Cd accumulates and how it is partitioned among tissues, plant species, and cultivars (Yang et al. 1995; Grant et al. 1998; Sánchez and Krieger 2006; Harris and Taylor 2013; Arduini et al. 2014; Perrier et al. 2016). Among the important agricultural crops for the human diet, the main species that uptake Cd and translocate it to the grain are durum wheat (*Triticum turgidum* L. var durum), corn (*Zea mays* L.), wheat, oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), and peas (*Pisum sativum* L.), all of which have Cd concentrations above the WHO limit for human health (Greger and Löfstedt 2004; Cajuste et al. 2006; Tanaka et al. 2007; Tsyganov et al. 2007; Wångstrand et al. 2007).

Legume crops generate many benefits for soil properties, but some species, such as white lupine (*Lupinus albus* L.) and narrow-leafed lupine (*Lupineus angustifolius* L.), present high Cd uptake and increase soil Cd availability (Trejo et al. 2016), which could affect the Cd uptake in the next crop, with a risk to the human health when this crop is used as food. When grown in soils that are deficient in available nutrients, white lupines exude chelating agents (organic anions and enzymes, such as phosphatase and probably phytase) and hydrogen (H) ions through roots, improving the absorption of phosphorus (P), iron (Fe), manganese (Mn), zinc (Zn) (Lambers et al. 2013), and also of Cd (Page et al. 2006). The white lupine root system has high Cd retention capacity through cell walls (Zornoza et al. 2002). Therefore, white lupines accumulate high concentrations of arsenic (As) and Cd in their roots (Vázquez et al. 2006) and, as a result, they effectively decrease the available fraction of As and Cd in acidic soils.

On the other hand, narrow-leafed lupines release large amounts of carboxylates from nonspecialized roots (Egle et al. 2003), which also can affect plant Cd availability and absorption (Bovet et al. 2006; Han et al. 2006; Grant et al. 2008). Oliver et al. (1993) indicate that the Cd concentration in wheat grain was higher when grown after lupines than in other rotations.

The importance of food consumption with a low risk for human health, and the cropping practices and cultivars that lead to low accumulation of heavy metals in foods derived from agriculture, could contribute to satisfy the growing demand for healthy foods. In this respect, little published information exists on how crop rotations could affect Cd uptake, characterized by both plant Cd accumulation and the translocation capacity to the grain. The objective of this study was to evaluate the effect of Cd application in the preceding crop on grain yield, dry matter (DM) production, and Cd accumulation in grain and whole plant in three durum wheat cultivars as second crop in four agricultural locations in Chile with three crop rotations that included white lupine, narrow-leafed

Juan Hirzel (corresponding author) and **Iván Matus** are researchers of the Instituto de Investigaciones Agropecuarias, the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) Quilamapu, Chillán, Chile. **Jorge Retamal-Salgado** is an assistant professor of the Universidad Adventista de Chile, Facultad de Ingeniería y Negocios, Chillán, Chile. **Ingrid Walter** is a senior researcher of INIA, Madrid, Spain.

lupine, and durum wheat as the preceding crops. Our hypothesis was that within a crop rotation, the preceding crop would affect both the biomass production and Cd uptake in durum wheat.

Materials and Methods

Four field experiments were conducted during the 2013 to 2014 and 2014 to 2015 crop season in different agricultural zones of Chile. Since the objective was to evaluate the effect of the application of Cd performed in the first crop of each rotation (2013 to 2014), the evaluations and analysis of results correspond to the durum wheat cultivars evaluated in the season 2014 to 2015.

Climatic and Soil Characteristics of Each Location. General features of the study sites were the following: (1) La Serena (30°3' S; 71°14' W), soil of alluvial-colluvial origin (Haplocambids), arid climate, maritime influence, 40 and 127 mm precipitation for the 2014 and 2015 seasons, respectively, and concentrated in winter; (2) Los Tilos (33°34' S; 70°37' W), soil of alluvial origin (Haploxeroll), semiarid and temperate Mediterranean climate with a hot and dry summer and cold winter with 163 and 345 mm of precipitation for the 2014 and 2015 seasons, respectively, and concentrated in winter; (3) Chillán (36°31' S; 71°54' W), soil of volcanic origin (Melanoxerand), temperate Mediterranean climate with a hot and dry summer and cold and wet winter with 672 and 978 mm of precipitation for the 2014 and 2015 seasons, respectively, and concentrated in winter and spring; and (4) Temuco (38°41' S; 72°25' W), soil of volcanic origin (Melanudands), Mediterranean climate with hot and dry summer and cold and rainy winter with 868 and 1,284 mm precipitation for the 2014 and 2015 seasons, respectively, and concentrated in winter and spring. The physical and chemical properties at the beginning of the first experimental year for each soil are shown in table 1.

Experiment Management. The crop rotations in the four locations were (1) durum wheat–durum wheat, (2) white lupine–durum wheat, and (3) narrow-leafed lupine–durum wheat. Agronomic practices were standardized for all locations and are those normally used in Chile for those crops. For durum wheat, the cultivars were Llareta-INIA, Corcolén-INIA, and Lleuque-INIA, developed by the wheat breeding program of the Agricultural Research Institute of Chile (Instituto de Investigaciones Agropecuarias, the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria [INIA]), and were selected according to their productivity and adaptability to the production area of durum wheat in Chile. For white and narrow-leafed lupines, the cultivars were Rumbo-Baer and Wonga, respectively.

Each experimental unit was a plot of 15 rows 3 m long and 0.2 m row spacing (9 m^2). For the first season, the experiment sowing dates for both white and narrow-leafed lupines were May 15, 2013, at La Serena and Los Tilos, and July 17, 2013, at Chillán and Temuco. The durum wheat was sown July 15, July 20, August 20, and August 25 in La Serena, Los Tilos, Chillán, and Temuco, respectively.

In the second season, 2014 to 2015 (only durum wheat), the experiments were sown July 18, July 25, August 22, and August 27 in La Serena, Los Tilos, Chillán, and Temuco, respectively.

