Universitat de Lleida

Ground vegetation covers as a tool for weed management in vineyards

Francisco Valencia Gradilla

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Universitat de Lleida

TESI DOCTORAL

Ground vegetation covers as a tool for weed management in vineyards

Francisco Valencia Gredilla

Memòria presentada per optar al grau de Doctor
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Resumen

La evolución hacia una agricultura más comprometida con el medio ambiente ha posibilitado el tránsito hacia técnicas de mantenimiento del suelo en viña más sostenibles. Si bien, el laboreo sigue siendo la técnica de manejo más empleada actualmente, la implantación de cubiertas vegetales está tomando protagonismo debido a las distintas ventajas que ofrece, entre las que destaca su papel opresor frente a malas hierbas. Este sistema de manejo del suelo se baraja como una opción interesante cuando en el cultivo encontramos especies de malas hierbas que no se controlan fácilmente mediante herramientas convencionales como son el laboreo o las aplicaciones herbicidas. La cubierta vegetal puede reducir la presencia de malas hierbas tanto por competencia (luz, agua, nutrientes) como por el efecto físico que ejercen cuando se siegan y aplican como acolchado, así como por la liberación de metabolitos secundarios (aleloquímicos) que pueden ejercer un efecto alelopático en ambas situaciones (cubierta y acolchado). La eficacia de este sistema de manejo depende de varios factores, siendo los más importantes la especie seleccionada como cubierta (la cual poseerá una serie de características y requerirá un determinado manejo) y las especies de malas hierbas presentes en el cultivo. En este escenario, el reto consiste en discernir entre el tipo de cubierta vegetal (vegetación natural o sembrada) y las especies que se pueden sembrar como cubierta vegetal, focalizando este manejo en gestionar malas hierbas considerando las limitaciones de la parcela y las condiciones climáticas.

En este contexto se plantea el trabajo que constituye la presente tesis doctoral, cuyos objetivos son: a) estudiar la influencia de distintos manejos de una cubierta vegetal así como de las distintas especies sembradas como cubierta, sobre la comunidad de malas hierbas presentes en un viñedo, especialmente frente a *Cynodon dactylon* (L.) Pers., una de las especies más difíciles de controlar; b) estudiar el comportamiento germinativo de *Conyza bonariensis* (L.) Cronquist, especie problemática en viña al no ser fácilmente controlada mediante aplicaciones herbicidas; c) estudiar el potencial fitotóxico de doce especies que se pueden utilizar como cubierta y/o acolchado vegetal frente a *C. bonariensis*, *Aster squamatus* (Spreng.) Hieron y *Bassia scoparia* (L.) A. J. con el fin de seleccionar las especies más alelopáticas y, a su vez, conocer qué aleloquímicos están presentes en ellas. Para llevar a cabo estos objetivos, se han

desarrollado distintos ensayos en condiciones tanto de campo como de laboratorio.

En un primer ensayo (Capítulo 2) se seleccionó una parcela de viña (*Vitis vinifera* L.) en Raimat (Lleida) donde se implementaron cuatro sistemas de manejo del suelo con cubierta vegetal durante las campañas 2015, 2016 y 2017: a) una cubierta vegetal espontánea controlada mediante siegas mecánicas; b) una cubierta espontánea combinada con aplicaciones herbicidas focalizadas en *C. dactylon*; c) una cubierta espontánea labrada anualmente y d) una cubierta de siembra anual (*Hordeum vulgare* L.). En esta parcela se estudió la evolución de *C. dactylon* así como la comunidad de malas hierbas bajo estos sistemas de manejo. Los resultados mostraron que los mejores manejos en cuanto a control de *C. dactylon* y otras malas hierbas, fueron, por un lado, la preparación del suelo y siembra de una cubierta de *Hordeum vulgare* y, por otro, el laboreo del suelo e instalación de una cubierta espontánea, con unos valores de reducción de la infestación *de C. dactylon*, al final del ensayo, en un 93,9% y un 82,6% respectivamente.

En un segundo ensayo (Capítulo 3) se seleccionó una parcela de viña, también en Raimat (Lleida) donde se sembraron como cubierta vegetal distintas gramíneas (*Festuca arundinacea* L., *H. vulgare,* una mezcla de *Bromus rubens* L. y *B. hordeaceus* L*., Vulpia ciliata* Dumort.) y una leguminosa (*Medicago rugosa* Desr.) durante las campañas 2016, 2017 y 2018. En este ensayo se evaluó la utilidad de estas especies para controlar y reducir la expansión de *C. dactylon,* así como su efecto sobre la comunidad de malas hierbas. También se evaluó el efecto de la labor de preparación del suelo, sobre el nivel de infestación de *C. dactylon*. Los resultados de este experimento mostraron que las labores del suelo, previas a la instalación de las cubiertas, redujeron el nivel de infestación de la mala hierba en un 60%, y la instalación de cubiertas de *F. arundinacea* y *H. vulgare*, redujeron la cobertura de mala hierba en un 38,8% y 21,3%, respectivamente, a lo largo de las tres campañas. Se confirmó también que el tipo de cubierta instalada influyó en la composición florística de la flora arvense. Los resultados de estos dos ensayos mostraron el efecto de las labores preparatorias del suelo y de las cubiertas instaladas, en reducir la expansión de *C. dactylon*, así como en limitar la presencia de otras malas hierbas.

En un tercer experimento (Capítulo 4) se recolectaron semillas de *C. bonariensis* en cuatro localidades distintas (tres españolas y una argentina) y se sometieron en cámaras a distintos test de germinación a temperaturas constantes y alternas. Los resultados mostraron que a temperaturas constantes (5, 10, 15, 20, 25, 30ºC), la germinación aumentaba conforme se acercaban a la temperatura óptima estimada (22ºC de media entre las poblaciones) obteniendo el máximo valor de germinación (99,2%) la población argentina a 20 y 25ºC. A temperaturas alternas alejadas de la temperatura óptima estimada (5/15ºC y 25/35ºC), los valores de germinación eran mayores que los observados a la temperatura constate equivalente al promedio de esos intervalos (10ºC y 30ºC). Asimismo, se observó también una clara influencia del origen de la población, obteniendo un mayor porcentaje de germinación (26,7%) a menor temperatura (5ºC) por parte de la población procedente de la zona invernal más fría (Lleida). Este hecho se ha constatado a su vez en las temperaturas bases obtenidas para las distintas poblaciones, siendo 4,9ºC para la población de Lleida, y 6,9ºC, 8,4ºC y 8,9ºC para las poblaciones de Bahía Blanca, Badajoz y Sevilla, respectivamente. Estos resultados definen el comportamiento germinativo y de emergencia de esta especie en función de la temperatura y del origen de las semillas, posibilitando su gestión en campo.

El cuarto ensayo (Capítulo 5) se planteó con el fin de determinar el potencial fitotóxico de distintas especies utilizadas como cubierta y/o acolchado vegetal en viña. Para ello, se seleccionaron doce especies (una mezcla de *B. hordeaceus y B. rubens, F. arundinacea* cv. `Firaces R1´*, Hoderum murinum* L.*, H. vulgare* cv. `Meseta´*, M. rugosa* cv. `Sapo´*, Medicago sativa* L. cv. `Victoria´*, Phacelia tanacetifolia* Benth.*, Sinapis alba* L.*, Trifolium incarnatum* L. cv. `Red´*, Trifolium subterraneum* L. cv. `Dalkeith´*, V. ciliata* y *Pinus sylvestris* L.). Los extractos acuosos de estas especies se ensayaron frente a tres especies de malas hierbas problemáticas en viña (*C. bonariensis, A. squamatus* y *B. scoparia*), en condiciones controladas (laboratorio) a fin de evitar la interacción de factores externos (tanto abióticos como bióticos). Los resultados de este experimento mostraron que *C. bonariensis* y *A. squamatus* vieron su germinación y crecimiento radicular prácticamente inhibido (100% de inhibición) para la mayoría de extractos y dosis ensayadas. Este efecto fue menos evidente en la especie *B. scoparia*, cuya germinación no se vio apenas inhibida (alcanzando valores de germinación cercanos al 100% para la mayoría de los extractos) pero, en cambio, sí se vio inhibido su crecimiento radicular, mostrando unos valores inferiores al 50% respecto al control. Los extractos fueron analizados y se identificaron los compuestos responsables del efecto fitotóxico (un total de 33 compuestos) tanto ácidos fenólicos como flavonoides. Los resultados de este experimento aportan información sobre el potencial efecto bioherbicida que tienen estos extractos en ciertas malas hierbas problemáticas en viña, así como la potencialidad de estas sustancias para ser liberadas en campo.

Los resultados de los diferentes experimentos muestran que tanto el tipo de cubierta como la gestión que se haga de la misma, es un aspecto clave a la hora de controlar ciertas especies de malas hierbas en un viñedo. A su vez, conocer el potencial fitotóxico de las distintas especies estudiadas como cubierta vegetal, así como las características biológicas de las malas hierbas que se desean controlar, nos permite poder definir un adecuado manejo de la cubierta. Estos resultados, en conjunto, verifican la capacidad de las cubiertas vegetales para controlar malas hierbas problemáticas, contribuyendo a la implementación de manejos integrados más completos.

Summary

The evolution of agriculture committed with the environment has enabled a transition towards more sustainable techniques for soil maintenance in vineyards. Although tillage is still the most widely used management nowadays, the implantation of ground vegetation covers is being considered due to the different advantages that it offers, among which its suppressive effect against weeds stands out. Ground vegetation covers are a really interesting soil management option when problematic weeds that cannot be controlled with tillage or herbicides become plentiful. Weed presence can be reduced by ground vegetation covers, either by competition (light, water, nutrients) and by the physical barrier they exert when mowed and applied as mulch; but also by releasing secondary metabolites (allelochemicals) that can cause an allelopathic effect in both situations (cover and mulch). The effectiveness of this management system depends on several factors, the most important being the species selected as cover crop (which will show a series of characteristics and will require certain management) and the weeds present in the crop. In this scenario, the selection between the type of ground cover (spontaneous vegetation or cover crop) and the species that can be sown as ground cover is a challenge, mainly when focussed on weed management, and considering the field limitations and the climatic conditions.

In this context, the objectives of the work that constitutes the present doctoral thesis are: a) to study the validity of different ground vegetation covers and the species used for these, for the control of the weed community present in a vineyard, and especially against *Cynodon dactylon* (L.) Pers., one of the most difficult-to-control species; b) to study the germination behaviour of *Conyza bonariensis* (L.) Cronquist, another problematic weed species in vineyard for the difficultness of its control with herbicide applications; c) to study the phytotoxic potential of twelve species that can be implemented as cover crops and/or mulching against *C. bonariensis, Aster squamatus* (Spreng.) Hieron and *Bassia scoparia* (L.) A.J., to select the species with the highest allelopathic potential, and to know their allelochemical composition.

To carry out these objectives, different experiments were designed under field and laboratory conditions.

In a first trial (Chapter 2), a vineyard plot (*Vitis vinifera* L.) in Raimat (Lleida) was selected, where four ground vegetation cover managements were implemented

during 2015, 2016 and 2017 campaigns: a) a no-till spontaneous vegetation ground cover managed by shredding; b) a no-till spontaneous vegetation ground cover managed by shredding plus herbicide application focusing on *C. dactylon*; c) spontaneous vegetation managed with yearly tillage; and d) barley seeded yearly as cover crop (*Hordeum vulgare* L.) after soil tillage. The evolution of the weed community, and particularly *C. dactylon,* was studied. The results showed that the installation of a seeded cover crop was the most appropriate management to control *C. dactylon* and other weeds, followed by an annually tilled spontaneous cover. In this two systems *C. dactylon* coverage was reduced by 93.9% and 82.6%, respectively, at the end of the study.

In a second trial (Chapter 3) another vineyard plot was also selected in Raimat (Lleida), where different grasses (*Festuca arundinacea* L., *H. vulgare*, a mixture of *Bromus rubens* L. and *B. hordeaceus* L., and *Vulpia ciliata* Dumort.) and a legume (*Medicago rugosa* Desr.) were implemented as a cover crops during the 2016, 2017 and 2018 campaigns. The ability of these species in controlling *C. dactylon* and reducing its expansion was evaluated, as well as their effect on the general weed community. The effect of tillage on *C. dactylon* was also evaluated. The results of this experiment showed that tillage prior to the cover crops' installation, reduced the weed infestation level by 60% and the implantation of *F. arundinacea* and *H. vulgare* reduced the weed coverage by 38.8% and 21.3%, respectively, throughout the three seasons. It was also observed that the floristic composition of the weed flora was affected by the type of cover crop. The results from the two experiments show that seedbed preparation followed by ground vegetation cover is a useful management to reduce the expansion of *C. dactylon* and to limit the presence of other undesired weeds.

In a third experiment (Chapter 4), *C. bonariensis* seeds were collected from four different locations (three from Spain and one from Argentina) and subjected to different germination tests in chambers at constant and alternating temperatures. The results showed that at constant temperatures (5, 10, 15, 20, 25, 30ºC), germination increased as they approached the estimated optimal temperature (22ºC on average between the populations) obtaining the maximum germination value (99.2%) the Argentinean population at 20 and 25ºC. At alternating temperatures far from the estimated optimum temperature (5/15ºC and 25/35ºC), the germination values were higher than those observed at the constant temperature equivalent to the average of those intervals (10ºC and 30ºC). Results were affected by the origin of the population, with a higher percentage of germination (26.7%) at the lower temperature (5ºC) shown by the population from the coldest winter zone (Lleida), which was also verified in the estimated base temperatures, being 4.9ºC for the population of Lleida, and 6.9ºC, 8.4ºC and 8.9ºC for the populations of Bahía Blanca (Argentina), Badajoz, and Seville, respectively. These results define the germination and emergence behaviour of this species depending on the temperature and origin, which will help improve its management at field conditions.

The fourth trial (Chapter 5) was proposed in order to determine the phytotoxic potential of different species used as cover crops and/or mulch in vineyards. For this, twelve species were selected (a mixture of *B. hordeaceus* and *B. rubens, F. arundinacea* cv. `Firaces R1*´, Hoderum murinum* L.*, H. vulgare* cv. `Meseta*´, M. rugosa* cv. `Sapo*´, Medicago sativa* L. cv. `Victoria*´, Phacelia tanacetifolia* Benth.*, Sinapis alba* L., *Trifolium incarnatum* L. cv. `Red*´, Trifolium subterraneum* L. cv. `Dalkeith*´, V. ciliata* and *Pinus sylvestris* L). The aqueous extracts of these species were tested against three problematic weed species in vineyards (*C. bonariensis, A. squamatus* and *B. scoparia*) under controlled conditions (laboratory) in order to avoid the interaction of external factors (both abiotic and biotic). Results showed that germination and root growth of *C. bonariensis* and *A. squamatus* were almost completely inhibited (100% inhibition) with most extracts and doses tested. This effect was less evident over *B. scoparia*, whose germination was hardly inhibited (reaching germination values close to 100% for most of the extracts assayed); on the contrary, its root growth was significantly inhibited, with root length values 50% shorter compared to the control. The extracts were analyzed and the compounds (a total of 33) responsible of the phytotoxic effect identified, both phenolic acids and flavonoids. The results of this experiment provide information on the bioherbicidal effect that these extracts have on certain problematic weed species in vineyards, as well as the potential of these substances to be released in the field.

The results of the different experiments show that both the type of ground vegetation cover and its management are key aspects when focused on controlling certain weed species in vineyards. At the same time, knowledge of the phytotoxic potential of the different species studied as cover crops, together with the biological characteristics of the weeds to be controlled, allow us to define an adequate ground cover management. These results, taken together, verify the capacity of the ground vegetation covers in controlling problematic weeds, contributing to the implementation of more complete integrated management systems.

Resum

L'evolució cap a una agricultura més compromesa amb el medi ambient ha possibilitat el trànsit cap a tècniques de manteniment del sòl en vinya més sostenibles. Si bé, el conreu continua sent la tècnica de maneig més emprada actualment, la implantació de cobertes vegetals està prenent protagonisme degut als diferents avantatges que ofereix, entre els quals destaca el seu paper opressor enfront de males herbes. Aquest sistema de maneig del sòl es planteja com una opció interessant davant espècies de males herbes que no es controlen amb facilitat mitjançant eines convencionals com són les labors del sòl o les aplicacions herbicides. La coberta vegetal pot reduir la presència de males herbes tant per competència (llum, aigua, nutrients) com per l'efecte físic que exerceixen quan es seguen i s'apliquen com encoixinat, així com per l'alliberament de metabòlits secundaris (al·leloquímics) que poden exercir un efecte al·lelopàtic en totes dues situacions (coberta i encoixinat). L'eficàcia d'aquest sistema de maneig depèn de diversos factors, sent els més importants l'espècie seleccionada com a coberta (la qual posseirà una sèrie de característiques i requerirà un determinat maneig) i les espècies de males herbes presents en el cultiu. En aquest escenari, el repte consisteix a destriar entre el tipus de coberta vegetal (vegetació natural o sembrada) i les espècies que la poden constituir, dirigint aquest maneig vers la gestió de males herbes presents, considerant les limitacions de la parcel·la i les condicions climàtiques.

En aquest context es planteja el treball que constitueix la present tesi doctoral, els objectius de la qual són: a) estudiar la validesa de diferents tipus de maneig d'una coberta vegetal així com de les diferents espècies sembrades com a coberta, sobre la comunitat de males herbes presents en una vinya, especialment enfront *Cynodon dactylon* (L.) Pers., una de les espècies major dificultat de control; b) estudiar el comportament germinatiu de *Conyza bonariensis* (L.) Cronquist, espècie problemàtica en vinya al no ser fàcilment controlada mitjançant aplicacions herbicides; c) estudiar el potencial fitotòxic de dotze espècies que es poden utilitzar com a coberta i/o encoixinat vegetal enfront de *C. bonariensis, Aster squamatus* (Spreng.) Hieron i *Bassia scoparia* (L.) A. J. amb la finalitat de seleccionar les espècies més al·lelopàtiques i, al seu torn, conèixer quins al·leloquímics estan presents en elles. Per a dur a terme aquests

objectius, s'han desenvolupat diferents assajos en condicions tant de camp com de laboratori.

En un primer assaig (Capítol 2) es va seleccionar una parcel·la de vinya (*Vitis vinifera* L.) a Raimat (Lleida) on es van implementar quatre sistemes de maneig del sòl amb coberta vegetal durant les campanyes 2015, 2016 i 2017: a) una coberta vegetal espontània controlada mitjançant segues mecàniques; b) una coberta espontània combinada amb aplicacions herbicides focalitzades en *C. dactylon*; c) una coberta espontània llaurada anualment i d) una coberta sembrada de forma anual (*Hordeum vulgare* L.). En aquesta parcel·la es va estudiar l'evolució de *C. dactylon* sota aquests sistemes de maneig així com la comunitat de males herbes present. Els resultats van mostrar que la instal·lació d'una coberta sembrada era el maneig més apropiat per a controlar *C. dactylon* i altres males herbes, seguit de la coberta espontània llaurada anualment, amb un valor de reducció de la superfície de *C. dactylon,* al final de l'assaig, del 93,9% i 82,6%, respectivament.

En un segon assaig (Capítol 3) es va seleccionar una parcel·la de vinya, també a Raimat (Lleida) on es van sembrar com a coberta vegetal diferents gramínies (*Festuca arundinacea* L., *H. vulgare*, una mescla de *Bromus rubens* L. i *B. hordeaceus* L., *Vulpia ciliata* Dumort.) i una lleguminosa (*Medicago rugosa* Desr.) durant les campanyes 2016, 2017 i 2018. En aquest assaig es va avaluar l'efecte d'aquestes cobertes per a controlar i reduir l'expansió de *C. dactylon*, així com el seu efecte sobre la comunitat de males herbes. També es va avaluar l'efecte de la labor del sòl sobre el nivell d'infestació de *C. dactylon*. Els resultats d'aquest experiment van mostrar que les labors del sòl, prèvies a la instal·lació de les cobertes, van reduir el nivell d'infestació de la mala herba en un 60%, i la instal·lació de cobertes de *F. arundinacea* i *H. vulgare*, van inhibir la cobertura de mala herba en un 38,8% i 21,3%, respectivament, al llarg de les tres campanyes. Es va confirmar també que el tipus de coberta instal·lada va influir en la composició florística de la flora arvense. Els resultats d'aquests dos assajos mostren la capacitat potencial de la preparació del sòl, així com el seu manteniment mitjançant una coberta vegetal, en reduir l'expansió de *C. dactylon*, així com el seu efecte en limitar la presència d'altres espècies de males herbes no desitjades.

En un tercer experiment (Capítol 4) es van recol·lectar llavors de *C. bonariensis* en quatre localitats diferents (tres espanyoles i una argentina) i es van sotmetre en cambres a diferents test de germinació a temperatures constants i alternes. Els resultats van mostrar que a temperatures constants (5, 10, 15, 20, 25, 30°C), la germinació augmentava a mesura que s'acostaven a la temperatura òptima estimada (22°C de mitjana entre les poblacions) obtenint el màxim valor de germinació (99,2%) la població argentina a 20ºC i 25°C. A temperatures alternes allunyades de la temperatura òptima estimada (5/15ºC i 25/35°C), els valors de germinació eren majors que els observats a la temperatura constat equivalent a la mitjana d'aquest interval (10°C i 30°C). També es va observar una influència de l'origen de la població, amb un major percentatge de germinació (26,7%) a menor temperatura (5°C) obtingut per a la població procedent de la zona hivernal més freda (Lleida), fet constatat, al seu torn, en les temperatures bases obtingudes, éssent 4,9°C per a la població de Lleida, i 6,9ºC, 8,4ºC i 8,9°C per a les poblacions de Bahía Blanca, Badajoz i Sevilla, respectivament. Aquests resultats defineixen el comportament germinatiu i d'emergència d'aquesta espècie en funció de la temperatura i el seu origen, possibilitant la seva gestió en el camp.

El quart assaig (Capítol 5) es va plantejar amb la finalitat de determinar el potencial fitotòxic de diferents espècies utilitzades com a coberta i/o encoixinat vegetal en vinya. Amb aquest objectiu es van seleccionar dotze espècies (una mescla de *Bromus hordeaceus* i *B. rubens*, *F. arundinacea* cv. `Firaces R1´, *Hoderum murinum* L., *H. vulgare* cv. `Meseta´, *M. rugosa* cv. `Sapo´, *Medicago sativa* L. cv. `Victoria´, *Phacelia tanacetifolia* Benth., *Sinapis alba* L., *Trifolium incarnatum* L. cv. `Red´, *Trifolium subterraneum* L. cv. `Dalkeith´, *V. ciliata* i *Pinus sylvestris* L). Els extractes aquosos d'aquestes espècies es van assajar enfront de tres espècies de males herbes problemàtiques en vinya (*C. bonariensis, A. squamatus* i *B. scoparia*) en condicions controlades (laboratori) a fi d'evitar la interacció de factors externs (tant abiòtics com biòtics). Els resultats d'aquest experiment van mostrar que *C. bonariensis* i *A. squamatus* van veure la seva germinació i creixement radicular pràcticament inhibit (100% d'inhibició) per a la majoria d'extractes i dosis assajades. Aquest efecte va ser menys evident en l'espècie *B. scoparia*, la germinació del qual no es va veure gairebé inhibida (assolint valors de germinació pròxims al 100% per a la majoria dels extractes) però en canvi sí que es va veure inhibit el seu creixement radicular, mostrant uns valors inferiors al 50% respecte al control. Els extractes van ser analitzats i es van identificar els compostos responsables de l'efecte fitotòxic (un total de 33 compostos), tant àcids fenòlics com flavonoides. Els resultats d'aquest experiment aporten informació sobre el potencial efecte bioherbicida que tenen aquests extractes en certes males herbes problemàtiques en vinya així com la potencialitat d'aquestes substàncies per a ser alliberades en camp.

Els resultats dels diferents experiments mostren que tant el tipus de coberta com la gestió que fem d'aquesta, és un aspecte clau a l'hora de controlar certes espècies de males herbes en vinya. Al seu torn, conèixer el potencial fitotòxic de les diferents espècies estudiades com a coberta vegetal, així com les característiques biològiques de les males herbes que es desitgen controlar, ens permet poder definir un adequat maneig de la coberta. Aquests resultats, en conjunt, verifiquen la capacitat de les cobertes vegetals per a controlar males herbes problemàtiques, contribuint a la implementació de manejos integrats més complets.

Capítulo 1

Introducción general y objetivos

Malas hierbas y malherbología

Desde el advenimiento de la agricultura, las malas hierbas (malezas) han sido fieles acompañantes de los cultivos, evolucionando y mostrando diversidad de estrategias adaptativas a los distintos métodos de manejo que se han ido implementando. Su presencia ha sido siempre motivo de rechazo, bien por la competencia ocasionada con el cultivo, por la posible contaminación de productos o por la dificultad que ofrecen a la hora de realizar tareas agrícolas. A pesar de la constante lucha ejercida contra las malas hierbas, su alta capacidad de resiliencia las hace continuamente presentes en nuestros cultivos, siendo necesario mantener siempre estrategias para su control. La eficacia de estos métodos de control dependerá de la idoneidad de su implementación, de la especie diana contra la que se lucha, momento y recurrencia de la actuación y posible integración de herramientas de control disponibles.

La Sociedad Española de Malherbología (SEMh) y la Sociedad Europea de Malherbología (European Weed Research Society, EWRS) definen a las malas hierbas como “cualquier planta o vegetación que interfiere con los objetivos del hombre”, siendo estas el objeto de estudio de lo que conocemos como “Malherbología”, la cual se define a su vez como “Ciencia pluridisciplinar que estudia la biología y el control de las malas hierbas” (SEMh).

El impacto generado por una mala gestión de las malas hierbas puede llegar a ser significativo y acarrear pérdidas cuantiosas, cuyo potencial se ha estimado, en promedio, en un 34% del rendimiento anual para los cultivos más extendidos a nivel mundial, valor muy superior al estimado para plagas (18%), patógenos (16%) o virus (3%) (Oerke 2006). A su vez, la implementación de medidas de control, permiten reducir las pérdidas causadas por malas hierbas en un 74%, cifra muy superior a la que aportan, en promedio, los métodos de control contra los agentes anteriormente citados (39%, 32% y 5%, respectivamente) (Oerke, 2006). Los esfuerzos para mantener las malas hierbas bajo control quedan reflejados en la cantidad de herbicidas que se aplican anualmente. Así, durante el año 2017, el 22,3% de los fitosanitarios comercializados en España fueron herbicidas, lo que se traduce a 16.077 toneladas, cifra que representa un incremento de un 2,5% respecto al año anterior (MAPA, 2017). La gestión de las malas hierbas depende de la especie o especies diana en cuestión, así como del sistema de producción del cultivo en el que se encuentre, donde podremos implementar unos u otros manejos. Independientemente del cultivo del que se trate, se deben tomar en consideración algunas cuestiones: ¿Cuál es el problema al que nos enfrentamos? ¿Su presencia supone realmente un problema? ¿Cómo lo podemos gestionar para tener una solución a largo plazo? La presencia de malas hierbas, *per se*, puede no suponer un problema si la infestación se encuentra en un nivel admisible o si su presencia es meramente puntual. Esta premisa es válida de forma general, pero la capacidad de dispersión y de infestación de algunas especies de malas hierbas hace que su sola presencia requiera de actuaciones para controlarlas o, incluso erradicarlas con el fin de evitar mayores problemas a largo plazo. Por esta razón, resulta imprescindible conocer con exactitud el comportamiento de la especie a controlar: sus requerimientos de germinación y crecimiento, su capacidad de reproducción, diseminación y establecimiento, su persistencia en el campo una vez establecida, los daños que puede producir para el cultivo y cómo responde frente a los diferentes manejos que puedan llevarse a cabo para su control.

Malas hierbas en viña

España encabeza la lista de países con mayor superficie dedicada al cultivo de la viña, con 967.234 hectáreas, de las cuales, un 58,8% se encuentran en secano y un 41,2% en regadío (MAPA, 2019) y que supone un 13% de la superficie a nivel mundial, seguido por China (12%), Francia (11%), Italia (9%) y Turquía (6%) (OIV, 2019).

En el cultivo de la viña, las malas hierbas compiten principalmente por agua y nutrientes, llegando a reducir el crecimiento de las cepas y la producción. La gestión de las malas hierbas en viña se suele llevar a cabo, de forma mayoritaria, a través del laboreo del suelo, aunque el mantenimiento del suelo del viñedo con cubiertas vegetales es una opción que está en auge (MAPA 2018). A su vez, el espacio bajo la fila de la viña, zona crítica por la posible competencia entre las cepas y las malas hierbas, se suele mantener libre de vegetación mediante intervenciones mecánicas o aplicaciones herbicidas. No obstante, en las últimas décadas, la implementación de acolchados orgánicos y de cubiertas vegetales vivas bajo la fila de la viña, ha tomado relevancia como sistema de manejo de malas hierbas debido a la presión que pueden ejercen sobre estas (Abad et al., 2018; DeVetter et al., 2015; Valencia et al., 2018). Como en otros cultivos, el manejo del suelo del viñedo puede condicionar la composición y estructura de la comunidad de malas hierbas. Gago et al. (2007) observó que el número de especies de malas hierbas en viña era claramente menor en los manejos que incorporaban la siembra de una cubierta vegetal y que las especies presentes variaban, a su vez, en función de cómo se gestionaba esa cubierta (mediante segadora o aplicaciones herbicidas sistémicos o de contacto). Estos resultados ponen de manifiesto la importancia de seleccionar un manejo del suelo adecuado teniendo en cuenta las especies de malas hierbas presentes.

De entre las distintas especies de malas hierbas que podemos encontrar en este escenario, algunas presentan ciertas particularidades que las hacen difíciles de controlar. El presente trabajo se centra en dos especies de malas hierbas que no sólo han alcanzado gran protagonismo en viñedos en nuestro país, sino también por mostrar particularidades biológicas singulares que obliga a planteamientos de programas de control específicos acorde a su biología, persistencia y dispersión. Estas especies son *Cynodon dactylon* y *Conyza bonariensis*.

Cynodon dactylon (L.) Pers.

