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Determinación del periodo crítico de interferencia de malezas en quinoa (*Chenopodium quinoa* Willd.) y evaluación de dos herbicidas, sobre el contenido de polifenoles totales

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RESUMEN

La escasa información sobre el periodo crítico de interferencia de malezas en quinoa (*Chenopodium quinoa* Willd.), la inexistencia de herbicidas postemergencia para el cultivo de quinoa, y el desconocimiento del comportamiento del contenido de polifenoles totales en el grano de quinoa y la fluorescencia de clorofila como indicadores de estrés, han llevado a plantear ésta investigación. El objetivo fue determinar el periodo crítico de interferencia de malezas y evaluar dos herbicidas, sobre indicadores de estrés en el cultivo de quinoa. Para cumplir con este objetivo se establecieron dos experimentos durante dos temporadas consecutivas en los predios de la Estación Experimental "El Nogal" de la Universidad de Concepción, campus Chillán. Para los dos experimentos se utilizó un diseño de bloques completos al azar con cuatro repeticiones. En el experimento para determinar el periodo crítico de interferencia de malezas, hubo dieciséis tratamientos que incluyeron ocho tratamientos con periodos crecientes de malezas y ocho tratamientos con periodos crecientes sin malezas, donde se evaluó población y biomasa de malezas; y en el cultivo de quinoa parámetros productivos, componentes del rendimiento y polifenoles totales. El número de granos por planta afectó al rendimiento debido a la competencia de malezas ($P < 0,05$), disminuyendo desde 4312 hasta 162 granos planta $^{-1}$ en periodos crecientes con malezas, y aumentando desde 181 hasta 5110 granos planta $^{-1}$ en periodos crecientes sin malezas. El contenido de polifenoles totales se afectó debido al estrés provocado por competencia de malezas ($P < 0,05$), aumentando desde 2,2 hasta 3,6 mg GAE g^{-1} en periodos crecientes con malezas, y disminuyendo desde 3,6 hasta 1,9 mg GAE g^{-1} en periodos crecientes sin malezas, mientras que la población se mantuvo constante ($P > 0,05$). El PCI se determinó entre los estados fenológicos de dos hojas verdaderas a floración, por lo tanto el cultivo de quinoa debe permanecer sin malezas entre estos dos estados fenológicos para descartar pérdidas superiores al 5 % en la producción. Por otra parte, para evaluar el efecto de dos herbicidas postemergentes sobre la producción y sobre los indicadores de estrés: contenido de polifenoles totales y fluorescencia de clorofila en quinoa, se implementó un experimento con siete tratamientos, que consistieron en la aplicación de dos herbicidas fomesafen y bentazon, a una dosis comercial pero en una, dos o tres aplicaciones secuenciales, más un control al cual no se aplicó herbicida. Todos los tratamientos se desmalezaron en tres oportunidades. Aquí se evaluó parámetros productivos, componentes del rendimiento e indicadores de estrés. El rendimiento fue afectado ($P < 0,05$) en algunos

tratamientos, por la aplicación de herbicidas, variando desde 1851,2 hasta 1235 kg ha^{-1} en el testigo sin herbicida y en el tratamiento con bentazon aplicado una sola vez, respectivamente. El número de granos por planta afectó al rendimiento ($P < 0,05$), disminuyendo desde 3984,6 hasta 2040,9 granos $planta^{-1}$ en el testigo sin herbicida y en el tratamiento con bentazon aplicado una sola vez, respectivamente. El contenido de polifenoles totales y el porcentaje de germinación en los granos de quinoa no fueron afectados por el estrés provocado por la aplicación de herbicidas ($P > 0,05$). El herbicida fomesafen no afectó al máximo rendimiento cuántico mientras que el herbicida bentazon sí lo afectó en cada momento de aplicación. Tres aplicaciones secuenciales de bentazon y fomesafen fueron suficientes para tener un rendimiento estadísticamente igual ($P > 0,05$) al testigo sin herbicida.

Palabras clave: *Chenopodium quinoa*, competencia de malezas, control químico de malezas, indicadores de estrés, fluorescencia de clorofila, metabolitos secundarios.

SUMMARY

The present research proposal stems from the scarce information available about the critical period of weed interference in quinoa (*Chenopodium quinoa* Willd.), the unavailability of post-emergence herbicides for this crop, and the lack of knowledge about total polyphenol content behavior in the grain and chlorophyll fluorescence as stress indicators. The objective was to determine the critical period of weed interference (CPWI) and evaluate two herbicides on quinoa crop stress indicators. Two experiments were established for two consecutive seasons in fields of the El Nogal Experimental Station of the Universidad de Concepción, Chillán campus. A completely randomized block design with four replicates was used in both experiments. The experiment to determine CPWI consisted of 16 treatments, including 8 treatments of weed-free growth periods and 8 treatments of weed growth periods, which evaluated the weed population and biomass. The production parameters, yield components, and total polyphenols of the quinoa crop were also evaluated. The number of grains per plant affected yield due to weed competition ($P < 0.05$), which decreased from 4312 to 162 grains plant^{-1} in weed growth periods and increased from 181 to 5110 grains plant^{-1} in weed-free growth periods. Total polyphenol content was affected by stress from weed competition ($P < 0.05$), which increased from 2.2 to 3.6 mg GAE g^{-1} in weed growth periods and decreased from 3.6 to 1.9 mg GAE g^{-1} in weed-free growth periods. The population remained constant ($P > 0.05$). The CPWI was determined between the 2 true leaf and flowering phenological stages; therefore, the quinoa crop must remain weed-free between these two phenological stages to rule out that production losses are more than 5%. On the other hand, an experiment with seven treatments was conducted to evaluate the effect of the two post-emergence herbicides on quinoa production and on the stress indicators (total polyphenol content and chlorophyll fluorescence). It involved the application one commercial dose of the herbicides fomesafen and bentazon at different application frequencies and rates, and a control with no herbicide application. Mechanical/manual weed removal was carried out three times with a hand hoe in all treatments, also production parameters, yield components, and stress indicators were evaluated. Yield was affected ($P < 0.05$) by herbicide application in some treatments; the control without herbicide and the treatment with only one bentazon application varied from 1851.23 to 1235.03 kg ha^{-1}, respectively. The number of grains per plant affected yield ($P < 0.05$) and decreased from 3984.60 to 2040.94 grains plant^{-1} in the control and the treatment with only one bentazon

application, respectively. Total polyphenol content and percentage of germination in quinoa grains were not affected by stress caused by herbicide application ($P > 0.05$). Fomesafen did not affect the maximum quantum yield, while bentazon had an effect during the application. Three sequential applications of bentazon and fomesafen were sufficient to achieve a yield that was statistically equal ($P > 0.05$) to the control.

Key words: Chenopodium quinoa, weed competition, chemical weed control, stress indicators, chorophyll fluorescence, secondary metabolites.

I. CAPITULO 1: Introducción

1.1 Cultivo de Quinoa

La quinoa *(Chenopodium quinoa* Willd*.),* es un pseudocereal que posee alto valor nutricional, perteneciente a la familia Amarantaceae, originaria de la región de los Andes. Se cultiva en varios países de Sudamérica donde se ha visto incrementado su superficie en los últimos veinte años, siendo su principal destino la alimentación humana (Abugoch, 2009). El cultivo de quinoa probablemente fue domesticado en la cuenca del lago Titicaca, luego sometido a un proceso extenso de selección bajo condiciones ambientales extremas; más tarde, el cultivo se desarrolló hasta los valles andinos del centro y centro norte y hacia el sur de Sudamérica (Jellen *et. al.*, 2014). Además de presentar un contenido óptimo de aminoácidos, minerales, vitaminas, polifenoles y flavonoides, el grano de quinoa contiene altas cantidades de proteína (Abugoch 2009; Repo-Carrasco-Valencia *et al*., 2010). Respecto a este tema se han realizado varios estudios, los cuales destacan el valor biológico de los granos de quinoa, especialmente por su alto contenido de proteínas, hasta un 23 % (Bhargava *et al*., 2006; Miranda *et al*., 2013). Se ha encontrado también en sus granos, cantidades importantes de componentes bioactivos como polifenoles (Álvarez-Jubete *et al*., 2010), principalmente ácidos fenólicos, como ácidos cafeico, ferúlico, p-cumárico, p-hidroxibenzoico, vinílico, gálico y cinámico (Repo-Carrasco-Valencia *et al*., 2010; Pasko *et al*., 2008); y, flavonoides, que se presentan generalmente como glucósidos de flavonoles de kaempferol y quercetina (Dini *et al*., 2004), además, estos compuestos bioactivos han reportado poseer efectos benéficos para la salud (Dini *et al*., 2010).

Por otra parte, el estrés causado por la interferencia entre malezas y cultivo, y la aplicación de herbicidas en quinoa, pueden repercutir negativamente en el contenido de metabolitos secundarios y/o fotosíntesis. Es así que los herbicidas pueden afectar la fisiología de la planta al inhibir la fotosíntesis o los procesos bioquímicos asociados (Miyazawa y Yahata, 2006), mientras que el estrés provocado en las plantas puede aumentar la concentración de polifenoles en los tejidos pero reducir la producción de biomasa (De Abreu y Mazzafera, 2005).

1.2 Control de malezas

Malezas son aquellas especies no cultivadas e indeseadas por el hombre, compitiendo con plantas cultivadas, por agua, luz, nutrientes y espacio físico. Afectan a varios factores del cultivo especialmente al rendimiento y la calidad del producto. Por lo tanto, el control de malezas se ha transformado en una práctica agronómica indispensable en cualquier cultivo (Pedreros, 2009). Los recursos limitados esenciales para el normal crecimiento de los cultivos son consumidos por las malezas, asimismo su interferencia además de causar pérdidas considerables en el rendimiento incrementa los costos de producción y reduce la calidad de los productos (Sardana *et al*., 2017). La intensidad y duración de la competencia de malezas influye directamente sobre el rendimiento final del cultivo (Swanton *et al*., 2015). Así, el manejo de malezas es un componente esencial en la producción, lo que permite incrementarla, satisfaciendo la demanda de alimentos de la población mundial actual (Oerke, 2006).

En la agricultura moderna, la utilización de herbicidas para el control de malezas se ha convertido en un instrumento necesario, debido a su eficacia sobre la mayoría de malezas, facilidad de aplicación, y mínimos requerimientos de mano de obra (Chauhan *et al*., 2012). Así los herbicidas se han agregado al control integrado, que es considerado como el enfoque más eficaz ya que los métodos individuales no aportan un control completo sobre las poblaciones de malezas (Datta y Knezevic, 2013). En estudios preliminares de aplicación de herbicidas en quinoa, Díaz *et al*. (2015), aplicaron herbicidas de preemergencia (metamitron y propizamida) y de postemergencia (propizamida), demostrando ser tolerados por el cultivo. También estos mismos autores han probado algunos herbicidas de la familia sulfonilureas aplicados en postemergencia. Por otra parte, Molina *et al*, (2014), probaron herbicidas de postemergencia (fomesafen y bentazon), mostrando también ser tolerados por la quinoa.