Seed rates were 140 and 120 kg ha^{-1} for white and narrow-leafed lupines, respectively. Irrigation in both lupine crops was applied for both seasons as indicated: in La Serena and Los Tilos, irrigation was applied at the tillering, preflowering, and flowering stages; in Chillán and Temuco, precipitation during the period of sowing to flowering was sufficient for the hydric requirements of the crops, so irrigation was not necessary. In both white and narrow-leafed lupines, fertilization with nitrogen (N) and P was not used because those species do not respond to the application of these elements. The available K in soil was sufficient for crop needs (table 1).

Durum wheat seed rate was 220 kg seed ha^{-1} in both seasons for each cultivar. Irrigation was applied at tillering (La Serena and Los Tilos), booting stage, heading, and milk to dough stage (all locations) according to crop demand so as not to generate any restriction. Total weed control was carried out, and disease control was not necessary. In durum wheat for both seasons, the N, P, and K fertilization rates were 240, 120, and 120 kg ha^{-1}, respectively, and fertilizer sources were urea, triple superphosphate, and potassium chloride (KCl). Nitrogen was applied 15%, 45%, and 40% at sowing, tillering, and flag leaf, respectively. Phosphorus and K were applied 100% at sowing.

At the beginning of the first season (crop preceding to second crop to evaluate in this experiment), Cd was applied as cadmium chloride ($CdCl_2$; 61.3% Cd) at rates of 0, 1, and 2 mg kg^{-1}, adjusted for soil depth from 0 to 0.2 m and the apparent density of each soil (table 1). The equivalent amount of Cd applied at rates of 1 and 2 mg kg^{-1} $CdCl_2$ at each location was (1) La Serena, 2,157.76 and 4,315.52 g ha^{-1}, respectively; (2) Los Tilos, 1,593.8 and 3,187.6 g ha^{-1}, respectively; (3) Chillán, 1,226 and 2,452 g ha^{-1}, respectively; and (4) Temuco, 980.8 and 1,916.6 g ha^{-1}, respectively. Application of $CdCl_2$ was performed in solution with distilled water at the rate of 20 mL m^{-2}. In the second season Cd was not applied.

Grain Yield, Soil, and Plant Tissue Analysis. Plots were harvested manually at maturity, and the whole yield of each plot was expressed on a megagram per hectare basis. Plant samples of 1 m^2 were collected at harvest time in each experimental unit at each location. Grain and tissue samples were oven-dried at 70°C for 72 hours.

Chemical soil analysis was performed before starting the experiment using the methodology indicated by Sadzawka et al. (2006). Soil pH was measured in a 1:2.5 soil:water ratio solution with a pH meter. Electrical conductivity was evaluated using a conductivity cell (soil:water ratio 1:5). Soil organic matter (OM) was measured by the Walkley-Black wet digestion method. Soil available N (nitrate [NO_3]-N and ammonium [NH_4]-N) was extracted with 2 M KCl and determined by colorimetry in an auto-analyzer (segmented flux spectrophotometer; Skalar, Breda, the Netherlands). Available P was determined by 0.5 M sodium bicarbonate ($NaHCO_3$) (Olsen P) using the ascorbic acid-molybdate method. Exchangeable Ca, Mg, K, and Na were determined by 1 M ammonium acetate (NH_4OAc) extraction followed by flame spectroscopy: absorption (Ca and Mg) and emission (K and Na). Soil exchangeable aluminum (Al) concentration was determined with 1 M KCl extraction using absorption spectroscopy. Iron, Mn, Zn, and copper (Cu) concentrations in soils were determined in diethylenetriaminepentaacetic acid (DTPA) extract (Lindsay and Norvell 1978) by atomic absorption spectrometry (AAS). Boron (B) was determined by colorimetry in a solution obtained by acid digestion. Soil total Cd was determined by extraction with HNO_3-HCl (*aqua regia*) and analyzed by inductively coupled plasma/optical emission spectrometry (ICP-OES) (table 1).

Table 1
Soil physical and chemical properties (0 to 20 and 20 to 40 cm depth) at four locations previous to establishing the preceding crop.

	Locations and depths (cm)							
	La Serena		Los Tilos		Chillán		Temuco	
Parameters	0 to 20	20 to 40	0 to 20	20 to 40	0 to 20	20 to 40	0 to 20	20 to 40
Clay (%)	20.2	20.3	21.5	27.3	20.7	15.9	25.3	23.1
Silt (%)	30.2	31.2	50.0	49.3	43.6	45.4	38.5	41.5
Sand (%)	49.6	48.5	28.5	23.4	35.7	38.7	36.2	35.4
Bulk density (g cm^{-3})	1.76	1.80	1.20	1.24	1.00	1.05	0.80	0.82
$pH_{(soil:water\ 1:5)}$	6.94	6.87	8.25	8.19	5.74	5.76	5.96	5.92
Organic matter (g kg^{-1})	11.6	11.3	19.6	21.7	63.0	56.2	155.6	143.7
EC (dS m^{-1})	0.15	0.23	0.11	0.15	0.11	0.07	0.09	0.09
Available N (mg kg^{-1})	18.0	20.0	11.0	14.0	40.0	38.0	30.0	28.0
Olsen P (mg kg^{-1})	51.3	44.9	3.9	5.1	35.2	25.3	24.5	20.9
Exchangeable K ($cmol_c$ kg^{-1})	0.85	0.67	0.35	0.41	0.65	0.39	0.67	0.41
Exchangeable Ca ($cmol_c$ kg^{-1})	8.12	8.22	20.70	19.66	6.74	5.89	7.40	7.77
Exchangeable Mg ($cmol_c$ kg^{-1})	2.41	2.61	0.92	0.86	0.95	0.72	0.71	0.76
Exchangeable Na ($cmol_c$ kg^{-1})	0.59	0.69	0.49	0.40	0.16	0.19	0.03	0.05
Exchangeable Al ($cmol_c$ kg^{-1})	0.05	0.05	0.04	0.04	0.21	0.10	0.06	0.05
Available Fe (mg kg^{-1})	21.5	20.8	16.7	17.0	59.8	46.5	55.9	59.9
Available Mn (mg kg^{-1})	36.3	34.3	11.8	11.5	9.8	5.4	3.5	2.8
Available Zn (mg kg^{-1})	4.5	4.4	1.1	1.2	0.7	0.6	0.8	0.6
Available Cu (mg kg^{-1})	9.2	9.3	8.9	8.8	1.4	1.2	1.0	0.9
Available B (mg kg^{-1})	2.3	2.4	0.8	0.8	0.5	0.4	0.3	0.3
Available S (mg kg^{-1})	40.8	64.9	11.9	13.5	14.2	15.4	14.6	9.6
Total Cd (mg kg^{-1})	1.33	1.49	0.52	0.51	0.21	0.18	0.14	0.14

Notes: EC = electrical conductivity. N = nitrogen. P = phosphorus. K = potassium. Ca = calcium. Mg = magnesium. Na = sodium. Al = aluminum. Fe = iron. Mn = manganese. Zn = zinc. Cu = copper. B = boron. S = sulfur. Cd = cadmium.