La grama (*Cynodon dactylon*) es una gramínea plurianual vivaz que está considerada como una de las más nocivas a nivel mundial (Holm et al., 1997). Esta especie plurianual muestra una alta capacidad de propagación a través de rizomas y estolones, que mantienen yemas latentes durante el invierno. Presenta un ciclo preferentemente estival y una alta tolerancia a la sequía (Fernández, 2003; Huang et al., 1997). Esta especie muestra un periodo de desarrollo comprendido entre primavera (con la brotación de rizomas y expansión de estolones tanto subterráneos como aéreos) y finales de otoño (con la parada vegetativa), por lo que su capacidad de competir con el viñedo resulta vigorosa e incluso agresiva. Al ser una gramínea C4, de la subfamilia de las Cloridoideas, le confiere una alta capacidad de desarrollo durante los periodos de alta temperatura (finales de primavera, verano y principios de otoño), mostrando un alto éxito en sus tasas fotosintéticas. Este comportamiento dificulta la posible eficacia de los métodos de control químico, los cuales pueden mostrar eficacias muy distintas en función de la materia activa, dosis, momento de aplicación y combinación con otras herramientas (Abdullahi, 2002; Bryson y Wills, 1985; Farthing et al. 2018; Gómez de Barreda et al., 2017). Por otro lado, esta especie garantiza su presencia en campañas siguientes gracias a mantener las yemas en estado latente cuando las temperaturas son inferiores a los 10 ºC (McCarty and Miller, 2002). Los métodos de control mecánico, tales como pases de picadora o laboreo, no llegan a ser totalmente eficaces en su control, e incluso pueden favorecer la expansión de los órganos vegetativos, por lo que resulta imprescindible combinar estos métodos con otras estrategias de control como aplicaciones herbicidas o manejos que intercepten la luz, como puede ser una cubierta vegetal vigorosa (Abdullahi, 2002; Fernandez, 2003; Recasens y Valencia, 2015).

Conyza bonariensis (L.) Cronquist.

Conyza bonariensis es una especie originaria de América del Sur, perteneciente a la familia de las asteráceas. Suele mostrar un comportamiento anual, aunque no resulta nada extraño observarla con un ciclo bienal, especialmente en cultivos de frutales y viñedos del área Mediterránea (Recasens y Conesa, 2009). Esta especie predomina en cultivos donde no se realizan labores del suelo y que su manejo resulta exclusivamente dependiente de la aplicación de herbicidas. Su nocividad resulta especialmente importante cuando se presenta bajo la línea en cultivos leñosos, como frutales o viñedos. La alta dispersión de sus aquenios por el viento, su fácil instalación en zonas no cubiertas por otras especies vegetales y su alto nivel de tolerancia a los tratamientos herbicida -según su estado fenológico-, constituyen elementos que la convierten en una especie altamente nociva. *Conyza bonariensis* está considerada como una de las más problemáticas a nivel mundial (Bajwa et al., 2016), habiendo mostrado una gran expansión en distintas zonas geográficas del globo. Su presencia en España está notificada a lo largo de todo el territorio nacional (Anthos, 2020). Sus altas tasas de fecundidad, su fácil dispersión anemócora y su germinación escalonada contribuyen de forma notable en su persistencia (Kempen y Graf, 1981; Savage et al., 2014). A ello hay que añadir la inherente capacidad de desarrollar resistencia a diferentes grupos de herbicidas, conociéndose hasta cuatro mecanismos de acción distintos, siendo el más relevante la mutación del enzima EPSPS que le confiere resistencia al herbicida glifosato (Heap, 2020).

Gestión de malas hierbas mediante cubiertas vegetales

Como hemos comentado anteriormente, la implementación de cubiertas vegetales en viñedo constituye una alternativa a los tradicionales métodos de laboreo del suelo. Una cubierta vegetal viva se define como aquella técnica consistente en mantener con hierba el suelo del cultivo, bien dejando crecer la vegetación natural o bien sembrando una especie vegetal para tal fin (EVENA, 2012). La cubierta puede abarcar una determinada zona del viñedo (cubierta localizada), estar combinada con otras técnicas como el laboreo (por ejemplo en calles alternas), o puede abarcar toda la superficie del viñedo (cubierta total). En el caso de cubiertas sembradas, destaca el uso preferente de especies gramíneas y leguminosas (Guerra y Steenwerth, 2011; Pardini et al., 2002). Por otro lado, la zona bajo la fila de cultivo se suele mantener libre de vegetación (Ibáñez, 2015), aunque estudios recientes demuestran el efecto de la siembra de cubiertas en estas zonas en el control de malas hierbas (Abad et al., 2018).

En España, la superficie de viñedo donde se mantiene el suelo mediante cubiertas vegetales abarca un porcentaje mucho menor en comparación con viñedos con laboreo. Durante el año 2018, la técnica de manejo de suelo más implementada en viñedo fue el laboreo mínimo (laboreo superficial a una profundidad menor de 20 cm) con 628.648 ha, seguido por el laboreo tradicional (con una profundidad igual o superior a 20 cm) con 250.327 ha. En cuanto a la superficie de viñedo con cubierta vegetal espontánea (suelo sin labor mecánica y con cubierta vegetal espontánea) fue de 47.570 ha, seguido por 38.399 ha sin mantenimiento (terreno que en la última campaña no ha recibido labor ni ningún tipo de control de la vegetación, bien sea mecánica, química o pastoreo). El no laboreo (mantenimiento del suelo sin labor mecánica y sin cubierta vegetal) se realizó en 3.929 ha y la cubierta inerte (suelo cubierto de restos de poda, piedra u otros compuestos inertes) representó 3.053 ha. La cubierta vegetal sembrada (suelo sin labor, con una cubierta sembrada) sigue estando muy alejada del resto de manejos, con 1.704 ha, aunque este valor es muy superior a años anteriores, si la comparamos con las 362 ha del año 2008 (MAPA 2008, 2018).

A pesar del menor uso de cubiertas vegetales en viñedo, su implementación conlleva una serie de ventajas. Una cubierta vegetal mejora la infiltración de agua en el suelo (Gaudin et al., 2010) y lo protege contra la erosión, siendo la viña uno de los cultivos más susceptibles a este fenómeno, especialmente aquellos viñedos que mantienen el suelo desnudo (Prosdocimi et al., 2016; Rodrigo-Comino et al., 2018). La cubierta vegetal mejora la estructura del suelo y a su vez, mejora la capacidad de retención de agua (Gabriel et al., 2019) y aumenta el contenido de materia orgánica (Morlat y Jacquet, 2003). Por otro lado, la cubierta vegetal es una herramienta que permite controlar el vigor de las cepas, lo que puede traducirse en una mejora de la sanidad de la cosecha y calidad del producto (Ibáñez et al., 2011, 2015). A su vez, la cubierta puede considerarse una herramienta más en la gestión de plagas ya que permite un incremento de la presencia de entomofauna beneficiosa en los viñedos (Sáenz-Romo *et al.,* 2019) además de ser eficaz en el control de malas hierbas dado que dificulta su emergencia y desarrollo (Gago et al., 2007; Miglécz et al., 2015; Olmstead et al., 2001).

En cuanto al beneficio de las cubiertas vegetales en el control de malas hierbas, su eficacia depende de varios factores, siendo clave el tipo de cubierta seleccionada (natural o sembrada), su temporalidad (permanente o temporal) y su posible integración con otros métodos de control.

Cubiertas naturales

Las cubiertas naturales son las constituidas por la propia flora que se desarrolla en el viñedo, siendo su composición variable en función del manejo que se lleva a cabo, pudiendo favorecer la selección de unas u otras especies. Para alcanzar una adecuada cubierta espontánea, se desea que el manejo favorezca la presencia de especies vegetales poco competitivas con el cultivo y con una estructura y composición lo más homogénea posible. Por otro lado, se desea que pueda ejercer un efecto opresor sobre especies de malas hierbas más agresivas, y/o de fácil expansión. Así, malas hierbas con distinta forma biológica requerirán de manejos de la cubierta distintos para poder ser controladas; por ejemplo, los constantes pases de picadora en una cubierta pueden favorecer la presencia no deseada de especies plurianuales que se regeneren a partir de yemas situadas en la base de la planta como *Aster squamatus* (Spreng.) Hieron. o *Plantago lanceolata* L. o bien de especies con órganos subterráneos de propagación como *Cynodon dactylon*. En estos casos, el manejo puede requerir además de aplicaciones herbicidas localizadas (Gago et al., 2007; Recasens et al., 2018). La combinación de una cubierta espontánea con aplicaciones localizadas de herbicidas allí donde se dé la presencia de especies no deseadas, puede favorecer la presencia de una vegetación en la calle del viñedo que ayude a configurar una cubierta vegetal adecuada. Sin embargo, debe tenerse también en cuenta la posible presencia de especies de difícil control químico (p.e. *Malva sylvestris* L., *Bassia scoparia* (L.) A. J.) o incluso de biotipos que han podido llegar a desarrollar resistencia a una materia activa concreta, caso ya conocido para *Conyza bonariensis* a glifosato, en campos de frutales y olivo en España (Urbano et al., 2007). Esta situación obliga a plantear de forma rigurosa la integración de métodos de control químico junto con métodos mecánicos de manejo de la cubierta no sólo para obtener la máxima eficacia de control de aquellas malas hierbas más nocivas, sino también para prevenir el posible riesgo de selección de resistencias (Recasens et al., 2018).

Cubiertas sembradas

Si en un viñedo se lleva a cabo la siembra de una cubierta concreta, es importante que la especie o especies seleccionadas cumplan una serie de requisitos para que pueda competir eficientemente frente a las malas hierbas. A la hora de seleccionar la especie a sembrar es necesario tener en cuenta varios atributos. Así, la posibilidad de un rápido establecimiento y que genere gran cantidad de biomasa será un factor positivo, que deberá acompañarse, en especies anuales, de una buena capacidad de autosiembra o, en el caso de plurianuales, de una alta persistencia y estabilidad. La posibilidad de liberación de sustancias aleloquímicas que inhiban la presencia de malas hierbas nocivas será también un valor añadido. En cualquier caso, resulta también importante valorar el coste de su instalación, la disponibilidad comercial de semillas, la gestión de la futura cubierta y el posible efecto sobre el viñedo (Ibáñez, 2015; Lemessa y Wakjira, 2015).

Como hemos comentado, las cubiertas vegetales permiten ejercer presión sobre las malas hierbas compitiendo con ellas por recursos, como agua, luz, espacio y nutrientes, pero también pueden ejercer una competencia indirecta mediante la liberación de compuestos aleloquímicos que interfieren con su desarrollo (Farooq et al., 2011; Sturm et al., 2018). Se define la alelopatía como “cualquier efecto (directo o indirecto, perjudicial o beneficioso, incluyendo microorganismos) causado por una planta sobre otra a través de la liberación al medio ambiente de compuestos químicos" (Rice, 1984). En este sentido, la alelopatía ha abierto, en los últimos años, un nuevo e interesante escenario en el control de malas hierbas. La implementación de cubiertas vegetales en la calle del viñedo, o de acolchados orgánicos bajo la línea del cultivo, no resulta tampoco ajeno a este hecho. En efecto, conocer el potencial alelopático de aquellas especies que pueden utilizarse como cubierta vegetal o acolchado, abre prometedoras expectativas en el planteamiento de un escenario de competencia tanto directa como indirecta contra las malas hierbas.

Estos antecedentes, permiten plantear el trabajo de investigación que configura la presente tesis doctoral, centrado en el manejo de malas hierbas en viñedos, profundizando en el comportamiento biológico de especies nocivas y de difícil control y en la integración de distintas herramientas –especialmente el uso de cubiertas vegetales- para alcanzar un manejo del suelo eficaz y sostenible de nuestros viñedos.

Hipótesis y objetivos

La presente tesis se centra en el manejo de malas hierbas problemáticas en viñedo mediante distintas herramientas que permitan optimizar su control. Con este fin, se han considerado dos de las especies de malas hierbas más importantes presentes en viñedos, *C. dactylon* y *C. bonariensis*, especies con distintas características biológicas: plurianual vivaz la primera y anual/bienal la segunda, hecho que permite plantear un manejo diferencial en función de su presencia.

Cynodon dactylon es una especie rizomatosa y estolonífera que le permite una rápida y agresiva ocupación del espacio. Resulta poco conocida su respuesta al distinto manejo del suelo mediante la realización de labores, pases de segadora, implementación de una cubierta vegetal o su combinación con aplicaciones herbicidas. Tampoco se dispone de información acerca su respuesta al posible efecto opresor ejercido por cubiertas vegetales con distinta composición florística. Especialmente ante aquellas constituidas por especies que pueden generar gran cantidad de biomasa y que tengan efecto alelopático sobre la mala hierba.

Por su parte, *C. bonariensis* es una especie de fácil dispersión y con una germinación y emergencia muy escalonada, hecho que dificulta la planificación de estrategias de control. Se dispone de escasa información acerca de los factores que rigen su germinación, y por ende, la emergencia, aspectos clave a la hora de tomar decisiones en la planificación de estrategias de manejo integrado. A su vez, la prácticamente nula información disponible acerca su respuesta a posibles efectos alelopáticos causados por especies vegetales potencialmente útiles como cubiertas o acolchados, constituye un área de investigación aún por profundizar. La obtención de toda esta información favorecerá la integración de distintas herramientas de control de esta especie mediante métodos químicos, mecánicos y culturales. A su vez, ante la proliferación y difícil control de esta especie bajo la línea del cultivo, la posible instalación de un acolchado generará un efecto tanto físico como químico sobre la emergencia de la mala hierba. Por todo ello, determinar el comportamiento germinativo y de emergencia de *C. bonariensis*, permitirá tomar una decisión en el momento más oportuno, bien mediante la realización de tratamientos herbicidas, o bien mediante la instalación de aquellos acolchados más efectivos.

Los objetivos que configuran el presente trabajo de investigación son:

1. Evaluar la influencia de distintos manejos del suelo (cubierta espontánea o la implementación de una cubierta sembrada), en la dispersión y desarrollo de *C. dactylon* en las calles de la viña, así como su efecto en la comunidad de malas hierbas.
2. Evaluar el efecto de distintas cubiertas vegetales sembradas en las calles de un viñedo, en la dispersión y desarrollo de *C. dactylon,* así como en la comunidad de malas hierbas, y evaluar, a su vez, el efecto de las labores de preparación del suelo para la instalación de las cubiertas, en el control y dispersión de esta especie.
3. Estudiar el comportamiento germinativo de *C. bonariensis* en función de la temperatura y evaluar posibles variaciones en función del origen o procedencia de la población.
4. Evaluar el potencial fitotóxico de distintas especies vegetales con potencial interés como cubiertas vegetales y/o acolchados orgánicos frente a malas hierbas de difícil control en viñedos como *C. bonariensis A. squamatus* o *B. scoparia*.

Metodología

Para alcanzar los distintos objetivos, se llevaron a cabo ensayos en campo y trabajos experimentales en laboratorio. Estos trabajos se desglosan en los siguientes capítulos:

Capítulo 2: Efecto del manejo del suelo sobre Cynodon dactylon *(objetivo 1).*

Para el objetivo 1, se planteó un ensayo en una viña (*Vitis vinifera* L.) variedad 'Cabernet Sauvignon' plantada en el año 1988 en la finca "Anatere" de Raimat (41º40'41"N, 0º28'29"E) donde se evaluaron cuatro manejos distintos del suelo. El ensayo se llevó a cabo durante el periodo 2015-2017. Esta parcela contaba con numerosos rodales de grama, seleccionando para el estudio, varias calles del viñedo con dicha presencia.

Figura 1. Vista general del ensayo del objetivo 1. Cubierta vegetal espontánea labrada anualmente (centro). Abril de 2016.

Los cuatro sistemas de manejo del suelo fueron: M1: Cubierta vegetal espontánea controlada mediante siegas mecánicas. M2: Cubierta vegetal espontánea controlada mediante siegas mecánicas combinada con aplicaciones herbicidas localizadas en los rodales de grama. M3: Cubierta vegetal espontánea labrada anualmente y controlada mediante siegas mecánicas. M4: Laboreo del suelo y siembra anual de una cubierta de cebada (*Hordeum vulgare* L.) a una dosis de 150 kg ha^{-1} y controlada mediante siegas mecánicas. Se seleccionaron distintas calles para cada manejo y en ellas se marcaron zonas de muestreo de 30 m^2 donde se estimó, durante la campaña, la cobertura de la grama en distintas fechas, con un total de cuatro repeticiones por manejo. En las parcelas de seguimiento, se realizaron, a su vez, inventarios florísticos para evaluar cambios en la composición de la flora en función de los distintos manejos implementados. Los resultados de este trabajo están recogidos en el capítulo 2.

Capítulo 3: Evaluar el efecto de distintas cubiertas vegetales sobre Cynodon dactylon *(Objetivo 2).*

Para este ensayo se seleccionó, en la parcela número 30 de Raimat (41º40’22”N, 0º29’23”), una parcela de *V. vinífera* variedad ‘Pinot noir’ plantada en el año 2015, que presentó a los pocos meses una alta infestación de grama.

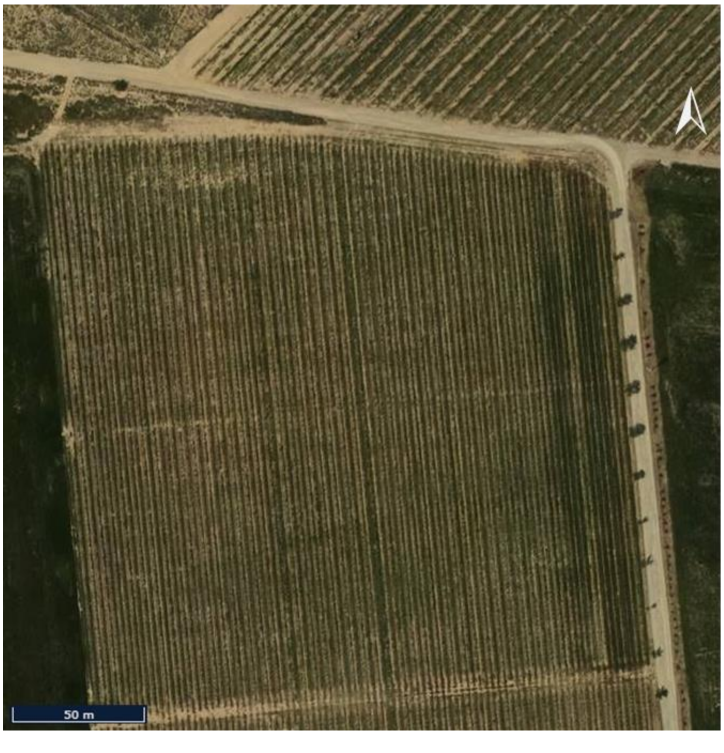

Figura 2. Parcela donde se instalaron las cinco cubiertas evaluadas en el objetivo 2.

Para valorar, de forma preliminar a la siembra de las cubiertas, el efecto de la labor sobre la grama y su posible dispersión en la parcela, se seleccionaron nueve zonas de muestreo de 30 m^{-2} en distintas calles y se muestrearon antes (octubre 2015) y después (noviembre 2015) de labrar la parcela.

Para valorar la presión ejercida sobre la grama por parte de las distintas especies implementadas como cubierta vegetal se sembrarona lo largo de distintas calles cinco cubiertas vegetales durante las campañas 2016-2017 y 2017-2018. En el total de calles sembradas se marcaron 40 zonas de muestreo de 30 m^2 (8 repeticiones por cubierta). En estas zonas se muestreó la presencia de grama en distintas fechas. Las cubiertas sembradas fueron: C1: *Festuca arundinacea* Schreber (40 kg ha^{-1}); C2: *Hordeum vulgare* (150 kg ha^{-1}); C3: Una mezcla de *Bromus rubens* L. y *Bromus hordeaceus* L. (20 kg ha^{-1}); C4: *Vulpia ciliata* Dumort (20 kg ha^{-1}); y C5: *Medicago rugosa* Desr. (35 kg ha^{-1}). Al final de la campaña, en las zonas de muestreo, se realizaron también inventarios florísticos para analizar la evolución de la flora silvestre. Los resultados de este ensayo están recogidos en el capítulo 3.

Figura 3. Vista general del ensayo (abril 2017) donde se evaluaron cinco cubiertas vegetales sembradas (objetivo 2). Calle central: *Hordeum vulgare*, a su izquierda: *Vulpia ciliata* y derecha: mezcla de *Bromus rubens* y *B. hordeaceus*.

Capítulo 4: Desarrollo de un modelo germinativo de Conyza bonariensis *(objetivo 3)*

Se estudió el comportamiento germinativo de cuatro poblaciones de *C. bonariensis* recolectadas en diferentes hábitats en España en septiembre de 2016 y en Argentina en noviembre de 2017. Las tres poblaciones españolas fueron recolectadas en un viñedo en Lleida (41º39'29"N, 00º31'26"E), en un jardín en Sevilla (37º21'12"N, 05º56'20"W) y en un olivar en Badajoz (38º09'13"N, 05º34'24"W); la población argentina se recolectó en un jardín en Bahía Blanca (38º41'43"S, 62º15'12"W). Las semillas de las distintas poblaciones se sometieron a diversos test a temperaturas tanto constantes como alternas con el fin de estudiar el comportamiento germinativo de esta especie bajo distintas condiciones térmicas y estimar las temperaturas cardinales (temperatura base, óptima y máxima) de cada población. Este estudio se llevó a cabo en el Departamento de Agronomía, Universidad Nacional del Sur y CONICET (Bahía Blanca, Argentina). Los resultados de este experimento están recogidos en el capítulo 4.

Capítulo 5: Evaluar el potencial fitotóxico de distintas especies vegetales con potencial interés como cubiertas vegetales y/o acolchados orgánicos (objetivo 4).

Se seleccionaron 12 especies vegetales con potencial interés para ser utilizadas como cubierta vegetal y/o acolchado orgánico vegetal en viñedo, con el fin de evaluar su posible efecto fitotóxico frente a tres especies de malas hierbas problemáticas en viña: *C. bonariensis, A. squamatus* y *B. scoparia*. Las semillas de estas tres especies se recolectaron en un viñedo en septiembre de 2016 (41º39'29"N, 0º31'26"E) en la localidad de Raimat (Lleida, España). Las especies seleccionadas para evaluar su efecto aleloquímico fueron: *Bromus* spp. *(B. hordeaceus y B. rubens*), *Festuca arundinacea* cv. \`Firaces R1´, *Hoderum murinum* L., *Hordeum vulgare* cv. \`Meseta´, *Medicago rugosa* cv. \`Sapo´, *Medicago sativa* L. cv. \`Victoria´, *Phacelia tanacetifolia* Benth., *Sinapis alba* L., *Trifolium incarnatum* L. cv. \`Red´, *Trifolium subterraneum* L. cv. \`Dalkeith´, y *Vulpia ciliata.* Estas especies se sembraron en parcelas de la Universidad de Lleida en noviembre 2017, obteniendo en el mes de abril de 2018 cuando comenzaba el inicio de espigado o plena floración –según especies-, suficiente biomasa vegetal para su posterior secado y procesado. Se consideró también corteza y hoja de pino de *Pinus sylvestris* L. que fue obtenido de empresas de desforestación locales. Los posteriores ensayos de fitotoxicidad así como los análisis químicos pertinentes se llevaron a cabo en el laboratorio de Agrobiología Ambiental de la Universidade de Vigo (Vigo, España) en julio de 2017. Los resultados de este estudio están recogidos en el capítulo 5.

Capítulo 6: Discusión general y conclusiones

En el capítulo 6, se realiza una discusión general de los distintos resultados obtenidos en los distintos capítulos, con el fin de integrar esta información en el planteamiento de estrategias de manejo integrado de malas hierbas en viñedo. Además, se enumeran las conclusiones que se han alcanzado en la presente tesis, haciendo énfasis en el nivel de consecución de los distintos objetivos planteados.

Bibliografía

Abad, F.J., Cibriáin, L.G., Santesteban, D., Marín, D., Sagüés, A. (2018). Manejo del suelo bajo la línea de cultivo de viñedo sin empleo de herbicida ni laboreo. Actas *III Jornadas del Grupo de Viticultura,* Palma: 164-167.

Abdullahi, A.E. (2002). *Cynodon dactylon* control with tillage and glyphosate. *Crop Prot.* 21, 1093-1100.

Anthos. (2011). [2020]. Information System of the plants of Spain. Real Jardín Botánico, CSIC - Fundación Biodiversidad. Electronic resource at www.anthos.es. Consultation carried out in March 2020[15/03/2020].

Bajwa, A. A., Sadia, S., Ali, H. H., Jabran, K., Peerzada, A. M., & Chauhan, B. S. (2016). Biology and management of two important Conyza weeds: a global review. *Environ. Sci. Pollut. Res.* 23, 24694-24710.

Bryson, C., Wills, G. (1985). Susceptibility of Bermudagrass (*Cynodon dactylon*) biotypes to several herbicides. *Weed Sci*. 33, 848-852.

DeVetter, L., Dilley, C., Nonnecke, G. (2015). Mulches Reduce Weeds, Maintain Yield, and Promote Soil Quality in a Continental-Climate Vineyard. *Am. J. Enol. Viticult.* 66, 54-64.

EVENA (2012). Ensayos de cubiertas vegetales en viña. 1995-2010. Departamento de Desarrollo Rural, Medio Ambiente y Administración Local. Litografía Ipar. Pamplona, España, 2012, 182p.

EWRS (2020). European Weed Research Society. Last access on April 10, 2020 Available: http://www.ewrs.org/

Farooq, M., Jabran, K., Cheema, Z., Wahid, A., Siddique, K. (2011). The role of allelopathy in agricultural pest management. *Pest Manag. Sci.* 67, 493-506.

Farthing, T.S., Muir, J.P., Falk, A., Murray, D. (2018). Efficacy of seven invasive Bermudagrass removal strategies in three Texas ecoregions. *Ecol. Restor*. 36, 306–314.

Fernandez, O.N. (2003). Establishment of *Cynodon dactylon* from stolon and rhizome fragments. *Weed Res.* 43, 130-138.

Gabriel, J. L., Quemada, M., Martín-Lammerding, D., and Vanclooster, M. (2019). Assessing the cover crop effect on soil hydraulic properties by inverse modelling in a 10-year field trial. *Agr. Water Manage.* 222, 62-71.

Gago, P., Cabaleiro, C., García, J. (2007). Preliminary study of the effect of soil management systems on the adventitious flora of a vineyard in northwestern Spain. *Crop Prot.* 26, 584-591.

Gaudin, R., Celette, F., Gary, C. (2010). Contribution of runoff to incomplete off season soil water refilling in a Mediterranean vineyard. *Agric. Water Manag.* 97, 1534-1540.

Gómez de Barreda, D., Izquierdo, D., de Luca, V. (2017). Evaluación del comportamiento de 20 materias activas herbicidas sobre la cespitosa "*Cynodon dactylon*" en diferentes épocas climáticas del año. XVI Congreso de la Sociedad Española de Malherbología. 25-27 octubre 2017, Pamplona-Iruña, España, 245-250.

Guerra, B., Steenwerth, K. (2011). Influence of floor management technique on grapevine growth, disease pressure, and juice and wine composition: A Review. *Am. J. Enol. Viticult.* 63, 149-164.

Heap, I.M. (2020). International Survey of Herbicide Resistant Weeds. Last access on April 10, 2020. Retrieved from: http://www.weedscience.org.

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P. (1977). The World's Worst Weeds. Distribution and Biology. University of Hawaii Press, Honolulu, USA, 1977.

Huang, B., Duncan, R.R., Carrow, R.N. (1997). Drought-resistance mechanisms of seven warm-season turfgrasses under surface soil drying: II. Root aspects. *Crop Sci.* 37, 1863-1869.

Ibáñez, S., Pérez, J.L., Peregrina, F., García-Escudero, E. (2011). "Utilización de cubiertas vegetales en viñedos de la DoC Rioja (España)". Le Bulletin de l'OIV, 84, 347-360.

Ibáñez, S. (2015). Mantenimiento del suelo en el viñedo mediante cubiertas vegetales. Ed. Gobierno de la Rioja. 2015, 167 p.

Kempen, H. M., Graf, J. (1981). Weed seed production. Proceedings of the Western Society of Weed Science, 34, 78–81.

Lemessa, F., Wakjira, M. (2015) Cover crops as a means of ecological weed management in agroecosystems. *J. Crop Sci. Biotech.* 18, 123-135.

MAPA (2008). Encuesta sobre superficies y rendimientos de cultivos. (ESYRCE) Resultados 2008. Ministerio de Agricultura, Pesca y Alimentación.

https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/boletin2008_tcm30-122322.pdf

MAPA (2017). Encuesta de Comercialización de Productos Fitosanitarios. Ministerio de Agricultura, Pesca y Alimentación. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/publicacion_2017web_26_12_2018_tcm30-500303.pdf

MAPA (2018). Encuesta sobre superficies y rendimientos de cultivos. (ESYRCE) Resultados 2018. Ministerio de Agricultura, Pesca y Alimentación. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/boletin2018_tcm30-504212.pdf

MAPA (2019). Encuesta sobre superficies y rendimientos de cultivos. (ESYRCE) Resultados 2019. Ministerio de Agricultura, Pesca y Alimentación. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/comentariosespana2019_tcm30-522390.pdf

McCarty, L.B., Miller, G. (2002). Managing bermudagrass turf. E. Ann Arbor Press. Chelsea, MI, 2002 pp. 67-86.

Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., Drexler, D., Tóthmérész, B. (2015). Establishment of three cover crop mixtures in vineyards. *Sci. Hortic.* 197, 117-123.

Morlat, R., Jacquet, A. (2003). Grapevine root system and soil characteristics in a vineyard maintained long-term with or without interrow sward. *Am. J. Enol. Viticult.* 54, 1-7.

Oerke, E. (2006). Crop losses to pests. *J. Agri. Sci.* 144, 31-43.

OIV (2019). International Organisation of Vine and Wine. Statistical report on world vitiviniculture (2019). http://www.oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf

Olmstead, M.A., Wample, R.L., Greene, S.L., Terara, J.M. (2001). Evaluation of potential cover crops for inland Pacific northwest vineyards. *Am. J. Enol. Vitic.* 52, 292-303.

Pardini, A., Faiello, C., Longhi, F. (2002). Cover crop species and their management in vineyards and olive groves. *Adv. Hortic. Sci.* 16, 225-234.

Prosdocimi, M., Cerdà, A., Tarolli, P. (2016). Soil water erosion on Mediterranean vineyards: A review. *Catena*, 141, 1-21.

Recasens, J., Conesa, J.A. (2009). Malas hierbas en plántula: Guía de identificación. Ed.

Universitat de Lleida y Bayer CropScience. 2009. 454 p.

Recasens, J., Valencia, F. (2015). Manejo de malas hierbas en viñedo mediantes cubiertas vegetales dirigidas. *Phytoma*, 274, 100-101.