1.3 Periodo crítico de interferencia de malezas

Es notorio el daño causado por las malezas en todos los cultivos, reflejado al final en la producción, pero además se debe considerar que el control de malezas a tiempo es una práctica importante para el éxito de la producción de cualquier cultivo. En un estudio realizado por Uremis *et al*. (2009), para determinar el periodo crítico de interferencia de malezas en maíz,

determinaron que además de afectar al rendimiento, la duración de competencia de las malezas y su tiempo de remoción también afectan al desarrollo de la panoja, la floración, la altura de planta, el diámetro del tallo, la altura de la primera mazorca y la cantidad de granos en la mazorca. En otro estudio realizado por Tursun *et al*., (2016), en tres tipos de maíz, comprobaron que el manejo de malezas en este cultivo se debe iniciar en la etapa V1 (una hoja desplegada) y mantenerse desmalezado hasta la etapa V12 (doce hojas desplegadas).

El período crítico de interferencia de malezas puede definirse como la etapa en el ciclo de crecimiento de cualquier cultivo durante la cual, las malezas presentes en el cultivo deben ser controladas para impedir pérdidas irrecuperables en el rendimiento. Este periodo sirve para una correcta toma de decisiones sobre el momento oportuno y la necesidad del control de malezas (Knezevic *et al*., 2002), proporcionando también una visión clara sobre el impacto de las poblaciones de malezas en diferentes etapas del cultivo, ya que marca la duración de la presencia de malezas en el cultivo y el momento de emergencia de las mismas (Hall *et al*., 1992). Determinar así el período crítico de interferencia de malezas, además ayuda a minimizar las pérdidas que el cultivo pueda manifestar en el rendimiento por la infestación de una maleza (Safdar *et al*., 2016).

La determinación del período crítico de interferencia de malezas es una herramienta indispensable para plantear estrategias efectivas de manejo de malezas en sistemas de producción de cualquier cultivo (Tursun *et al*., 2016). En el cultivo de quinoa, la información sobre el periodo crítico de interferencia de malezas y su efecto sobre el rendimiento es muy escaso, por lo tanto, los hallazgos realizados en otros cultivos incentivan a investigar el efecto de malezas en quinoa y así poder contribuir a incrementar la rentabilidad y ratificar el momento en que el manejo de malezas sea una actividad más eficiente.

1.4 Fluorescencia de clorofila

Se producen grandes cambios en la fluorescencia de la clorofila cuando las plantas se encuentras sometidas a diferentes condiciones ambientales (Müller *et al*., 2001). El principio del análisis se basa en que las moléculas de clorofila absorben la energía luminosa y esta energía es capaz de someterse a cualquiera de estos tres procesos: ser utilizada para conducir la

fotosíntesis, el exceso puede disiparse en forma de calor o se puede remitir como luz fluorescente de clorofila (Maxwell y Johnson, 2000; Müller *et al*., 2001). Así, el análisis de fluorescencia de clorofila es una herramienta que permite medir el impacto del estrés ambiental sobre la capacidad fotosintética en plantas superiores (Baker y Rosenqvist, 2004; Calatayud *et al*., 2006).

Para la investigación vegetal el parámetro de medición de fluorescencia de clorofila es el máximo rendimiento cuántico del fotosistema II (Fv/Fm), tanto a la luz como en un estado de adaptación a la oscuridad (Baker y Rosenqvist, 2004; Broetto *et al*., 2007). Este parámetro mide la eficiencia cuántica máxima de la actividad fotoquímica del PSII mientras están abiertos los centros de reacción del PSII (Baker y Rosenqvst, 2004). Para medirlo, las muestras se adaptan a la obscuridad durante 30 minutos antes de realizar las mediciones a temperatura ambiente, utilizando un clip de hojas y un fluorometro (Mehta *et al*., 2010)

1.5 Polifenoles

Los polifenoles son metabolitos secundarios que se encuentra presentes en alimentos de origen vegetal y que frecuentemente son consumidos en la dieta. Los principales tipos de polifenoles son tres: flavonoides, ácidos fenólicos y taninos, y su función principal es actuar como potentes antioxidantes, aportando beneficios para la salud (Han *et al*., 2007; Scalbert *et al*., 2005). En numerosos estudios epidemiológicos, se ha encontrado que los compuestos fenólicos presentes en los alimentos, tienen actividad benéfica sobre la salud, incluyendo: anticancerígeno, potencial antioxidante, actividad antiviral, actividad antimicrobiana y actividad antimutagénica (Ssonko y Wenshui, 2005; Benavente-García y Castillo, 2008).

La acumulación de metabolitos secundarios en plantas es influenciado por el estrés provocado por factores bióticos y abióticos como es el caso de los herbicidas (Ksouri *et al*., 2007). Existe una relación directa entre el contenido de polifenoles con los mecanismos de resistencia y defensa de los vegetales, existiendo factores que motivan la formación y acumulación de polifenoles en las plantas. Estos factores pueden ser: condiciones de estrés como bajas temperaturas, radiación ultravioleta, heridas en el tejido, estrés osmótico, provocadores abióticos, como fungicidas, sales de metales pesados, enzimas, antibióticos, iones

metálicos, fosfito, etileno, glutatión y provocadores bióticos, como infección bacteriana o viral (Maestro-Durán *et al.*, 1993). No obstante, el estrés provocado en las plantas tiene dos efectos contrarios sobre la producción de polifenoles: aumenta la concentración de polifenoles en los tejidos pero reducen la producción de biomasa (De Abreu y Mazzafera, 2005).

Valores promedios obtenidos reportados por algunos estudios realizados, indican que el contenido de fenoles totales en granos de quinoa es de 1,1 mg de equivalente de ácido gálico g^{-1}. Estos valores son superiores a los obtenidos en semillas de cereales tradicionales como cebada, trigo, arroz y mijo (valores entre 0,16 a 0,36 de equivalente de ácido gálico g^{-1}). Estos datos ayudan a corroborar el potencial de la quinoa como sustituto de cereales (Asao y Watanabe, 2010; Djordjevic *et al*., 2010).

1.6 HIPOTESIS

- La interacción de las malezas con el cultivo de quinoa en sus diferentes etapas de desarrollo, disminuyen el rendimiento y aumentan el contenido de polifenoles totales.
- La aplicación de los herbicidas fomesafen y bentazon para el control de malezas, alteran el contenido de polifenoles totales y la fluorescencia de clorofila; y disminuyen el rendimiento en el cultivo de quinoa.

1.7 OBJETIVOS

1.7.1 General

Determinar el periodo crítico de interferencia de malezas y evaluar el efecto de las malezas y de dos herbicidas sobre la producción y sobre indicadores de estrés en el cultivo de quinoa (*Chenopodium quinoa* Willd.).

1.7.2 Específicos

- Determinar el periodo crítico de interferencia de malezas y su efecto sobre el rendimiento y el contenido de polifenoles totales.
- Evaluar el efecto de una, dos y tres aplicaciones secuenciales de dos herbicidas de post emergencia: fomesafen y bentazon, sobre la fluorescencia de clorofila.
- Analizar el efecto de la aplicación de herbicidas sobre la germinación de la semilla cosechada.

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II. CAPITULO 2: CRITICAL PERIOD OF WEED INTERFERENCE ON TOTAL POLYPHENOL CONTENT IN QUINOA

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2.1 ABSTRACT

There is limited information about the critical period of weed interference (CPWI) in quinoa (*Chenopodium quinoa* Willd.) and the effect produced by the weed-crop interaction in secondary metabolite accumulation. The objective of the present study was to determine the CPWI and its effect on total polyphenol content in quinoa. The experiments were conducted during two consecutive seasons using a randomized complete block design with 16 treatments consisting of 8 weed growth periods and 8 weed-free growth periods in which weed population and biomass were evaluated; productive parameters, yield components, and total polyphenols were determined in the quinoa crop. Grain number per plant affected yield because of weed interference ($P < 0.05$), which decreased from 4312 to 162 grains plant^{-1} in weed growth periods and increased from 181 to 5110 grains plant^{-1} in weed-free growth periods. Total polyphenol content was affected by stress from weed interference ($P < 0.05$), which increased from 2.2 mg gallic acid equivalents (GAE) g^{-1} to 3.6 mg GAE g^{-1} in weed growth periods and decreased from 3.6 mg GAE g^{-1} to 1.9 mg GAE g^{-1} in weed-free growth periods, while the population remained constant ($P > 0.05$). The CPWI was determined between the phenological stages of two true leaves to flowering; therefore, the quinoa crop must remain weed-free between these two phenological stages to rule out production losses greater than 5%.

Key words: *Chenopodium quinoa*, stress from weed interference, total polyphenols, critical period of weed interference, weed control in quinoa.

2.2 INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal belonging to the *Amaranthaceae* family with high nutritional value originating from the Andes region. It is grown in several countries of South America where the cultivated area has increased in the last 20 years and is destined to food for humans (Abugoch, 2009). The quinoa grain also contains high amounts of protein and exhibits an optimum content of amino acids, minerals, vitamins, polyphenols, and flavonoids (Abugoch, 2009; Repo-Carrasco-Valencia et al., 2010). Important amounts of bioactive components such as polyphenols (Alvarez-Jubete et al., 2010), mainly phenolic acids such as caffeic, ferulic, p-coumaric, p-hydroxybenzoic, vinyl, gallic, cinnamic acids (Repo-Carrasco-Valencia et al., 2010; Pasko et al., 2008), and flavonoids have also been found in its grains. All these bioactive compounds have been reported to provide benefits for human health (Dini et al., 2010).

Although quinoa is a traditionally low-yield crop in the regions of origin, increased demand for its abovementioned benefits has led to increased production. Among the pending problems to be studied are those related to weeds because production decreases as density and duration of weed interference increases. Another factor that interferes with yield is the relationship between weed emergence and the pressure exerted on the crop (Fahad et al., 2014). Yield losses caused by ineffective weed control are usually higher than losses caused by pests and diseases (Oerke, 2006). In this way weeds are a limiting factor in the quinoa crop; they directly affect yield because they compete for factors such as water, nutrients, and light. The intensity and duration of weed interference are factors that determine the extent of yield losses (Swanton et al., 2015).

The critical period of weed interference (CPWI) can be defined as the stage in the growth cycle of any crop during which weeds must be controlled to prevent unrecoverable yield losses. Knowledge of crop CPWI also contributes to minimize yield losses that this crop can exhibit due to weed infestation (Safdar et al., 2016); determining CPWI is an indispensable tool to

propose effective weed management strategies in any crop production system (Tursun et al., 2016). Information about CPWI in quinoa and its effect on yield is very limited; therefore, findings in other crops encourage research on the effect of weeds on quinoa and define when weed management is more efficient. However, weed interference not only affects crop yield but also alters the amount of plant secondary metabolites, which accomplish important functions within the plants (Olivoto et al., 2016). Provoked stress in the plants also increases polyphenol concentration in the tissues but reduces biomass production (De Abreu and Mazzafera, 2005).