To determine Cd content in plant tissues of durum wheat in the second season, dried samples were ground with a mill and passed through a 2 mm sieve. Tissue samples were digested with nitric acid concentrate (in a microwave), and Cd in the solution was determined by ICP-OES (Sadzawka et al. 2006).

Experimental Design and Statistical Analysis. The experimental design was a split-split-split plot, where the main plot was the location (four), the split plot was the crop rotation, the split-split plot was the Cd rate (three), and the split-split-split plot was the durum wheat cultivar (three) with three replicates.

Results were analyzed by ANOVA and Tukey's test ($p = 0.05$) using the SAS PROC MIXED Model procedure (SAS Institute, Cary, North Carolina), considering the locations as nonrandom effects. For the significant interactions, contrast analysis was used to compare the treatment effects separately.

Results and Discussion

Statistical Analysis. The analysis of significance (table 2) indicated a location effect and crop rotation effects in all the evaluated characteristics. At the same time, the Cd dose had a significant effect in most of the evaluated characteristics, with the exception of total DM production and grain yield. On the other hand, the cultivar did not have an effect on any of the evaluated characteristics (table 2). A significant effect was found for the simple interaction between location × crop rotation for all of the evaluated characteristics (table 2). The interaction location × Cd dose significantly affected the content and extraction of Cd in total DM production, and the content and extraction of Cd in the grain (table 2). The interaction location × Cd dose only affected the total DM production (table 2). The interaction crop rotation × Cd dose affected all of the evaluated characteristics, except grain yield (table 2). The interaction crop rotation × Cd dose only affected the grain yield (table 2). The interaction Cd dose × cultivar affected the Cd content in total DM production and the concentration and extraction of Cd in the grain (table 2). In the triple interactions, a significant effect was found for the location × rotation × Cd dose in both the content and extraction of Cd in the whole plant, as well as the interaction location × rotation × cultivar in the production of total DM production and grain yield, and the interaction rotation × Cd dose × cultivar in the grain yield (table 2). Finally, the interaction of all the sources of variation only presented a significant effect in the content and Cd uptake in total DM production (table 2). Since the significance analysis indicated interactions among the majority of the sources of variation, highlighting the effect of location and rotation, a contrasting analysis was carried out separating the effects by locality (figure 1) and crop rotation (figure 2).

Effect of the Location on the Production, Concentration, and Extraction of Cadmium. The whole plant production in both white and narrow-leafed lupines for the first season of the experiment was discussed by Trejo et al. (2016) with normal values for total DM yield and differences between locations. The total DM production and grain yield for durum wheat in the first season of the experiment were discussed by Hirzel et al. (2017) with values within the normal ranges for the locations in which the experiments were performed.

Table 2
Parameters' significance levels and interactions.

Variation sources	Total dry matter production	Dry matter Cd content	Dry matter Cd extraction	Grain yield	Grain Cd content	Grain Cd extraction	DF
L*	***	***	***	***	***	***	3
C†	***	***	***	***	***	***	2
R‡	NS	***	***	NS	***	***	2
W§	NS	NS	NS	NS	NS	NS	2
L × C	***	***	***	***	***	**	6
L × R	NS	***	***	NS	***	***	6
L × W	**	NS	NS	NS	NS	NS	6
C × R	**	***	***	NS	**	**	4
C × W	NS	NS	NS	**	NS	NS	4
R × W	NS	**	NS	NS	***	**	4
L × C × R	NS	***	***	NS	NS	NS	12
L × C × W	**	NS	NS	**	NS	NS	12
L × R × W	NS	NS	NS	NS	NS	NS	12
C × R × W	NS	NS	NS	**	NS	NS	8
L × C × R × W	NS	***	***	NS	NS	NS	24

Notes: NS = nonsignificant. DF = degrees of freedom.

*L = locations (4); La Serena, Los Tilos, Chillán, and Temuco.

†C = crop rotation (3); durum wheat–durum wheat (DW), white lupin (WL) –DW, and narrow-leafed lupin (NL) –DW.

‡R = $CdCl_2$ rates (3); 0, 1, and 2 mg kg^{-1}.

§W = durum wheat cultivars (3); Llareta-INIA, Corcolén-INIA, and Lleuque-INIA.