Recasens, J., Valencia, F., Montull, J.M., Taberner, A. (2018). Malas hierbas problemáticas en viñedos con cubiertas vegetales y métodos químicos para su control. *Vida rural*, 4, 48-58.

Rice, E.L. (1984). Allelopathy, 2nd ed. Academic Press, Orlando. pp: 189.

Rodrigo-Comino, J., Keesstra, S., Cerdà, A. (2018). Soil erosion as an environmental concern in vineyards. The case study of Celler del Roure, Eastern Spain, by means of rainfall simulation experiments. *Beverages*. 4, 31.

Sáenz-Romo, M.G., Veas-Bernal, A., Martínez-García, H., Campos-Herrera, R., Ibáñez-Pascual, S., Martínez-Villar, E., Pérez-Moreno, I., Marco-Mancebón, V.S. (2019). Ground cover management in a Mediterranean vineyard: Impact on insect abundance and diversity. *Agric. Ecosyst. Environ.* 283.

Savage, D., Borger, C. P., Renton, M. (2014). Orientation and speed of wind gusts causing abscission of wind-dispersed seeds influences dispersal distance. *Funct. Ecol.* 28, 973-981.

SEMh (2020). Sociedad Española de Malherbología https://semh.net/ Con acceso el 30-3-2020

Sturm, D.J., Peteinatos, G., Gerhards, R. (2018). Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res.* 58, 331-337.

Urbano, J., Borrego, A., Torres, V., Leon, J., Jimenez, C., Dinelli, G., Barnes, J. (2007). Glyphosate-resistant Hairy Fleabane (*Conyza bonariensis*) in Spain. Weed Technol. 21, 396-401.

Valencia, F., Mas, N., Arbonés, A., Cabrera, C., Recasens, J (2018). Uso de acolchados orgánicos y bioherbicidas en el control de malas hierbas en viña ecológica. Actas *III Jornadas del Grupo de Viticultura*, Palma: 177-181.

Chapter 2

Different ground vegetation cover management systems to manage *Cynodon dactylon* in an irrigated vineyard

Different ground vegetation cover management systems to manage *Cynodon dactylon* in an irrigated vineyard

Abstract

Ground cover management in vineyards in NE Spain is focused on minimizing soil erosion and compaction. Such practices have influenced the weed community structure in the inter-rows, contributing to the spread of the highly noxious weed *Cynodon dactylon* (L.) Pers. This fact highlights the need for further investigation of the interaction between ground cover practices and weed control techniques. In this study, the effect of four different ground cover managements in the inter-rows on *C. dactylon* population dynamics (evolution of coverage and frequency) was assessed over three seasons (2015-2017): M1) a no-till spontaneous vegetation ground cover managed by shredding; M2) a no-till spontaneous vegetation ground cover managed by shredding plus herbicide application; M3) tilled soil and spontaneous vegetation growing; and M4) tilled soil and a barley cover crop seeded (*Hordeum vulgare* L.). The various weed control methods responded differently to *C. dactylon* evolution but also other weeds presence. Management practices were compared in terms of efficacy, the barley cover crop being the most efficient management to control *C. dactylon* and other weeds development. Managements affected the weed coverage differently, with final percentage values of 0.5 and 1.1% for barley cover crop and tilled soil with spontaneous vegetation respectively, and 3.7 and 7.7% obtained by spontaneous vegetation mowed plus herbicide application and spontaneous vegetation only mowed. Total weed frequency also varied from 9.7% for barley cover crop to 45.8% for spontaneous vegetation only mowed. Weed community composition changed due to the pressure exerted by each management and the adaptive strategy of the different species. This study highlights the importance of knowledge of the ground management technique affecting weed flora to improve the sustainability of wine grape production systems.

Keywords: Integrated Weed Management; Bermudagrass; Cabernet Sauvignon; *Vitis vinifera*; barley.

Introduction

Grapes are at present, one of the most important and profitable crops in the world. Wine production increased by 17% from 2017 to 2018, with 292 million hL (global wine production in 2018), and the global vineyard cover was about 7.4 million hectares in 2018 (OIV, 2019). However, grape production can be reduced by the presence of weeds, which negatively affect the productivity of crops (Oerke, 2006) by competing for water and nutrients. Weed control in vineyards has usually been carried out by tillage or herbicide applications; however, the establishment of ground covers has gained interest and has been implemented for different reasons (Pardini et al., 2002; García et al., 2018). In general, vineyard soil management involves different procedures focused on organic matter improvement, water availability, biodiversity enhancement, reduction of soil erosion, vine vigour and weed control (Guerra and Steenwerth, 2012). However, tillage and herbicide applications have been changing over time for different reasons. On the one hand, leaving the soil surface without any ground cover increases the risk of erosion, with vines being one of the most affected crops (Cerdan et al., 2010; Prosdocimi et al., 2016). Furthermore, the use of herbicides as the main tool to control weeds can not only contaminate the environment but can also have a negative effect on the vines, such as reducing root mycorrhization or altering nutrient composition in grapevine roots, leaves or grape juice (Zaller et al., 2018). Reiterative use of herbicide increases the risk of weed herbicide resistance evolution, with 500 cases of herbicide-resistant weeds globally, and many of these species appear in the vineyard, such as *Conyza bonariensis*, *Lolium rigidum* or *Amaranthus retroflexus*, among others (Heap, 2020). In addition, environmentally-friendly agronomic techniques are desired because wine consumers and producers are more aware of the environmental impacts of wine and consumers have a positive image of organic cultivation methods (Stolz and Schmid, 2007; Viers et al., 2013).

Alternative soil managements to mechanical cultivation or herbicide applications are ground covers. Spontaneous ground covers or planted cover crops are sustainable floor management techniques that offer a variety of agronomic and environmental advantages, i.e. reduction of soil erosion and water runoff, increase of organic matter, nutrient availability or benefiting soil biota (Le Bissonnais et al., 2004; Steenwerth and Belina, 2008; Vukicevich et al., 2016). Moreover, weed management practices that promote higher plant diversity and density have shown to favor arthropod weed seeds predation, promoting beneficial entomofauna and contributing to weed growth suppression (Gago et al., 2007; Miglécz et al., 2015; Sáenz-Romo et al., 2019; Sanguankeo et al., 2011). Cover crops can compete with weeds for resources, but they can also release allelochemicals that interfere with weed development (Farooq et al., 2011; Sturm et al., 2018), that can be implemented to control difficult weed species, such as the case of *Cynodon dactylon* L. (Pers).

Cynodon dactylon is a stoloniferous and rhizomatous perennial grass considered as one of the world´s worst weeds (Holm et al., 1997). Its water stress tolerance makes it very efficient in water and nutrient use and its vegetative structures contribute to the colonization of new areas, especially when soil is disturbed (Guglielmini and Satorre, 2002, 2004; Kim et al., 2009). The base temperature for bud sprouting is 10 ºC (McCarty and Miller, 2002), below this temperature the weeds become dormant and control measures can be less effective. *Cynodon dactylon* (var "princess 77") has been reported as not controlled after a single glyphosate (N-(phosphonomethyl) glycine) application (36% at 4 L ha^{-1}) although different phytotoxic effects were observed on that species (Gómez de Barreda et al., 2017). However, different applications can reduce weed biomass and groundcover more than 99% after multiple herbicide applications times during the warm season with glyphosate (48.7% at 9 L ha ha^{-1}) (Farthing et al., 2018). Tillage can be performed to control *C. dactylon*, but it can be ineffective as several interventions are required. Furthermore, the soil disturbance performed for its control, achieved by reducing the regrowth capacity of the weed, contributes to the fragmentation and dispersal of its rhizomes and stolons, and from them, new plants can be formed (Abdullahi, 2002; Fernandez, 2003). In addition, it has been found that shading can reduce *C. dactylon* infestations due to the lack of tolerance to shade of this weed (Guglielmini and Satorre, 2002; McCarty and Miller, 2002).

When the establishment and management of the ground vegetation cover are focused on controlling *C. dactylon*, a dense canopy should be encouraged to cover this shade-intolerant species. In addition, because the ground cover management can influence weed flora composition (Gago et al., 2007), it is essential to select the most appropriate management (type of cover and the combination with other methods such as herbicide applications or tillage) to avoid the proliferation of other problematic weeds in vineyards, such as *Conyza bonariensis* or *Bassia scoparia* (Recasens et al., 2018).

Due to the different efficacy of tillage or herbicide applications on *C. dactylon* control, its integration with a ground cover could improve the control of this noxious weed. The hypothesis is that the presence of a vegetation cover, either natural (spontaneous) or implemented (barley, as a representative cover crop) can offer a competitive pressure against *C. dactylon*, modifying this growth and making the plant in a medium/long term more susceptible to other tools as shredding, tillage and herbicide applications. Different management methods could also affect the composition of weed communities.

Materials and methods

Study area

The experiment was conducted in a commercial vineyard in Raimat (Lleida, NE Spain) from 2015 to 2017. The climate is semi-arid Mediterranean with an annual rainfall of 342 mm and a mean annual temperature of 15 ºC (Spanish Meteorological Agency, AEMET). The vineyard was planted in May 1988 with Cabernet Sauvignon variety (*Vitis vinifera* L.). Rows were separated by 3 m and vine spacing of 1.5 m. Vines were trained as bilateral cordons and had drip irrigation.

The traditional soil management on the plantation consists of shredding the inter-rows spontaneous flora 2-3 times per season and 4-5 herbicide intra-row applications during the season. Herbicide applications were glyphosate (36 % at 3.5 L ha^{-1}), twice in winter and once in summer, and two applications of a mixture of glyphosate (36 % at 3.5 L ha^{-1}) and oxyfluorfen (24% at 3 L ha^{-1}), once in spring and once in summer. Herbicide applications were carried out with a tractor-mounted crop-sprayer with two fan-type nozzles. Initially, there was an important infestation, both intra- and inter-row of *C. dactylon*.

Experimental design

A completely randomized design was carried out, with four different soil management systems (treatments) placed in different inter-rows with four replications. There were not two consecutive inter-rows with the same soil system. Each management required different interventions (Table 1.):

Management 1 (M1): a no-till spontaneous vegetation ground cover managed by shredding. The cover was shredded three times per season (May, July, and September) according to biomass presence.

Management 2 (M2): a no-till spontaneous vegetation ground cover managed by shredding plus regular herbicide application focused on *C. dactylon* (glyphosate 36% at 3.5 L ha^{-1}). Each season the ground cover was shredded three times as in M1 and two herbicide applications were performed, one in May, when the weed was actively growing and another in November, when the plant stopped its development due to mean temperature dropping to below 10 ºC. Applications were focused in areas where *C. dactylon* was present. Herbicide was applied with a tractor-mounted crop-sprayer with five low drift nozzles (HARDI LD-110).

Management 3 (M3): inter-row tilling. In April 2015, the ground cover was shredded and the soil sub-soiled and tilled. The spontaneous cover was shredded in July and October and tilled in October. In 2016 the cover was shredded twice (June and October) and tilled once (October). In 2017, the ground cover was also shredded twice (June and October) but not tilled as the trial ended. Tillage was performed with a chisel plow at a soil depth of 20 cm.

Management 4 (M4): inter-row tilling and barley cover crop (*Hordeum vulgare* L.) sown in autumn. Management activities were similar to M3 plus sowing of a barley cover crop in November 2015 and 2016. Barley was shredded when its life cycle finished in June 2016 and 2017, providing a mulch of straw. Seed-bed preparation was performed with a chisel plow at a soil depth of 20 cm and barley var. Meseta was sown with a vineyard seeder. This barley variety was selected for its good coverage, with low height and high tillering capacity (Ibáñez, 2015). The sowing rate of barley was 150 kg ha^{-1}. No fertilization was applied.

Table 1. Timing of agricultural practices in the inter-rows for each management.

	2015			2016			2017		
	SPR	SUM	AUT	SPR	SUM	AUT	SPR	SUM	AUT
M1	Sh	Sh	Sh	Sh	Sh	Sh	Sh	Sh	Sh
M2	Sh + H	Sh	Sh + H	Sh+ H	Sh	Sh + H	Sh + H	Sh	Sh + H
M3	Sh+ S + T	Sh	Sh + T		Sh	Sh + T		Sh	Sh
M4	Sh + S + T	Sh	Sh + T + So		Sh	Sh + T + So		Sh	Sh

Abbreviations: SPR: Spring, SUM: Summer, AUT: Autumn, Sh: Shredding, H: Herbicide, S: Subsoiling, T: Tilling, So: Sowing.

Weed sampling

Cynodon dactylon coverage was monitored in 16 rectangular plots (four for each management) of 2 x 10 m situated in the center of the inter-rows, covering the area occupied by the ground vegetation cover. To carry out the surveys each plot was subdivided into 80 sub plots of 0.5 x 0.5 m. Weed coverage of each subplot and weed frequency (mean percentage of the 80 subplots per plot with weed presence) were recorded. Sampling was done in March (previously to management implementation) and July 2015, February, May and September 2016, and February, June and September 2017.

With the aim of reflecting the spatial and temporal dynamics of *C. dactylon* in the experiment, one plot of each ground management was represented using the graph builder of JMP Pro 14 software with the option “contour: shows regions of data density” (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, USA. SAS Institute, Inc.).

At the end of the experiment, weed surveys were performed in the plots in April of 2017.

Statistical analyses

Weed evolution (*C. dactylon* percentages of coverage and frequency between managements and sampling dates) and the final variation, were compared by several one-way ANOVAs and Tukey’s honestly significant difference (HSD) ($P < 0.05$). As field operations (till *vs* no till, herbicide *vs* no herbicide, and so on) affected *C. dactylon* coverage and frequency, it was decided to compare these variables separately for each management by date, and between managements for each date, discarding a two-way ANOVA. The original data was log or square-root transformed if needed to achieve normality and homoscedasticity. In the case of heteroscedasticity data were subjected to Kruskal-Wallis *H* test and post-hoc Dunn's test at $P \leq 0.05$. Analysis and graphs were made with JMP Pro 14 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, USA. SAS Institute, Inc.) and SigmaPlot 11.0 (Systat Software, San Jose, CA). In order to evaluate differences in weed species composition among the four management systems, a Redundancy Analysis (RDA) was performed. Furthermore, as a supplement of the RDA analysis, an analysis of the diagnostic species for each management system was performed, using the ϕ coefficient of association, which is independent of the number of samples, and is little affected by the relative size of the sample unit (Chytry et al., 2002). Both analyses were performed with CANOCO 5.0 (Ter Braak and Smilauer, 2012).

Results

Climatic conditions

Average monthly temperatures were similar during the period when *Cynodon dactylon* was actively growing (temperature above 10 ºC), but precipitations differed (Figure 1). Temperatures averaged 12-14 ºC between March and May (spring), 23-24 ºC between Jun and Aug (summer), 14-15 ºC Sep.-Nov (autumn) and 5-7 ºC between Dec.-Feb (winter). Total spring precipitation was lower in 2015 (40 mm) than 2016 and 2017, with 133 and 149 mm respectively. During the summer period, 2015 presented the highest precipitation (90 mm) followed by 2017 (78 mm), and the lowest being in 2016 (12 mm). In autumn 87 mm were registered in 2015 and 107 in 2016. It must be pointed out that autumn rains were concentrated in November, 64.5 mm and 72.0 mm falling, respectively in 2015and in 2016. Winter precipitation in 2015-16 doubled that of 2016-17 (92 mm *vs* 46 mm) (Figure 1).

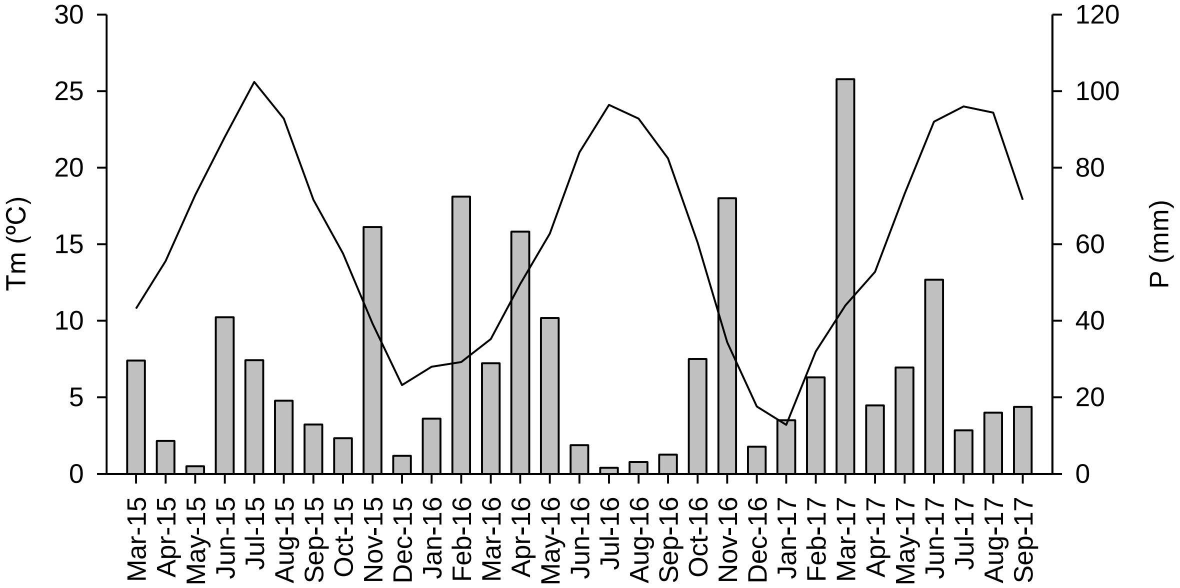

Figure 1. Climatic conditions for the experiment period (March 2015-September 2017). The black line indicates the mean monthly temperature (Tm) and the grey bars show total precipitation (P) in each month (AEMET).

Cynodon dactylon *evolution*

Infestation levels of *C. dactylon* varied among managements among the sampling dates, both in percentages of soil coverage and frequency (Tables 2 and 3).

Barley cover crop (M4) significantly reduced the *C. dactylon* coverage; from 7.4% to 0.5%, which means a reduction of 93.9% (Table 2). The spontaneous tilled cover also had a significant reduction, initially 6.6% of coverage that decreased to 1.1% at the last sampling date (82.6% of coverage reduction). The spontaneous cover crop plus herbicide application management (M2) produced a 62.4% reduction (coverage values that varied from 9.7% to 3.7%). In the case of M1, no-till spontaneous vegetation ground cover managed by shredding, the initial weed coverage value (4.2%) increased to 7.7%, which means an 82.5% increase, a value significantly different to those obtained by the managements with soil disturbance (M3 and M4) (Table 2).

Managements affected weed coverage differently depending on the season. Before management implementation, there were no significant differences (March 2015) between the plots selected for the four management systems. In July 2015, after tillage for M3 and M4, significant differences were observed between M1 and M2

(>3.2%) and M3 and M4 (≤0.5%). In February 2016, there were statistical differences between M1 (2.9%) in respect to M4 (0%). In May 2016, significant differences were only observed between M1 (7.4%) and M4 (0.1%). One year after the *H. vulgare* cover crop implantation (September 2016), M3 and M4 maintained low infestation levels (0.6%), significantly lower than in M1 (5.9%) but not than in M2 (4.9%). These differences were maintained until June 2017, but in September 2017 M4 showed the lowest *C. dactylon* coverage (0.5%), statistically similar to M3 (1.1%), but differing from M2 (3.7%) and M1 (7.7%).

Table 2. Soil covered (%) by *C. dactylon* in each management across the different sampling dates. Mean values of soil covered ± standard errors of the mean.

M	Mar.15	Jul.15	Feb.16	May 16	Sep.16	Feb.17	Jun.17	Sep.17	Variation
1	4.2±0.9 Aa	5.5±0.7 Aa	2.9±0.7 Aa	7.4±1.6 Aa	5.9±0.8 Aa	4.2±1.4 Aa	6.9±1.6 Aa	7.7±2.1 Aa	82.5±82.6 a
2	9.7±2.5Aa	3.2±1.2 Aa	1.8±1.1 Aab	1.3±0.5 Aab	4.9±2.4 Aab	4.3±2.1 Aa	3.7±1.2 Aa	3.7±1.1 Aa	-62.4±9.6 ab
3	6.6±3.2 Aa	0.5±0.1 Ab	0.1±0.1 Aab	0.6±0.2 Aab	0.6±0.4 Ab	0.4±0.2 Aab	1.2±0.6 Aab	1.1±0.8 Aab	-82.6±11.1 b
4	7.4±1.3 Aa	0.4±0.1 BCb	0.0±0.0 Cb	0.1±0.0 BCb	0.6±0.2 Bb	0.1±0.0 BCb	0.8±0.3 Bb	0.5±0.1 BCb	-93.9±2.3 b

Different letters represent significant differences at $p < 0.05$; capital letters, differences among sampling dates; lowercase letters: differences among managements.

Regarding the percentage of *C. dactylon* frequency, it decreased noticeably in those managements with soil disturbance (M3 and M4) (Table 3). The comparison between the first and the last sampling date during the three seasons showed a decrease of the presence of *C. dactylon* in M2 (14.6% coverage reduction), M3 (52%) and M4, which varied from 36.3% to 9.75% and was the only significant reduction (73.3% of frequency reduction). In no-till spontaneous vegetation ground cover managed by shredding (M1) the presence of the weed increased by 89.7%, a value significantly different from M3 and M4.

Before management implementation, (March 2015) the frequency of *C. dactylon* was similar among the plots selected. In July 2015, after tillage for M3 and M4, the weed frequency was greatly reduced for M3 and M4, with low values that are maintained until the last sampling date. At the end of the experiment (September 2017) shredding (M1) and shredding plus herbicide applications (M2) showed the highest percentages of frequency (45.8% and 36.6% respectively), followed by M3 (15.3%). The lowest value was obtained by barley cover crop management, at 9.7%.

Table 3. Frequency (%) of *C. dactylon* in each management across the different dates. Mean values of number of subplots with presence ± standard errors of the mean.

M	Mar.15	Jul.15	Feb.16	May 16	Sep.16	Feb.17	Jun.17	Sep.17	Variation
1	24.2±3.6 Aa	37.1±8.6 Aab	18.8±3.3 Aa	38.3±7.9 Aa	37.9±7.9 Aa	24.6±4.6 Aa	40.9±6.7 Aa	45.8±9.9 Aa	89.7±23.4 a
2	42.8±11.5 Aa	46.3±10.4 Aa	12.9±5.2 Aab	23.8±10.5 Aab	34.1±9.2 Aab	28.5±10.7 Aa	33.8±11.2 Aa	36.6±9.5 Aab	-14.6±17.6 ab
3	31.9 ±16.4 Aa	10.9±3.1 Ab	2.5±1.4 Abc	13.8±3.8 Aab	9.1±4.8 Ab	14.1±6.0 Aa	13.8±4.6 Aa	15.3±7.3 Aab	-52.0±16.7 b
4	36.3±5.7 Aa	7.8±0.6 Bb	0.6±0.6 Cc	3.1±1.7 BCb	9.7±0.6 Bab	3.8±1.5 BCa	12.8±3.6 Ba	9.7±3.0 Bb	-73.3±12.6 b

Percentage calculated as the number of the 80 subunits of each plot with presence of *C. dactylon*. Different letters in the same column mean significant differences at $p<0.05$; capital letters, differences among initial and final dates; lowercase letters: differences among managements.

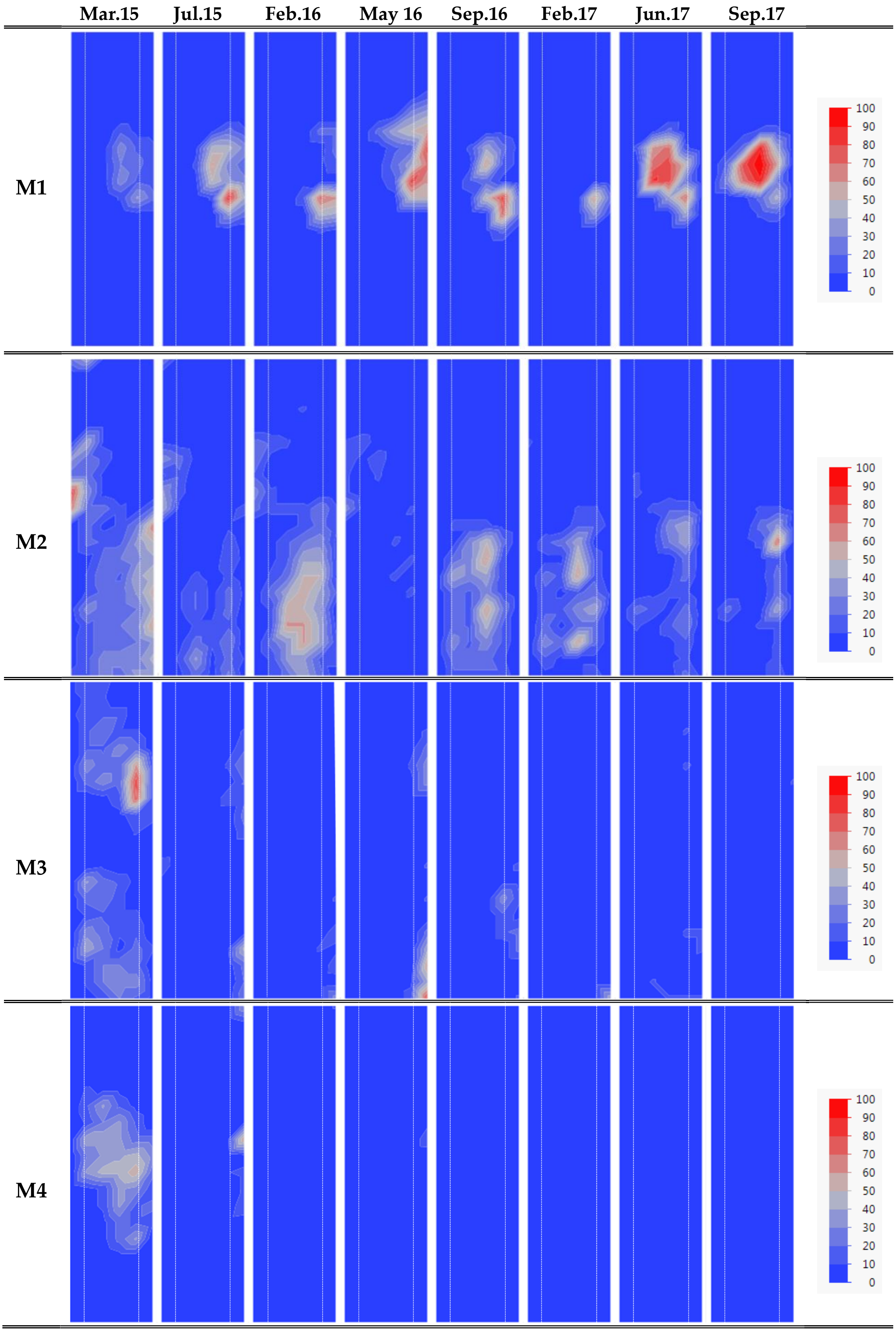

Figure 2. *Cynodon dactylon* evolution (percentage of soil covered) of one plot selected of each management across the different sample dates. Each plot is divided by two white lines in each lateral to differentiates the central part (the inter-rows), where the management is completely done from the borders (the intra-rows), where management is not complete and also mixed with intra-rows herbicide applications.

Weed flora evolution

Excluding *C. dactylon*, a total of 39 weed species were recorded in the weed flora surveys, with 31 species in M1, 14 in M2, 23 in M3 and 6 in M4. The RDA analyses showed a variance of 46.6 % on the weed community composition after three years of management (Figure 3).

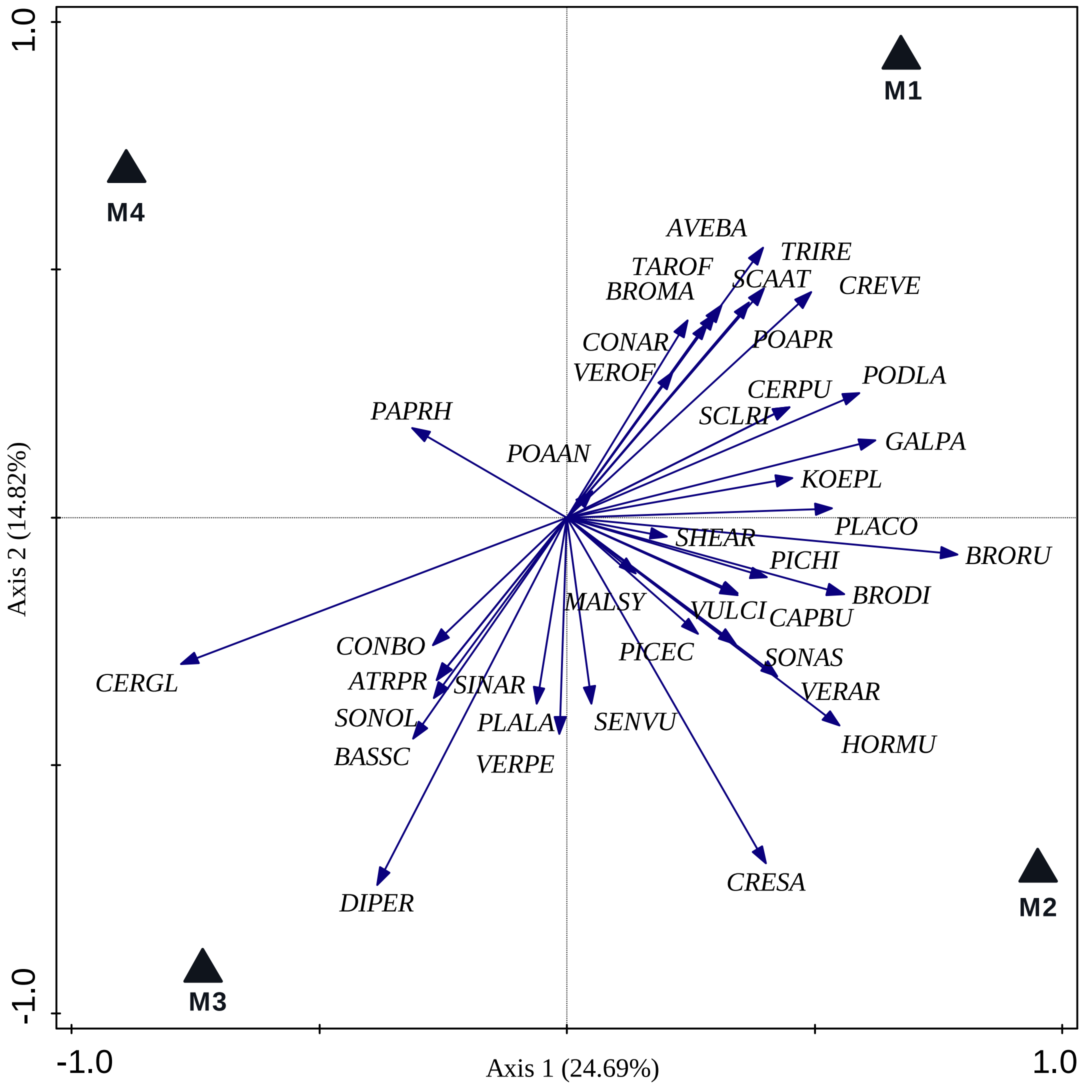

Figure 3. Redundancy Analysis of species composition. Black labels denote managements abbreviated. Arrows show the weed species present in the analysis. Weed species abbreviations are listed in Table 4.