Manual weed control methods in quinoa are an important factor that directly interferes with yield; the effect of weed-crop interaction on total polyphenol content in the grain such as stress indicators are still unknown. Therefore, the objective of the present study was to determine the CPWI in quinoa and its effect on total polyphenol content.

2.3 MATERIALS AND METHODS

2.3.1 Plant material and experimental design

The experiments were conducted during two consecutive seasons in the El Nogal Experimental Station (36º34' S and 74º06' W) in Ñuble Region, Chile. A randomized complete block experimental design was used with four replicates. The experimental unit was 3 × 2 m and consisted of four rows of quinoa 'Regalona' plants with 0.5 m spacing. A modified version of the methodology proposed by Karkanis et al. (2012) was used; each block contained 16 treatments at 0, 15, 30, 45, 60, 75, 90, and 105 d after emergence consisting of 8 weed growth periods and 8 weed-free growth periods (Table 1). This allowed determining the beginning and the end of the period needed to eliminate weeds.

Table 1. Description of treatments in weed and weed-free growth periods in the 2015-2016 and 2016-2017 seasons.

Treatment	Weed growth periods	Treatment	Weed-free growth periods
1	0 d with weeds	9	0 d weed-free
2	15 d with weeds	10	15 d weed-free
3	30 d with weeds	11	30 d weed-free
4	45 d with weeds	12	45 d weed-free
5	60 d with weeds	13	60 d weed-free
6	75 d with weeds	14	75 d weed-free
7	90 d with weeds	15	90 d weed-free
8	105 d with weeds	16	105 d weed-free

Fuente: Elaboración propia.

2.3.2 Agronomic management

The soil was prepared using one pass with a moldboard plow, two passes with a disc harrow plow, and two passes with a vibro-cultivator in each season. Continuous manual sowing was performed in mid-October at a 12 kg ha^{-1} seeding rate and 0.5 m row spacing. The soil at the experimental site exhibited 6.8 neutral pH, 8.6 mg kg^{-1} available N, 6 mg kg^{-1} P, and 626.0 mg kg^{-1} K according to soil analysis; therefore, fertilization was uniformly applied as 100 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1} before sowing together with 160 kg N ha^{-1} of which 50% was applied at the 4 true leaf stage and 50% at the beginning of branching. Furrow irrigation was used to facilitate homogeneous water flow, and mechanical/manual weed removal was carried out with a hand hoe in accordance with the treatments at biweekly intervals.

2.3.3 Determination of critical period of weed interference (CPWI)

Quinoa population and growth were evaluated at 0, 15, 30, 45, 60, 75, 90, and 105 d after emergence. Measurements were taken in two linear meters in the two central rows of each plot; plant height (PH) was measured from the root collar to the apex of the panicle in the first seven plants from the selected section. Each treatment was associated with the crop phenological

stages proposed by Mujica and Canahua (1989). Likewise, yield per unit area (kg ha^{-1}) was determined from the two linear meters of the two central rows of each plot. To estimate grain number per plant and biomass per plant (g), the mean of the seven first plants in the two linear meters of the two central rows of each plot was considered. The 1000 grain weight (g) variable was determined by recording the weight of 1000 quinoa seeds without considering the perigonium, and this was accomplished by separating the grains from the chaff.

2.3.4 Determination of total polyphenols in quinoa seeds

Quinoa samples (20 g) from each of the 16 treatments that included both weed and weed-free growth periods were ground in a grinder (1093, Cyclotec, Barcelona, Spain). Extracts were prepared according to a modified version of the method described by Fischer et al. (2013). The supernatant was filtered, placed in amber glass jars, and stored at 4 °C until analyzed. A modified version of the method described by Miranda et al. (2010) was used to determine total polyphenols. Results were expressed as mg gallic acid equivalents (GAE) g^{-1} and all measurements were performed in triplicate.

2.3.5 Weed evaluations

Quinoa weed population and DM were evaluated by sampling according to a modified version of the method proposed by Stagnari and Pisante (2011); a quadrant with 0.5 × 0.5 m was traced 1 m inside between two central rows of each experimental unit and weed population and DM were extracted with weed growth periods for weeding at 0, 15, 30, 45, 60, 75, 90, and 105 d after emergence. Weeds were cut at soil level, placed in paper bags, and oven-dried (FP 115, Binder, Germany) at 65 °C for 72 h.

2.3.6 Statistical analysis

Data was subjected to ANOVA at $P < 0.05$ after verifying the assumptions of normal distribution and homogeneity of variances. These assumptions were not fulfilled in yield data

(data not shown and necessary to calculate the CPWI) and transformations were performed with the ln (x+1) function. Means were compared by the LSD test at the 95% significance level.

The CPWI was determined by non-linear regressions adjusted to the Gompertz and simple logistic models. The Gompertz equation was used to model the effect of weed-free growth periods on relative grain yield, while the logistic equation was used to model the effect of weed growth periods on relative grain yield (Singh et al., 2014).

The Gompertz equation $Y = a*exp(-b*exp(-X_0*X))$ expresses Y as yield, a the asymptote, b and X_0 are constants, and X days after emergence. On the other hand, the logistic equation $Y = a/(1 + b*exp(-X_0*X))$ expresses Y as yield, a the asymptote, b and X_0 are constants, and X days after emergence.

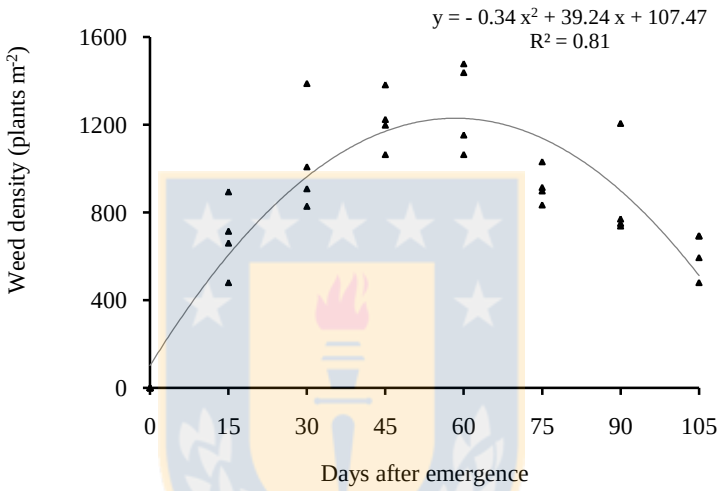
The statistical analysis and the curves to estimate the critical period of weed interference were performed with the INFOSTAT statistical program (Di Rienzo et al., 2017). To compare the results of the variables between the first and second season, a combined experiment analysis was performed in which the interaction between year and treatment for all the variables was not significant ($P > 0.05$); therefore, data were grouped and analyzed together.

2.4 RESULTS AND DISCUSSION

2.4.1 Weed population and biomass

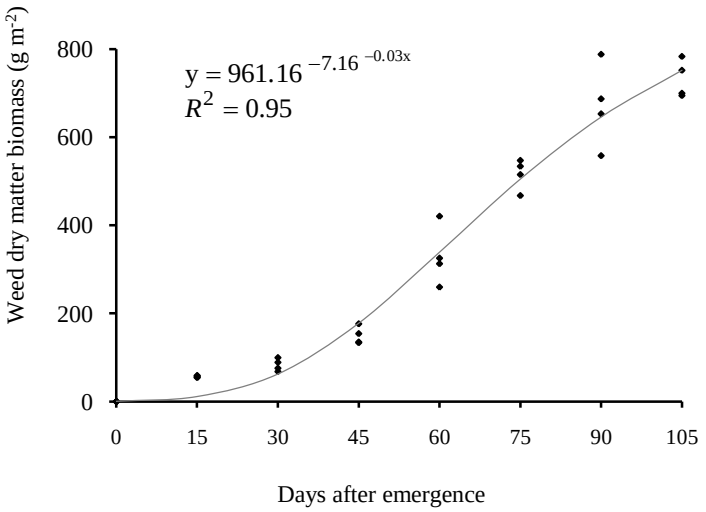
Weed density was adjusted to a quadratic polynomial model related to days after emergence (DAE) with a significant ($P < 0.05$) coefficient of determination and a highly significant ($R^2 = 0.81$) relationship. The lowest weed density (0 plants m^{-2}) was observed in the treatments that were weed-free during the whole experiment (105 d weed-free and 0 d with weeds). After 60 DAE, weed density reached its maximum value and decreased until 105 DAE (Figure 1), while weed DM at this point continued to increase (Figure 2). The decrease in density could be caused by intra- and interspecific competition in which the environmental load capacity was unable to sustain the whole initial population; the remaining plants continued to accumulate biomass, which allowed them to continue their vegetative cycle until harvest. In a similar study

with peas, Singh et al. (2016) reported increased weed density until 60 DAE and then observed a downward trend and concluded that most weeds emerge up to 60 DAE. Weed density was also affected by environmental conditions in both seasons (Table 2); weeds faced high precipitation in the early stages, which could cause their higher germination and emergence and result in higher weed density (Tursun et al., 2012). There was also an increase in temperature during the two seasons (Table 2), and this increase can accelerate weed seed germination and increase competition between different species (Giménez et al., 2013).



Fuente: Elaboración propia

Figure 1. Weed density response to increase in days of duration of weed interference after emergence in the quinoa 'Regalona' crop adjusted to a quadratic polynomial model $Y = a_0 + (a_1 * X) + (a_2 * X^2)$.



Fuente: Elaboración propia

Figure 2. Dry matter biomass response to increase in days of duration of weed interference after emergence. The Gompertz equation adjusted to dry matter biomass was used.

Table 2. Meteorological data for mean temperature (T) and precipitation at the experimental site for two seasons.