**Significant at $p < 0.05$.

***Significant at $p < 0.01$.

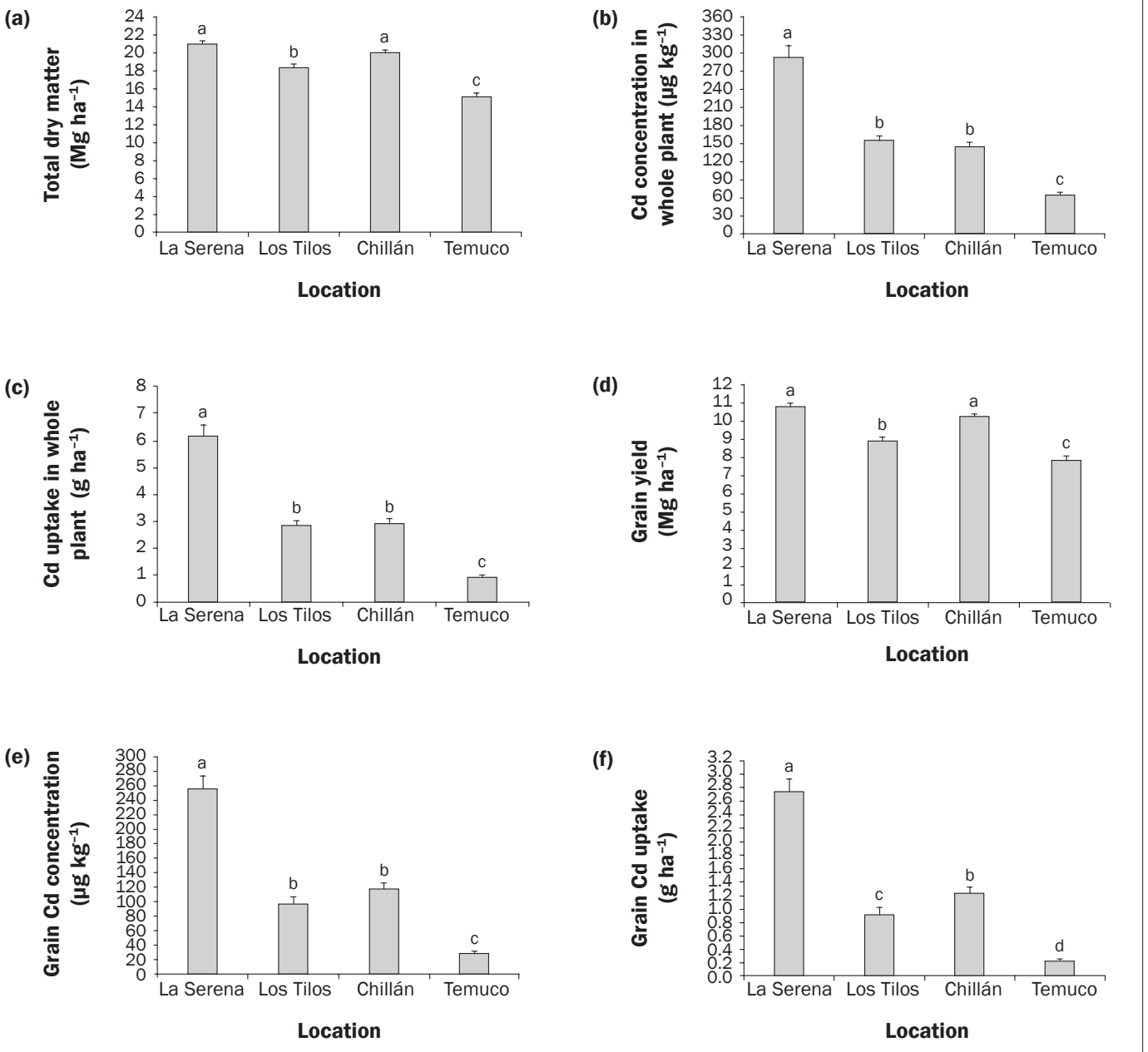
On comparing locations, the production of DM was highest in La Serena and Chillán ($p < 0.05$), followed by Los Tilos, and the lowest value was obtained in Temuco ($p < 0.05$) (figure 1a). The values obtained for DM fluctuated between 15.1 and 20.9 Mg ha^{-1} (figure 1a) and were similar to those indicated by Hirzel et al. (2010) and Hirzel et al. (2017). The differences existing in DM production between the study locations were discussed previously by Hirzel et al. (2017). The Cd concentration in the whole plant (figure 1b) fluctuated between 63.6 and 292.3 μg kg^{-1}, and was lower than the values reported by Arduini et al. (2014) in aerial residues of two durum wheat cultivars. The largest value was obtained in La Serena ($p < 0.05$), followed by Los Tilos and Chillán, and the lowest value was obtained in Temuco ($p < 0.05$).

For its part, the Cd uptake in whole plant fluctuated between 0.9 and 6.1 g ha^{-1} (figure 1c), slightly lower values than those reported by Hirzel et al. (2017), with a behavior similar to that obtained for the Cd concentration in the whole plant—that is, the highest in La Serena ($p < 0.05$), followed by Los Tilos and Chillán, and the lowest in Temuco ($p < 0.05$). The grain yield fluctuated between 7.8 and 10.8 Mg ha^{-1} (figure 1d), and was similar to that reported by Hirzel et al. (2010) and Hirzel et al. (2017). The largest grain yields were obtained in La Serena and Chillán ($p < 0.05$), followed by Los Tilos, and the lowest value was found in Temuco ($p < 0.05$) (figure 1d). These differences in grain yield were discussed previously by Hirzel et al. (2017). The concentration of Cd in the grain (figure 1e) fluctuated between 28.9 and 256.1 μg kg^{-1}, and was much lower than the values reported by Arduini et al. (2014) for two Italian durum wheat cultivars, and similar to the values reported by Perrier et al. (2016) for 10 French durum wheat lines, except for the values obtained in La Serena. As observed for the whole plant, the largest value was obtained in La Serena ($p < 0.05$), followed by Los Tilos and Chillán, and the smallest value was obtained in Temuco ($p < 0.05$) (figure 1e). The grain Cd uptake fluctuated between 0.23 to 2.74 g ha^{-1} (figure 1f), being highest in La Serena ($p < 0.05$), followed by Chillán ($p < 0.05$), which was higher than Los Tilos ($p < 0.05$), with the lowest value in Temuco. These values were slightly lower to those reported by Hirzel et al. (2017).