Table 4. Plant species analyzed in Figure 2 and diagnostic species for individual management classes.

Abbr: Abbreviation; PG: Preferred Group.

Abbr.	Name	PG	Abbr.	Name	PG
ATRPR	*Atriplex prostrata* Boucher ex DC.	M3	PICHI	*Picris hieracioides* Sibth. & Sm.	M2
AVEBA	*Avena barbata* Pott ex Link	M1	PLACO	*Plantago coronopus* L.	-
BASSC	*Bassia scoparia* (L.) A.J.Scott	M3	PLALA	*Plantago lanceolata* L.	-
BRODI	*Bromus diandrus* Roth	M2	POAAN	*Poa annua* L.	-
BROMA	*Bromus madritensis* L.	M1	POAPR	*Poa pratensis* L.	M1
BRORU	*Bromus rubens* L.	M2	PODLA	*Podospermum laciniatum* (L.) DC.	M1
CAPBU	*Capsella bursa-pastoris.* (L.) Medik	-	SCAAT	*Scabiosa atropurpurea* L.	M1
CERGL	*Cerastium glomeratum* Thuill.	M3	SCLRI	*Scleropoa rigida* (L.) Griseb.	M1
CERPU	*Cerastium pumilum* Curtis	M1	SENVU	*Senecio vulgaris* L.	-
CONAR	*Convolvulus arvensis* L.	-	SHEAR	*Sherardia arvensis* L.	-
CONBO	*Conyza bonariensis* (L.) Cronquist	-	SINAR	*Sinapis arvensis* L.	M3
CRESA	*Crepis sancta* (L.) Bornm.	-	SONAS	*Sonchus asper* (L.) Hill	M2
CREVE	*Crepis vesicaria* L.	M1	SONOL	*Sonchus oleraceus* L.	M3
DIPER	*Diplotaxis erucoides* (L.) DC.	M3	TAROF	*Taraxacum officinale* Weber	M1
GALPA	*Galium parisiense* L.	M2	TRIRE	*Trifolium repens* L.	M1
HORMU	*Hordeum murinum* L.	-	VEROF	*Verbena officinalis* L.	M1
KOEPL	*Koeleria phleoides* (Vill.) Pers.	M1	VERAR	*Veronica arvensis* L.	M2
MALSY	*Malva sylvestris* L.	-	VERPE	*Veronica persica* Poir.	M2
PAPRH	*Papaver rhoeas* L.	-	VULCI	*Vulpia ciliata* Dumort.	-
PICEC	*Picris echioides* L.	M1			

The first axis clearly separates those managements based on shredding from those based on soil disturbance, leading to the selection of different weed communities. Shredding (M1) favored the dominance of perennial species, such as *Trifolium repens*, *Crepis vesicaria* or *Convolvulus arvensis. Bromus rubens, Crepis sancta*, among others, were related to the second management, where shredding and herbicide applications were performed; while soil disturbance (M3 and M4) selected, in general, annual species, such as *Papaver rhoeas, Cerastium glomeratum, Bassia scoparia* or *Diplotaxis erucoides* (Figure 3).

The diagnostic species for individual management showed that some problematic species for crop yield, such as *Conyza bonariensis*, *Convolvulus arvensis* or *Malva sylvestris* had no preferred group, which indicates that any of the management methods favour the increase of those species. Also, any of the species recorded showed preference to M4 (*H. vulgare* cover crop) (Table 4).

Discussion

Coverage and frequency of *C. dactylon* varied depending on the management system. Shredding solely (M1) was not only ineffective for controlling this weed but also increased weed infestation level. However, the other strategies (herbicide, tillage or sowing a *H. vulgare* cover crop) partially controlled *C. dactylon*. Nevertheless, significant fluctuations of the weed coverage and frequency were observed over time (Tables 2 and 3).

The inconsistent effect of spontaneous ground cover shredded (M1) can be explained by characteristics of the *C. dactylon* and the removal of other weeds that could compete with *C. dactylon*. On the one hand, shredding cannot disturb the underground rhizomes and the ground stolons that spread horizontally to the soil surface. In addition, shredding decreases the presence of other species (whether beneficial or not for the vines) that could generate more shading, especially in spring and summer. As a consequence, light could reach the soil surface more efficiently, benefiting horizontally growing species, such as *C. dactylon* (Guglielmini and Satorre, 2002).

The combination of shredding and herbicide applications (M2) reduced *C. dactylon* coverage (62.4%), and slightly its frequency (14.6% reduction). As described previously, shredding alone increased weed infestation, and some authors have reported different weed control levels after glyphosate applications: Abdullahi (2002) reported a relatively good control (60%) of this species by glyphosate application at 2.16 kg ha^{-1} in summer (when the weed is actively growing), with its effect remaining for up to 16 weeks. Gómez de Barreda et al. (2017) observed that a single application of glyphosate (36% at 4 L ha-1) did not control *C. dactylon* (var "princess 77") although some phytotoxic effects were observed. Bryson and Wills (1985) showed variations in the effectiveness of glyphosate application between rates (1.12 and 2.24 kg ha^{-1}) across 17 different *C. dactylon* biotypes and observed that 13 weeks after application, the highest rate maintained 70% of control. These results are in accordance with our results and highlight the need to combine herbicide applications with other methods or to make more frequent applications. Farthing et al. (2018) observed 72.13% of *C. dactylon* canopy cover reduction with a single glyphosate application during the warm season (48.7% at 9 L ha ha^{-1}) and biomass and groundcover reduction after multiple applications (more than 98.60%, with or without previous shredding). Mau-Crimmins (2007) also reported the effectiveness of glyphosate on *C. dactylon* removal but that herbicide applications proved to increase other noxious weeds species.

Several factors can affect the herbicide efficacy, but the timing of herbicide application is crucial because the weed becomes dormant below 10 ºC (McCarty and Miller, 2002) and herbicide applications are not so effective. In the present study, glyphosate caused phytotoxic effects on *C. dactylon*, but the weed recovered and the infestation level was unaffected. This result could, again, be explained by the death of the rest of the spontaneous cover species, which reduced competition against *C. dactylon*. On the other hand, Campbell (2008) reported that in sugarcane in the absence of tillage, glyphosate applications at rates between 1.14 and 2.88 kg ha^{-1}, obtained more than 80% control at 20 weeks after treatment, but when combined with a previous tillage, the control was reduced to 60% at the highest rate assayed (2.88 kg ha^{-1}).

Tillage is known to be effective in reducing weed infestation level, as was shown in M3 and M4, where its coverage was reduced below 1% after three seasons. This result agrees with the recommendations of MAGRAMA (2014) which endorses soil tillage so that the rhizomes and stolons are fragmented to the smallest possible size and remain in or close to the soil surface.

Guglielmini and Satorre (2004) reported that mechanical cultivation cuts and spreads the structures of the weed, and although these fragments are then dispersed; only a small proportion of them (which are in contact with the soil) were able to survive and establish. In our case, the fragments that survived the tillage effect also had to face the spontaneous vegetation competition. On the other hand, Dalley et al. (2013) reported that in sugarcane conventional tillage (four cultivations of row sides each spring) and reduced tillage (two cultivations) significantly reduced *C. dactylon* coverage compared to no-till (no cultivation), with mean ground cover values of 60%, 70%, and 90%, respectively. These results agree with Phillips (1993) in sorghum crop, who observed that treatments with winter and spring double-ploughing reduced *C. dactylon* regrowth by 60% and that double spring ploughing reduces it by 33% compared with a single pass.

The management where tillage was combined with *H. vulgare* cover crop (M4) was the most effective in reducing the coverage and frequency (93.9% and 73.3% respectively) of *C. dactylon*. In this situation, an additional effect seems to be created by seedbed preparation (tillage) with a competitive pressure exerted by *H. vulgare* as a cover crop. At the end of the experiment, there were no significant differences between M3 and M4 but the different percentages, 1.1% and 0.5% respectively (very low in both cases) can be explained by the higher and more homogeneous biomass production obtained by barley cover crop than with the spontaneous vegetation cover. Moreover, *H. vulgare* is a winter cereal that produces a dense shading canopy when *C. dactylon* begins its growing activity, creating more unfavorable conditions for weed development. The influence of the shade provided by the cover crop could be a crucial factor in the weed management, and shading has previously been reported to contribute to the reduction of *C. dactylon* infestation (Guglielmini and Satorre, 2002).

Dong and de Kroon (1994) studied the influence of the availability of light and nutrients in *C. dactylon* development in terms of morphology plasticity and biomass allocation. They observed that under a low level of light and nutrients, stolon and rhizome branching was reduced which could explain the influence of the *H. vulgare* cover crop competing for light, nutrients and other resources and reducing weed level infestation.

Furthermore, shade treatments obtained plants that produced leaves twice as long as the unshaded plants (Dong and de Kroon 1994) which could partly explain our results. *Hordeum vulgare* cover crop provides shade for *C. dactylon* during some months, and during this time, the weed can change its growth pattern producing more orthotropic buds instead of lateral stolons (Dong and de Kroon, 1994) making *C. dactylon* more susceptible to shredding. Dong and de Kroon (1994) also reported that plants under low-intensity light, which could have been achieved in our experiment by the vegetation cover canopy, failed to develop rhizomes, and stolons biomass allocation was slightly lower. Other cover crop species had shown an effect on *C. dactylon* development, as reported by Farthing et al. 2018 who observed that shredding *C. dactylon* and overseeding *Vicia villosa* Roth reduced weed biomass 50% although canopy cover was not decreased.

In addition to the shade and competition exerted by the cover crop, the effect on weed suppression can be explained by the release of allelochemicals and by a physical mulch effect once the cover crop is shredded. Ormeño-Núñez et al. (2008) reported that rye (*Secale cereale* L.) mulch on the vine rows reduced 82% of *C. dactylon* dry matter compared to chemical plus mechanical control. The weed suppression effect by different species implemented as mulch or cover crop has been studied by other authors: DeVetter et al. (2015) studied the effect of straw and living mulching (*Festuca rubra* L. Pennlawn) in vineyards and obtained better weed control than with cultivation and herbicides. Steinmaus et al. (2008) obtained good results in weed control in vineyards by different mulched cover crops. Bioassay study revealed several weeds' germination and growth reduction, including *Echinochloa crus-galli* (L.) P.Beauv. and *Setaria verticillata* (L.) P.Beauv., by extracts of *H. vulgare* and other cereals usually implemented as cover crops in Mediterranean areas (Dhima et al., 2006). This suppressive effect of *H. vulgare* and *S. cereale* cover crops has also been observed on *Solanum ptychanthum* Dun. and *Setaria glauca* (L.) P.Beauv. in the United States (Creamer et al., 1996).

The effect of different cover crop species to suppress weed development described in these studies is in agreement, in part, with our results: *H. vulgare* cover crop management has been the method with the lowest number of registered weed species (6 versus 32, 15 and 29 for M1, M2 and M3, respectively. This result is also in accordance with Gago et al. (2007), who observed a higher number of weed species in unsown plots than in those sown with cereals. *Hordeum vulgare* cover crop (M4) and spontaneous ground cover plus herbicide applications (M2) were related with lower species richness than in shredding (M1) or tilling a spontaneous cover crop (M3). A high quantity of hemicryptophyte species, such as *Taraxacum officinale* or *Trifolium repens*, was related to the shredding management (M1), as described in Figure 3., which demonstrates that the weed community was really influenced by the different three-year managements. The quantity of species related to M1 is also found in orchards and can be explained because shredding can favor seedling recruitment, the survival and reproductive ability of the species (Mas et al., 2007; Juárez-Escario et al., 2017). Furthermore, the diagnostic species for individual management classes showed that any of the species recorded obtained *H. vulgare* cover crop (M4) as preferred group which reinforces the implementation of this cover crop as the most appropriate management for weed control. Some of the most problematic weeds (*C. bonariensis, C. avernsis* or *M. sylvestris*) did not show to be related to any group indicating that those species are not favored by any management. The implementation of the four ground cover managements systems is not discouraged in the presence of these species. Focusing on *B. scoparia*, also a very problematic species, the diagnostic species showed M3 as the preferred group. In this case, this result could be explained because *B. scoparia* is a very fast-growing species that can outgrow the cover in a few days if the cover is not competitive and vigorous enough. Therefore, in the presence of this species, M3 is not recommended or more shredding interventions in order to stop its growth could be required.

Shredding alone (M1) a spontaneous ground cover has not been effective enough, but its combination with other tools such as herbicide applications or tilling increased the control of the *C. dactylon*, although the best management was the implementation of a barley cover crop. In this study, the cover crop has been limited to the inter-row but in some vineyards, the cover crop is implemented intra-row, with effective weed control with cover crops species such as *Trifolium fragiferum* L. (Abad et al., 2018). The installation and management of the cover crop under the vines can be crucial for the management of *C. dactylon*, where this weed frequently gets shelter. Further installation of the cover crop in intra-row areas would reduce herbicide applications and inter-vine interventions, providing easier soil management as well.

As cover crops are non-chemical alternatives for weed management, our results enhance their implementation in a vineyard, which is in accordance with the Directive 2009/128/EC (European Union, 2009) where Integrated Pest Management is promoted.

Conclusions

Cover crop implementation can be a useful tool in weed management. In the vineyards where soil maintenance is carried out through ground vegetation covers, it is important to know which weed species are present to carry out proper management. If *C. dactylon* is present, repeated use of the shredding interventions is discouraged because not only does it not control this weed species but it also encourages its development and expansion. This work demonstrates that the best strategy for the control of this species is to perform a mechanical intervention that disturbs the soil, such as tillage, combined with the implementation of a vegetation cover. The species selected for this purpose must meet several characteristics, such as rapid implementation and generation of a large amount of biomass. In this sense, although tillage combined with spontaneous cover has given good results controlling *C. dactylon*, the species that can constitute this type of cover, as well as the amount of biomass that they can generate is uncertain. It is advisable, therefore, to sow known species. In addition, through the Redundancy Analysis of species composition as well as the analysis of the diagnostic species, it has been observed how the weed species community is quickly affected by implemented management. Thus, knowing the weed species present in the field, especially those that may become more problematic is crucial before deciding the management to implement.

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References

Abad, F.J., Cibriáin, F., Santesteban, L.G., Marín, D., Sagüés, A. (2018). Manejo del suelo bajo la línea de cultivo de viñedo sin empleo de herbicida ni laboreo. III Jornadas del Grupo de Viticultura, Palma, Spain, 28-29 November, Libro de actas. 2018, 164-167.

Abdullahi, A.E. (2002). *Cynodon dactylon* control with tillage and glyphosate. *Crop Prot*. 21, 1093-1100.

Agencia Estatal de Meteorología (AEMET). Ministerio para la Transición Ecológica. Gobierno de España. Accessed on 2 March 2020. Available online: www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos

Bryson, C., Wills, G. (1985). Susceptibility of Bermudagrass (*Cynodon dactylon*) biotypes to several herbicides. *Weed Sci*. 33, 848-852.

Campbell, PL. (2008). Efficacy of glyphosate, alternative post-emergence herbicides and tillage *for control of Cynodon dactylon. S. Afr. J. Plant Soil*. 25:4, 220-228.

Cerdan, O., Govers, G., Le Bissonnais, Y., Van Oost, K., Poesen, J., Saby, N., Gobin, A., Vacca, A., Quinton, J., Auerwald, K., et al. (2010). Rates and spatial variations of soil erosion in Europe: A study based on erosion plot data. *Geomorphology*, 122, 167–177.

Chytrý, M., Tichý, L., Holt, J., Botta-Dukát, Z. (2002). Determination of Diagnostic Species with Statistical Fidelity Measures. *J. Veg. Sci.* 13, 79-90.

Creamer, N.G., Bennett, M.A., Stinner, B.R., Cardina, J., Regnier, E.E. (1996). Mechanisms of weed suppression in cover crop-based production systems. *Hort Sci.* 31, 410-413.

Dalley, C. D, Viator, R. P., Richard, E. P. (2013). Integrated management of bermudagrass (*Cynodon dactylon*) in sugarcane. *Weed Sci*. 61, 482-490.

DeVetter, L.W., Dilley, C.A., Nonnecke G.R. (2015). Mulches reduce weeds, maintain yield, and promote soil quality in a continental-climate vineyard. *Amer. J. Enol. Viticult.* 66, 54–64.

Dhima, K.V., Vasilakoglou, I.B., Eleftherohorinos, I.G., Lithourgidis, A.S. (2006). Allelopathic potential of winter cereals and their cover crop mulch effect on grass weed suppression and corn development. *Crop Sci*. 46, 345–352.

Dong, M., De Kroon, H. (1994). Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*. 70, 99–106.

European Union. (2009). Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009. Establishing a framework for Community action to achieve the sustainable use of pesticides. Official Journal of the European Union 2009, L 309, 71–86. https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:en:PDF

Farooq, M., Jabran, K., Cheema, Z., Wahid, A., Siddique, K. (2011). The role of allelopathy in agricultural pest management. *Pest. Manag. Sci*. 67, 493-506.

Farthing, T.S., Muir, J.P., Falk, A., Murray, D. (2018). Efficacy of seven invasive Bermudagrass removal strategies in three Texas ecoregions. *Ecol. Restor*. 36, 306–314. https://doi.org/10.3368/er.36.4.306.

Fernandez, O.N. (2003). Establishment of *Cynodon dactylon* from stolon and rhizome fragments. *Weed Res*. 43, 130-138.

García, L., Celette, F., Gary, C., Ripoche, A., Valdés-Gómez, H., Metay, A. (2018). Management of service crops for the provision of ecosystem services in vineyards: A review. *Agric. Ecosyst. Environ*. 251, 158-170.

Gago, P., Cabaleiro, C., García, J. (2007). Preliminary study of the effect of soil management systems on the adventitious flora of a vineyard in northwestern Spain. *Crop Prot*. 26, 584-591.

Gómez de Barreda, D., Izquierdo, D., de Luca, V. (2017). Evaluación del comportamiento de 20 materias activas herbicidas sobre la cespitosa "*Cynodon dactylon*" en diferentes épocas climáticas del año. XVI Congreso de la Sociedad Española de Malherbología. 25-27 October 2017, Pamplona-Iruña, España, 2017, 245-250.

Guerra, B., Steenwerth, K. (2012). Influence of floor management technique on grapevine growth, disease pressure, and juice and wine composition: A review. *Am. J. Enol. Vitic*. 63, 149-164.

Guglielmini, A.C., Satorre, E.H. (2002). Shading effects on spatial growth and biomass partitioning of *Cynodon dactylon*. *Weed Res*. 42, 123–134.

Guglielmini, A.C., Satorre, E.H. (2004). The effect of non-inversion tillage and light availability on dispersal and spatial growth of *Cynodon dactylon*. *Weed Res*. 44, 366–374.

Heap, I. (2020). The International Survey of Herbicide Resistant Weeds. Online. Internet. Last access on January 8, 2020 Available: www.weedscience.org

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P. (1977). The World's Worst Weeds. Distribution and Biology. University of Hawaii Press, Honolulu, USA, 1977.

Ibáñez, S. (20115). Mantenimiento del suelo en el viñedo mediante cubiertas vegetales. Ed. Gobierno de la Rioja. 2015, 167p.

Juárez-Escario, A., Conesa, J.A., Solé-Senan, X.O. (2017). Management as a driver of functional patterns and alien species prominence in weed communities of irrigated orchards in Mediterranean areas. *Agr. Ecosyst. Environ*. 249, 247-255.

Kim, C., Lemke, C., Paterson, A.H. (2009). Functional dissection of drought-responsive gene expression patterns in *Cynodon dactylon* L. *Plant Mol Biol*. 70, 1–16.

Le Bissonnais, Y., Lecomte, V., Cerdan, O. (2004). Grass strip effects on runoffand soil loss. *Agronomie*. 24, 129–136. http://dx.doi.org/10.1051/agro:2004010

MAGRAMA (2014). Guía de gestión integrada de plagas. Uva de transformación. Ministerio de Agricultura, Alimentación y Medio Ambiente, España, Madrid, 2014.

Mas, M.T., Poggio, S.L., Verdú, A.M.C. (2007). Weed community structure of mandarin orchards under conventional and integrated management in northern Spain. *Agr. Ecosyst.Environ*. 119, 305–310.

Mau-Crimmins, T.M. (2007). Effects of removing *Cynodon dactylon* from a recently abandoned agricultural field. *Weed Res*. 47, 212-221.

McCarty, L.B., Miller, G. (2002). Managing bermudagrass turf. E. Ann Arbor Press. Chelsea, MI, 2002 pp. 67-86.

Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., Drexler, D., Tóthmérész, B. (2015). Establishment of three cover crop mixtures in vineyards. *Sci. Horti*. 197, 117-123.

Oerke, E. (2006). Crop losses to pests. *J.Agr.Sci*. 144, 31–43.

OIV (International Organisation of Vine and Wine) (2019). Statistical report on world vitiviniculture. April 2019. http://www.oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf

Ormeño-Núñez, J., Pino-Rojas, G., Garfe-Vergara, F. (2008). Inhibition of yellow nutsedge (*Cyperus esculentus* L.) and bermudagrass (*Cynodon dactylon* (L.) Pers.) by a mulch derived from rye (*Secale cereale* L.) in grapevines. *Chil. J. Agric. Res*. 68, 238-247.

Pardini, A., Faiello, C., Longhi, F. (2002). Cover crop species and their management in vineyards and olive groves. *Adv. Hortic. Sci*. 16, 225-234.

Phillips, M.C. (1993). Use of tillage to control *Cynodon dactylon* under small-scale farming conditions. *Crop Prot*. 12, 267-272.

Prosdocimi, M., Cerdà, A., Tarolli, P. (2016). Soil water erosion on Mediterranean vineyards: A review. *Catena*, 141, 1–21.

Recasens, J., Valencia, F., Montull, J.M., Taberner, A. (2018). Malas hierbas problemáticas en viñedos con cubiertas vegetales y métodos químicos para su control, *Vida rural*, 444, 48-58.

Sáenz-Romo, M.G., Veas-Bernal, A., Martínez-García, H., Campos-Herrera, R., Ibáñez-Pascual, S., Martínez-Villar, E., Pérez-Moreno, I., Marco-Mancebón, V.S. (2019). Ground cover management in a Mediterranean vineyard: Impact on insect abundance and diversity. *Agr. Ecosyst. Environ*. 283.

Sanguankeo, P., León, R. (2011). Weed management practices determine plant and arthropod diversity and seed predation in vineyards. *Weed Res*. 51, 404–412.

Steenwerth, K., Belina, K.M., (2008). Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. *Appl. Soil Ecol*. 40, 359–369.

Steinmaus, S., Elmore, C.L., Smith, R.J., Donaldson, D., Weber, E.A., Roncoroni, J.A., Miller, P.R.M. (2008). Mulched cover crops as an alternative to conventional weed management systems in vineyards. *Weed Res*. 48, 273-281.

Stolz, H., Schmid, O. (2007). Organic viticulture and wine-making: development of environment and consumer friendly technologies for organic wine quality improvement and scientifically based legislative framework. D 2.7 Public report about first round qualitative consumer research and market needs. 2007 https://orgprints.org/10608/1/stolz-schmid-2007-ORWINE_d2.7_consumers_research.pdf

Sturm, D.J., Peteinatos, G., Gerhards, R. (2018). Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res*. 58, 331–337.

Ter Braak, C.F., Smilauer, P. (2012). Canoco Reference Manual and User's Guide: Software for Ordination, Version 5.0. Microcomputer Power. Ithaca, USA.

Viers, J.H., Williams, J.N., Nicholas, K.A., Barbosa, O., Kotzé, I., Spence, L., Leanne, B.W., Merenlender, A., Reynolds, M. (2013). Vinecology: Pairing wine with nature. *Conservation Letters*. 6, 287–299.

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J.R., Hart, M. (2016). Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agron. Sustain. Dev*. 36, 48.

Zaller, J.G., Cantelmo, C., Santos, G.M., Muther, S., Gruber, E., Pallua, P., Mandl, K., Friedrich, B.Q., Hofstetter, I., Schmuckenschlager, B., Faber, F. (2018). Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice. *Environ. Sci. Pollut. Res. Int.* 25, 23215-23226.

Chapter 3

Evolution of *Cynodon dactylon* and other weeds under different cover crops species in vineyards

Evolution of ***Cynodon dactylon*** and other weeds under different cover crops species in vineyards

Abstract

Cover crop implementation in vineyards is an environmentally-friendly soil management technique that has several advantages, including weed suppression. The effectiveness of the management depends on both the weed community characteristics and the implemented cover crop species. The objective of this study was to evaluate the evolution of *Cynodon dactylon* patches and the weed community composition under five different cover crop species in a commercial vineyard in Spain: perennial *Festuca arundinacea*, and annuals *Hordeum vulgare, Vulpia ciliata, Medicago rugosa* and a *Bromus* mixture. Management was carried out in the inter-row vineyard over three seasons (2015-2018). Results showed that cover crops affected the evolution of *C. dactylon* in terms of soil coverage and its distribution across the field. Initially, tillage from the seedbed preparation significantly reduced the percentage of the weed soil coverage (60%). *Festuca arundinacea* and *H. vulgare* were the best cover crops controlling *C. dactylon* expansion, which reduced the weed coverage by 38.8% and 21.3%, respectively. Canonical Correspondence Analysis (CCA) showed that the weed community composition changed depending upon the implemented cover crop, revealing the importance of management in the weed assemblage, which was in accordance with the selected species.

Keywords: Bermudagrass, Integrated Weed Management, Pinot noir, *Vitis vinifera, Hordeum vulgare, Festuca arundinacea, Vulpia ciliata, Medicago rugosa, Bromus rubens, Bromus hordeaceus.*

Introduction

Among the many advantages that ground vegetation cover implementation offers in vineyards, such as soil protection, improvement of soil fertility or habitat for beneficial predators (Garcia et al., 2018; Guerra and Steenwerth, 2012; Ibáñez, 2015), weed control is one of the most interesting for Integrated Pest Management (IPM). Weed community composition in vineyards can vary depending upon the soil management system that is implemented (Gago et al., 2006). The operations required in vineyards that maintain the soil surface with vegetation ("ground vegetation covers"), such as tillage for seedbed preparation or mowing/shredding to control cover development, as well as the species selected as ground cover/cover crop, can affect weed development in different ways.

In general, cover crops suppress weed growth by competition (den Hollander et al., 2007; Gago et al., 2007; Miglécz et al., 2015), but can also affect weed germination and growth by releasing allelochemicals (Álvarez-Iglesias et al., 2014; Bettoni et al., 2012; Sturm et al., 2018). This weed suppression capability depends on the characteristic of both the selected cover crop and the target weed species (Lemessa & Wakjira, 2015; Recasens et al., 2018). Methods to prevent the cover crop from competing with the vines, especially where water is a limiting factor, include herbicide application, mowing/shredding, tillage or the implementation of cover species with a short life cycle (Sullivan, 2003). These operations can also eliminate weeds that are exposed to them, and surviving weeds have to face the mulching effect provided by the mowed cover crop, which acts as a physical barrier, but can also release allelochemicals (Lemessa and Wakjira, 2015). For these reasons, the installation and management of a cover crop in vineyards can be a useful tool in weed control.

However, to achieve successful weed control, it is essential to know the most problematic weed species present in the crop to select the correct management (Recasens et al., 2018), and to consider, in the case of vines, plant age, soil type and climatic conditions (Garcia et al., 2018; Ripoche et al., 2010). After these considerations, the selection of the appropriate cover crop species focusing on weed control is determined by growth rates or leaf area development, time of establishment, self-sowing capacity, amount of biomass provided, competitive ability or allelopathic potential (Ibáñez, 2015; Lemessa & Wakjira, 2015).

In the particular case of the weed *Cynodon dactylon* (L.) Pers., the different operations related to soil management can modulate the infestation level. On the one hand, mechanical soil disturbance can break rhizomes and stolons, and scatter them throughout the field, thereby increasing levels of weed infestation. On the other hand, the effect of shading (in this case, provided by a cover crop) can reduce weed biomass (Guglielmini and Satorre, 2002, 2004). Valencia-Gredilla et al. (2020) reported that the implantation of a *Hordeum vulgare* L. cover was more effective in reducing levels of weed infestation than spontaneous vegetation cover managed by tillage, herbicide applications or shredding. But the effect of other cover crop species implemented in vineyards in reducing *C. dactylon* level is unknown.

A frequently implemented cover crop is *Festuca arundinacea* L., which is commonly called tall fescue. It is a persistent perennial that limits erosion, excessive tree vigour and promotes vine balance (Chou et al., 2019; Coniberti et al., 2018; Pardini et al., 2002). The implementation of this species is desirable because it does not require annual seeding, thus reducing costs. Besides, some studies have reported on its allelopathic potential (Bertoldi et al., 2012; Fujii, 2001), making this species a suitable cover crop for reducing weed germination and development.

On the other hand, annual species with a short lifecycle are also suitable choices when water is the most limiting factor. The implantation of barley (*H. vulgare*) as a cover crop in a vineyard has proven to be an effective tool to reduce both *C. dactylon* infestation levels and the presence of other weeds (Valencia-Gredilla et al., 2020). Considering this, the implantation of *H. vulgare* as a cover crop is highly desirable when cover crop management is focused on weed control, but generally, *H. vulgare* has a limited reseeding capacity. This was observed by Ibáñez (2015), who implemented different species as cover crops in vineyards and observed that *H. vulgare* showed poor reseeding capacity (<25%) compared to other species such as *Medicago truncatula* Gaertn. (50-75%), *Vulpia myuros* (L.) C. C. Gmel. (75-90%) or *Bromus catharticus* Vahl (>90%). The same author reported different weed control levels from these species, which was high in the case of *H. vulgare*, *V. myuros* and *B. catharticus* (<5% weed invasion) medium-high in the case of *F. arundinacea* (5-15%) and low in the case of *M. truncatula* (>25%). For this reason, knowledge of how the management of similar species can influence weed community and *C. dactylon* expansion is needed.