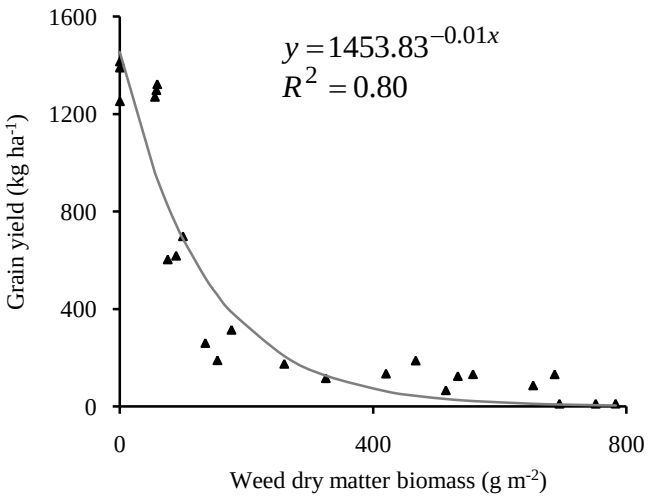
Month	Mean temperature		Precipitation	
	2015-2016	2016-2017	2015-2016	2016-2017
	°C		mm mo^{-1}	
October	12.06	13.27	98.60	65.00
November	15.13	16.65	7.40	11.60
December	18.24	17.86	0.00	31.50
January	20.50	21.50	7.30	3.50
February	19.95	20.40	0.00	13.00

Fuente: Elaboración propia

The weed DM biomass growth curve was adjusted to the Gompertz model, which showed a highly significant (R^2 = 0.95) relationship in which the coefficients to determine model parameters a, b, and X_0 were adjusted to the model ($P < 0.05$) (Figure 2). Maximum weed biomass was recorded in the treatments that had weeds during the whole experiment, that is, 105 d with weeds and 0 d weed-free; therefore, weed DM biomass was directly influenced

by the increase in the duration of periods of weed interference and increased until harvest. This could be caused by the shade of the highest weeds and of the crop on the germination of new weed populations. These results concur with data reported by Tursun et al. (2016), who detected increased weed biomass until harvest in three types of corn. On the other hand, Stagnari and Pisante (2011) determined that weed biomass increased as the duration of weed infestation increased in a bean crop. Ahymadvand et al. (2009) also found increased weed DM biomass until harvest in a potato crop and reported that weed biomass was higher in low sowing densities; they concluded that open spaces with low sowing densities could increase weed biomass and that early canopy closure at high sowing densities impede weed growth.

The relationship between quinoa grain yield and weed biomass was adjusted to an exponential model with a significant ($P < 0.05$) coefficient of determination and highly significant ($R^2 = 0.80$) relationship. Figure 3 illustrates that grain yield decreased as weed DM biomass increased; therefore, maximum grain yield was obtained in the treatments that remained weed-free during the whole experiment (0 d with weeds and 105 d weed-free). On the other hand, the lowest grain yields were obtained in the treatments with high weed biomass, that is, the treatments that had weeds during the whole experiment (105 d with weeds and 0 d weed-free). This implies that higher weed biomass maintained until later stages can damage crop production because of the weed-crop competition for water, light, and nutrients, which directly affected quinoa yield. Similar studies in other crops have demonstrated yield reduction due to weed interference. Qasem (2009) determined that mean cauliflower yield decreased and was directly affected by weed interference during the crop season. Meanwhile, Singh et al. (2014) reported a negative linear relationship between grain yield and weed DM biomass in rice where treatments with higher weed DM biomass caused a loss of up to 100% of crop yield compared with treatments with lower weed biomass; therefore, weeds would constitute an important restrictive biotic factor that influences yield.



Fuente: Elaboración propia

Figure 3. Grain yield response to increase in weed dry matter biomass in the quinoa 'Regalona' crop adjusted to an exponential model Y = a∗exp(b∗X).

2.4.2 Critical period of weed interference (CPWI)

The quinoa population remained constant (P > 0.05) in all periods, whereas PH varied (P < 0.05) in the weed (Table 3) and weed-free (Table 4) growth periods. Thus, quinoa PH increased as the duration of weed interference decreased, that is, as the number of days increased in which treatments remained weed-free. In the weed growth periods (Table 3), PH did not show much difference between treatments (P > 0.05) up to 30 DAE; then at 45 DAE, treatments with only 0 and 15 d with weeds exhibited higher values (68.3 and 64.1 cm, respectively) than the mean of the other treatments. At 60 DAE, the same treatments with weeds at 0 and 15 d maintained the trend and showed higher PH (80.5 and 75.1 cm, respectively) than the other treatments. In the same period, the weed biomass growth curve, adjusted to the Gompertz equation (Figure 2), continued its upward trend, while weed density adjusted to a quadratic model (Figure 1) reached its maximum value. Finally, the same trend was observed at 105 DAE when the two treatments (0 and 15 d with weeds) were significantly different with values of 83.9 and 79.4 cm, respectively, and these PH values were higher than for the other

treatments. The treatments with lower PH at 105 DAE were those for 105 and 90 d with weeds; there were no differences between the two and PH was 41.4 and 41.0 cm, respectively.

Table 3. Plant height response to weed growth periods expressed as days after emergence of the quinoa 'Regalona' crop.

Treatments	0 DAE	15 DAE	30 DAE	45 DAE	60 DAE	75 DAE	90 DAE	105 DAE
0 d with weeds	1.8a	6.1a	15.2a	68.3a	80.5a	81.1a	83.3a	83.9a
15 d with weeds	1.8a	5.3b	15.0a	64.1a	75.1b	76.3a	77.1a	79.4b
30 d with weeds	1.9a	5.2b	12.9b	42.5b	46.1c	47.7b	49.3b	49.5c
45 d with weeds	1.9a	5.2b	12.7b	40.5bc	43.3cd	45.0bc	45.2bc	45.7cd
60 d with weeds	1.8a	5.3b	12.6b	38.8bc	42.7cde	44.1bc	44.2bcd	45.0de
75 d with weeds	1.9a	5.3b	12.6b	38.0c	39.3de	41.0c	43.2cd	44.7de
90 d with weeds	1.8a	5.3b	12.6b	36.8c	38.8e	40.3c	40.7d	41.4e
105 d with weeds	1.8a	5.3b	12.5b	36.5c	38.5e	40.2c	40.5d	41.0e

Fuente: Elaboración propia

The lower-case letters indicate significant differences between treatments (LSD test $P \leq 0.05$).

In the weed-free growth periods (Table 4), trends were similar to the weed growth periods, and PH up to 30 DAE did not exhibit much difference between treatments. At 45 DAE, the 105 and 90 d weed-free treatments had higher values than the mean of the other treatments, 72.3 cm and 71.0 cm, respectively, and there were no differences between the two. At 60 DAE, the same 105 and 90 d weed-free treatments maintained the trend and exhibited the highest PH with 92.1 and 91.3 cm, respectively; there were no differences between the two but were higher than the other treatments. A similar trend was maintained at 105 DAE in which the two treatments (105 and 90 d weed-free) did not differ one from the other with values of 93.0 and 92.2 cm, respectively, and they were significantly higher than the other treatments. The treatment that had significantly lower PH was the 0 d weed-free with a value of 55.0 cm at 105 DAE.

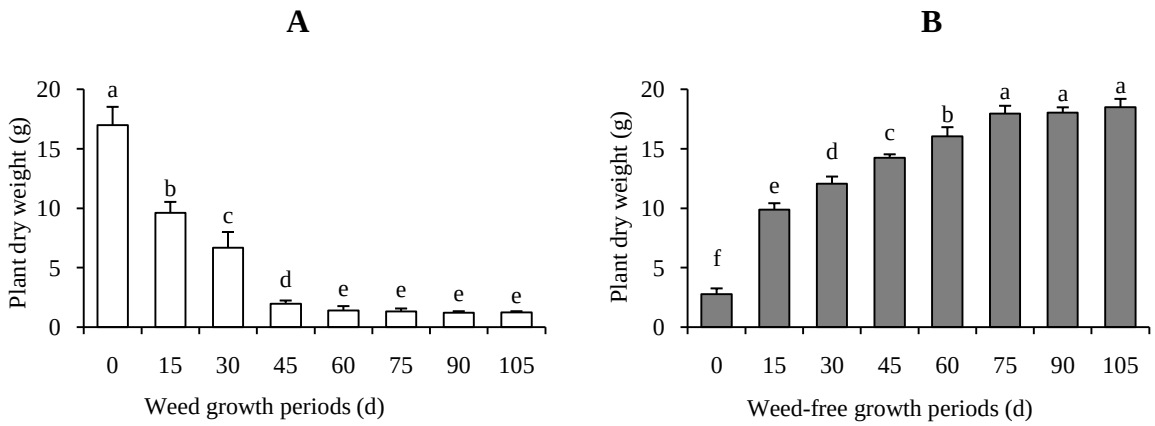
Table 4. . Plant height response to weed-free growth periods expressed as days after emergence of the quinoa 'Regalona' crop.

Treatments	0 DAE	15 DAE	30 DAE	45 DAE	60 DAE	75 DAE	90 DAE	105 DAE
0 d weed-free	1.8a	5.0b	12.8b	49.2c	54.0f	54.6f	54.9f	55.0f
15 d weed-free	1.8a	5.9a	13.0b	64.9b	69.3e	70.6e	70.6e	70.9e
30 d weed-free	1.9a	5.9a	14.6a	66.9ab	76.7d	78.2d	79.8d	80.0d
45 d weed-free	1.8a	5.8a	14.7a	68.4ab	78.9cd	80.2d	80.5d	80.7d
60 d weed-free	1.8a	5.9a	14.9a	69.3ab	83.4bc	84.0c	84.2c	84.4c
75 d weed-free	1.8a	5.8a	15.0a	70.3ab	86.4b	87.7b	88.1b	88.2b
90 d weed-free	1.8a	5.9a	15.1a	71.0a	91.3a	91.9a	92.1a	92.2a
105 d weed-free	1.9a	5.8a	15.2a	72.3a	92.1a	92.8a	92.8a	93.0a

Fuente: Elaboración propia

The lower-case letters indicate significant differences between treatments (LSD test $P \leq 0.05$).

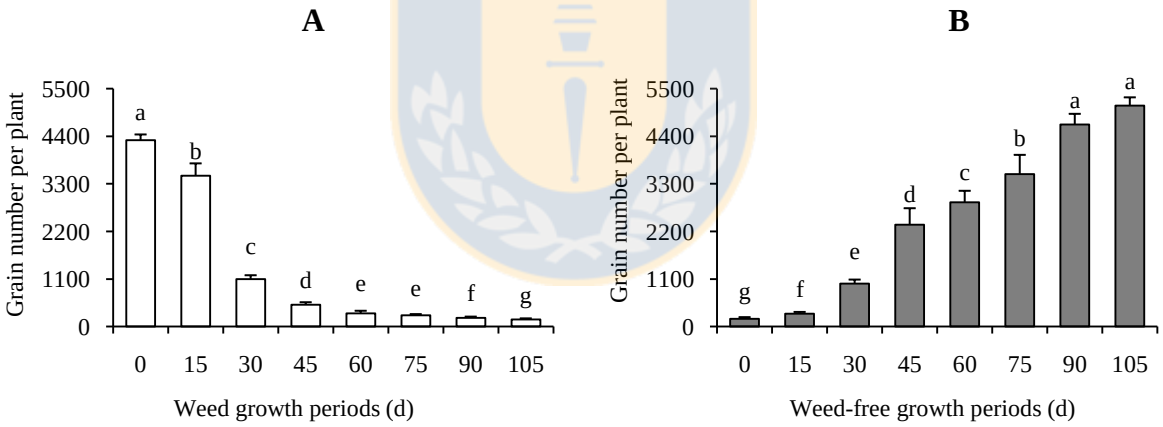
Plant biomass and the grain number per plant variable were significantly ($P < 0.05$) influenced by the treatments with weed and weed-free growth periods. For plant biomass, the treatment with 0 d with weeds (Figure 4A) was significantly higher than the rest of the treatments and had a value of 16.99 g in the weed growth periods, while in the weed-free growth periods (Figure 4B), treatments with 105, 90, and 75 d weed-free were significantly equal and had values of 18.5, 18.0, and 18.0 g, respectively. The highest value for the grain number per plant variable occurred in the treatment at 0 d with weeds (Figure 5A); the value of 4312 grains plant^{-1} was significantly different from the other treatments in the weed growth periods. Meanwhile, in the weed-free growth periods (Figure 5B), the highest values were found in treatments with 105 and 90 d weed-free; values were significantly equal at 5110 and 4671 grains plant^{-1}, respectively. On the other hand, the 1000 grain weight variable was constant ($P > 0.05$) in the weed growth periods (Figure 6A) and in the weed-free growth periods (Figure 6B); values varied between 3.1 and 2.9 g in weed growth periods and between 3.0 and 2.9 g in weed-free growth periods.