Effect of the Crop Rotation on the Production, Concentration, and Extraction of Cadmium. The effect of crop rotation (figure 2) generated differences in all the variables evaluated, although of lower magnitude to those obtained as an effect of the study sites (figure 1). In general, the use of narrow-leafed lupines prior to durum wheat (figure 2) generated the largest values of all the evaluated variables, being higher than the effects of white lupines and durum wheat as previous crops ($p < 0.05$). For its part, the use of white lupines as a prior crop to durum wheat had higher values than use of durum wheat alone in the production of total DM, Cd uptake in whole plant, and grain yield ($p < 0.05$) (figure 2). The values obtained in the evaluated variables were within the range reported by Hirzel et al. (2017). Oliver et al. (1993) indicated that the lupine cultivar previous to spring wheat generated an increase in the Cd concentration in the wheat and proposed that the effect was due to increased Cd availability by the rhizosphere and root release of citric acid in lupines. The effects of crop rotation on the Cd concentration in durum wheat grain have also been reported by Gao and Grant (2012), comparing the

Figure 1

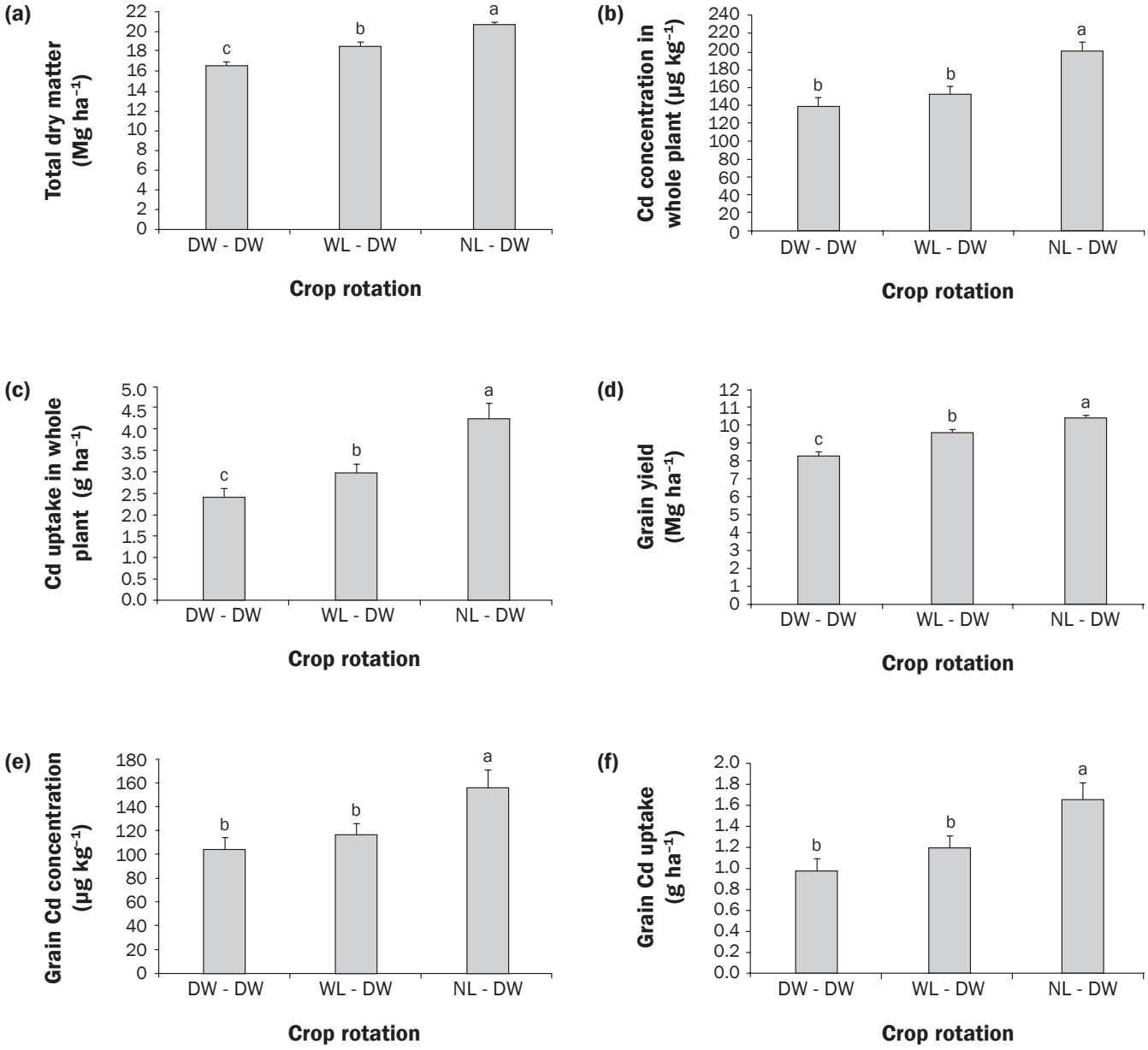
(a) Dry matter production, (b) cadmium (Cd) content and (c) Cd extraction in whole plant, (d) grain yield, (e) Cd content, and (f) Cd extraction in grain of durum wheat (DW) as average of different preceding crops (DW, white lupine [WL], and narrow-leafed lupine [NL]), cadmium chloride ($CdCl_2$) rates in the preceding crop (0, 1, and 2 mg kg^{-1}), and cultivars (Llareta-INIA, Corcolén-INIA, and Lleuque-INIA), in four locations. Different letters on bars indicate significant differences according to Tukey's test ($p < 0.05$). Lines over the bars correspond to the standard error.



rotations (1) wheat–flax (*Linum usitatissimum* L.)–durum wheat, and (2) canola (*Brassica napus* L.)–flax–durum wheat, reporting differences in only one of three seasons, and for one of the two locations evaluated, with a greater Cd concentration in durum wheat grain when the preceding crops were canola–flax. Grant et al. (2010) also noted an increase in Cd concentration in flax grain when preceded by canola, and with respect to bread wheat, which was attributed to an increase in mycorrhizal colonization or to the lower Cd concentration in the decomposing wheat residue relative to canola residue. It should be noted that in spite of the higher grain Cd concentration and Cd uptake obtained in durum wheat after narrow-leafed lupines (figure 2e and 2f), this did not affect the grain yield (figure 2d), coinciding with the report of Grant et al. (1998). Finally the average concentration of Cd in durum wheat grain after narrow-leafed lupines crop (55.3 µg kg^{-1}) was lower than that reported by Arduini et al. (2014) as the maximum concentration permitted in this cereal (figure 2d).

Effect of Each Location × Crop Rotation × Cultivar Combination on the Dry Matter

Figure 2
(a) Dry matter production, (b) cadmium (Cd) content and (c) Cd extraction in whole plant, (d) grain yield, (e) Cd content and (f) Cd extraction in grain of durum wheat (DW) as average of different locations (La Serena, Los Tilos, Chillán, and Temuco), cadmium chloride ($CdCl_2$) rates in the preceding crop (0, 1, and 2 mg kg^{-1}), and cultivars (Llareta-INIA, Corcolén-INIA, and Lleuque-INIA), after different preceding crops (DW, white lupine [WL], and narrow-leafed lupine [NL]). Different letters on bars indicate significant differences according to Tukey's test ($p < 0.05$). Lines over the bars correspond to the standard error.