The objective of the study was to determine the effects of different cover crop species on the infestation levels of *C. dactylon* in vineyards, and its possible spread across the field, as well as assessing the competitive effect of the cover crop with other weeds. We hypothesized that the weed community composition would change in time depending on the cover crop.

Materials and methods

Study area

An experiment was conducted in a commercial wine grape vineyard in Raimat (Lleida, NE Spain, 41º 40’22”N, 0º 29’23”) from 2015 to 2018. The vineyard was established in February 2015 with Pinot noir (*Vitis vinifera* L.). Rows were separated by 2.40 m and vine spacing of 1.7 m. Vines were trained as bilateral cordons and drip irrigation was established. At the beginning of the experiment, there was a high infestation level of *C. dactylon* in the entire field (Figure 1.). Previous to vineyard establishment, alfalfa (*Medicago sativa* L.) was grown for several seasons.

Figure 1. General view of the *Cynodon dactylon* infestation level in September 2015.

Experimental design

Two experiments were set in October and November 2015:

1. Tillage effect on *C. dactylon*: a complete randomized design was set in different inter-rows with tillage as the main factor. In the inter-rows, 2 x 10 m^2 plots were selected and established in their centre. Each of these plots was subdivided in 80 0.5 x 0.5 m^2 subplots. Plots were tilled twice in November 2015. Weed coverage percentage and frequency (percentage of subplots with presence of *C. dactylon*) were measured in nine plots before tillage (October 2015) and after two tillage interventions with a chisel plow at a depth of 20 cm (November 2015).

2. Effect of cover crop managements on *C. dactylon*: 20 plots of 2 x 10 m^2 with *C. dactylon* presence, and 20 more without the presence of this weed were randomly selected to follow its evolution over three years under the effect of the management of different cover crops. The experiment started in November 2015 and finished in September 2018. Five cover crop species were sown in different, randomly selected inter-rows (Figure 3): C1: *F. arundinacea* (40 kg ha^{-1}); C2: *H. vulgare* (150 kg ha^{-1}) (Figure 5.); C3: a mixture of *Bromus rubens* L. and *Bromus hordeaceus* L. (20 kg ha^{-1}); C4: *Vulpia ciliata* Dumort. (20 kg ha^{-1}) (Figure 6.); and C5: *Medicago rugosa* Desr. (35 kg ha^{-1}) (Figure 7.). Seed-bed preparation was performed with four interventions of a chisel plow at a soil depth of 20 cm in November 2015 of the first year, and one intervention in October/November (according to the soil conditions) the following years, except for the perennial cover crop (*F. arundinacea*) that only required pre-sowing tillage. *Festuca arundinacea* and *H. vulgare* were sown with a vineyard seeder. The mixture of *Bromus* species, *V. ciliata* and *M. rugosa* were sown by hand. All the cover crops were shredded once a year, in June, except for *F. arundinacea* that was shredded again in September 2017 and 2018. As in experiment 1, all plots were subdivided into 80, 0.5 x 0.5 m2 subplots, where the percentage and frequency (presence/absence) of *C. dactylon* were monitored at the beginning of the experiment (November 2015), and in June and September the following three years (2016-18) for the plots with initial weed presence, and in June and September 2017 and 2018 in the plots that were initially weed-free.

During the study, intra-row weed control method consisted of herbicide applications with glufosinate (15% at 4 L ha^{-1}) in April, June, July, and August, and glyphosate (36% at 4 L ha^{-1}) in December.

In order to analyze the influence of the assessed managements in the weed floristic composition of the plant-cover, floristic surveys were performed in the plots in April 2018. For these, the percentage of soil in the whole plot covered by each species was recorded.

Statistical analyses

Cynodon dactylon evolution (percentage of *C. dactylon* coverage and its frequency values between the different management systems and sampling dates) as well as the final variation were compared by one-way ANOVA analysis and post-hoc comparisons by Tukey’s honestly significant difference (HSD) and t-test ($P < 0.05$). When necessary, data were previously square-root or log transformed to achieve the normality assumption. In the case of heteroscedasticity, data were subjected to Kruskal-Wallis H test and post-hoc Dunn's test ($P \leq 0.05$). Analysis and graphs were performed with SigmaPlot 11.0 (Systat Software, San Jose, CA). To analyze treatment effects on weed species composition, a Canonical Correspondence Analysis (CCA) was performed with CANOCO 5.0 (Ter Braak and Smilauer, 2012). Cover crop species were not considered for the analysis. Previous to the CCA analysis, a forward selection of explanatory variables test was performed.

Results

Climatic conditions

During the experiment, temperatures were similar across years (Figure 2), with an average of 5-7 ºC between December and February, 12-14 ºC from March to May, 23-24ºC between June and August, and 14-15 ºC between September and November. Differences were, however, observed in precipitation. In November 2015, at the beginning of the experiment, 64.5 mm were recorded, similar to November 2016 (72 mm), and much more than in November 2017 (1.4 mm). Winter 2015-16 (December-February) was wettest of the three years, with 92 mm, followed by 2017-18 (62 mm) and

2016-17 (46 mm). On the other hand, all three springs (March-May) were wet, with the highest precipitation recorded in 2018 (184 mm), followed by 2017 and 2016, with 149 and 133 mm, respectively. From June to September, when *C. dactylon* is very active, 17 mm were recorded in 2016 and 96 and 74 mm in 2017 and 2018, respectively.

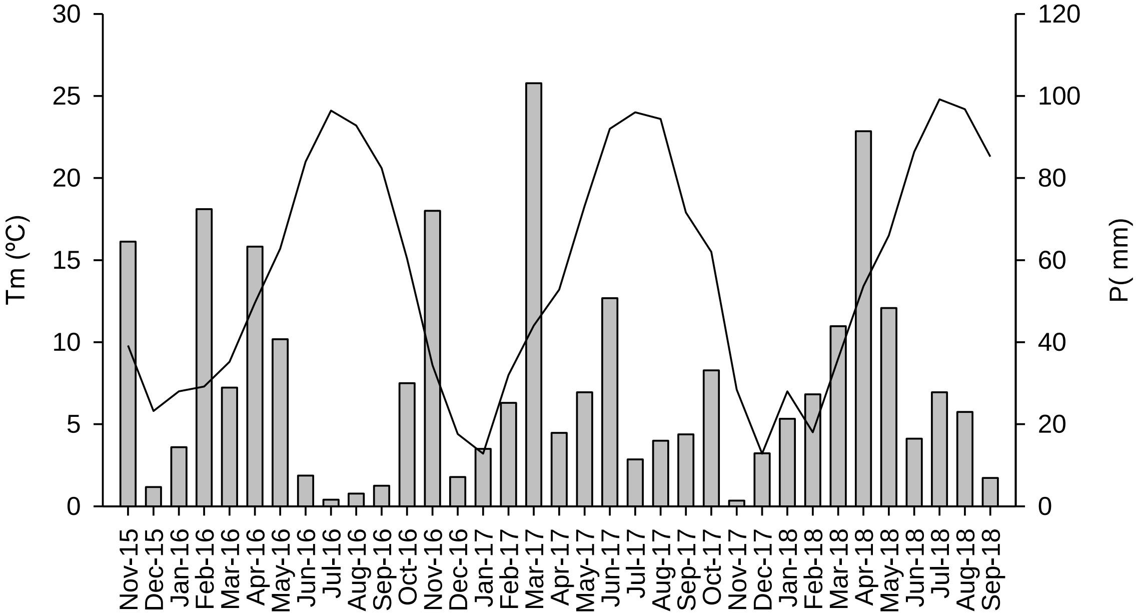

Figure 2. Climatic conditions during the experiment period (March 2015-September 2017). The grey bars show total precipitation (P) and the black line indicates the mean monthly temperature (Tm) in each month (AEMET).

Figure 3. General view of the experiment in April 2017.

Tillage effect on C. dactylon

Tillage significantly reduced the *C. dactylon* infestation level. The initial *C. dactylon* coverage across the field was close to 30% and decreased down to 12% after tillage interventions (60% reduction). Infestation frequency also reduced from 61% to 52%, but this difference was not significant (Figure 4).

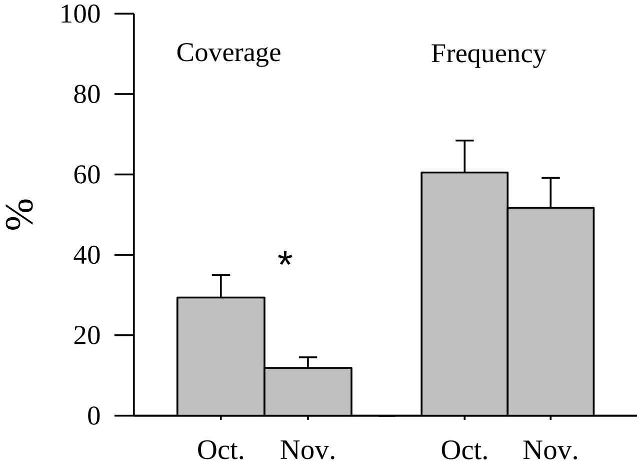

Figure 4. *Cynodon dactylon* coverage and frequency (%) before tillage (October 2015) and after double tillage (November 2015). Mean values represented percentage of soil covered by the weed. Error bars represents standard errors of the means. * statistically significant differences between dates at $p < 0.05$ (t-test).

Cover crop effect on C. dactylon

Percentage of coverage and frequency values of *C. dactylon* fluctuated within each campaign, with lower values persistent until June due to the annual tillage of annual cover species, and the pressure exerted by the cover crops until the summer period. Then, these values increased (from June to September) because the annual cover crops are dead and no longer compete with *C. dactylon*. These fluctuations are not so evident in the case of *F. arundinacea*, a cover crop that generally maintained the lowest values throughout the experiment.

From November 2015 to June 2016, coinciding with the cover crops' growth period, *C. dactylon* soil coverage decreased in *F. arundinacea* (C1), *Bromus* mixture (C3), and *V. ciliata* (C4) (7.6% to 3.3%, 4.1% to 3.5% and 2.0% to 1.1% respectively). Conversely, the presence of *C. dactylon* under *H. vulgare* (C2) and *M. rugosa* (C5) increased up to 7% and 10.6%, respectively. In September 2016 only the perennial cover crop (C1) was able to maintain a low infestation level (5%), whereas it increased in the rest of the cover crops until a range comprised between 10.4% and 19.5% (Table 1).

Figure 5. *Hordeum vulgare* (left) and *Festuca arundinacea* (right) cover crops.

Similar to the previous season, from September 2016 to June 2017, almost all the cover crops competed successfully against *C. dactylon*, and low weed coverage percentages were maintained (2.5%-7.3%), except for *M. rugosa*, where the percentage of *C. dactylon* coverage increased up to 11%. From June to September 2017, the increase was even higher than in previous season, with values of weed coverage ranging from 8.3% in *F. arundinacea* to 40.6% in *M. rugosa*, respectively (Table 1). In June 2018, there were low coverage percentages with values that ranged from 0.6% in *H. vulgare* to 3.7% for *M. rugosa* without significant differences among them. At the end of the study (September 2018), *H. vulgare* had the lowest infestation levels of *C. dactylon* (3%), followed by *F. arundinacea* (4.6%; Table 1).

Figure 6. Mixture of *bromus* (left) and *Vulpia ciliata* (right) cover crops.

The comparison between the sampling dates revealed that *F. arundinacea* and *H. vulgare* were the only cover crops that decreased the percentage of *C. dactylon* coverage, from 7.6% to 4.6% and 3.9% to 3.0%, supposing a 38.8% and 21.3% reduction, respectively, though without significant differences between them. Under the rest of the cover crops, the weed coverage infestation increased. Despite the low values obtained by *H. vulgare*, *F. arundinacea* was the cover crop that maintained low values with little fluctuation throughout all sampling dates, unlike the rest of the cover crops.

Table 1. *Cynodon dactylon* coverage (%) in each cover crop across the different sampling dates in plots with initial weed presence. Mean values of weed cover ± standard errors of the mean. C1, *Festuca arundinacea*; C2, *Hordeum vulgare*; C3, mixture of *Bromus*; C4, *Vulpia ciliata*; C5, *Medicago rugosa.*

	Nov. 15	June 16	Sept. 16	June 17	Sept. 17	June 18	Sept. 18	% Variation
C1	7.6±1.2 ABa	3.3±0.8 BCa	5.0±1.5 ABCa	2.5±0.8 Ca	8.3±0.7 Aa	2.1±0.8 Ca	4.6±1.2 ABCa	-38.8±35.1 a
C2	3.9±1.1 Aab	7.0±4.5 Aa	10.4±2.6 Aab	2.8±1.5 Aa	9.6±5.1 Aa	0.6±0.3 Aa	3.0±1.2 Aa	-21.3±15.6 ab
C3	4.1±1.3 Bab	3.5±1.3 Ba	19.5±4.1 ABb	7.3±3.8 Ba	37.5±9.7 Aa	3.6±2.1 Ba	20.7±7.8 ABa	408.7±199.7 bc
C4	2.0±0.2 Bb	1.1±0.2 Ba	13.2±1.3 Abab	3.4±1.1 Ba	31.3±12.3 Aa	2.4±1.1B a	19.4±7.4 ABa	882.4±428.2 c
C5	2.5±0.7 Bb	10.6±3.5 Ba	12.6±3.9 Bab	11.0±2.1 Ba	40.6±8.1 Aa	3.7±0.3 Ba	13.9±5.5 Ba	450.4±93.8 c

Different letters represent significant differences at p <0.05; capital letters, differences among sampling dates; lowercase letters: differences among managements.

Cynodon dactylon frequency changed across the sampling dates in plots where it was present at the beginning (Table 2). The initial frequency was higher than 49% in all cover crops, without differences between them (November 2015). In general, the frequency of *C. dactylon* was reduced from autumn to summer (tillage and cover crop competition) and increased during summer (June-September) in all seasons (from 2015 to 2018; Table 2). When comparing the sampling dates, *H. vulgare* and *F. arundinacea* were the cover crop species that reduced weed frequency from 69.2% to 37.1% and from 71.9% to 44.1%, supposing a 46.4% and 38.7% of reduction, respectively. Despite this, differences were not significant. In the case the mixture of *Bromus* frequency values barely varied between the first and the last sampling date. During this same time, the frequency of *C. dactylon* significantly increased from 52.5% to 77.2% in *V. ciliata* and from 49.4% to 78.5% in the case of *M. rugosa.*

Figure 7. *Medicago rugosa* cover crop

Table 2. ***Cynodon dactylon* frequency (%) in each cover crop across the different dates in plots with initial weed presence.** Mean values of soil cover ± standard errors of the mean. C1, *Festuca arundinacea*; C2, *Hordeum vulgare*; C3, mixture of *Bromus.*; C4, *Vulpia ciliata*; C5, *Medicago rugosa.*

	Nov. 15	June 16	Sept. 16	June 17	Sept. 17	June 18	Sept. 18	% Variation
C1	71.9 ±5.2 Aa	30.3±4.3 BCab	43.1±6.2 ABCa	30.0±6.2 BCb	55.0±7.1 ABb	23.8±4.7 Ca	44.1±9.0 ABCa	-38.7±12.4 a
C2	69.2±13.1 Aa	47.5±16.3 Aab	58.0±5.8 Aa	53.8±15.7 Aab	62.2±13.5 Aab	18.3±5.5 Aa	37.1±22.7 Aa	-46.4±9.3 a
C3	69.4±7.1 ABa	46.3±9.1 ABab	56.2±6.6 ABa	64.5±11.5 ABab	89.4±4.6 Aab	43.5±12.6 Ba	69.7±13.6 ABa	0.5±17 ab
C4	52.5±2.8 ABCa	18.8±3.2 Cb	46.3±4.0 BCa	47.8±7.9 BCab	86.9±7.6 Aab	46.0±15.4 BCa	77.2±8.2 ABa	47.1±12.4 bc
C5	49.4±6.3 Da	53.5±6.5 CDa	59.9±5.9 CDa	85.3±3.5 ABa	98.6±1.0 Aa	59.1±8.1 BCDa	78.5±6.0 ABCa	58.9±12.7 c

Different letters represent significant differences at $p < 0.05$; capital letters, differences among sampling dates; lowercase letters: differences among managements.

Tillage dispersion effect and cover crop competitiveness in initially weed-free plots

Initially, weed-free plots were colonized by *C. dactylon* between November 2015 and June 2017, after one tillage intervention that was performed in autumn 2016 for annual cover crops, one shredding intervention that was conducted in spring 2017 for annual covers, and shredding twice for *F. arundinacea* in spring and autumn 2017 (Table 3 and Table 4). After one and a half years of cover crop management, coverage percentages of *C. dactylon* were lower than 4.2% (June 2017). That summer (June to September 2017) all frequency percentages increased, as in the previous experiment, with the lowest value for *F. arundinacea* (3%) and the highest for *M. rugosa* (20%). Then tillage carried out in autumn 2017 for the annual species and the competitive effect of the cover crops reduced weed occurrence in all cases. In June 2018, percentages of weed coverage remained low, but then increased progressively until the end of the experiment (September 2018). At this date, coverage values ranged from 3.9% for *Bromus* mixture to 6.9% for *M. rugosa*. Despite that none of the cover crops were able to avoid weed colonization and there were low weed coverage percentages in all cases at the end of the experiment.

Table 3. ***Cynodon dactylon* cover (%) in each management across the different sampling dates in plots without initial weed presence.** Mean values of soil cover ± standard errors of the mean. C1, *Festuca arundinacea*; C2, *Hordeum vulgare*; C3, mixture of *Bromus*; C4, *Vulpia ciliata*; C5, *Medicago rugosa.*

	June 17	Sept. 17	June 18	Sept. 18
C1	0.5±0.3 Aa	3.0±1.6 Aa	1.5±1.3 Aa	5.5±3.1 Aa
C2	2.1±0.9 Aa	8.7±4.6 Aa	0.7±0.6 Aa	4.3±2.5 Aa
C3	0.5±0.2 Aa	5.2±3.0 Aa	1.0±0.5 Aa	3.9±1.8 Aa
C4	0.9±0.3 Aa	10.7±4.9 Aa	2.3±0.4 Aa	6.1±2.3 Aa
C5	4.2±2.3 Aa	20.0±12.4 Aa	3.2±0.7 Aa	6.9±4.1 Aa

Different letters represent significant differences at p <0.05; capital letters, differences among sampling dates; lowercase letters: differences among managements.

With regard to weed frequency (Table 4) one and a half years after the start of the experiment (June 2017), *C. dactylon* was present in all plots of different cover crops with values ranging from 7.5% in the case of *F. arundinacea* up to 60.6% for *M. rugosa*. The presence of *C. dactylon* increased again in September 2017 (21%-79%), and after tillage in November 2017 (in the case of annual species), it was reduced by June 2018 in all cover crops. At the end of the study (September 2018), frequency values varied from 18% in *F. arundinacea* up to 44% in *M. rugosa*, but without significant differences between them (Table 4).

Table 4. ***Cynodon dactylon*** **frequency (%) in each management across the different dates in plots without initial weed presence.** Mean values of soil cover ± standard errors of the mean. C1, *Festuca arundinacea*; C2, *Hordeum vulgare*; C3, mixture of *Bromus*; C4, *Vulpia ciliata*; C5, *Medicago rugosa.*

	June 17	Sept. 17	June 18	Sept. 18
C1	7.5±4.2 Ab	20.6±9.5 Aa	19.1±12.7 Aa	17.8±10.2 Aa
C2	35.6±8.5 Aab	56.4±15.6 Aa	19.0±17.0 Aa	36.0±19.2 Aa
C3	15.3±6.9 Ab	41.6±14.7 Aa	17.9±8.3 Aa	29.0±8.4 Aa
C4	22.8±4.5 Ab	60.9±14.1 Aa	31.6±3.0 Aa	40.6±14.3 Aa
C5	60.6±10.2 Aa	78.7±9.8 Aa	46.3±10.4 Aa	43.7±18.7 Aa

Different letters represent significant differences at $p < 0.05$; capital letters, differences among sampling dates; lowercase letters: differences among managements.

Cover crop effect on the weed flora composition

Regarding weed community composition, a total of 20 species were recorded in the floristic surveys (excluding *C. dactylon*). *Festuca arundinacea* (C1) and *Bromus spp* (C3) were the cover crops with the lowest number of species (nine in each treatment) followed by *H. vulgare* (C2) with 11, 13 in *V. ciliata* (C4) and 14 in *M. rugosa* (C5). Forward selection of explanatory variables revealed that the initial presence or absence of *C. dactylon* in the plots were not significant to explain species composition variance (p=0.42). All cover crops showed significant effects ($p \leq 0.01$), thus Correspondence Analysis (CCA) was performed with management as the only explanatory variable. An influence of the cover crop management on the weed flora composition was revealed by the CCA (Figure 8). The first axis, which explains 24% of the species composition variance, separates C1 from the rest of cover crops. *Festuca arundinacea* (C1) was related to *Malva sylvestris* and *Picris echioides*. The second axis (12% of variance explained) mainly separates C2 and C3 that were associated to *Papaver rhoeas* and *Beta vulgaris*, and *Hordeum murinum* and *Phalaris brachystachys*, respectively. The managements C4 and C5 showed similar weed community composition, with the presence of *Sisymbrium irio, Sinapis alba, Sonchus asper* or *Polygonum aviculare*.

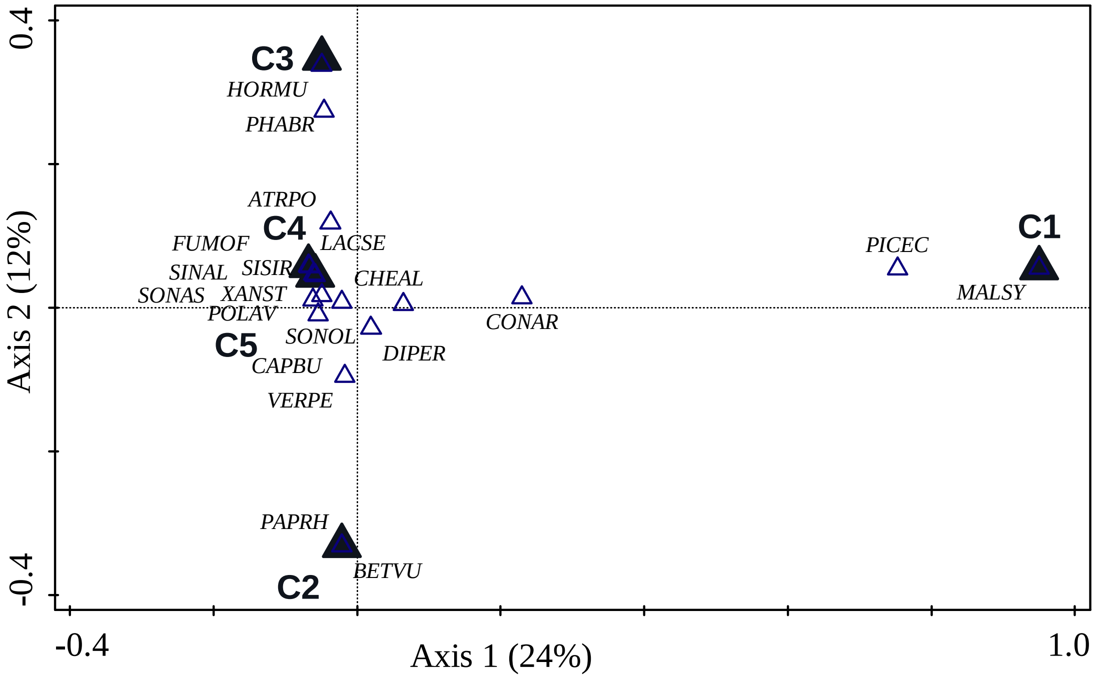

Figure 8. Canonical Correspondence Analysis (CCA) biplot of weed species. Black labels denote management codes. C1, *Festuca arundinacea*; C2, *Hordeum vulgare*; C3, mixture of *Bromus*; C4, *Vulpia ciliata*; C5, *Medicago rugosa***.** ATRPO: *Atriplex prostrata Boucher ex DC.*, BETVU: *Beta vulgaris* L., CAPBU: *Capsella bursa-pastoris* (L.) Medik., CHEAL: *Chenopodium album* L., CONAR: *Convolvulus arvensis* L., DIPER: *Diplotaxis erucoides* (L.) DC., FUMOF: *Fumaria officinalis* L., HORMU: *Hordeum murinum* L., LACSE: *Lactuca serriola* L., MALSY: *Malva sylvestris* L., PAPRH: *Papaver rhoeas* L., PHABR: *Phalaris brachystachys* Link, PICEC: *Picris echioides* L., POLAV: *Polygonum aviculare* L., SONAS: *Sonchus asper* (L.) Hill, SONOL: *Sonchus oleraceus* L., SINAL: *Sinapis alba* L., SISIR: *Sisymbrium irio* L., VERPE: *Veronica persica* Poir., XANST: *Xanthium strumarium* L.

Discussion

Tillage effect on C. dactylon

Two tillage interventions reduced weed infestation rates in a highly infested field. For the control of perennial weed species, soil disturbance that brings the subterranean organs up to the soil surface and cuts them into smaller fragments (Magrama, 2014) is the most desirable. The mortality of the stolons and rhizomes fragments is increased when they are buried deep compared to those that remain buried close to the soil surface, especially the smallest fragments, but also, soil disturbance when the weed is not dormant (autumn) increase weed control level compared to winter (when the weed is dormant) interventions (Valencia-Gredilla et al., 2019). In our experiment, soil disturbance significantly reduced weed coverage by about 60%. Our results are in accordance to Phillips (1993), who observed that double ploughing treatments reduced weed re-growth by 60%, a higher value of reduction compared to a single pass (33%). Also, Abdullahi (2002) reported that double ploughing led to better control of *C. dactylon* than single ploughing. On the other hand, the frequency of the weed was reduced by 14.8% after double tillage in autumn 2015. This result disagrees with Guglielmini and Satorre (2004), who observed that stolons and rhizomes were cut into smaller units and spread by tillage, although the establishment of these units was very low since only the segments in contact with the soil were able to survive, while the rest dried up and died. Our results might differ between studies because of the different machinery used for tillage and/or the soil type, as well as the timing of the intervention. Moreover, in our experiment, due to the high initial weed infestation level, *C. dactylon* was very compacted and the plough dragged weed plant material out of the plot and the inter-rows (personal observation), reducing weed infestation level. On the other hand, the colonization of the weed free plots can be related both to the plant dissemination through stolons and rhizomes and to the machinery interventions, especially tillage. Accordingly, the untilled plots (C1, *F. arundinacea*) had the lowest weed frequency in the initially weed free plots at the end of the experiment.

Cover crop effect on C. dactylon *and weed flora composition*

The different cover crops had an unequal effect over *C. dactylon* infestation levels. *Festuca arundinacea* (C1) and *H. vulgare* (C2) maintained or slightly reduced *C. dactylon*, both in terms of percentage of weed coverage and frequency, maintaining these variables low until the end of the experiment. This effect was observed in plots with or without initial presence of *C. dactylon*. Conversely, *Bromus* mixture (C3), *V. ciliata* (C4) and *M. rugosa* (C5) were not able to avoid the increase of these values, mainly in terms of weed coverage and especially in the plots with initial weed presence. The observed differences between *H. vulgare* and *F. arundinacea* versus the other three cover crop species could be explained by the dense canopy and biomass produced by *F. arundinacea* and *H. vulgare*, as shading has previously been reported to contribute to a reduction in *C. dactylon* biomass (Guglielmini and Satorre, 2002). Moreover, shade induces orthotropic shoot production and leaf elongation (Dong and de Kroon, 2006), so the weed could have been forced to invest more resources on its aerial parts in order to search for light while decreasing the growth of stolons and rhizomes. Although the presence of *C. dactylon* can be selected by managements with mowing/shredding interventions (Gago et al., 2007; Valencia-Gredilla et al., 2020), because of the characteristic of the weed (rhizomes and stolons), the pressure exerted by *F. arundinacea* cover crop was able to control its expansion. In *H. vulgare,* similarly to the rest of the annual cover crops, the pressure lasted every year until its death in June, but the annual tillage before seeding compensated this lack of competition during summer. On the other hand, and in spite of the fact that tillage was able to reduce *C. dactylon* infestations, annual tillage may have favoured the dissemination of small fragments across the field, thereby forming new infestation points, as observed in the plots without initial weed presence, and mainly in the cover crops that did not compete successfully.

The lack of tillage in the management of *F. arundinacea* explains the low frequency obtained in June 2017 in the plots without initial presence (7.5%), comparing to the rest of the annual cover crops (from 15.3% to 60.6%). This cover crop species requires time to stabilize, so the competition exerted during the first year may not have been enough to avoid new fragment establishments from outside the plot, and could explain the initial 7.5% of the weed presence. A combination of *F. arundinacea* with *H. vulgare*, a species with faster growth development, could probably solve this initial handicap. On the other hand, the weed flora composition under *F. arundinacea* was different in respect to the annual cover crop species. This cover crop is commonly implemented in vineyards for different goals (Chou et al., 2019; Coniberti et al., 2018; Pardini et al., 2002), and has been reported to control several weeds such as *Anagallis* spp., *Picris echioides* or *Solanum nigrum* after fescue aboveground biomass incorporation into the soil (Bertoldi et al., 2012). In our study, *F. arundinacea* successfully controlled other weed species, resulting in a weed community comprised of few species adapted to its management, where *Malva sylvestris* and *Picris echioides* stand out. The presence of these species can be related to the lack of tillage on this management and their tolerance to shredding.

Hordeum vulgare has already been reported to control *C. dactylon*, resulting in 0.5% weed coverage and 9.7% weed presence (Valencia-Gredilla et al., 2020). In the present study, these final values were higher, probably because of the high initial weed infestation level (over 25%), compared to that (7.4%) reported by Valencia-Gredilla et al. (2020). With respect to other weeds, *H. vulgare* prevented the establishment of a rich weed community assemblage, which could be explained by the dense canopy it provided, or by an allelopathy effect, as previously reported (Bertholdsson 2004; Bouhaouel et al., 2019; Dhima et al., 2006; Kremer and Ben-Hammouda, 2009).