Fuente: Elaboración propia

The error bars represent the standard deviation of the mean in each treatment and lower-case letters indicate significant differences between treatments (LSD test $P \leq 0.05$).

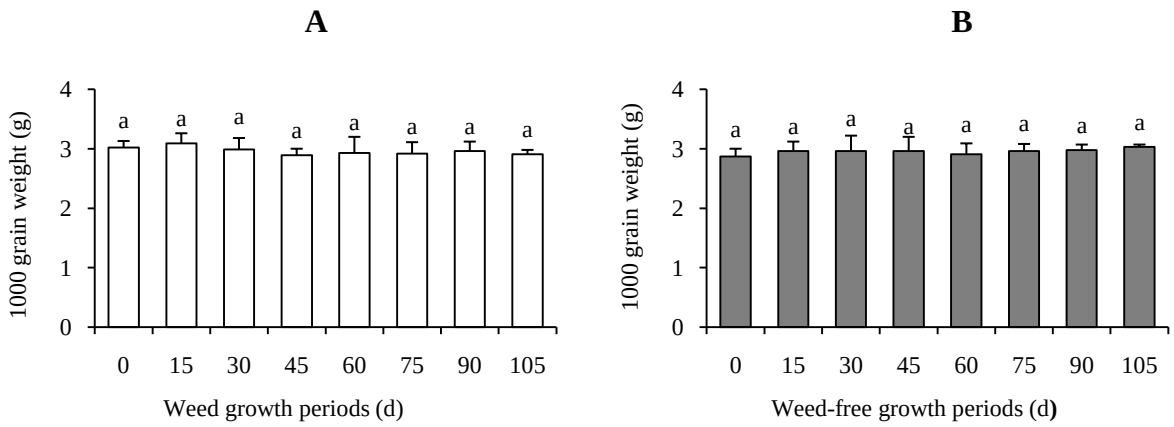
Figure 4. Plant biomass response to weed interference in the quinoa 'Regalona' crop in the growth periods (A) with weeds and (B) weed-free.



Fuente: Elaboración propia

The error bars represent the standard deviation of the mean in each treatment and lower-case letters indicate significant differences between treatments (LSD test $P \leq 0.05$).

Figure 5. Grain number per plant response to weed interference in the quinoa 'Regalona' crop in the growth periods (A) with weeds and (B) weed-free.



Fuente: Elaboración propia

The error bars represent the standard deviation of the mean in each treatment and lower-case letters indicate significant differences between treatments (LSD test $P \leq 0.05$).

Figure 6. 1000 grain weight response to weed interference in the quinoa 'Regalona' crop in the growth periods (A) with weeds and (B) weed-free.

The PH, plant biomass, and grain number per plant showed their highest values in those treatments that remained weed-free during the experiment, while the lowest values were recorded in the treatments that were weed-infested. This is due to the effect of weeds on the development of crops, competing for water, light, nutrients, CO_2 and space, behaving as hosts of pests and diseases (Page et al., 2009), also by the shade effect caused by the highest weeds that reduce available light for photosynthesis and thus biomass production, resulting in decreased yield components (Vasilakoglou and Dhima, 2012).

Akhter et al. (2009) reported a decrease in the yield components of a pea crop under weed conditions. Similar studies in other crops have demonstrated decreased yield components due to weed-crop interference. Safdar et al. (2016) reported decreased yield components in a corn crop and Singh et al. (2016) in a pea crop. Weed interference did not affect grain filling, so that the 1000 grain weight variable was the most stable. It is possible that the quinoa plants that were exposed to a longer period of weed interference responded by only forming the number of grains they were able to fill.

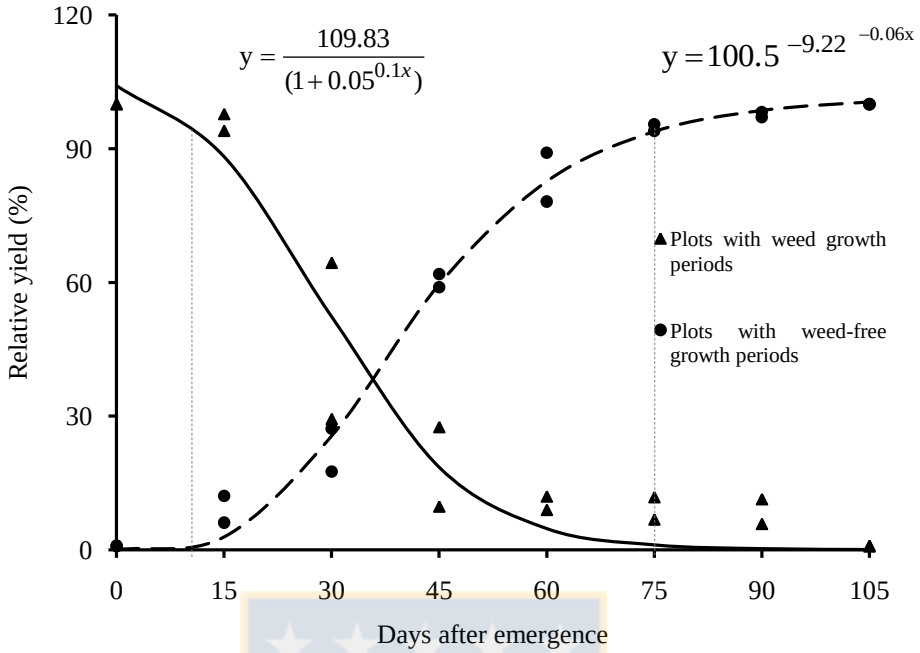
Finally, by the logistic and Gompertz regression equations adjusted for grain relative yield (%), CPWI was determined for quinoa 'Regalona' at the experimental site between 10

and 75 days after the emergency corresponding to the phenological stages of 2 true leaves and flowering respectively (Table 5) to not have production losses greater than 5% (Figure 7). The estimated parameters used in the equations were adjusted to the model ($P < 0.05$); parameters for the Gompertz equation were a = 100.5, b = 9.2, and X_0 = 0.06. Meanwhile, parameters for the logistic equation were a = 109.8, b = 0.05, and X_0 = -0.01. In similar studies with other crops, Ahymadvand et al. (2009) reported that weight of tubers per plant and total tuber production in a potato crop decreased when the duration of weed interference increased, calculated on the basis of 10% yield losses by the logistic and Gompertz equations. Tursun et al. (2016) reported that the production of three types of corn was influenced by the duration of weed-free and weed-infested periods and CPWI was determined from the development V1 (1 unfolded leaf) stage and maintained until the V12 (12 unfolded leaves) stage.

Table 5. . Phenological stages of quinoa 'Regalona' crop at each treatment application in two seasons.

Days after emergence	Phenological stages
0	Emergence
15	2 true leaves
30	6 true leaves
45	Start of panicle formation
60	Start of flowering
75	Flowering
90	Milky grain
105	Physiological maturity

Fuente: Elaboración propia



Fuente: Elaboración propia

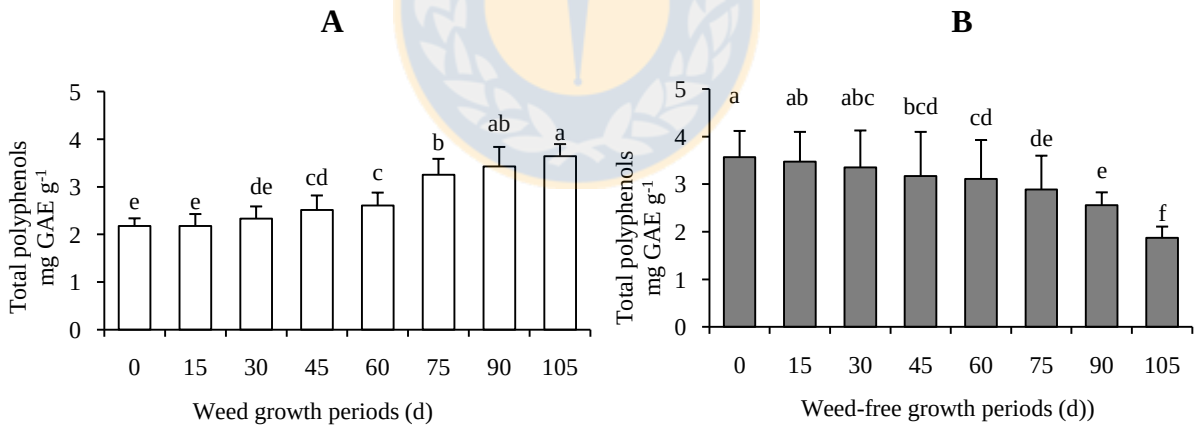
The Gompertz equation was used in weed-free plots and the logistic equation adjusted to grain yield (% yield) was used in weed-infested plots. The weed-free critical periods to achieve 95% of the maximum yield are shown between the vertical dashed lines.

Figure 7. Effect of weed control periods on quinoa 'Regalona' yield.