Production and Grain Yield of Durum Wheat. The statistical results indicate significant effects on location × crop rotation × cultivar (table 2); tables 3 and 4 show the effects of those interactions on DM production and grain yield, respectively.

The effect of the preceding crop on the DM production and grain yield was found to be different depending on the cultivar and the location (tables 3 and 4). The use of durum wheat as preceding crop was often significantly associated ($p < 0.05$) to lower DM production (for the three cultivars in Los Tilos and Temuco and for cv. Llareta-INIA in La Serena and Chillan) and to lower grain yield (for the three cultivars in Temuco and Los Tilos, for cv. Llareta-INIA in La Serena, and for cv. Lleuque-INIA in Chillan). In turn, when the previous crop was narrow-leafed lupine, the production was often the greatest (for the three cultivars in Temuco, for cv. Corcolen-INIA and Lleuque-INIA in Chillan, and for cv. Lleuque-INIA in Los Tilos) and the grain yield was often the greatest (for the three cultivars in Los Tilos and Temuco).

Table 3
Total dry matter production (Mg ha^{-1}) in locations, crop rotation, and cultivar.

		Cultivar		
Location	**Crop rotation**	**Llareta**	**Corcolén**	**Lleuque**
La Serena	Durum wheat–durum wheat	15.4Bb	19.0Aa	23.9Aa
	White lupin–durum wheat	22.3Aa	21.0Aa	21.4Aa
	Narrow-leafed lupin–durum wheat	21.4Aa	21.7Aa	22.2Aa
Los Tilos	Durum wheat–durum wheat	16.7Ba	15.8Ba	15.9Ca
	White lupin–durum wheat	19.5Aa	17.4AB	17.8Ba
	Narrow-leafed lupin–durum wheat	19.9Aa	19.9Aa	21.8Aa
Chillán	Durum wheat–durum wheat	18.4Ba	19.9Ba	18.5Ba
	White lupin–durum wheat	20.2Aa	19.9Ba	18.6Ba
	Narrow-leafed lupin–durum wheat	20.9Aa	21.6Aa	22.1Aa
Temuco	Durum wheat–durum wheat	11.8Ca	11.4Ca	11.0Ca
	White lupin–durum wheat	15.9Ba	14.3Ba	14.6Ba
	Narrow-leafed lupin–durum wheat	18.5Aa	19.1Aa	19.0Aa

Notes: Different capital letters in a same column for the same durum wheat cultivar indicate significant differences between crop rotation for each location according to Tukey's test ($p < 0.05$). Different lowercase letters in a same row for the durum wheat cultivars indicate significant differences according to Tukey's test ($p < 0.05$).

Table 4
Grain yield (Mg ha^{-1}) in locations, crop rotation, and cultivar.

		Cultivar		
Location	**Crop rotation**	**Llareta**	**Corcolén**	**Lleuque**
La Serena	Durum wheat–durum wheat	7.8Bb	10.4Aa	12.3Aa
	White lupin–durum wheat	11.5Aa	10.9Aa	11.3Aa
	Narrow-leafed lupin–durum wheat	10.7Aa	10.8Aa	11.1Aa
Los Tilos	Durum wheat–durum wheat	8.0Ba	7.7Ca	7.6Ba
	White lupin–durum wheat	9.2Aa	8.6Ba	8.5Ba
	Narrow-leafed lupin–durum wheat	9.7Aa	9.7Aa	10.8Aa
Chillán	Durum wheat–durum wheat	9.4Aa	9.9Aa	9.5Ba
	White lupin–durum wheat	10.7Aa	10.3Aa	9.9AB
	Narrow-leafed lupin–durum wheat	10.4Aa	11.0Aa	11.3Aa
Temuco	Durum wheat–durum wheat	5.6Ba	5.5Ca	5.5Ca
	White lupin–durum wheat	8.7Aa	7.7Ba	7.8Ba
	Narrow-leafed lupin–durum wheat	9.7Aa	9.8Aa	10.1Aa

Notes: Different capital letters in a same column for the same durum wheat cultivar indicate significant differences between crop rotation for each location according to Tukey's test ($p < 0.05$). Different lowercase letters in a same row for the durum wheat cultivars indicate significant differences in according to Tukey's test ($p < 0.05$).

Effect of Each Crop Rotation × Cadmium Chloride Rate × Cultivar Combination on the Dry Matter Production and Grain Yield of Durum Wheat. The results presented in table 2 show significant effects on crop rotation × $CdCl_2$ rate × cultivar, so tables 5 and 6 show the effects of those interactions on DM production and grain yield, respectively.

The cultivar and the location also affected the effect of the $CdCl_2$ dose on the production and grain yield (tables 5 and 6). Increasing $CdCl_2$ doses were clearly associated to decreased production of DM and grain yield only for cv. Corcolen-INIA when the preceding crop was durum wheat. We found the lowest production and grain yield for intermediate $CdCl_2$ doses for cv. Lleuque-INIA when the preceding crop was durum wheat and for cv. Llareta-INIA when the previous crop was narrow-leafed lupin. Finally, we found no effects of the $CdCl_2$ dose on the production and grain yield per cultivar when the preceding crop was white lupin.

Effect of Each Location × Crop Rotation × Cadmium Chloride Rate × Cultivar Combination on Both the Cadmium Content and Extraction in the Dry Matter Production of Durum Wheat. Regarding the effect of the dose of $CdCl_2$ in each rotation and locality on the Cd concentration in the total DM for each cultivar of durum wheat (table 7), in general there was a significant effect ($p < 0.05$) directly proportional to the increasing dose of $CdCl_2$. However, this effect was not observed in certain locations, rotations, and cultivars: La Serena in the rotation white lupine–durum wheat for the cvs. Llareta-INIA and Lleuque-INIA, and Los Tilos in the rotation white lupine–durum wheat and narrow-leafed lupine-durum wheat for the cv. Corcolen-INIA. As an average of the three cultivars of durum wheat, a significant ($p < 0.05$) and directly proportional effect of the dose of $CdCl_2$ on the Cd concentration in the total DM was also observed (table 7).