The other two grass species, *Bromus* spp. and *V. ciliata*, were not able to effectively reduce *C. dactylon* coverage. Similar species implemented as cover crops in vineyards, such as *B. catharticus* and *V. myuros,* have been demonstrated to be suitable cover crops, with a high capacity for self-seeding and good persistence against weeds (Ibáñez, 2015). In the present study, however, the competition exerted by *Bromus* spp. and *V. ciliata* was not enough to reduce *C. dactylon* infestation. On the other hand, weed flora composition varied between *Bromus* spp. and *V. ciliata*, the former being related to a lower species richness than the later.

Finally, other *Medicago* spp,. such as *M. polymorpha `Anglona´*, has been reported to produce high quantity of biomass when implemented as cover crops in vineyard (Corleto and Cazzato, 2008), and an inverse relationship between the cover crop and weed biomass has been reported (Baumgartner et al., 2008). This was not the case in our experiments, as *M. rugosa* was not able to control *C. dactylon*. Moreover, this species was related to a higher number of weed species, as revealed by CCA analysis. Our results agree with Fujii (2001), who observed the allelopathic potential of *M. rugosa* in laboratory conditions (74% inhibition of lettuce plants) but the same species implemented as cover crops only controlled 17% of the weeds in field experiments.

Cynodon dactylon was not completely eliminated by any cover crop but the reduction achieved by *F. arundinacea* and *H. vulgare* coverss highlights its implantation of a very useful tool in an integrated weed management system. Moreover, to increase the efficacy of the managements, site-specific herbicide applications could be performed to reduce weed expansion in the field. To this respect, de Castro et al. (2020) developed an algorithm using aerial images taken with an Unmanned Aerial Vehicle (UAV) combined with decision tree and automatic object-based image analysis for mapping *C. dactylon* infesting cover crops in vineyards. This procedure allows the vine-growers to obtain precise *C. dactylon* infestation maps to perform site-specific interventions (such as herbicide applications). For this reason, the implantation of *H. vulgare* or *F. arundinacea* cover crops in vineyards is a suitable tool to control weeds, but its efficacy could be improved by combination with other tools that do not compromise cover crop development.

Conclusions

Tillage reduced *C. dactylon* coverage when infestation levels were high, and the plants that did survive had to face the suppressive effect of the cover crop. Moreover, all the cover crops maintained a low percentage of infestation in the plots that were not initially infested by *C. dactylon*, which verifies cover crops as a useful management tool in reducing the survival of disseminated fragments. In addition, *F. arundinacea* and *H. vulgare* impeded the expansion of *C. dactylon* in already infested plots, so they could be deemed the most suitable species for this purpose. Finally, general weed flora composition was affected by cover crop species, notably *F. arundinacea* was the cover crop with the lowest number of related weed species.

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References:

Abdullahi, A.E. (2002). *Cynodon dactylon* control with tillage and glyphosate. *Crop Prot.* 21, 1093-1100.

AEMET (2020). Agencia Estatal de Meteorología. Ministerio para la Transición Ecológica. Gobierno de España. Available online: www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos (accessed on 2 March 2020).

Álvarez-Iglesias, L., Puig, C.G., Garabatos, A., Reigosa, R.M., Pedrol, N. (2014). *Vicia faba* aqueous extracts and plant material can suppress weeds and enhance crops. *Allelopathy J.* 34, 299-314.

Baumgatner, K., Steenwerth, K.L., Veilleux, L. (2008). Cover-crop systems affect weed communities in California vineyards. *Weed Sci*. 56, 596-605.

Bertholdsson, N. O. (2004). Variation in allelopathic activity over 100 years of barley selection and breeding. *Weed Res*. 44, 78-86.

Bertoldi, C., De Leo, M., Ercoli, L., Braca, A. (2012). Chemical profile of *Festuca arundinacea* extract showing allelochemical activity. *Chemoecology,* 22, 13-21.

Bettoni, J.C., Gardin, J.P.P., Feldberg, N.P., Dalbó, M.A. (2012). Potencial alelopático de plantas de cobertura verde de inverno sobre o crescimento do porta-enxerto VR 043-43. *Rev. Ceres*, 59, 136-141.

Bouhaouel, I., Richard, G., Fauconnier, M. L., Ongena, M., Franzil, L., Gfeller, A., Amara, H.S., Du Jardin, P. (2019). Identification of barley (*Hordeum vulgare* L. SubSp. Vulgare) root exudates allelochemicals, their autoallelopathic activity and against *Bromus diandrus* Roth. germination. *Agronomy* 9, 1-18.

Chou, M.Y., Vanden Heuvel, J. (2019). Annual under-vine cover crops mitigate vine vigor in a mature and vigorous cabernet franc *vineyard. Am. J. Enol. Vitic.* 70, 98-108.

Coniberti, A., Ferrari, V., Disegna, E., Garcia Petillo, M. (2018). Under-trellis cover crop and planting density to achieve vine balance in a humid climate. *Sci. Hortic*. 227, 65-74.

Corleto, A., Cazzato, E. (2008). Adaptation of annual and perennial legumes and grasses utilised as cover crops in an olive grove and a vineyard in southern Italy. *Acta Hortic*. 767, 89-96.

De Castro, A., Peña-Barragán, J. M., Torres-Sánchez, J., Jiménez-Brenes, F., Valencia-Gredilla, F., Recasens, J., López-Granados, F. (2019). Mapping *Cynodon Dactylon* infesting cover crops with an Automatic Decision Tree-OBIA procedure and UAV imagery for precision viticulture. *Remote Sens.* 12, 56.

Den Hollander, N.G., Bastiaans, L., Kropff, M.J. (2007). Clover as a cover crop for weed suppression in an intercropping design. II: Competitive ability of several clover species. *Europ. J. Agronomy,* 26, 92-103.

Dhima, K.V., Vasilakoglou, I.B., Eleftherohorinos, I.G., Lithourgidis, A.S. (2006). Allelopathic potential of winter cereals and their cover crop mulch effect on grass weed suppression and corn development. *Crop Sci.* 46, 345-352.

Dong, M., De Kroon, H. (1994). Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*, 70, 99-106.

Fujii, Y. (2001). Screening and future exploitation of allelopathic plants as alternative herbicides with special reference to hairy vetch. *J. Crop Prod.* 4, 257-275.

Gago, P., Cabaleiro, C., García, J. (2007). Preliminary study of the effect of soil management systems on the adventitious flora of a vineyard in northwestern Spain. *Crop Prot.* 26, 584-591.

Garcia, L., Celette, F., Gary, C., Ripoche, A., Valdés-gómez, H., Metay, A. (2018). Management of service crops for the provision of ecosystem services in vineyards: a review. *Agric Ecosyst Environ*. 251, 158-170.

Guerra, B., Steenwerth, K. (2012). Influence of floor management technique on grapevine growth, disease pressure, and juice and wine composition: a review. *Am. J. Enol. Vitic*. 63, 149-164.

Guglielmini, A.C., Satorre, E.H. (2002). Shading effects on spatial growth and biomass partitioning of *Cynodon dactylon*. *Weed Res*. 42, 123-134.

Guglielmini, A.C., Satorre, E.H. (2004). The effect of non-inversion tillage and light availability on dispersal and spatial growth of *Cynodon dactylon*. *Weed Res*. 44, 366–374.

Ibáñez, S. (2015). Mantenimiento del suelo en el viñedo mediante cubiertas vegetales: aspectos generales y experiencias. Ed. Gobierno de la Rioja. 168 p.

Kremer, R.J., Ben-Hammouda, M. (2009). Allelopathic Plants. 19. Barley (*Hordeum vulgare* L). *Allelopathy J.* 24, 225-242.

Lemessa, F., Wakjira, M. (2015). Cover crops as a means of ecological weed management in agroecosystems. *J. Crop Sci. Biotech.* 18, 123-135.

MAGRAMA (2014). Guía de gestión integrada de plagas. Uva de transformación. Ministerio de Agricultura, Alimentación y Medio Ambiente, España, Madrid, 2014.

Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., Drexler, D., Tóthmérész, B. (2015). Establishment of three cover crop mixtures in vineyards. *Sci. Hortic.* 197, 117-123.

Pardini, A., Faiello, C., Longhi, F., Mancuso, S., Snowball, R. (2002). Cover crop species and their management in vineyards and olive groves. *Adv. Hort. Sci.* 16, 225-234.

Phillips, M.C. (1993). Use of tillage to control *Cynodon dactylon* under small-scale farming conditions. *Crop Prot*. 12, 267-272.

Recasens, J., Valencia, F., Montull, J.M., Taberner, A. (2018). Malas hierbas problemáticas en viñedos con cubiertas vegetales y métodos químicos para su control, *Vida rural*, 444, 48-58.

Ripoche, A., Celette, F., Cinna, J.-P., Gary, C. (2010). Design of intercrop management plans to fulfil production and environmental objectives in vineyards. *Eur. J. Agron*. 32, 30-39.

Sturm, D.J., Peteinatos, G., Gerhards, R. (2018). Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res*. 58, 331-337.

Sullivan, P. (2003). Overview of cover crops and green manures. ATTRA, National sustainable Agricultural information Center. Fayetteville.

Ter Braak, C.F., Smilauer, P. (2012). Canoco Reference Manual and User's Guide: Software for Ordination, Version 5.0. Microcomputer Power, Ithaca, USA.

Valencia-Gredilla, F, Recasens, J, Royo-Esnal, A (2019). Supervivencia de rizomas y estolones de Cynodon dactylon en función de su estado vegetativo, tamaño y profundidad de enterramiento. Actas XVII Congreso de la Sociedad Española de Malherbología, Vigo: 249-253.

Valencia-Gredilla, F., Royo-Esnal, A., Juárez-Escario, A., Recasens, J. (2020). Different ground vegetation cover management systems to manage *Cynodon dactylon* in an irrigated vineyard. *Agronomy* (under review).

Chapter 4

Germination behaviour of *Conyza bonariensis* to constant and alternating temperatures across different populations

Germination behaviour of *Conyza bonariensis* to constant and alternating temperatures across different populations

Abstract

Conyza bonariensis is one of the most problematic weed species throughout the world. It is considered highly noxious due to its interference with human activities, and especially the competition it poses with economically important crops. This research investigated the temperature requirements for seed germination of four populations of *C. bonariensis* with distinct origin and the influence of daily alternating temperatures. For this, a set of germination tests were performed in growth chambers to explore the effect of constant and alternating temperatures. Seeds of the four populations (from Lleida, Badajoz and Seville, Spain and Bahía Blanca, Argentina) were maintained at constant temperatures ranging from 5–35ºC. The final germination and cardinal temperatures (base, optimum and maximum) of each population were obtained. We also tested the influence of daily alternating temperatures on final germination. To do so, seeds were exposed to two temperature regimes: 5/15, 10/20, 15/25, 20/30 and 25/35ºC night/day temperature (intervals increasing 5ºC, with constant oscillation of 10ºC) and to 18/22, 16/24, 14/26, 12/28 and 10/30ºC night/day temperature (intervals with average of 20ºC, but increasing the oscillation in 4ºC between intervals). In general, all populations behaved similarly, with the highest germination percentages occurring in the optimum temperature range (between 21.7ºC and 22.3ºC) for both constant and alternating temperatures. In general, climatic origin affected germination response, where seeds obtained from the coldest origin exhibited the highest germination percentage at the lowest temperature assayed. In addition, we observed that the alternating temperatures can positively affect total germination, especially in oscillations that were further from the average optimum temperature (20ºC), with high germination percentage for the oscillations of 15/25, 20/30, 18/22, 16/24, 14/26, 12/28 and 10/30ºC in all populations. The cardinal temperatures obtained were significantly different across the populations. These results provide information that will facilitate a better understanding of the behaviour of *Conyza* and improve current field emergence models.

Keywords

Weed biology, hairy fleabane, germination response, cardinal temperatures, weed management.

Introduction

Weed management is one of the most important issues in crop production, especially in conservation cropping systems. Some weeds are difficult to control due to their specific biological characteristics and the lack of information about them.

Conyza bonariensis (L.) Cronquist, (hairy fleabane, flaxleaf fleabane) is one of the most problematic weed species throughout the world (Bajwa et al., 2016), appearing in more than 40 crops in 70 countries (Holm et al., 1997). In Spain, it is one of the most competitive introduced noxious weeds (Zambrano-Navea et al., 2013) that harms crops and leads to yield loss (Davis & Johnson, 2008; Trezzi et al., 2013, 2015; Urbano et al., 2007). *Conyza bonariensis* is an annual or short-lived perennial weed native to South America (Thebaud & Abbott, 1995; Wu et al., 2007). Its invasive behaviour is due to high plant fecundity that varies from 85,000 (Dauer et al., 2007) to 375,500 seeds per plant (Kempen & Graf, 1981). Moreover, the anemochorous dispersion of the seeds permits their long-distance spread and establishment in new fields (Savage et al., 2014).

Conyza bonariensis is difficult to control in minimum tillage and conservation cropping systems (Somervaille & McLennan, 2003; Wicks et al., 2000), but is easily controlled with tillage (Brown & Whitwell, 1988). Herbicide control depends on the growth stage of the plants (Shrestha et al., 2008) and if the population presents herbicide resistance, given that *C. bonariensis* has been cited as evolving resistance to herbicides with different action sites (EPSP synthase inhibitors, PSI Electron Diverter, ALS inhibitor, etc.) in several countries (Argentina, Australia, United States or Spain), in addition to evolving multiple resistance (PSI Electron Diverter and EPSP synthase inhibitors) (Heap, 2019).

Conyza is photoblastic, emerging from the upper layers of the soil surface (0–2 cm) with limited persistence, as it has very low dormancy levels and the viability of the ungerminated seeds is severely lost in the first year (Wu et al., 2007). Annual weed species survival is highly dependent on seedling emergence and recruitment (Forcella et al., 2000). Thus, it is important to know both timing and magnitude of seedling emergence in the field in order to implement successful control measures for weeds (García et al., 2013; Royo-Esnal et al., 2015). In this respect, Zambrano-Navea et al. (2013) modelled the emergence of *C. bonariensis* and developed a cohort-based stochastic model of the population dynamics (Zambrano-Navea et al., 2016). However, studying the germination response of more populations and at more temperatures and intervals would expand upon these existing models. *Conyza bonariensis* biology is well understood, but additional information regarding germination temperature thresholds is required to implement integrated management control measures. An added complexity is that the variation of threshold parameters between populations can be significant due to local adaptations (Tozzi et al., 2014; Bajwa et al., 2016). For example, in *C. bonariensis*, Karlsson & Milberg (2007) established cardinal temperatures of 4.2ºC (base temperature, T_b), 20ºC (optimum temperature, T_o) and 35ºC (ceiling temperature, T_c), while Wu et al., (2007) cited a T_b of 10.6ºC.

This research investigated thermal requirements for seed germination of four different *C. bonariensis* populations collected from contrasting environments. The final aim was to compare the total germination percentage of each population at constant temperatures and at different alternating temperatures. An additional objective was to compare the cardinal temperatures (T_b, T_o, T_c) of each population.

Materials and methods

Plant material collection

Conyza bonariensis seeds were harvested at maturity in September 2016 in Spain and in November 2017 in Argentina. Seeds from Spain were collected from three different habitats: a vineyard in Lleida (41.658010, 0.523766), a garden in Seville (37.352824, -5.933194) and an olive orchard in Badajoz (38.702537, -5.573246). The population from Argentina belonged to a garden in Bahía Blanca (-38.695394, -62.253302). The four locations have specific climatic conditions (Table 1). According with Torra et al. (2016), seeds were collected from different plants throughout the field, were air-dried under laboratory conditions for one week and dry stored in the dark in paper bags at 4ºC until the beginning of the experiment.

Table 1. Climatic characterization of the origin locality of each population. T_{Mean}: Annual Mean Temperature; T_{Warm}: Mean temperature of warmest month; T_{Cold}: Mean temperature of coldest month

Climatic characterization							
Location	Macro Bioclimate	Ombrotype	Thermotype	Bioclimate	T_{mean}	T_{warm}	T_{cold}
Lleida	Mediterranean	Xeric	Mesomediterranean	Continental	15.0	25.2	5.5
Badajoz	Mediterranean	Xeric	Mesomediterranean	Oceanic	17.1	26.1	8.6
Seville	Mediterranean	Xeric	Thermomediterranean	Oceanic	19.2	28.2	10.9
B.Blanca	Temperate	Xeric	Mesotemperate	Oceanic	15.3	23.6	7.5

Temperature means calculated for the period 1983-2010 for the Spanish locations and 1981-2010 for the Argentinian

Experimental design

Three germination tests were established at different temperature conditions and repeated twice: the first one at constant temperatures, the second at constant day/night temperature oscillations (T_{osc}) and different mean temperatures (T_m), and the third one at different day/night T_{osc} but with the same T_m. All the experiments were performed at the Departamento de Agronomía, Universidad Nacional del Sur and CONICET (Bahía Blanca, Argentina). In all three tests, batches of 30 seeds were sown on 9-cm Petri dishes lined with a Nº1 filter paper layer wetted with distilled water. Four replicates per population and temperature were used following a completely randomized design. Germinated seeds were counted on a daily basis until 21 days or until no further germination occurred during five consecutive days.

Germination test at constant temperatures (Test 1):

Seeds from each population were incubated, in November 2017, at constant temperatures of 5, 10, 15, 20, 25, 30 and 35ºC with a 12/12 day/night photoperiod. Temperatures were maintained at ±0.2ºC and monitored with digital temperature data loggers (Thermochron Ibuttons, Model DS1921G-F50; Maxim Integrated Products, Inc., San Jose, California, USA).

Germination test at alternating temperatures (variable T_m/constant T_{osc}) (Test 2):

In this assessment, seeds from each population were incubated at different alternating temperatures with 10ºC of T_{osc} 5/15, 10/20, 15/25, 20/30 and 25/35ºC night/day temperatures using an Electronic Gradient Plate Germinator (SECELEC, CCT-Bahía Blanca, CONICET). Seeds were placed inside independent germination chambers with automatic temperature control (±0.1ºC).

Germination test at alternating temperatures (constant T_m/variable T_{osc}) (Test 3):

In this test, T_m was maintained constant (20ºC) while T_{osc} was varied. The T_m considered was the optimum temperature (T_o) obtained by Wu et al. (2007). Seeds were then placed at constant 20ºC (in a growth chamber) and at 18/22, 16/24, 14/26, 12/28 and 10/30ºC night/day alternating temperatures. Thus, incubation temperature amplitudes (T_{osc}) were 0, 4, 8, 12, 16 and 20°C.

For the different tests, a seed was considered germinated when the radicle had extended more than 1mm beyond the seed coat (Steinmaus et al., 2000; Wu et al., 2007). Germinated seeds were removed from the dish once counted. Seed viability at the end of the germination tests was assessed by counting the number of germinated seeds after incubation at 20ºC (Wu et al., 2007) under a 12-h photoperiod for five days.

Statistical analysis

Total germination percentages between populations and incubation temperatures as well as cardinal temperatures were subjected to analysis of variance (ANOVA). The SED and LSD are provided.

Estimation of cardinal temperatures (base temperature, T_b, optimum temperature, T_o and ceiling temperature, T_c)

In order to estimate the cardinal temperatures for each population, a three parameter logistic function was first fitted to the cumulative emergence of each replication of each population at each constant temperature (Eq. [1]).

$$Y = \frac{a}{1+e^{\left(\frac{-(x-d50)}{b}\right)}} \qquad \text{Eq. [1]}$$

Where *Y* is the germination percentage, *a* is the maximum germination percentage, d_{50} is the time in days to achieve 50% of germination and b is the germination rate at d_{50}.

Estimation of the optimum temperature (T_o):

Once d_{50} was defined, its inverse value ($1/d_{50}$) where represented in a figure and a three-parameter Lorentzian function was fitted, equally, to each replicate of each population (Eq. [2]).

$$Y = \frac{a}{1+\left(\frac{x-d50}{b}\right)^2} \qquad \text{Eq. [2]}$$

Where Y is $1/d_{50}$ value at each temperature x, a is the maximum$1/d_{50}$ value, x_0 is the temperature at which the highest value of $1/d_{50}$ is obtained, and coincides with the centre of the peak and the optimum temperature at the same time; and parameter b is the mean width of the peak.

T_b and T_c estimation:

Once T_o was defined, sub-optimal temperatures were used to obtain T_b and supra-optimal temperatures were used to obtain T_c, and regression lines were fitted, respectively, to each (Eq. [2]) (Guillemin et al., 2013; Torra et al., 2016).

$$Y = ax + b \qquad \text{Eq. [3]}$$

Where Y is the $1/d_{50}$ value at each temperature x, a is the slope and b is a constant value. The point where the regression lines intercept the X axis, estimated with the mathematical approach of the regression line, was considered as the T_b and T_c respectively for each population (Holt & Orcutt, 1996; Steinmaus et al., 2000; Wu et al., 2007), assuming there are no intra-population variations.

All statistical analyses were performed with JMP Pro 14 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, USA. SAS Institute, Inc.) and all linear and non-linear regression analysis were conducted with SigmaPlot 11.0 (Systat Software, San Jose, CA).

Results

At constant temperatures, the highest germination percentages for all populations were obtained between 15 and 25ºC (Table 2, Test 1), being maximum at 20ºC. Seeds from Seville population had significantly different germination response comparing to the rest of the populations. A decrease in the germination percentage was observed at much lower (10ºC and 5ºC) or higher (30ºC) constant temperatures (Table 2). Each population was affected similarly by the gradient of temperatures assessed, but the observed germination percentage was different depending on the biotype. Statistical differences were found between populations and incubation temperatures at all constant temperatures ($P < 0.001$) (Test 1, Table 2). At constant 5ºC, the population from Lleida showed >25% of germination, value significantly higher than those observed in the population from Badajoz (1.3%), Seville (2.9%) and Bahía Blanca (9.6%). At constant 10ºC, the populations from Lleida and Bahía Blanca showed significantly higher germination percentages (82.1% and 86.3%, respectively) than the population from Seville (48.8%) and Badajoz (55.4%). At 20ºC, the population from Seville showed the lowest germination percentage (81.3%) significantly different from the rest of the populations, with values between 99.2% for Bahía Blanca and 96.3% for Badajoz population. On the other hand, the population from Badajoz showed the highest germination percentage (25.0%) at constant 30ºC, with values significantly different from the rest of the populations. Finally, no germination was observed at 35ºC in any of the populations.

With respect to test 2, the seed exposure to a constant T_{osc} and at different T_m significantly affected the germination percentage. Statistical differences were found between populations and incubation temperatures at all constant temperatures ($P < 0.001$) except for 20-30ºC ($P < 0.397$). Higher germination percentages were observed at 20-30ºC for populations from Lleida and Seville, with germination percentages higher than 93% for all of them, at 5-15ºC for Badajoz and at 15-25ºC for Bahía Blanca (Test 2, Table 2). At 10-20ºC and at 5-15ºC, the population from Seville showed lower germination percentages (58.3% and 64.6%, respectively) than the rest of the populations, which always showed values above 82%. At 15-25ºC, the population from Bahía Blanca showed a 98.3%, with significant different to those observed in population from Seville (76.7%) and Badajoz (87.9%) but not with Lleida (97.1%).

Table 2. Total percentage of germination for the *Conyza bonariensis* populations at each experiment. Test 1, constant temperatures; Test 2, alternating temperatures (variable mean/constant oscillation); Test 3, alternating temperatures (constant mean/variable oscillation).

	T (ºC)	Lleida	Badajoz	Seville	Bahía Blanca	SED	LSD	d.f.
Test 1	5	26.7	1.3	2.9	9.6	4.93	9.81	31
	10	82.1	55.4	48.8	86.3	8.98	18.39	31
	15	92.5	86.3	74.6	97.1	6.48	13.27	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	25	93.3	90.0	77.9	99.2	3.35	6.87	31
	30	15.8	25.0	7.1	5.4	3.78	7.75	31
	35	0.0	0.0	0.0	0.0	-	-	-
	SED	5.80	5.44	5.09	3.78			
	LSD	11.60	10.92	10.23	7.58			
	d.f.	55	55	55	55			
Test 2	5-15	90.0	94.2	64.6	82.1	5.84	11.96	31
	10-20	92.5	83.3	58.3	89.2	5.90	12.09	31
	15-25	97.1	87.9	76.7	98.3	4.55	9.32	31
	20-30	98.3	93.3	95.0	94.6	2.99	6.12	31
	25-35	21.3	45.0	19.6	10.0	5.07	10.38	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	SED	2.98	4.77	6.49	3.94			
	LSD	6.01	9.63	13.10	7.96			
	d.f.	47	47	47	47			
Test 3	18-22	98.3	93.8	83.3	94.6	3.74	7.65	31
	16-24	97.1	90.8	71.7	94.6	5.10	10.45	31
	14-26	95.0	94.2	85.0	99.2	4.14	8.49	31
	12-28	99.1	93.3	79.6	100.0	4.09	8.38	31
	10-30	93.8	95.4	75.8	92.5	5.12	10.48	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	SED	2.76	3.79	6.45	3.11			
	LSD	5.57	7.64	13.01	6.28			
	d.f.	47	47	47	47			

SED, standard error of the difference between two means; LSD, least significant difference between two means at $P = 0.05$; d.f., degrees of freedom associated with LSDs and SEDs.

At 25-35ºC, the population from Badajoz showed the highest germination percentage (45.0%), which was significantly higher than the populations from Lleida (21.3%), Seville (19.6%) and Bahía Blanca (10.0%).

In the case of test 3, where a same T_m and different T_{osc} was assessed, no significant differences in germination percentage were observed inside each population (*P* = 0.327 for Lleida, *P* = 0.780 for Badajoz, *P* = 0.334 for Seville and *P* = 0.09 for Bahía Blanca), but there were differences between the different T_{osc} considered ($P < 0.001$), (Test 3, Table 2). At all temperatures assayed, significantly lower germination percentages were observed between populations from Seville (with percentages between 71.7% and 85.0%) and the rest of the populations.

The effect of temperature did not only affect the final germination percentage, but also the germination timing and rate (Figure 1).

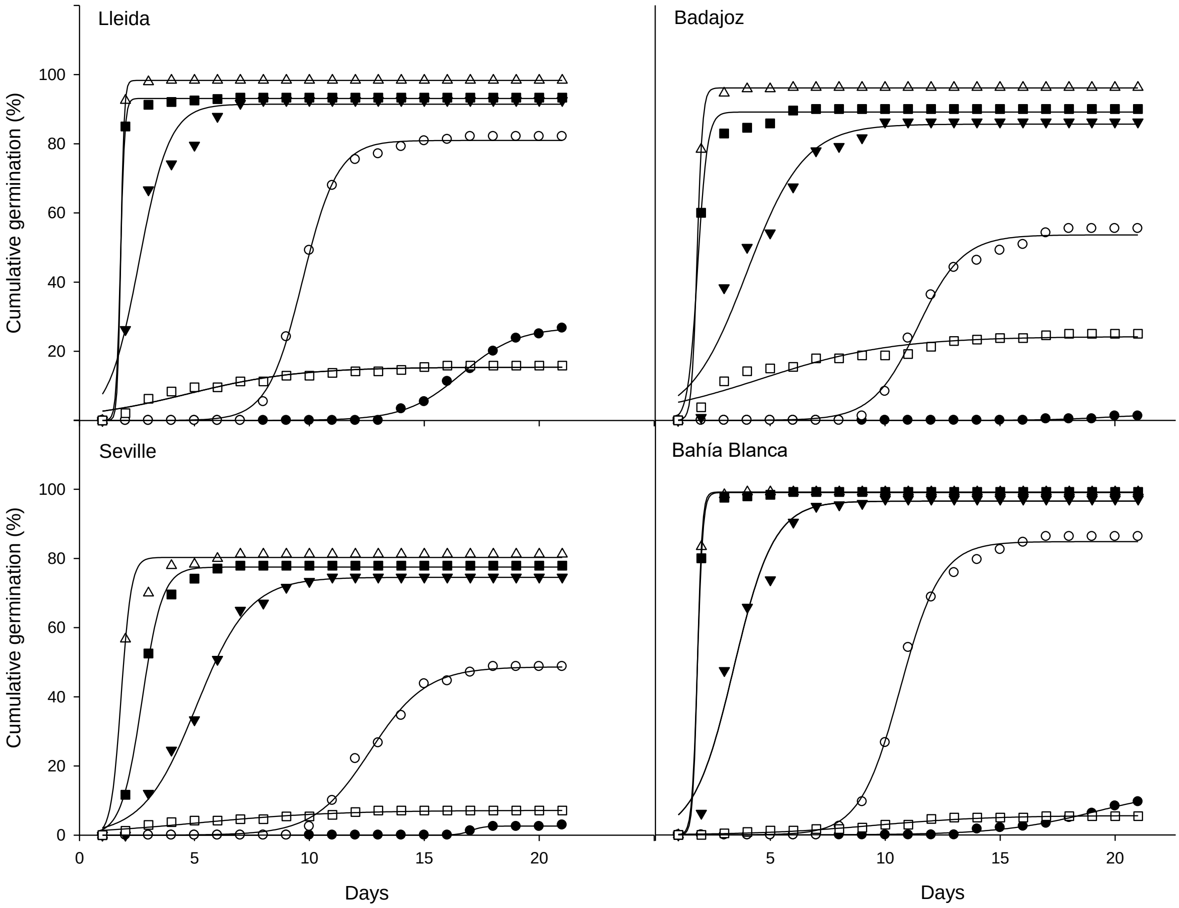

Figure 1. Cumulative germination percentage (%) of *Conyza bonariensis* at constant temperatures of 5ºC (●), 10ºC (○), 15ºC (▼), 20ºC (Δ), 25ºC (■), 30ºC (□) across the different populations.

Table 3. Values of parameters for the log-logistic models obtained from the cumulative germination data for each population assessed at every constant temperature.