2.4.3 Total polyphenols

Total polyphenols varied and increased as the duration of weed interference increased and decreased when the duration of weed interference decreased ($P < 0.05$). In the weed growth periods (Figure 8A), polyphenols were increased between 45 and 105 d with weeds, the treatments at 105 and 90 d with weeds were significantly equal and exhibited the same high total polyphenol values, 3.6 and 3.4 mg GAE g^{-1}, respectively, while the lowest values were observed in the totally or partially weeded treatments. The at 0, 15, and 30 d with weeds were significantly equal and exhibited the lowest total polyphenol values, 2.2, 2.2, and 2.3 mg GAE g^{-1}, respectively. In the weed-free growth periods (Figure 8B), polyphenols were decreased between 0 and 90 d weed-free, the weed-free treatments at 0, 15, and 30 d were statistically equal and exhibited the highest total polyphenol values, 3.6, 3.5, and 3.4 mg GAE g^{-1}, respectively, while the lowest value was 1.9 mg GAE g^{-1} in the totally weeded treatment (105 d

weed-free). Most secondary metabolites found in plants accomplish important functions such as protecting against parasites, conferring attractive characteristics for pollinators and seed dispersers, as well as an important role in plant-plant competition in plant-microorganism symbiosis (Olivoto et al., 2016). In the present study, stress caused by weed interference increased total polyphenol concentration as weedy periods increased. Polyphenols are secondary metabolites that are important in plants and accomplish functions in response to stress conditions (Miranda et al., 2013). Reported mean values in some studies indicate that total phenol content in quinoa grains is 1.1 mg GAE g^{-1}, and these values are higher than those obtained in traditional cereal seeds such as barley, wheat, rice, and millet that varied between 0.16 and 0.36 mg GAE g^{-1} (Asao and Watanabe, 2010; Djordjevic et al., 2010). Meanwhile, Fischer et al. (2013) determined the variation of the antioxidant capacity in quinoa subjected to different water stress levels and found an increase in total polyphenol content between 3.3 and 4.5 mg GAE g^{-1} as water restriction increased in the quinoa 'Regalona' crop under field conditions. Miranda et al. (2010) also determined a notable reduction in total polyphenol content when quinoa seed was subjected to high temperatures with hot air.



Fuente: Elaboración propia

The error bars represent the standard deviation of the mean in each treatment and lower-case letters indicate significant differences between treatments (LSD test $P \leq 0.05$).
GAE: Gallic acid equivalent.

Figure 8. Total polyphenol response to weed interference in the quinoa 'Regalona' crop in the growth periods (A) with weeds and (B) weed-free.

2.5 CONCLUSIONS

The critical period of weed interference was determined between 10 and 75 days after the emergency, which corresponded to the phenological stages of two true leaves and flowering, respectively. The determination of this period will allow crop management decision-making to minimize losses produced by weed interference. Stress caused by weed interference altered polyphenol contents and affected quinoa production. Among the yield components, the grain number per plant showed the greatest differences and directly affected yield. Total polyphenols varied and increased with a longer weed interference period, while they decreased with a shorter weed interference period. Thus, in the weed growth periods, polyphenols increased between 45 and 105 d with weeds, whereas polyphenols decreased between 0 and 90 d weed-free in the weed-free growth periods. Higher amount total polyphenols in the quinoa crop and lower crop yield were due to stress caused by weed interference.

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III. CAPITULO 3: EFFECT OF POST-EMERGENCE HERBICIDES ON STRESS INDICATORS IN QUINOA

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3.1 ABSTRACT

The impact of herbicide application on the accumulation of secondary metabolites and photosynthesis as stress indicators in the quinoa (*Chenopodium quinoa* Willd.) crop is unknown. The objective of the present study was to evaluate the effect of two post-emergence herbicides on production, total polyphenol content, and chlorophyll fluorescence in quinoa. The experiments were conducted for two consecutive seasons using a completely randomized block design with seven treatments, including one commercial dose of the herbicides fomesafen and bentazon at three application frequencies, as well as a control without herbicide application. Production parameters, yield components, and stress indicators were evaluated. Yield was affected ($P < 0.05$) by herbicide application in some treatments; the control and the treatment with only one bentazon application varied from 1851.23 to 1235.03 kg ha^{-1}, respectively. The number of grains per plant affected yield ($P < 0.05$); the control and the treatment with only one bentazon application decreased from 3984.60 to 2040.94 kg ha^{-1}, respectively. Total polyphenol content and percentage of germination in quinoa grains were not affected by stress caused by herbicide application ($P > 0.05$). The herbicide fomesafen did not affect the maximum quantum yield, while the herbicide bentazon had an effect during the application. Three sequential applications of bentazon and fomesafen were sufficient to achieve a yield that was statistically equal ($P > 0.05$) to the control.

Key words: *Chenopodium quinoa*, stress indicators, total polyphenols, chlorophyll fluorescence, chemical weed control in quinoa.

3.2 INTRODUCTION

Quinoa (*Chenopodium quinoa*, Wild.) has been cultivated in the cool and semiarid regions of various South American countries because of its nutritional properties with high concentrations of proteins, essential amino acids, unsaturated fat acids, vitamins, minerals, and other beneficial compounds (Bazile et al., 2016). It is a crop that has recently attracted a great deal of attention (Navruz-Varli and Sanlier, 2016). Quinoa has high genetic variability that allows growth and adaptation to adverse environmental conditions due to its great abiotic stress resistance (Abderrahim et al., 2015, Navruz-Varli and Sanlier, 2016; Li and Zhu, 2018). Due to current inefficient results obtained with herbicides in the quinoa crop, weed control is manual or mechanical; however, some options are being studied for herbicide application at pre-emergence (metamitron and propyzamide) and post-emergence (propyzamide), which have been tolerated by quinoa. Some sulphonylurea-type herbicides have also been tested at post-emergence and have demonstrated selectivity in quinoa; however, further evaluation is necessary to draw sound conclusions (Díaz et al., 2015). On the other hand, herbicides applied at post-emergence cause stress in crops by negatively influencing photosynthesis, the opening and closing of stomata, and increasing susceptibility to attack by fungi and/or altering physiological and metabolic functions (Das and Mondal, 2014). Thus, plant metabolism is altered by herbicide application and produces changes in fluorescence emission that are detected in the leaves before any other visual effect (Barbagallo et al., 2003). Chlorophyll fluorescence has also proven to be a basic non-invasive technique to study and quantify damage to the photosynthetic apparatus in leaves as a response to stress caused by abiotic factors (Baker and Rosenqvist, 2004). Chlorophyll fluorescence is affected by herbicides that depend on light, such as inhibitors of glutamine synthetase, protoporphyrinogen oxidase, and carotenoid biosynthesis; they also affect the stability of the photosynthetic apparatus and can indirectly alter chlorophyll fluorescence (Hess, 2000). An important component to measure chlorophyll fluorescence is the Fv/Fm parameter, which is the maximum quantum yield of the photosystem II (PSII) and used to evaluate the herbicide toxicity in plants. The Fv/Fm

measures the maximum quantum efficiency of the PSII photochemical activity while the PSII reaction centers are open (Baker and Rosenqvst, 2004). On the other hand, reduced crop growth, especially decreased yield, can be influenced by a decrease in photosynthesis (Ali and Honermeier, 2016); however, this stress on the crops alters the quantity of secondary plant metabolites and also affects yield (Olivoto et al., 2016). Plant stress also reduces biomass production, but it increases the polyphenol concentration in tissues (De Abreu and Mazzafera, 2005).

Chemical methods to control weeds in the quinoa crop are therefore important factors that can directly affect yield; the effect on total polyphenol content in the grain and chlorophyll fluorescence as stress indicators is as yet unknown. Therefore, the objective of the present study was to evaluate the effect of two post-emergence herbicides on production, total polyphenol content, and chlorophyll fluorescence in quinoa.

3.3 MATERIALS AND METODOS

3.3.1 Plant material and experimental design

The experiments were conducted for two consecutive seasons at the El Nogal Experimental Station (36º 34’ S and 74º 06’ W) in Ñuble Region, Chile. A randomized complete block experimental design was used with four replicates. The experimental unit was 4 x 2 m and consisted of four rows of quinoa ‘Regalona’ plants with 0.5 m spacing. Seven treatments were established in each replicate to which two herbicides, fomesafen and bentazon, were applied at one commercial dose and three application frequencies; a control without herbicide application was also used (Table 6).

Table 6. Description of treatments in the 2015-2016 and 2016-2017 seasons.

Treatments	Active ingredient (a.i.)	Commercial product (c.p.)	Rate a.i. (g o cc ha^{-1})	Rate c.p (L ha^{-1})	Application frequency
TSH	Control				Without herbicide
Fom3	Fomesafen	Flex	83	0.33	3 applications, once every 9 d
Fom2	Fomesafen	Flex	125	0.50	2 applications, once every 9 d
Fom1	Fomesafen	Flex	250	1	Single application
Bent3	Bentazon	Basagran	317	0.66	3 applications, once every 9 d
Bent2	Bentazon	Basagran	480	1	2 applications, once every 9 d
Bent1	Bentazon	Basagran	960	2	Single application

Fuente: Elaboración propia

a.i.: active ingredient; c.p.: commercial product

Fomesafen (Flex ®, 250 g/L SL, Bayer); bentazon (Basagran ® 480 g/L SL, Basf).

3.3.2 Agronomic management

The soil was prepared in each season using one pass with a moldboard plow, two passes with a disc harrow plow, and two passes with a vibro-cultivator. Continuous manual sowing was performed in mid-October at a 12 kg ha^{-1} seeding rate and 0.5 m row spacing. After soil analysis, fertilization was uniformly applied as 100 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1} before sowing together with 160 kg N ha^{-1} of which 50% was applied at the 4 true leaf stage and 50% at the beginning of branching. Furrow irrigation was used to facilitate homogeneous water flow; mechanical/manual weed removal was carried out three times with a hand hoe.

3.3.3 Agronomic variables and yield components

The population was standardized at the beginning of the experiments to 18 plants m^{-1} and measurements were taken in two linear meters of the two central rows of each plot. Population and crop growth were evaluated at 7, 30, and 60 days after the first application (DFA). Each population and growth evaluation was associated with the crop phenological stages proposed by Mujica and Canahua (1989). Plant height was determined by averaging the seven first plants of the selected section. Likewise, yield per unit area (kg ha^{-1}), number of grains per plant, and

1000-grain weight (g) were determined. The methodology proposed by Carciochi et al. (2014), with some modifications, was used to evaluate seed germination.

3.3.4Stress indicators: chlorophyll fluorescence and total polyphenols

The maximum quantum yield (Fv/Fm) was measured as a chlorophyll fluorescence parameter. The methodology proposed by Mehta et al. (2010), with some modifications, was used to measure Fv/Fm on the surface of the quinoa leaves with a leaf clip and fluorometer OS5+ (Opti-sciences, USA). One plant from each plot was marked and Fv/Fm was measured for three consecutive days after herbicide application. Samples adapted to darkness for 30 min before taking the measurements at ambient temperature.

To determine total polyphenols in quinoa seeds, 20 g quinoa seed samples from each treatment were ground with a mill (1093, Cyclotec, Barcelona, Spain). Extract preparation was carried out according to the method described by Fischer et al. (2013) with some modifications. The supernatant was filtered and placed in amber glass jars and stored at 4º C until analysis. Total polyphenols were determined by the Folin-Ciocalteu method described by Miranda et al., (2010) with some modifications. Results were expressed as mg gallic acid equivalents (GAE) g^{-1} and all measurements were in triplicate. Two samples with the two herbicides at 100% commercial rate were included to verify the possible reaction of the herbicides on the Folin-Ciocalteu reagent and rule out false positives.