The effects of the $CdCl_2$ dose on the Cd extraction were similar, showing increasing values with increasing extraction with increasing doses, except for La Serena, when the preceding crop was white lupine in cvs. Llareta-INIA and Lleuque-INIA; Los Tilos, when the predecessor crop was durum wheat and white lupine in cv. Corcolén-INIA, and when the preceding crop was narrow lupine in cvs. Llareta-INIA and Corcolén-INIA; and in Temuco, when the preceding crop was white lupine in cv. Llareta-INIA. In general, for the average values of cultivars, there was a directly proportional and significant effect ($p < 0.05$) between the dose of $CdCl_2$ and the extraction of Cd from the total DM (table 8).

Summary and Conclusions

The data obtained in this study indicate a clear location effect on the production properties of DM and grain yield, and on their respective concentrations and uptake of Cd, associated mainly to the initial concentration of Cd in the soil.

At the same time, there was a preceding crop effect, with a greater grain yield and a greater concentration and uptake of Cd in the grain after narrow-leafed lupine, as well as greater production, concentration, and extraction of Cd in the whole plant after the same crop, which indicates that a greater accumulation of Cd in the durum wheat crop did not affect its productivity. A residual effect was also noted in the $CdCl_2$ dose applied in the crop previous to the durum

Table 5
Dry matter production (Mg ha^{-1}) in combinations of crop rotation, cadmium chloride ($CdCl_2$) rates, and cultivar.

Crop rotation	$CdCl_2$ rates (mg kg^{-1})	Cultivar		
		Llareta	Corcolén	Lleuque
Durum wheat–durum wheat	0	15.4Aa	18.1Aa	17.7Aa
	1	15.6Aa	16.5Ba	16.3Ba
	2	15.7Aa	14.9Ca	18.0Aa
White Lupin–durum wheat	0	19.5Aa	17.6Aa	18.8Aa
	1	18.9Aa	17.9Aa	17.7Aa
	2	20.0Aa	18.8Aa	17.8Aa
Narrow-leafed lupin–durum wheat	0	20.4AB	20.6Aa	21.3Aa
	1	19.0Bb	19.7Ab	22.0Aa
	2	21.1Aa	21.4Aa	20.5Aa

Notes: Different capital letters in same column for the same durum wheat cultivar indicate significant differences between $CdCl_2$ rates for each crop rotation according to Tukey's test ($p < 0.05$). Different lowercase letters in a same row for the durum wheat cultivars indicate significant differences according to Tukey's test ($p < 0.05$).

Table 6
Grain yield (Mg ha^{-1}) in combinations of crop rotation, cadmium chloride ($CdCl_2$) rates, and cultivar.

Crop rotation	$CdCl_2$ rates (mg kg^{-1})	Cultivar		
		Llareta	Corcolén	Lleuque
Durum wheat–durum wheat	0	7.5Aa	9.1Aa	8.8Aa
	1	7.7Aa	8.6AB	8.1Aa
	2	7.9Aa	7.5Ba	9.3Aa
White lupin–durum wheat	0	9.8Aa	9.3Aa	9.7Aa
	1	10.0Aa	9.1Aa	9.1Aa
	2	10.2Aa	9.7Aa	9.3Aa
Narrow lupin–durum wheat	0	10.4Aa	10.4Aa	10.7Aa
	1	9.2Bb	9.9Ab	11.3Aa
	2	10.7Aa	10.6Aa	10.5Aa

Notes: Different capital letters in a same column for the same durum wheat cultivar indicate significant differences between $CdCl_2$ rates for each crop rotation according to Tukey's test ($p < 0.05$). Different lowercase letters in a same row for the durum wheat cultivars indicate significant differences according to Tukey's test ($p < 0.05$).

wheat, which was dependent on the location and preceding crop. With respect to differences between the durum wheat cultivars evaluated, in general there was no effect on the combinations of location × crop rotation in the different characteristics evaluated. Finally, it is suggested that narrow-leafed lupine should not be used as the preceding crop in soils where there was a Cd concentration over the critical level for the durum wheat crop.

As a general conclusion, a significant environmental effect was obtained, associated mainly with the initial concentration of Cd in the soil as well as the prior crop. The residual effect of the $CdCl_2$ dose applied in the preceding crop was partial and dependent on both location and preceding crop. The cultivars did not present differences for the characteristics evaluated. Consequently, the data obtained in this study suggest that it is not advisable to cultivate durum wheat in those soils with a Cd concentration higher than the critical level designated for durum wheat crop when the preceding crop is narrow-leafed lupines.

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Table 7
Total dry matter cadmium (Cd) content (µg kg^{-1}) in combinations of location, crop rotation, cadmium chloride ($CdCl_2$) rates, and cultivar.