Population	T (ºC)	a	b	x_0	F	P
Lleida	5	269.957	12.307	166.264	37.029.921	<0.0001
	10	809.581	0.7598	97.044	78.662.978	<0.0001
	15	914.461	0.6621	25.887	3.669.728	<0.0001
	20	983.114	0.0724*	1.7998*	502.481.464	<0.0001
	25	930.921	0.0911*	1.7858*	130.975.736	<0.0001
	30	154.061	24.003	47.235	1.656.618	<0.0001
Badajoz	5	1.7422*	1.2499*	195.611	1.352.574	<0.0001
	10	536.136	0.9767	114.118	16.594.462	<0.0001
	15	856.338	12.452	39.909	2.649.829	<0.0001
	20	961.187	0.1165*	18.273	288.319.755	<0.0001
	25	891.273	0.2082	18.518	9.188.929	<0.0001
	30	242.102	28.306	46.569	980.223	<0.0001
Seville	5	26.267	0.2815*	170.325	16.387.769	<0.0001
	10	486.567	12.155	126.461	22.070.097	<0.0001
	15	745.380	11.501	50.842	21.164.081	<0.0001
	20	802.973	0.2188*	18.141	4.639.906	<0.0001
	25	775.177	0.4436	27.171	26.146.267	<0.0001
	30	71.728	29.543	52.191	1.438.073	<0.0001
Bahía Blanca	5	135.797	20.785	191.641	10.586.897	<0.0001
	10	847.909	0.8799	106.539	49.653.598	<0.0001
	15	965.026	0.8747	34.091	4.595.015	<0.0001
	20	991.229	0.1018*	18.307	1.293.769.258	<0.0001
	25	989.696	0.1173*	18.312	199.244.810	<0.0001
	30	56.035	24.995	95.902	4.154.343	<0.0001

* Parameters not fitted ($P<0.05$) without incidence in the log-logistic function.

The cumulate germination of all populations at all temperatures successfully fitted to log–logistic function, except for those temperatures at which germination was too low or null (Table 3). At the lowest and highest constant temperatures, higher values were estimated for parameter x_o, indicating a delay in germination (Table 3; Figure 1). This behaviour is similar in all the populations. In general, the germination rate, identified as parameter b (Table 3) was faster between 15ºC and 25ºC, compared to at 5ºC, 10ºC and 30ºC, except for the population from Badajoz. Parameter b could not be significantly fitted ($P<0.05$) for populations from Lleida and Bahía Blanca at 20 and 25ºC, due to the fast germination rate, though the log-logistic function was significantly fitted (Table 3).

Estimation of T_b, T_o and T_c

Lorentzian function was significantly adjusted to the *$1/d_{50}$* values (Figure 2) for every population with high accuracy ($R^2 > 0.86$). The optimal temperature (T_o) for populations from Lleida and Seville was established at 21.7ºC, whereas these values were higher for Bahía Blanca and Badajoz: 22.2ºC and 22.3°C, respectively (Table 4).

Regression lines were successfully fitted for the estimation of the T_b and T_c, with R^2 values ranging between 0.90 and 0.99 (Figure 3) in seven of the eight cases, and being $R^2 = 0.81$ for the T_c of the Badajoz population. The lowest T_b (4.9°C) was estimated for the population from Lleida (Table 4), while the highest values (8.9ºC and 8.4ºC) were obtained for populations from Seville and Badajoz, respectively. An intermediate T_b value (6.9°C) was observed in the Bahía Blanca population (Table 4). The lowest T_c value was also obtained from the Bahía Blanca population (31.5°C), followed by that from Seville (31.7ºC) and Lleida (32.3ºC). Finally, the highest value was observed in the Badajoz population (34.0ºC). Statistical differences were found between populations ($P < 0.001$) for T_b but not for T_o and T_c ($P < 0.246$ and $P < 0.103$, respectively).

Table 4. Estimated base temperatures (T_b), optimal temperature (T_o), and ceiling temperatures (T_c), for each population of *Conyza bonariensis* assessed. Mean values are presented in ºC.

	Lleida	Badajoz	Sevilla	B. Blanca	SED	LSD	d.f.
T_b	4.9	8.4	8.9	6.9	0.45	0.92	31
T_o	21.7	22.3	21.7	22.2	0.31	0.64	31
T_c	32.3	34.0	31.7	31.5	1.05	2.17	27

SED, standard error of the difference between two means; LSD, least significant difference between two means at $P = 0.05$; d.f., degrees of freedom associated with LSDs and SEDs.

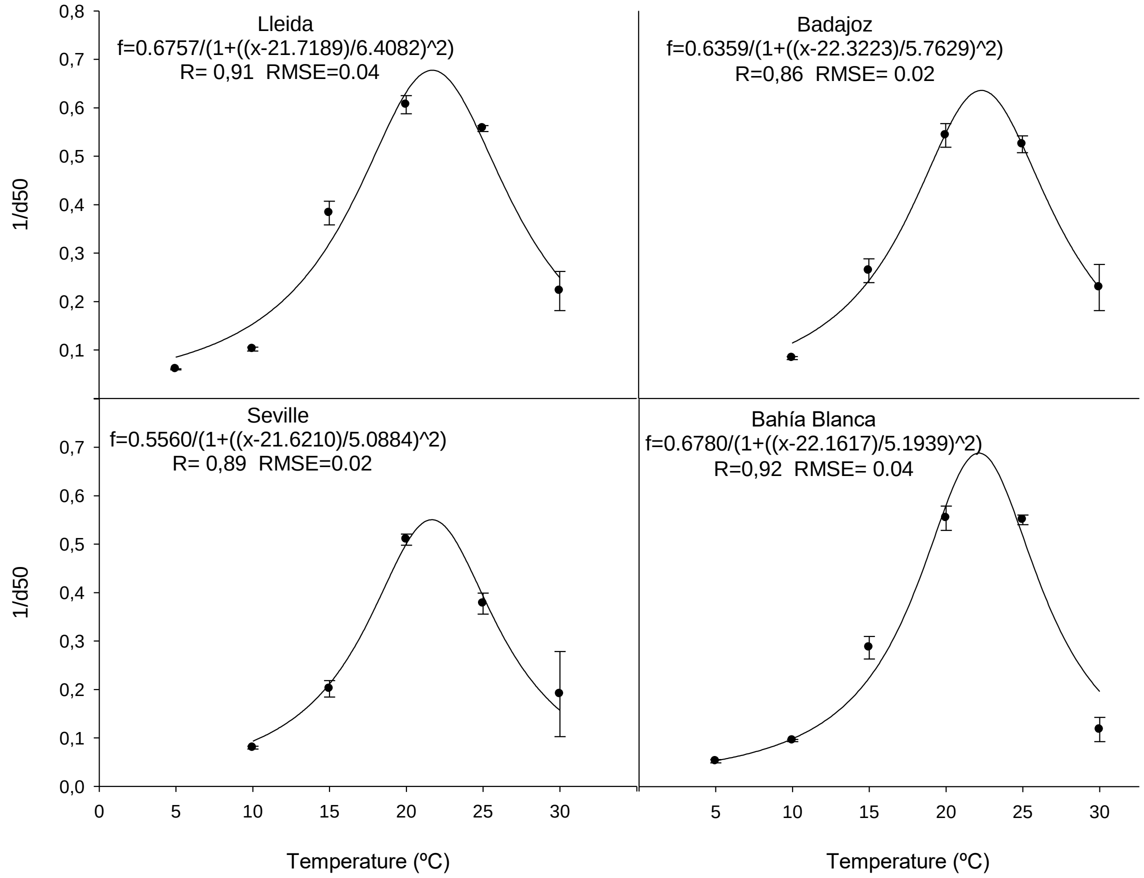

Figure 2. Lorentzian functions adjusted to the *1/d₅₀* obtained at each temperature by each population assessed. Symbols represent means and bars represent standard errors. R^2 of the functions and RMSE are provided.

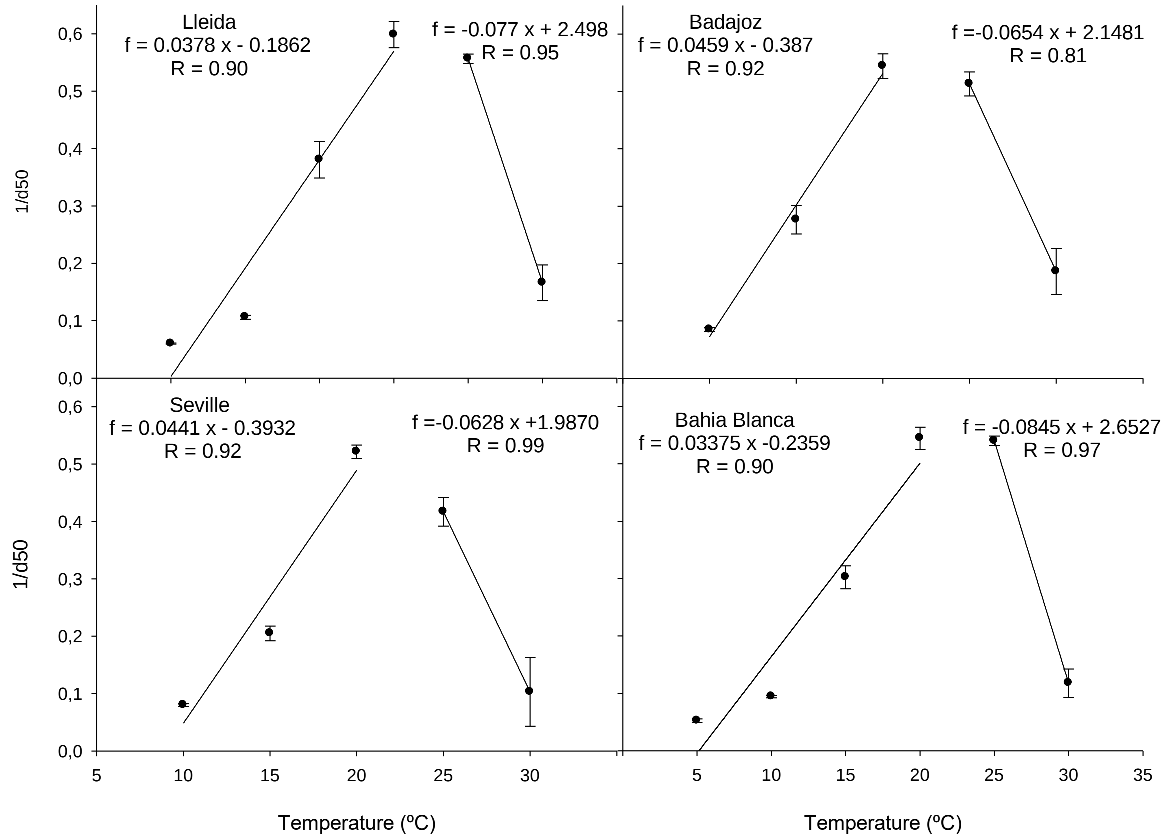

Figure 3. Estimation of the base temperatures (T_b) on the left and ceiling temperatures (T_c) on the right for each of the *Conyza bonariensis* populations. Symbols represent means and bars represent standard errors. Regression lines are presented, together with the lineal functions for each T_b and T_c, followed by the R^2.

Discussion

All populations showed similar germination behaviour. Germination percentage was highest near the optimum temperature and there were significant differences in the final germination percentages, which appeared mainly at lower temperatures. These differences could be explained, in part, by the climate of the original localities, but also by a possible maternal effect. The environmental conditions under which the mother plant produced the seeds, and also the position of the seed in the plant can impact seed germination. Likewise, water deficit, the age of the plant, the day length, the parental photo-thermal environment, light quality, altitude, and temperature are known, among other factors, to affect germinability (and dormancy in some cases) in other species (Gutterman, 2000; Menegat et al., 2018).

Effect of temperature on germination

For all populations, the maximum germination percentage was reached near 20ºC. The estimation of the optimal temperature (T_o) (21.7ºC to 22.3ºC) allowed for little distinction between populations (Table 4). When the temperatures moved away from the optimal, the final germination percentage decreased. This decrease was faster for supra-optimal than for sub-optimal temperatures (Table 2, Test 1). These results are similar to those found by Hardegree (2006) for various gramineous species.

The alternating temperatures did not have a significant effect when the mean temperature was close to the optimum (20ºC). Similar trend was observed by Ottavini et al. (2019) with *C. canadensis*, who did not observed significant differences between constant and alternating temperatures of 15, 20 and 25ºC on average. Moving away from the optimal, the alternating temperatures (Table 2, Test 2) favoured the germination of the seeds compared to the corresponding constant mean temperature (Test 1, Table 2). For example, at 10ºC, an oscillation of 10ºC (5-15ºC) increased the total germination percentage in Lleida, Badajoz and Seville, in between 8% and up to 39%. The Bahía Blanca population was apparently the only one not affected by the oscillation at such low temperature. Similarly, at constant temperatures of 30ºC, germination percentage was, on average, 13.3%; whereas at oscillation, the germination percentage was 24% at 25-35ºC. This increasing germination percentage is also observed by Vidal et al. (2007) when comparing constant 25ºC to 20-30ºC, which increased from less than 50% up to nearly 85%. Differences could be explained by alternating temperatures, which enhance germination in photoblastic species (Roeder et al., 2013) such as *C. bonariensis*. Moreover, temperature changes are more pronounced at the soil surface where *C. bonariensis*, a very small seeded species, germinates better. Furthermore, the germination of this species is null at depths deeper than 2 cm (Wu et al., 2007), similar to *C. canadensis* which germination is reduced to 0% between 0.5 cm and 1cm (Ottavini et al., 2019).

In the intervals with constant mean temperatures and variable oscillation (Table 2, Test 3), there are not differences between the intervals and with the constant 20ºC, with similar percentage germination with the intervals with variable mean and constant oscillation when the mean is close to 20ºC.

The differences observed between germination percentages at constant and alternating temperatures could correspond to depth- and gap-sensing mechanism: temperature oscillations are more pronounced at or near to the soil surface and the amplitude of these fluctuations decreased with burial depth (Ren et al., 2002). This could be an adaptation that staggers the germination with the changing temperatures throughout the seasons (Vidal et al., 2007).

Effect of the origin of the population on germination

Differences in germination percentages are accentuated between populations (Table 2). The population from Lleida, which is a comparatively colder location (Table 1), showed more germination (26.7%) at the lowest temperature assayed (5ºC). The populations from warmer climates had lower germination percentage at this temperature (1.3% and 2.9% for Badajoz and Seville, respectively). These differences are not so clear when comparing the populations at 30ºC, wherein germination percentages from Badajoz and Seville populations showed statistical differences of 25.0% and 7.1%, respectively.

Under the 10ºC oscillation treatment (Table 2, Test 2), excluding the higher one (25/35ºC), there were a high germination percentages and significant differences between the intervals across the populations. Similarly, there were high germination percentages for seeds subjected to temperature oscillations that were 20ºC on average (Table 2, Test 3).

Higher germination percentages with 10ºC oscillation occurred between 15/25ºC and 20/30ºC in all populations, except for that of Badajoz which had highest germination percentages at 5/15ºC. Except for Seville population, these results are not in accordance with those from Travlos & Chachalis (2013), who found differences between 15/25ºC and 20/30ºC in populations of *C. bonariensis* from Greece. On the other hand, the lack of differences between 10/20ºC and 15/25ºC intervals in populations from Lleida and Badajoz agrees with other authors finding that there is less variation for these temperature ranges in climatically closer biotypes. Despite this, the Spanish populations from Lleida and Badajoz seem to be more adapted to colder winters (exhibiting more winter-summer oscillation) than those from Greece, as at 5/15ºC the Spanish ones obtained over 90% germination, while the Greek ones did not exceed 35%.

Our results also agree with those from Karlsson & Milberg (2007), for populations from Ethiopia, Mexico and Morocco, for the 10/20, 15/25 and 20/30ºC intervals, but not for the 5/15ºC, where Lleida, Badajoz and Bahía Blanca populations obtained higher germination percentages (82.1-94.6%) than in previous studies (below 75%). The low germination percentages obtained by Karlsson & Milberg (2007) at 5/15ºC could be explained by the local climatic conditions of these populations, which are classified as: Tropical pluviseasonal (Mexico), Tropical xeric (Ethiopia), and Mediterranean xeric-oceanic (Morocco) (Rivas-Martinez & Rivas-Saenz, 1996-2018). Population differences could be due to the adaptation to the climatic characteristics of each original site where the seeds were produced (Clements & DiTommaso, 2011), as reported for *C. canadensis* (Tozzi et al., 2014).

Germination patterns and threshold values

The germination patterns of all population at all constant temperatures were, in general, successfully fitted to a log-logistic sigmoidal function (Table 3, Figure 1). The lack of this adjustment in some cases (Table 3) could be partially explained by the exceedingly high or low germination rates of the populations: 20ºC and 25ºC for Lleida and Bahía Blanca, and 5ºC and 20ºC for Badajoz and Seville.

The calculation of the x_0 with the log-logistic model allowed the estimation of the threshold values of T_b and T_c (Figure 3), while T_o was estimated with the Lorentzian model applied to the total germination percentages (Figure 2). Our results for the population from Lleida agree with Wu et al. (2007), which estimated that *C. bonariensis* can germinate between 4.2ºC and 30ºC, even if the T_o is 20ºC. The T_b of one of our populations (Lleida) are close to that from Wu et al. (2007), and the values are in accordance with the germination response explained above: T_b in Lleida shows the lowest value (4.9ºC) followed by Bahía Blanca (6.9ºC), which agrees with their local climatic origin. In accordance with this, the T_b in Seville (8.9ºC) and Badajoz (8.4ºC) are higher than in Lleida and Bahía Blanca, and not considerably different from that estimated by Zambrano-Navea et al. (2013) (10.6ºC). The differences in the T_b from Lleida and Bahía Blanca (4.9ºC and 6.9ºC) compared to the T_b obtained by Zambrano-Navea et al. (2013) can be explained by the variations in the experimental design, as these authors used constant 15ºC as the lowest temperature, while we also experimented with 10 and 5ºC, which obtained 48.8 and 2.9% germination, respectively. The idea that

C. bonariensis is a summer weed could have led to the thought that its T_b was similar to other summer weeds, such as *Amaranthus retroflexus* which is estimated to be between 10.0ºC and 12.9ºC (Loddo et al., 2018), or *C. canadensis*, which is between 8ºC and 14ºC, depending on the population (Tozzi et al., 2014).

The estimated ceiling temperature (T_c) varied from 31.5ºC to 34.0ºC, which agrees with the lack of germinated seeds at constant 35ºC in any population. These results also agree, in part, with those from Yamashita & Guimaraes (2011), who only obtained 6% germination at a constant 35ºC and 1% germination at 40ºC. Similar to the tendency in the other threshold values, the lowest T_o was obtained in the population from Lleida, but also in Seville (21.7ºC), while the highest one was obtained in the population from Badajoz (22.3ºC). These T_o values, which could in general be established at about 22 ºC (±0.3ºC), differ from that selected from the literature (Wu et al., 2007) to set the Test 2 and Test 3 of the experiment, and could be considered for future experiments.

Implications for developing emergence models

The emergence model for *C. bonariensis* from Zambrano-Navea *et al.* (2013) has been proven to be valuable in several sites. This model was developed and validated with populations from the South of Spain, and has less relevance for other climatic biotypes and regions since the germination behaviour and the threshold values of other populations are different. The genetic variation of the *Conyza* species (Ren et al., 2010) could explain, in part, these results. Even if *C. bonariensis* has the ability to spread and disperse its seeds long distances (Savage et al., 2014), which could diminish the variations between populations, these differences are still important according to the results of the present and past work (Karlsson & Milberg, 2007). In addition to genetic origin, the maternal effect is another factor which could have enhanced differences between local populations.

In our study, there were four degrees (ºC) of difference in T_b between the seeds from different origins, thereby impeding the development of a common model. In order to develop a model that could be widely applied, the next step is to test differences in the base parameters and germination behaviour of populations coming from different geographical sites, but belonging to the same climatic biotype. If there are not any differences between them, a more precise model could be developed or the current one created by Zambrano-Navea et al. (2013) could be readjusted to the populations of a certain climatic area.

Conclusions

The germination percentage of *C. bonariensis* was higher when close to the optimal temperature obtained (22ºC), both for constant and alternating temperatures. In the intervals with same T_{osc} and different T_m, some obtained higher germination percentages than at constant temperature. In the intervals with different T_{osc} but the same T_m, there are high germination percentages for all temperatures, without significant differences from the constant temperature. The different populations responded to the tests according to the apparent influence of their climatic origin. The biotype adapted to the coldest winter site (Lleida) had more germinated seeds at lower temperatures, while the biotypes adapted to warmer climatic sites (Badajoz and Seville) were more acutely affected by a temperature decrease. The differences in the threshold values for the cardinal parameters, as well as the in the germination behaviour of the different climatic biotypes prevents us from developing a common germination/emergence model. Thus, there is further need of investigation to achieve the goal of obtaining accurate models for each climatic region.

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Conflicts of interests

Authors declare that there are not conflicts of interest.

References

Bajwa, A.A., Sadia, S., Ali H.H., Jabran, K., Peerzada, A.M., Chauhan, B.S. (2016). Biology and management of two important *Conyza* weeds: a global review. *Environ. Sci. Pollut. Res.* 23, 24694–24710.

Brown, S.M., Whitwell, T. (1988). Influence of tillage on horseweed (*Conyza canadensis*). *Weed Technol.* 2, 269–270.

Clements, D.R., Ditommaso, A. (2011). Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? *Weed Res.* 51, 227–240.

Dauer, J.T., Mortensen, D.A., Vangessel, M.J. (2007). Temporal and spatial dynamics of long-distance *Conyza canadensis* seed dispersal. *J. Appl. Ecol.* 44, 105–114.

Davis, V.M., Johnson, W.G. (2008). Glyphosate-resistant horseweed (*Conyza canadensis*) emergence, survival, and fecundity in no-till soybean. *Weed Sci.* 56, 231–236.

Forcella, F., Benech-Arnold, R.L., Sánchez, R., Ghersa, C.M. (2000). Modeling seedling emergence. *Field Crops Res.* 67, 123–139.

García, A., Recasens, J., Forcella, F., Torra, J., Royo-Esnal, A. (2013). Hydrothermal emergence model for Ripgut brome (*Bromus diandrus*). *Weed Sci.* 61, 146-153.

Guillemin, J.P., Gardarin, A., Granger, S., Reibel, C., Munier-Jolain, N., Colbach, N. (2013). Assessing potential germination period of weeds with base temperatures and base water potentials. *Weed Res.* 53, 76–87.

Gutterman, Y. (2000). Maternal effects on seeds during development. In Fenner, M (Ed.), Seeds: The ecology of regeneration in plant communities (pp. 59-84) New York, NY: CABI.

Hardegree, S.P. (2006). Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Ann. Bot.* 97, 1115–1125.

Heap, I.M. (2019). International Survey of Herbicide Resistant Weeds. Available at: http://www.weedscience.org (last accessed 7 March 2019).

Holm, L., Doll, J., Holm, E., Pancho, J., Herberger, J. (1997). World Weeds, Natural

Histories and Distribution; J. Wiley, New York, USA, 226–235.

Holt, J.S., Orcutt, D.R. (1996). Temperature thresholds for bud sprouting in perennial weeds and seed germination in cotton. *Weed Sci.* 44, 523–533.

Karlsson, L.M., Milberg, P. (2007). Comparing after-ripening response and germination requirements of *Conyza canadensis* and *Conyza bonariensis* (Asteraceae) through logistic functions. *Weed Res.* 47, 433–441.

Kempen, H.M., Graf, J. (1981). Weed seed production. *Proceedings of the Western Society of Weed Science*, 34, 78–81.

Loddo, D., Ghaderi-Far, F., Rastegar, Z., Masin, R. (2018). Base temperatures for germination of selected weed species in Iran. *Plant Prot. Sci.* 54, 60–66.

Menegat, A., Milberg, P., Nilsson, A., Andersson, L., Vico G. (2018). Soil water potential and temperature sum during reproductive growth control seed dormancy in *Alopecurus myosuroides* Huds. *Ecol. Evol.* 8, 7186–7194.

Ottavini, D., Pannacci, E., Onofri, A., Tei, F., Jensen, P. (2019). Effects of light, temperature, and soil depth on the germination and emergence of *Conyza canadensis* (L.) Cronq. *Agronomy*, 9, 533.

Ren, J., Tao, L., Liu, X.M. (2002). Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. *J. Arid Environ.* 51, 603-611.

Ren, M.-X., Li, X.-Q., Ding, J.-Q. (2010). Genetic variation and spread pattern of invasive *Conyza sumatrensis* around China’s Three Gorges Dam. *Acta Oecologica*, 36, 599–603.

Rivas-Martinez., Rivas-Saenz. (1996-2018). Worldwide Bioclimatic Classification System. Phytosociological Research Center, Spain. http://www.globalbioclimatics.org

Roeder, M., Ferraz, I.D.K., Hölscher, D. (2013). Seed and germination characteristics of 20 amazonian liana species. *Plants, 2,* 1–15.

Royo-Esnal, A., García, A., Torra, J., Forcella, F., Recasens, J. (2015). Describing *Polygonum aviculare* emergence in different tillage systems. *Weed Res.* 55, 387–395.

Savage, D., Borger, C.P., Renton, M. (2014). Orientation and speed of wind gusts causing abscission of wind-dispersed seeds influences dispersal distance. *Funct. Ecol.* 28, 973–981.

Shrestha, A., Hembree, K., Wright, S. (2008). Biology and management of horseweed and hairy fleabane in California. Publication No. 8314. University of California, Division of Agricultural and Natural Resources, Fresno, California.

Somervaille, A., McLennan, B. (2003). The 2nd Fallow Weed Management Guide. Conservation Farmers, Toowoomba, Australia.

Steinmaus, S.J., Prather, T.S., Holt, J.S. (2000). Estimation of base temperatures for nine weed species. *J.Exp. Bot.* 51, 275–286.

Thebaud, C., Abbott, R. (1995). Characterization of invasive *Conyza* Species (Asteraceae) in Europe: quantitative trait and isozyme analysis. *Am. J. Bot.* 82, 360–368.

Torra, J., Royo-Esnal, A., Recasens, J. (2016). Temperature and light requirements for germination and emergence of three arable papaveraceae species. *Weed Sci.* 64, 248–260.

Tozzi, E., Beckie, H., Weiss, R., Gonzalez-Andujar, J.L., Storkey, J., Cici, S.Z.H., Acker, R.C. (2014). Seed germination response to temperature for a range of international populations of *Conyza canadensis*. *Weed Res.* 54, 178–185.

Travlos, I.S, Chachalis, D. (2013). Relative competitiveness of glyphosate- resistant and glyphosate-susceptible populations of hairy fleabane, *Conyza bonariensis*. *J. Pest Sci.* 86, 345–351.

Trezzi, M.M., Balbinot-Jr, A.A., Benin, G., Debastiani, F., Patel, F., Miotto-Jr, E. (2013). Competitive ability of soybean cultivars with horseweed (*Conyza bonariensis*). *Planta Daninha,* 31, 543–550.

Trezzi, M.M., Vidal, R.A., Patel, F., Miotto, E., Debastiani, F., Balbinot, A.A., Mosquen, R. (2015). Impact of *Conyza bonariensis* density and establishment period on soyabean grain yield, yield components and economic threshold. *Weed Res.* 55, 34–41.

Urbano, J.M., Borrego, A., Torres, V., Leon, J.M., Jimenez, C., Dinelli, G., Barnes, J. (2007). Glyphosate-resistant hairy fleabane (*Conyza bonariensis*) in Spain. *Weed Technol.* 21, 396–401.

Vidal, R.A., Kalsing, A., Goulart, I., Lamego, F., Christoffoleti, P. (2007). Impact of temperature, light and seed depth on emergence and germination of *Conyza bonariensis* e *Conyza canadensis* resistant to glyphosate. *Planta Daninha*, 25, 309-315.

Wicks, G.A., Felton, W.L., Murison, R.D., Martin, R.J. (2000). Changes in fallow weed species in continuous wheat in northern New SouthWales, 1981-90. *Aust. J. Exp. Agric.* 40, 831-842.

Wu, H., Walker, S., Rolling, M.J., Yuen, D.K., Robinson, G., Werth, J. (2007). Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist). *Weed Biol. Manag.* 7, 192–199.

Yamashita, O., Guimaraes, S. (2011). Germination of *Conyza canadensis* and *Conyza bonariensis* seeds under different conditions of temperature and light. *Planta Daninha,* 29, 333-342.

Zambrano-Navea, C., Bastida, F., Gonzalez-Andujar, J.L. (2013). A hydrothermal seedling emergence model for *Conyza bonariensis*. *Weed Res.* 53, 213–220.

Zambrano-Navea, C., Bastida, F., Gonzalez-Andujar, J. L. (2016). A cohort-based stochastic model of the population dynamic and long- term management of *Conyza bonariensis* in fruiting tree crops. *Crop Prot.* 80, 15-20.

Chapter 5

In vitro assay of herbicide effects of aqueous extracts from plants implemented as cover crop and/or mulch in vineyards

In vitro assay of herbicide effects of aqueous extracts from plants implemented as cover crop and/or mulch in vineyards

Summary

Cover crops and mulches have become an alternative for soil management in vineyards due to the agronomic, environmental and economic advantages that they offer, especially the possibility of weed control. Implicitly to this objective lies the idea of assessing the potential herbicide effect of the allelochemicals released by different species implemented as a cover crop and/or mulch in vineyards. With this objective, the present work evaluated the phytotoxic effect of 12 aqueous extracts of selected species with potential use as a cover crop or mulch: a *Bromus* mixture *(B. hordeaceus and B. rubens), Festuca arundinacea, Hordeum murinum, H. vulgare, Medicago rugosa, M. sativa, Phacelia tanacetifolia, Sinapis alba, Trifolium incarnatum, T. subterraneum, Vulpia ciliata* and *Pinus sylvestris*, on the germination and early growth of three troublesome weeds (*Conyza bonariensis, Aster squamatus* and *Bassia scoparia*). The different in-vitro bioassays showed that aqueous extracts of some species greatly inhibited and/or reduced germination and radicle and hypocotyl growth of the target weed species, especially *A. squamatus* and *C. bonariensis*. Also, the chemical profiles of phenolic compounds of the aqueous extracts were also obtained and identified by HPLC-DAD. These results support cover crop and mulch implementation in weed management but can also help to select the most suitable species to implement as a cover crop or mulch according to the target weed species.

Keywords: Phytotoxicity, Phenolic compounds, Weeds, Natural herbicides, Organic agriculture.