3.3.5 Statistical analysis

Data was subjected to ANOVA at $P < 0.05$ after verifying the assumptions of normal distribution and homogeneity of variances. When these assumptions were not fulfilled, transformations were performed with the ln (x+1) function. Means were compared by the LSD test at the 95% significance level. The statistical analysis was performed with the INFOSTAT statistical program (Di Rienzo et al., 2017). To compare the results of the variables between the first and second season, a combined experiment analysis was performed in which the

interaction between year and treatment for all the variables was not significant ($P > 0.05$); data were therefore grouped and analyzed together.

3.4 RESULTS AND DISCUSSION

3.4.1 Agronomic variables and yield components

Precipitation in the 2015-2016 and 2016-2017 seasons (October to February) was 113.30 and 124.60 mm, respectively; precipitation in October of each season was 98.60 and 65.00 mm $month^{-1}$, respectively. The crop faced environmental conditions of high precipitation and a gradual temperature increase in the early stages, which caused higher weed density; plots were weeded three times during the experiment, which allowed evaluating the direct effect of the herbicides on the crop.

The quinoa population was constant ($P > 0.05$) in all the treatments, whereas plant height varied ($P < 0.05$) with the treatments (Table 7). Plant height was not different between treatments up to 7 DFA ($P > 0.05$); however, treatments with bentazon at 30 DFA exhibited the highest plant height that was equal to the control ($P > 0.05$) but higher than the treatment with fomesafen in two sequential applications. Finally, the same trend was observed at 60 DFA in treatments with bentazon, which exhibited the same plant height ($P > 0.05$) as the control and higher than the three treatments with fomesafen. Two applications of fomesafen resulted in quinoa plants with lower height (75 cm) and it was equal only to the same herbicide with three sequential applications.

Table 7. Plant height response to the application of two post-emergence herbicides at different number of days after the first application (DFA) in the 'Regalona' quinoa crop.

Treatments	0 DFA	7 DFA	30 DFA	60 DFA
TWH	14.0a	42.0a	80.7abc	86.1a
Fom3	13.7a	41.6a	77.2bc	79.1bc
Fom2	14.2a	41.7a	74.6c	75.0c
Fom1	14.5a	41.6a	78.9bc	80.6b
Bent3	14.2a	41.3a	83.0ab	85.5a
Bent2	13.8a	43.0a	86.9a	88.0a
Bent1	13.8a	41.6a	83.4ab	86.7a

Fuente: Elaboración propia

Different lowercase letters indicate significant differences between treatments (LSD test at $P \leq 0.05$).
TWH: Treatment without herbicide application.

Crop yield (Table 8) was affected ($P < 0.05$) by herbicide application in the absence of weeds. The highest value was obtained in the control and the bentazon and fomesafen treatments, both with three sequential applications and values of 1851.23, 1772.55, and 1698.78 kg ha^{-1}, respectively, and values were statistically equal. The lowest yield occurred in the treatments with both herbicides and two sequential applications and a single application; values were 1431.90 and 1427.37 kg ha^{-1} for fomesafen and 1439.98 and 1235.03 kg ha^{-1} for bentazon, respectively, and values were statistically equal. In a similar study of bean, Pedreros and Tay (2003) used fomesafen and bentazon sequential applications and determined that dry grain yield increased when herbicides were fractionalized in three applications rather than a single application. This is because herbicides were applied in plots with weeds; therefore, when the herbicides controlled the weeds, interspecific competition decreased and the crop was able to express its potential. In contrast, herbicides in the present study were applied in manually weeded quinoa plots to evaluate the direct effect of the herbicide on the crop. In a study of chemical weed control in quinoa, Molina et al. (2014) used three post-emergence herbicides: fomesafen, bentazon, and metsulfuron-methyl, as well as two controls (manual weed control y no weed control). They obtained the highest yield in the manual weed control, which was no

different from the three fomesafen applications, whereas the lowest yield was obtained with a single application of metsulfuron-methyl.

Similar studies conducted in other crops have demonstrated reduced yield components as a consequence of herbicide application. Ali and Honermeier (2016) evaluated the influence of post-emergence herbicides on artichoke yield with treatments that included five herbicides applied after germination; when these were compared with mechanical weeding, the herbicide pyridate (phenyl-pyridazine) was the one that most affected yield in the two harvests of the first production cycle and the second harvest of the second production cycle compared with the control. Karimmojeni et al. (2013) suggested that bentazon was a good option to chemically control broadleaf weeds in flaxseed because it did not reduce yield and also produced acceptable phytotoxicity in the crop.

Table 8 shows that the variable number of grains per plant was affected by herbicide application ($P < 0.05$); the control exhibits the highest value, 3984.60 grains plant^{-1}, which is statistically different from the rest of the treatments. Meanwhile, the lowest value, 2040.94 grains plant^{-1}, is exhibited in the treatment with a single bentazon application. This would indicate that stress produced in the plant by a single bentazon application during the early physiological stages of the quinoa crop directly affected this variable and therefore yield. There is a direct influence of factors such as irradiance levels, water potential, nutrients, and duration of the formation of potential harvest organs on the number of these organs; moreover, not all organs begin effective filling and those that do are established in a period going from anthesis to the beginning of the effective growth of the harvest organ (Hall, 1980). On the other hand, it is possible that the reactive oxygen species (ROS) generated by quinoa plants exposed to early bentazon application at an initial physiological stage impeded adequate herbicide metabolism and ROS that were not metabolized and interfered at some stage of quinoa grain formation to form only the number of grains that they would be able to fill.

The 1000-grain weight variable was constant ($P > 0.05$) in all treatments (Table 8) with values ranging between 2.83 and 2.89 g, which would indicate that herbicide application did not affect this variable that was the most stable. Mellado and Pedreros (2005) evaluated the effect of four herbicides on yield and quality of wheat grain during grain maturity and concluded that from the semi-hard grain stage onwards any of the herbicides used in those

experiments can be applied without affecting the 1000-grain weight variable. This due to the fact that from the semi-hard grain stage onwards, the wheat grain condition is a semi-hard mass that is physiologically mature, and has completed the transport of nutrients from the leaves, stems, and spikes to the grain. Therefore, the maturity stage impedes the displacement of the applied systemic herbicides.

Height, yield, and number of grains per plant were affected in the present study when two herbicides were applied in the absence of weeds: we also demonstrated that the measurement of agronomic variables after herbicide application treatments has been a procedure used to characterize different herbicides (Pavlovic et al., 2008). In addition, herbicide application frequency was an important factor that influenced the variables; therefore, treatments with three sequential applications exhibited better crop response and allowed quinoa to effectively detoxify the herbicides. Thus, detoxification based on the metabolism of toxic compounds in plants aims to reduce the compound's toxic capacity, isolate or expel it, and prevent the damage it can cause. Bentazon and fomesafen belong to the group of photosynthesis inhibitor and photosynthetic pigment inhibitor herbicides, respectively; plants exposed to these herbicides produce ROS but mainly cause an excess of active forms of O_2, which generate lipid destruction by forming lipid radicals in polyunsaturated fatty acids (Hess, 2000; Lascano et al., 2003). Lipid peroxidation increases with herbicide stress and is correlated with the damage to the lipid membrane and amount of stress (Ekmekci and Terzioglu, 2005; Lukatkin et al., 2013). To eliminate these ROS, plants activate compounds in their tissues that act as antioxidant defense systems, such as ascorbic acid, and enzymes that interact with ROS, such as catalase, ascorbate peroxidase, and peroxidase (Blokhina et al., 2003; Lascano et al., 2003; Lukatkin et al., 2013). Pitty (2018) mentions that the metabolism in herbicides can be caused by an acceleration of the herbicide metabolism that forms nonphytotoxic compounds, decreases absorption or translocation, or places the herbicide in organelles in which it does not damage the plant. Pavlovic et al., (2008) evaluated the morphophysiological traits and sensitivity to atrazine in *Chenopodium album* L. and point out that the herbicide metabolism is usually an inherited quantitative characteristic; they concluded that the metabolism of the PSII inhibitor herbicides could have contributed to atrazine tolerance of a *Chenopodium álbum* biotype.

Finally, the evaluated percentage of germination showed that herbicide application did not affect germination and was constant (P > 0.05) in all treatments (Table 8) with values ranging between 95% and 93%. In a similar study, Rinella et al. (2010) applied three herbicides (2,4-D, dicamba, and picloram) to *Bromus japonicus* Thunb.; they observed a negative effect on seed germination when the herbicides were applied during the entire plant cycle and concluded that the final reproductive stages can often be more sensitive to herbicide application than other growth stages.

Table 8. Yield, number of grains per plant, 1000-grain weight, percentage of germination, and total polyphenol response to the application of two post-emergenc herbicides in the 'Regalona' quinoa crop.

Tratamientos	Yield (kg ha^{-1})	Number of grains per plant	1000-grain weight (g)	Germination (%)	Total polyphenols mg GAE g^{-1}
TWH	1851.2a	3984.6a	2.8a	94.4a	2.9a
Fom3	1698.8a	3388.8b	2.8a	93.3a	2.9a
Fom2	1431.9b	2545.8c	2.9a	93.6a	2.9a
Fom1	1427.4b	2544.5c	2.8a	92.6a	2.9a
Bent3	1772.6a	3678.9b	2.9a	94.6a	3.0a
Bent2	1440.0b	2679.4c	2.9a	93.5a	3.0a
Bent1	1235.0b	2040.9d	2.8a	93.0a	3,1a

Fuente: Elaboración propia

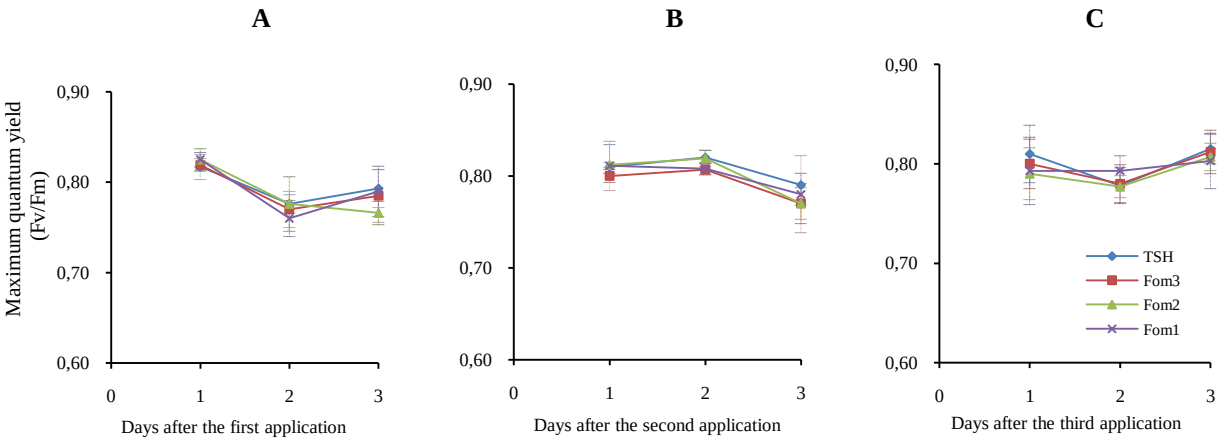
Different lowercase letters indicate significant differences between treatments (LSD test at P ≤ 0.05).
TWH: Treatment without herbicide application.