Location	Crop rotation	$CaCl_2$ rates (mg kg^{-1})	Cultivar			
			Llareta	Corcolén	Lleuque	X_1
La Serena	Durum wheat–durum wheat	0	137.37Ca	119.77Ca	96.45Ca	117.86B
		1	266.96Ba	203.32Ba	265.89Ba	245.39AB
		2	320.29Aa	286.99Aa	420.70Aa	342.66A
	White lupin–durum wheat	0	196.79Ba	189.49Ba	196.83Ba	194.37A
		1	139.92Cb	196.85Bb	322.85Aa	219.87A
		2	359.09Aa	329.58Aa	123.12Cb	270.60A
	Narrow-leafed lupin–durum wheat	0	183.57Ba	272.38Ba	170.61Ca	208.85C
		1	499.95Aa	294.75Ba	385.38Ba	393.36B
		2	573.07Aa	640.58Aa	698.96Aa	637.54A
Los Tilos	Durum wheat–durum wheat	0	90.51Ba	112.54Ba	96.54Ca	99.86B
		1	116.25Ba	109.40Ba	152.16Ba	125.94B
		2	201.33Aa	191.83Aa	184.46Aa	192.54A
	White lupin–durum wheat	0	210.08Aa	170.39Aa	104.29Ba	161.59A
		1	205.19Aa	152.56Ba	170.28Aa	176.01A
		2	153.73Ba	185.20Aa	186.32Aa	175.08A
	Narrow-leafed lupin–durum wheat	0	138.84Aa	153.87Ba	109.45Ca	134.06A
		1	146.54Aa	120.99Ca	166.04Ba	144.52A
		2	108.98Ba	231.69Aa	210.77Aa	183.81A
Chillán	Durum wheat–durum wheat	0	77.03Ba	74.69Ca	71.71Ba	74.48B
		1	124.91Aa	102.58Ba	98.08Ba	108.53A
		2	112.31Aa	131.86Aa	130.37Aa	124.84A
	White lupin–durum wheat	0	123.95Ba	105.75Ca	106.45Ca	112.05B
		1	189.86Aa	153.64Ba	139.08Ba	160.86AB
		2	195.47Aa	191.43Aa	170.01Aa	185.64A
	Narrow-leafed lupin–durum wheat	0	107.50Ca	126.62Ba	107.13Ba	113.75B
		1	184.44Ba	146.76Ba	181.41Aa	170.87B
		2	282.10Aa	243.28Aa	204.77Aa	243.38A
Temuco	Durum wheat–durum wheat	0	29.86Ba	20.87Ca	24.58Ba	25.10B
		1	103.07Aa	63.37Ba	90.78Aa	85.74A
		2	113.70Aa	136.74Aa	108.04Aa	119.50A
	White lupin–durum wheat	0	19.76Ba	25.67Ca	20.45Ca	21.96B
		1	91.68Aa	44.57Ba	65.33Ba	67.19A
		2	78.38Aa	75.95Aa	86.92Aa	80.42A
	Narrow-leafed lupin–durum wheat	0	17.41Ba	29.65Ba	22.60Ca	23.22B
		1	87.22Aa	30.36Ba	46.39Ba	54.66B
		2	99.61Aa	100.92Aa	84.20Aa	94.91A

Notes: Different capital letters in same column for the same durum wheat cultivar indicate significant differences between $CdCl_2$ rates for each location and crop rotation according to Tukey's test ($p < 0.05$). Different lowercase letters in same row for the durum wheat cultivars indicate significant differences according to Tukey's test ($p < 0.05$). X_1 = mean Cd concentration as average of cultivars for the same Cd rate.

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Table 8
Total dry matter cadmium (Cd) extraction (g ha^{-1}) in combinations of location, crop rotation, cadmium chloride ($CdCl_2$) rates, and cultivar.

Location	Crop rotation	$CaCl_2$ rates (mg kg^{-1})	Cultivar			
			Llareta	Corcolén	Lleuque	X_1
La Serena	Durum wheat–durum wheat	0	2.347Ba	2.791Ca	2.530Ca	2.556B
		1	4.463Aa	3.914Ba	5.731Ba	4.703AB
		2	4.147Aa	5.224Aa	10.131Aa	6.500A
	White lupin–durum wheat	0	4.361Ba	3.950Ba	4.368Ba	4.227A
		1	2.967Cb	4.155Bb	6.690Aa	4.604A
		2	8.508Aa	6.653Aab	2.703Cb	5.955A
	Narrow-leafed lupin–durum wheat	0	4.028Ba	6.177Ba	3.954Ca	4.720C
		1	10.329Aa	6.232Ba	8.678Ba	8.413B
		2	12.356Aa	13.431Aa	14.848Aa	13.545A
Los Tilos	Durum wheat–durum wheat	0	1.523Ba	2.079ABa	1.604Ca	1.735B
		1	1.851Ba	1.776Ba	2.389Ba	2.006AB
		2	3.459Aa	2.540Aa	3.003Aa	3.001A
	White lupin–durum wheat	0	3.993Aa	3.029ABa	2.071Ba	3.031A
		1	3.550Aa	2.535Ba	3.077Aa	3.054A
		2	3.518Aa	3.834Aa	2.782Aa	3.378A
	Narrow-leafed lupin–durum wheat	0	2.767Aa	3.024Ba	2.480Ca	2.757A
		1	2.842Aa	2.140Ca	3.737Ba	2.906A
		2	2.184Ba	4.863Aa	4.465Aa	3.837A
Chillán	Durum wheat–durum wheat	0	1.378Ba	1.452Ca	1.450Ba	1.427B
		1	2.262Aa	2.020Ba	1.642Ba	1.974AB
		2	2.182Aa	2.720Aa	2.341Aa	2.415A
	White lupin–durum wheat	0	2.803Ba	2.035Ca	1.875Ca	2.238B
		1	3.709Aa	2.962Ba	2.580Ba	3.084AB
		2	3.777Aa	3.842Aa	3.582Aa	3.734A
	Narrow-leafed lupin–durum wheat	0	2.171Ca	2.654Ba	2.396Ba	2.408B
		1	3.580Ba	2.987Ba	4.318Aa	3.628B
		2	6.633Aa	5.545Aa	4.238Aa	5.472A
Temuco	Durum wheat–durum wheat	0	0.294Ba	0.278Ca	0.274Ca	0.282B
		1	1.260Aa	0.754Ba	0.799Ba	0.938A
		2	1.513Aa	1.141Aa	1.380Aa	1.345A
	White lupin–durum wheat	0	0.326Ca	0.354Ca	0.321Ca	0.334B
		1	1.565Aa	0.688Ba	0.901Ba	1.051A
		2	1.072Ba	1.146Aa	1.252Aa	1.157A
	Narrow-leafed lupin–durum wheat	0	0.326Ca	0.571Ba	0.414Ca	0.437B
		1	1.524Ba	0.542Ba	0.902Ba	0.989B
		2	2.098Aa	2.118Aa	1.624Aa	1.946A

Notes: Different capital letters in a same column for the same durum wheat cultivar indicate significant differences between $CdCl_2$ rates for each location and crop rotation according to Tukey's test ($p < 0.05$). Different lowercase letters in same row for the durum wheat cultivars indicate significant differences according to Tukey's test ($p < 0.05$). X_1 = Cd mean concentration as average of cultivars for the same Cd rate.

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