3.4.2 Stress indicators: chlorophyll fluorescence and total polyphenols

Table 3 shows that the quantity of total polyphenols is not affected by herbicide application and is constant (P > 0.05) in all treatments with values ranging between 2.85 and 3.06 mg GAE g^{-1}; the two herbicides, fomesafen and bentazon at the100 % commercial rate, do not react with the procedure and exhibit a value of 0 mg GAE g^{-1}. Polyphenols are secondary metabolites important in plants because they perform functions in response to stress conditions (Miranda et al. 2013). In the present study, although stress caused by bentazon and fomesafen affected yield, it was not sufficient to alter the total polyphenol concentration. Ali and Honermeier

(2016) applied five post-emergence herbicides in artichoke and observed that all the treatments increased caffeoylquinic acid content in leaves compared to the control during the first growth stage of the first period. No significant differences were detected in the second growth stage. As for flavonoids, they found that no herbicide affected their accumulation; finally, the control was the treatment that produced the lowest quantity of polyphenols (caffeoylquinic acid and flavonoids) during the two growth stages.

The maximum quantum yield related to fluorescence was not affected by fomesafen application (Figure 1) at any commercial rate. On the first day after the first application (Figure 1A), all treatments exhibited similar Fv/Fm values ($P > 0.05$) of 0.82, 0.82, 0.83, and 0.83 in the control and treatments with three, two, and a single fomesafen application, respectively. On the second and third after the first application, the trend was maintained, that is, all treatments had Fv/Fm values near 0.80. On the first day after the second application (Figure 1B), all treatments had equal Fv/Fm values ($P > 0.05$) of 0.81, 0.80, 0.81, and 0.81 in the control and treatments with three, two, and a single fomesafen application, respectively; treatments behaved similarly to the control on the second and third day. Finally, the first day after the third application (Figure 1C), all treatments exhibited similar Fv/Fm values ($P > 0.05$) of 0.81, 0.80, 0.79, and 0.79 in the control and treatments with three, two, and a single fomesafen application, respectively; all treatments behaved similarly to the control on the second and third day. Therefore, fomesafen did not affect the photosynthetic apparatus of the quinoa plants.



Fuente: Elaboración propia

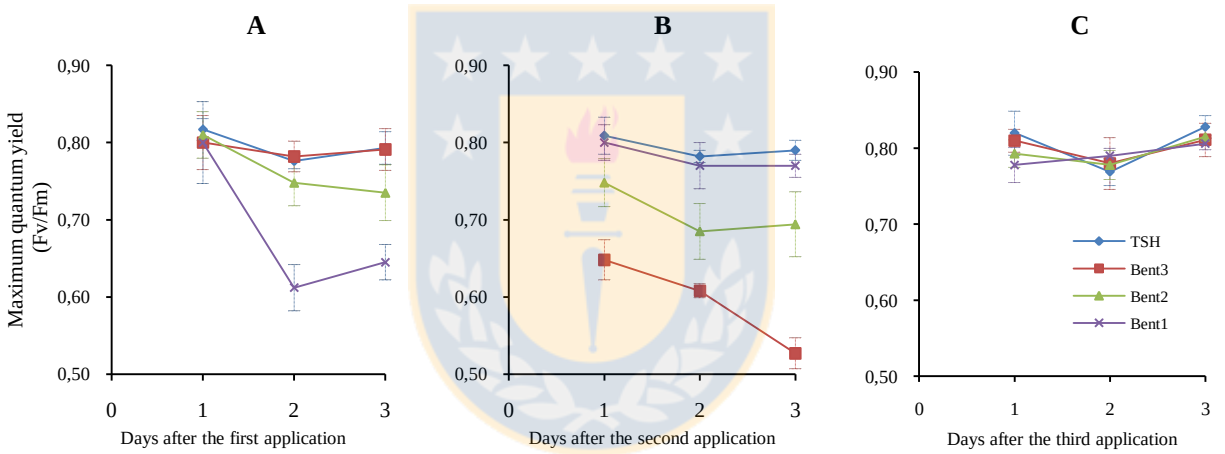
Bars represent the standard deviation of the mean in each treatment.

Figure 9. Response of maximum quantum yield to post-emergence herbicide application of fomesafen in the 'Regalona' quinoa crop in the first (A), second (B), and third (C) applications.

The bentazon application revealed changes ($P < 0.05$) in the maximum quantum yield values (Figure 2). On the first day after the first application (Figure 2A), all treatments exhibited similar Fv/Fm values ($P > 0.05$) of 0.82, 0.80, 0.81, and 0.80 in the control and treatments with three, two, and a single bentazon application, respectively. On the second after the first application, treatments with two and a single bentazon application showed variations in the Fv/Fm values; the single bentazon application Fv/Fm value (0.61) significantly decreased compared to the control. On the other hand, the bentazon treatment with two applications exhibited a variation in Fv/Fm with a value of 0.75, while the control and treatment with three bentazon applications maintained values near 0.80. Finally, on the third after the first application, the trend was maintained and the bentazon treatment with a single application managed to recover a little by increasing its Fv/Fm value to 0.65. On the other hand, the bentazon treatment with two applications had an Fv/Fm value of 0.74, which was lower than the control, whereas the control and the bentazon treatment with three applications maintained a value near 0.80. On the first day after the second application (Figure 2B), the Fv/Fm values of the bentazon treatments with three and two applications were different ($P < 0.05$) and lower than the control and the bentazon treatment with a single application that maintained values near 0.80; however, the highest stress occurred in the bentazon treatment with three applications since it exhibited an Fv/Fm value of 0.65. On the second day after the second

application, these trends persisted, that is, the control and bentazon treatment with a single application maintained values near 0.80, whereas Fv/Fm values in the bentazon treatments with three and two applications decreased to 0.61 and 0.69, respectively. On the third day after the second application, only the Fv/Fm value of the bentazon treatment with three applications continued to decrease to 0.53, while the bentazon treatment with two applications remained at 0.69.

Finally, for the third application (Figure 2C), Fv/Fm values on the first, second, and third days were constant ($P > 0.05$) and near 0.80 in all treatments. Under normal conditions, healthy superior plants have an optimum Fv/Fm near 0.83 (Maxwell y Johnson, 2000); however, reported values in quinoa leaves range from 0.78 to 0.84 (Winkel et al., 2002).



Fuente: Elaboración propia

Bars represent the standard deviation of the mean in each treatment.

Figure 10. Response of maximum quantum yield to post-emergence herbicide application of bentazon in the 'Regalona' quinoa crop in the first (A), second (B), and third (C) applications.

For the fomesafen application, Fv/Fm values were stable during the experiment, whereas Fv/Fm values decreased and then recovered in the bentazon application, which indicates that the plant tolerated stress caused by the herbicide. The effect can be related to the mode of action of the herbicides; fomesafen specifically inhibits the protoporphyrinogen oxidase enzyme in chlorophyll biosynthesis and acts indirectly in photosynthesis. On the other

hand, bentazon is absorbed by the plant through the leaves, prevents protein D1 production, and slowly acts during several days in a direct way in PSII. Lootens and Vandecasteele (2000) conducted studies with similar crops and herbicides; they analyzed the effect of the herbicide diuron in a maize crop, measured chlorophyll fluorescence 4 min after it was applied on the adaxial side of the leaf, and then this effect was heightened after 30 min with Fv/Fm decreasing by approximately 40%. This effect is possibly due to the fact that phenylurea herbicides, such as linuron, diuron, or isoproturon, block the electron flow between the primary and secondary electron acceptors of PSII. Ali and Honermeier (2016) studied an artichoke crop and indicated that the damage to the photosynthetic apparatus was caused by the herbicides pyridate and phenmedipham because the greatest alterations in chlorophyll fluorescence were generated by these two herbicides.

3.5 CONCLUSIONS

Stress produced by the application of the herbicides fomesafen and bentazon in the absence of weeds affected quinoa crop yield; however, when dividing the dose into three sequential applications of both herbicides, once every 9 days, it was sufficient to achieve a yield equal to the control. For both herbicides, the number of grains per plant was the variable that exhibited the greatest differences and directly affected yield in some treatments. Total polyphenol content was not altered by herbicide application despite affecting crop yield in some treatments. The herbicide fomesafen did not affect maximum quantum yield, whereas the herbicide bentazon affected it in each application.

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IV. CONCLUSION GENERAL

El estrés ocasionado por competencia interespecífica para determinar el periodo crítico de interferencia de malezas, alteró el contenido de polifenoles y afectó a la producción del cultivo de quinoa, mientras que el estrés ocasionado por la aplicación de los herbicidas fomesafen y bentazon sin presencia de malezas, afectó al rendimiento del cultivo de quinoa en algunos tratamientos, pero no alteró el contenido de polifenoles totales en el cultivo.

El período crítico de interferencia de malezas se determinó entre 10 y 75 días después de la emergencia, que correspondió a las etapas fenológicas de dos hojas verdaderas y floración, respectivamente. Además tres aplicaciones secuenciales de bentazon y fomesafen, una cada nueve días, fueron suficientes para tener un rendimiento igual al testigo sin herbicida.

La respuesta de la variable número de granos por planta a los dos tipos de estrés inducidos al cultivo de quinoa, fue la que evidenció mayores diferencias y afectó directamente al rendimiento del cultivo en algunos tratamientos.

El contenido de polifenoles totales varió y aumentó en períodos de interferencia de malezas más largo, mientras que disminuyó con un período de interferencia de malezas más corto, por lo tanto el aumento del contenido de polifenoles totales en el cultivo de quinoa y el menor rendimiento del cultivo se debió al estrés causado por la interferencia de las malezas. Por otro lado, con la aplicación de herbicidas, el contenido de polifenoles totales no fue alterado a pesar de haber afectado al rendimiento del cultivo.

El herbicida fomesafen no afectó al máximo rendimiento cuántico mientras que el herbicida bentazon si afectó, en cada momento de aplicación.

El contenido de polifenoles totales fue un indicador eficaz del estrés inducido por la competencia de malezas con el cultivo de quinoa, mientras que la fluorescencia de clorofila fue el indicador más efectivo para cuantificar el estrés inducido por la aplicación de los herbicidas postemergentes en quinoa.