Introduction

Grape production can be compromised by different factors, including weed presence. The high competition for water and nutrients exerted by weeds (Oerke, 2006) makes weed management an important challenge in viticulture. For weed control, winegrowers usually till and/or apply herbicides but these managements have a series of disadvantages, such as erosion, land contamination or development of herbicide resistance risk, among others (Cerdan et al., 2010; Heap, 2019; Prosdocimi et al., 2016). An alternative to these soil managements is the implementation of a cover crop or mulching. Cover crops bring several advantages from an agronomic, environmental or economic point of view (Gómez et al., 2011; Ibáñez 2015; Morlat and Jacquet, 2003), weed suppression being one of them (Garcia et al., 2018; Gago et al., 2007; Miglécz et al., 2015). Cover crops can reduce weed presence for the competition exerted for light, water and nutrients but can also release secondary metabolites (allelochemicals) that can interfere with weed development (Álvarez-Iglesias et al., 2018; Bettoni et al., 2012; Farooq et al., 2011; Sturm et al., 2018; Weston and Duke, 2003). The efficacy and magnitude of the allelopathic effect depend on both the implemented cover crop and the target weeds to be controlled. Some weeds such as *Aster squamatus* (Spreng.) Hieron, *Bassia scoparia* (L.) A. J. and *Conyza bonariensis* (L.) Cronquist, have become difficult to manage in vines and other crops due to their biological characteristics. *Aster squamatus* is a species native to central South America and widely distributed in many European countries (Sajna et al., 2014). This species is difficult to control in vineyards and orchards in Spain, due to its adaptation to mechanical shredding (Recasens et al., 2018), its high fecundity and its airborne dispersion (Sajna et al., 2014). In addition, the presence of resistant biotypes to three imidazolinone herbicides along with cross-resistance to sulfonylurea herbicides has been confirmed in Spain (Osuna et al., 2003). *Bassia scoparia* is also a noxious weed in vineyards (Recasens et al., 2018) displaying an effective mechanism of seed dispersal by plant rotation, with a high potential fecundity (Osipitan, 2016; Osipitan et al., 2019). Herbicide control in vineyards is not easy and this can be explained by different factors: in general, herbicide weed control is reduced as plant size increases, and *B. scoparia* presents rapid growth (Friesen et al., 2009). Furthermore, this species presents difficult-to-wet leaf characteristics (such as pubescent leaves) which can reduce herbicide absorption and consequently, decrease herbicide efficacy (Friesen et al., 2009). *Bassia scoparia* is also resistant to several herbicides, and some biotypes present multiple resistance (in some cases, 4 sites of action) (Heap, 2019). *Conyza bonariensis* is also a noxious species difficult to control (Bajwa et al., 2016). It presents a high fecundity and an efficient airborne dispersion of seeds (Kempen & Graf, 1981; Savage et al., 2014). Herbicide effectiveness depends on both the growth plant stage and if the biotype is herbicide-resistant. *Conyza bonariensis* resistance to herbicide with different action sites and also multiple resistance have been confirmed (Heap, 2019). In Spain, populations of *C. bonariensis* sampled from perennial crop locations have been confirmed simazine-resistant (de Prado et al., 1989) and glyphosate-resistant (Urbano et al., 2007).

After the evidence of harm caused by these weeds and the difficulty to control them by mechanical or chemical methods, the need to develop new strategies that inhibit seed germination and emergence has arisen. In this context, it is promising to study the phytotoxic effect of different aqueous extracts of species that can be implemented as a cover crop and/or mulch in vineyards. In order to isolate the experiment from the effect of biotic and abiotic factors, an *in vitro* assay under controlled conditions was carried out to evaluate the phytotoxic potential of the aqueous extracts of 12 different species implemented as cover crop and/or mulch in vineyards on germination and radicle and hypocotyl growth of three noxious weeds: *C. bonariensis, A. squamatus* and *B. scoparia*. According to this trial, a complementary analysis of the chemical profile of water-soluble compounds (phenolic acids and flavonoids) was also carried out.

Material and methods

Plant material

Different species implemented as a cover crop and/or mulch in the vineyard were selected for the experiment: a *Bromus* mixture *(B. hordeaceus* L. *and B. rubens* L.*), Festuca arundinacea* Schreb. cv. `Firaces R1´*, Hoderum murinum* L.*, H. vulgare* L. cv. `Meseta´*, Medicago rugosa* Desr. cv. `Sapo´*, M. sativa* L. cv. `Victoria´*, Phacelia tanacetifolia* Benth., *Sinapis alba* L., *Trifolium incarnatum* L. cv. `Red´*, T. subterraneum* L. cv. `Dalkeith´*, Vulpia ciliata* Dumort. and *Pinus sylvestris* L. Most species were sown in experimental fields of the University of Lleida (Lleida, Spain) in autumn (November 2016), and collected in spring (April-May 2017) (Figure 1.) when legume-species were at flowering stage and gramineous had started spike development. The entire plant, including flowers, leaves, and roots, was collected. In the case of *P. sylvestris* only branches smaller than 25 cm were considered. All material was dried at constant 20 ºC in semi-darkness in the laboratory until constant weight. Then, the material was packed until the bioassay.

Figure 1. *Trifolium incarnatum* (left) and *Sinapis alba* (right) at the collection moment.

Seeds of the target weed species *C. bonariensis, A. squamatus*, and *B. scoparia* were collected in a commercial organic vineyard in Raimat (41º39'29"N, 0º31'26"E) (Lleida, Spain) in September 2016 from different plants throughout the field. Then, seeds were air-dried under laboratory conditions and stored in the dark in paper bags.

Plant aqueous extracts

In July 2017 the plant material was processed at the laboratory of Plant ecophysiology of the University of Vigo. All plant material was slashed in 1cm^2-sized pieces. Each species was placed in an independent 2-L Erlenmeyer flask at plant dry weight/distilled water volume ratio of 66.7g L^{-1} (Puig et al., 2013, 2018). Erlenmeyer flasks were left in a dark room at constant 20 ºC for 24 h and soaked every 6 hours to increase homogenization. The obtained aqueous extracts were vacuum filtered through 45µm cellulose membrane to clean impurities and then through 0.45 µm to achieve almost total asepsis of the extract and thus avoid contamination by microorganisms.

Then, all extracts were frozen at -20 ºC in sterile plastic bottles until bioassayed. A portion of each frozen aqueous extract was freeze-dried in a lyophilizer (Telstar CRYODOS) for better conservation until the chemical analyses.

Bioassays: germination and early growth

When the bioassays started, crude extracts were defrosted and diluted in distilled water at 100, 50 and 0% (v/v) corresponding to concentrations of 66.7, 33.3 and 0 g dw $\cdot$ L^{-1}, respectively. Values for osmolarity (Gonotec OSMOMAT 030 cryoscopic osmometer), electrical conductivity (EC, Crison CDTM-523 conductivity meter) and pH (Crison MicropH 2001 pHmeter) were measured for each extract.

Seeds of the different weed species were surface-sterilized as described by Kruidhof et al., (2014) by shaking the seeds for 5 min in a 0.5% bleach solution and then rinsing them for 2 min with de-ionized water.

For germination bioassays, weed seeds were incubated in 6-well plates of 3.48 cm diameter/well at the rate of 15 seeds per well placed on a layer of filter paper wetted with 600 µL of solution. For each target weed species, solution and concentration, six replicates were placed. Seeds were incubated in growth chambers at different temperatures: 30/20ºC for *C. bonariensis* and *B. scoparia* and 15/5ºC for *A. squamatus* with a 12/12h day/night photoperiod. The selected temperatures were close to the optimal reported by Wu et al., (2007) for *C. bonariensis* (20ºC) and by Al-Alahmadi and Kafi (2006) for *Kochia scoparia* (*B. scoparia*) with 24ºC. In the case of *A. squamatus*, different temperature tests were performed in growth chambers in order to obtain the best temperature range for germination, which was set at 5/15ºC.

Germinated seeds were counted every 12 h for *B. scoparia* and every 24h for *C. bonariensis* and *A. squamatus* until a complete germination was achieved or no germination was observed in control seeds in five consecutive days. A seed was considered germinated when the radicle exceeded 1 mm (Figure 2.) (Mayer and Poljakoff-Mayber, 1963).

For *in vitro* radicle and hypocotyl growth (early growth) bioassays, ten pre-germinated seeds of each target weed species (1mm root length) were considered. Pre-germinated seeds were placed in a Petri dish of 9 cm diameter on a layer of filter paper wetted with 4 mL of solution and incubated under the same conditions as for germination bioassays. For each target weed species, solution and concentration, four replicates were placed. After 48 hours of incubation, radicle and hypocotyl lengths were measured (Figure 3.).

Quantification and identification of phenolic compounds from the cover crops aqueous extracts

The analysis and characterization of the phenolic compounds of each species were performed following the methodology described by Souto et al. (2001).

Extraction Procedure: The lyophilized aqueous extracts were re-dissolved in 15 mL of distilled water. Afterwards, three sequential extractions with 15 mL of diethyl ether were performed. The mixture was extracted with an extraction funnel by shaking vigorously for one minute each time, waiting until the complete separation of two phases: the aqueous one, in the lower part, and the organic one, in the upper part of the funnel, containing the ether extracted phenolics. This phase was removed and saved, collecting the three ethereal phases (approximately 45 mL) to an Erlenmeyer flask. Another three sequential extractions with 15 mL of ethyl acetate on the aqueous phase were then performed, obtaining three new organic phases that were collected and combined with the ethereal ones. Subsequently, the total organic fraction was evaporated to dryness in the rotary evaporator. The final residue containing the phenolics was reconstituted in 1 mL of methanol.

HPLC analysis: The analysis was performed using an HPLC (Shimadzu chromatograph) equipped with a UV-DIODE ARRAY detector to identify flavonoids and phenolic acids. Identification was achieved by using a reverse-phase Waters Nova-Pak C-18 (4.6 mm x 250 mm) column with a 4 µm particle size. For flavonoids, the extracts were analyzed using two mobile phases: (A) methanol: phosphoric acid 999:1 and (B) water: phosphoric acid 999:1. HPLC grade solvents were used. Linear gradients starting at 20% (A) and ending at 100% (A) were used over the first 50 min with an additional 5 min at 100% (A). The flow rate of the mobile phase was 1 mL/min and the eluate was analyzed at 250–400 nm (Hussain *et al.* 2011). For phenolics, linear gradient elution was carried out at a flow rate of 1.5 mL/min. Solvent A was 0.5% acetic acid in pure water, and solvent B, acetonitrile with 0.5% acetic acid. A gradient from 0% to 20% B over 45 min, followed by 15 min re-equilibration with A was used (Gallet, 1994).

Identification and quantification of both flavonoids and phenolic acids were performed by comparing retention times, wave length detection, and peak areas to those of standard compounds. Derivatives were quantified using peak areas of the correspondent aglycones.

Statistical analyses

A replicated experiment was performed and followed in a completely randomized design. Obtained values were expressed as a percentage of the respective control. Data were tested for normality and homogeneity by Kolmogorov-Smirnov and Levene´s test. Differences between each treatment and the control were tested by independent samples Student´s t-test ($P = 0.05$). Differences between treatments were tested by one-way ANOVA and means were separated by Waller-Duncan test at $P \leq 0.05$ in case of normality and homoscedasticity. In the case of heterocedasticity, data were subjected to Kruskal-Wallis *H* test and post-hoc Tamhane´s *T2* test at $P \leq 0.05$. All statistical analyses were performed with IBM SPSS Statistics 19.0 software package (IBM SPSS Inc., Chicago, IL, USA) and graphs were conducted with SigmaPlot 11.0 (Systat Software, San Jose, CA).

Results

pH, EC and osmolarity values

Values of pH, EC and osmolarity of the plant extracts ranged from 4.43 to 7.02, from 0.43 to 7.93 dS m^{-1}, and from 0.018 to 0.208 osmol kg^{-1}, respectively (Table 1).

Table 1. Physicochemical properties of the aqueous plant extracts at two different concentrations: pH, Electrical conductivity (dS m^{-1}) and Osmolarity (osmol kg^{-1}).

	pH		EC		Osmolarity	
	50%	100%	50%	100%	50%	100%
Hordeum murinum	4.47	4.43	2.03	3.14	0.075	0.158
Hordeum vulgare	5.66	5.70	2.13	3.57	0.091	0.162
Bromus sp.	4.60	4.59	2.03	3.60	0.070	0.140
Vulpia ciliata	5.71	5.72	1.40	2.53	0.051	0.102
Festuca arundinacea	4.86	4.84	1.58	2.80	-	-
Pinus sylvestris	5.24	5.13	0.43	0.81	0.018	0.035
Sinapis alba	6.40	6.17	1.90	7.93	0.073	-
Phacelia tanacetifolia	6.02	5.91	1.85	3.22	0.067	0.130
Medicago sativa	6.30	6.16	1.18	2.16	-	-
Medicago rugosa	7.02	6.61	1.83	3.06	0.093	0.176
Trifolium subterraneum	6.30	6.44	1.75	2.93	0.081	0.145
Trifolium incarnatum	4.72	4.68	2.50	4.15	0.113	0.208

Germination and early growth bioassays

The high germination percentage of the controls revealed the suitability of the conditions to which seeds were subjected as well as the viability of them, with germination values of 92.2±12% for *C. bonariensis*, 85±14.2% for *A. squamatus* and 98.3±1.2% for *B. scoparia* (Mean ± SD).

Aqueous extracts from the different species selected as a cover crop and/or mulch produced phytotoxic effects on the three assayed target weed species, both in terms of germination or/and shoot and root elongation, being more accentuated when the extracts were more concentrated.

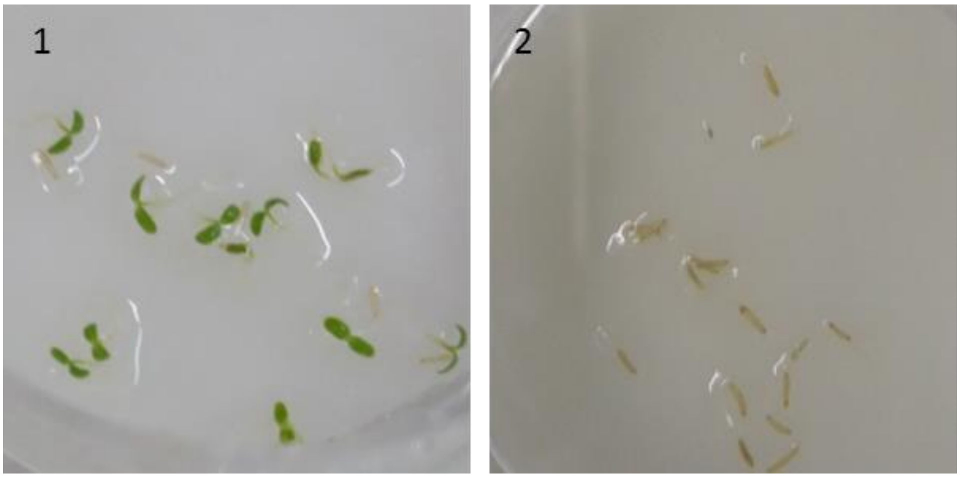

Figure 2. Example of one of the germination assays: germination of *Conyza bonariensis* control (1) and *Festuca arundinacea* at 50% dose (2).

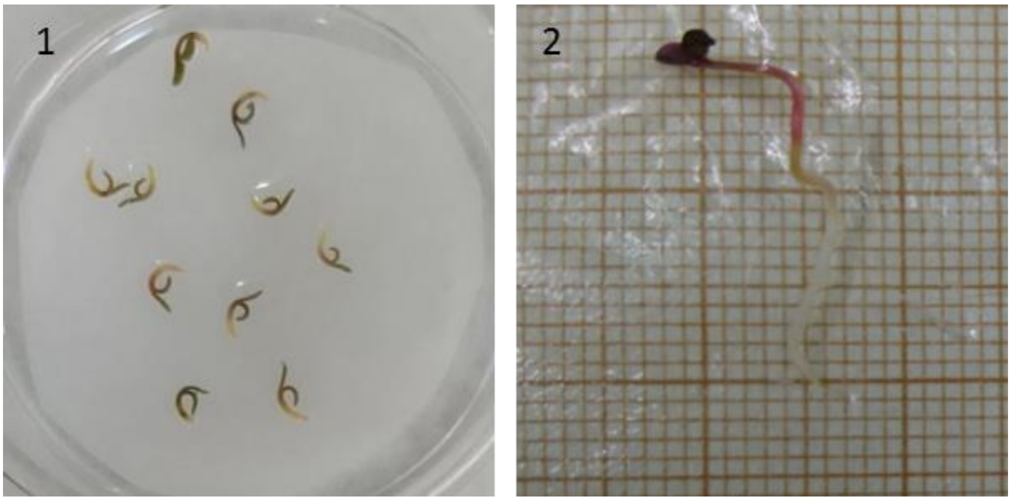

Figure 3. Example of one of the growth assays: *Bassia scoparia* control growth before (1) and after 48 h of incubation (2).

The effects of the aqueous extracts on the germination and growth of *C. bonariensis* are represented in Figure 4a. The germination percentage of this species was significantly reduced by all of the assayed aqueous extracts. Germination was inhibited by the solution of six of the species (*Bromus* sp., *F. arundinacea, H. murinum, H. vulgare, M. sativa and T. incarnatum*) with both doses (50% and 100%) at $P \leq 0.001$.

The rest of the plant extracts also inhibited *C. bonariensis* germination at 100% dose, with exception of *M. rugosa* where a value of about 15% of total germination was obtained. At 50% dose of *P. silvestrys*, *P. tanacetifolia, S. alba* and *T. subterraneum* the percentage of germinated seeds ranged from 4% to 20% compared to control.

Conyza bonariensis radicle growth was also significantly inhibited by the different aqueous extracts and doses, except for *P. sylvestris* at 50% dose (Figure4b). Extracts from seven of the twelve assayed species (*Bromus* mixture*, H. murinum, H. vulgare, M. rugosa, P. tanacetifolia, S. alba* and *T. incarnatum*) completely inhibited root elongation at both doses. Regarding *M. sativa* and *T. subterraneum*, whereas at 50% dose, the root length values were 4% and 14% respectively, at 100% dose, the root length was inhibited completely. *Vulpia ciliata* at 50% dose and *P. sylvestris* and *F. arundinacea* at 100% dose also significantly reduced root elongation, with values of 62%, 21% and 6.5% respectively.

Regarding hypocotyl (Figure 4c), almost all aqueous extracts at both doses significantly stimulated growth, or no statistical differences compared to control were found, of the shoot length of *C. bonariensis.* However, *H. murinum* (at 100% dose) and *P. sylvestris* (at 50% dose) significantly reduced shoot elongation by 17% and 32% compared to control, respectively.

The effects of the aqueous extracts from the species assayed on the germination and growth of *A. squamatus* are represented in Figure 5. All plant extracts had a significant inhibitory effect on seed germination ($P \leq 0.001$). Those solutions from *Bromus* mixture*, F. arundinacea, H. murinum, M. sativa, P. sylvestris, P. tanacetifolia* and *T. incarnatum* completely inhibited germination at both doses (Figure 5a). At 100% dose, all species completely inhibited seed germination with exception of *M. rugosa*, where 5% of total germination was obtained. At 50% dose, the percentages of germination were variable, with values between 40% for *M. rugosa* and 11% for *V. ciliata*. Root elongation for this species was completely inhibited by the effect of all assayed extracts and at both doses (Figure 5b).

Shoot elongation of *A. squamatus* was significantly reduced ($P \leq 0.001$) by all aqueous extracts at both doses compared to control, with the exception of *M. sativa* and *T. subterraneum*, both at the dose of 50%, with no differences from the control (Figure 5c). The lowest shoot length values were observed by the extract of *P. tanacetifolia* at 100% and 50% doses, with values of 26% and 40%, respectively. The other extracts showed final values of shoot length ranging between 48% for *H. murinum* and *T. incarnatum* and 83% for *V. ciliata*, at doses of 50% in all cases.

The phytotoxic effects of the aqueous extracts from the species tested on the germination and growth of *B. scoparia* are represented in Figure 6. *Trifolium subterraneum* was the species that showed more phytotoxic effect, being significantly different from the rest of the species, and with final values of 44% and 23% of germination for 50 and 100% doses, respectively. The other species significantly reduced the final germination with values ranging from 65% from *H. murinum* to 94% from *H. vulgare*. *Festuca arundinacea* and *T. incarnatum* at both doses, and *H. vulgare, S. alba* and *V. ciliata* at 50% showed no effect on *B. scoparia* germination (Figure 6a).

Root elongation was greatly inhibited by all the extracts and doses ($P \leq 0.001$) with values lower than 50% compared to the control, (Figure 6b). Aqueous extracts of *Bromus* mixture*, H. murinum, H. vulgare, M. rugosa, P. tanacetifolia* and *T. incarnatum* showed at both doses root length percentages close to 20% compared to the control. Similar values were observed for *F. arundinacea, M. sativa* and *S. alba* at 100% dose.

Shoot elongation was also reduced by almost all extracts (Figure 6c). The lowest percentages of shoot length (around 30%) were observed with extracts of *H. murinum, M. sativa* and *T. incarnatum* at 100% dose, whereas higher values (about 60%) were observed for the same species extracts at 50% doses. No differences in shoot elongation were observed compared to the control for the extracts of *F. arundinacea* and *V. ciliata* at 50% dose, an even greater value of 100% was observed for *S. alba* at 50% dose.

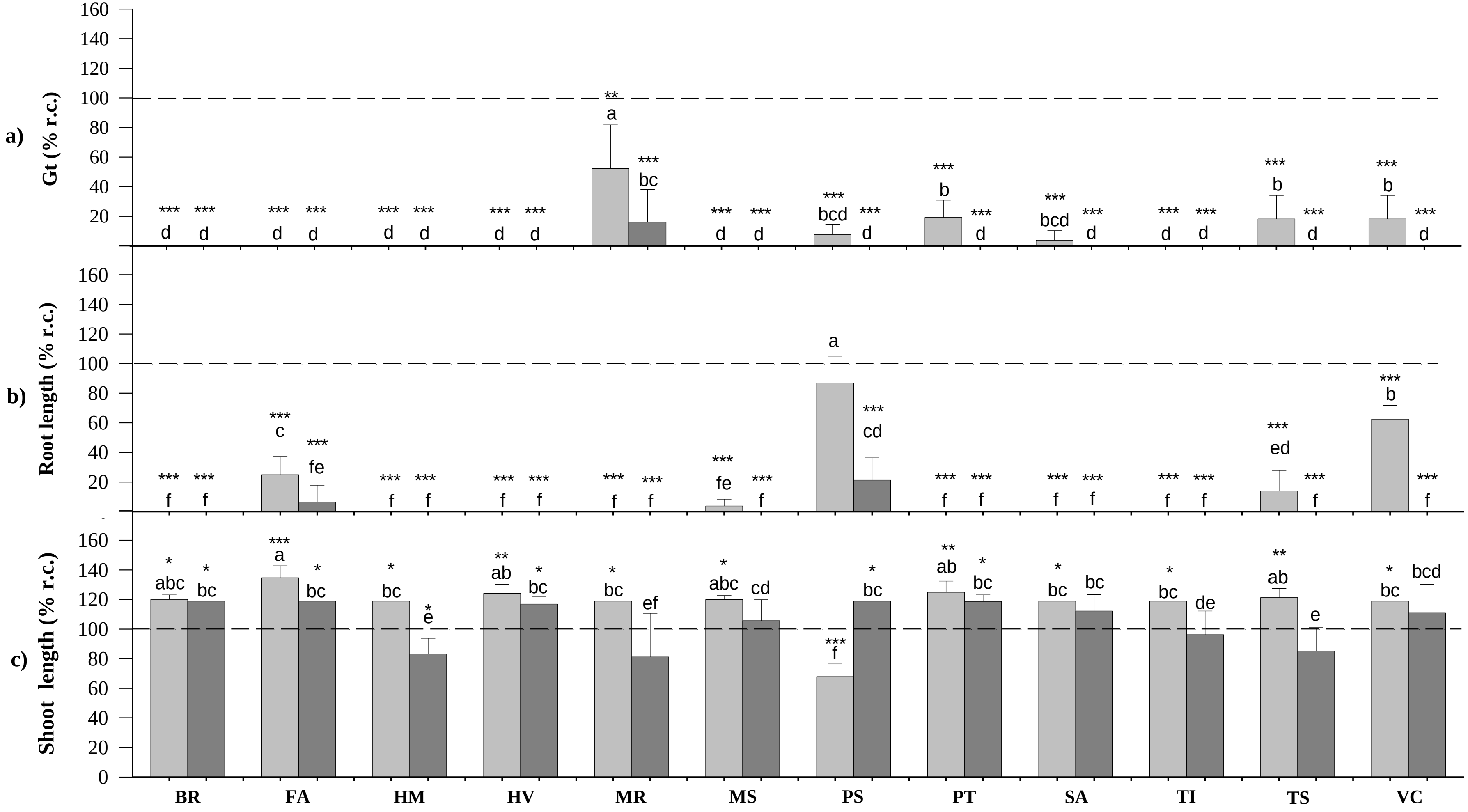

Figure 4. Effects of the aqueous extracts from the species assayed on the germination and growth of *Conyza bonariensis* at the doses of 50% (33,3 g/L)■ and 100% (66,7 g/L)■. Gt: total percentage of germinated seeds; BR: *Bromus* mixture (*B. hordeaceus* and *B. rubens*); FA: *Festuca arundinacea*; HM: *Hordeum murinum*; HV: *Hordeum vulgare*; MR: *Medicago rugosa*; MS: *Medicago sativa*; PT: *Phacelia tanacetifolia*; SA: *Sinapis alba*; TI: *Trifolium incarnatum*; TS: *Trifolium subterraneum*; VC: *Vulpia ciliata*; PS: *Pinus sylvestris*. Mean values are represented as percentages relative to the control. Error bars represent standard deviation (SD). For each species, asterisks denote statistically significant differences between control (100%) and species at $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$ (independent samples t-test). Mean values labeled with distinct letters are significantly different at $P \leq 0.05$ (ANOVA or Kruskal-Wallis *H* test and post-hoc Waller-Duncan or Tamhane‘s T2 test).

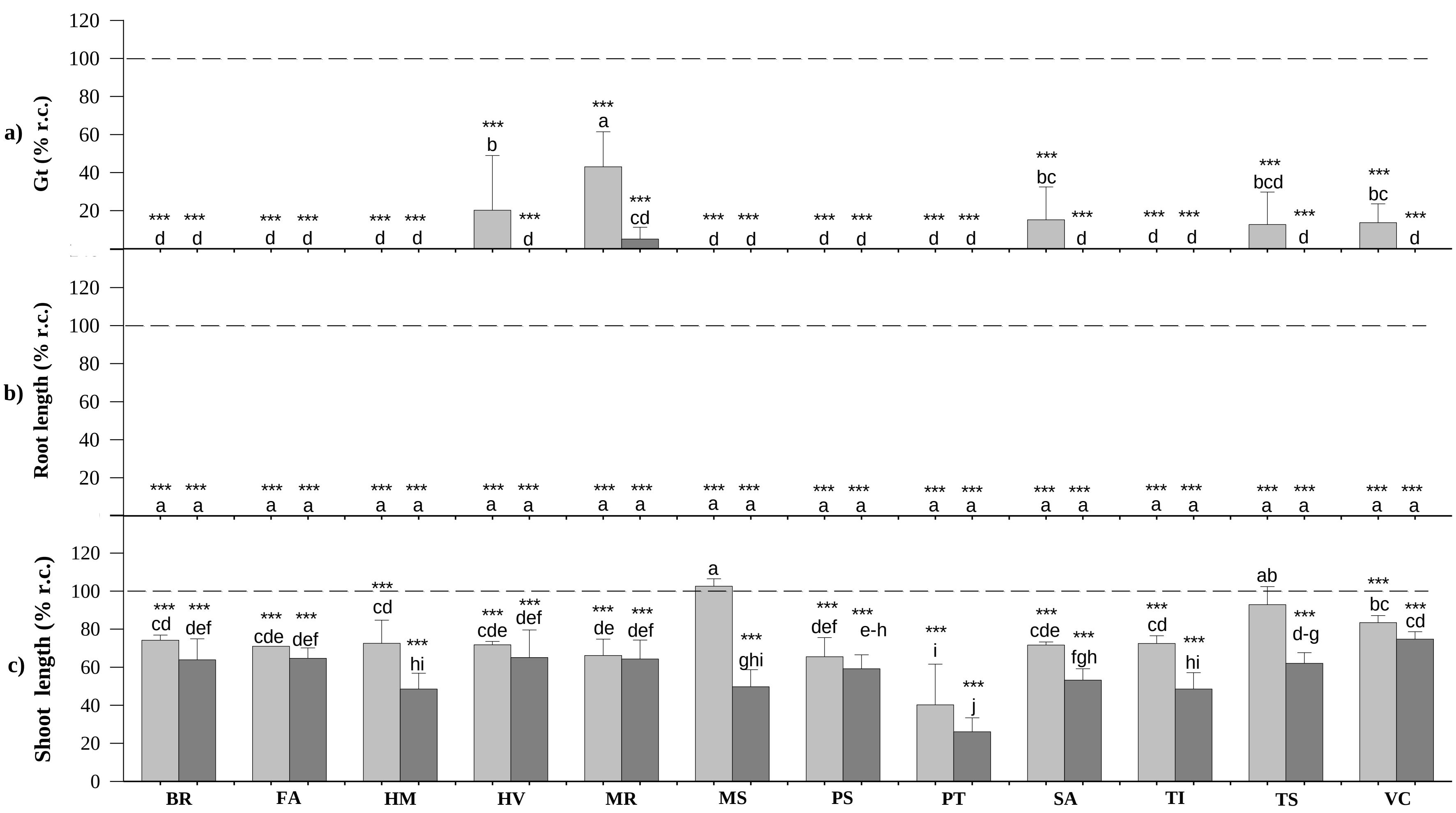

Figure 5. Effects of the aqueous extracts from the species assayed on the germination and growth of *Aster squamatus* at the doses of 50% (33,3 g/L)■ and 100% (66,7 g/L)■. Gt: total percentage of germinated seeds; BR: *Bromus* mixture (*B. hordeaceus* and *B. rubens*); FA: *Festuca arundinacea*; HM: *Hordeum murinum*; HV: *Hordeum vulgare*; MR: *Medicago rugosa*; MS: *Medicago sativa*; PT: *Phacelia tanacetifolia*; SA: *Sinapis alba*; TI: *Trifolium incarnatum*; TS: *Trifolium subterraneum*; VC: *Vulpia ciliata*; PS: *Pinus sylvestris*. Mean values are represented as percentages relative to the control. Error bars represent standard deviation (SD). For each species, asterisks denote statistically significant differences between control (100%) and species at $^{*}P\leq 0.05$, $^{**}P\leq 0.01$ and $^{***}P\leq 0.001$ (independent samples t-test). Mean values labeled with distinct letters are significantly different at $P \leq 0.05$ (ANOVA or Kruskal-Wallis *H* test and post-hoc Waller-Duncan or Tamhane‘s T2 test).

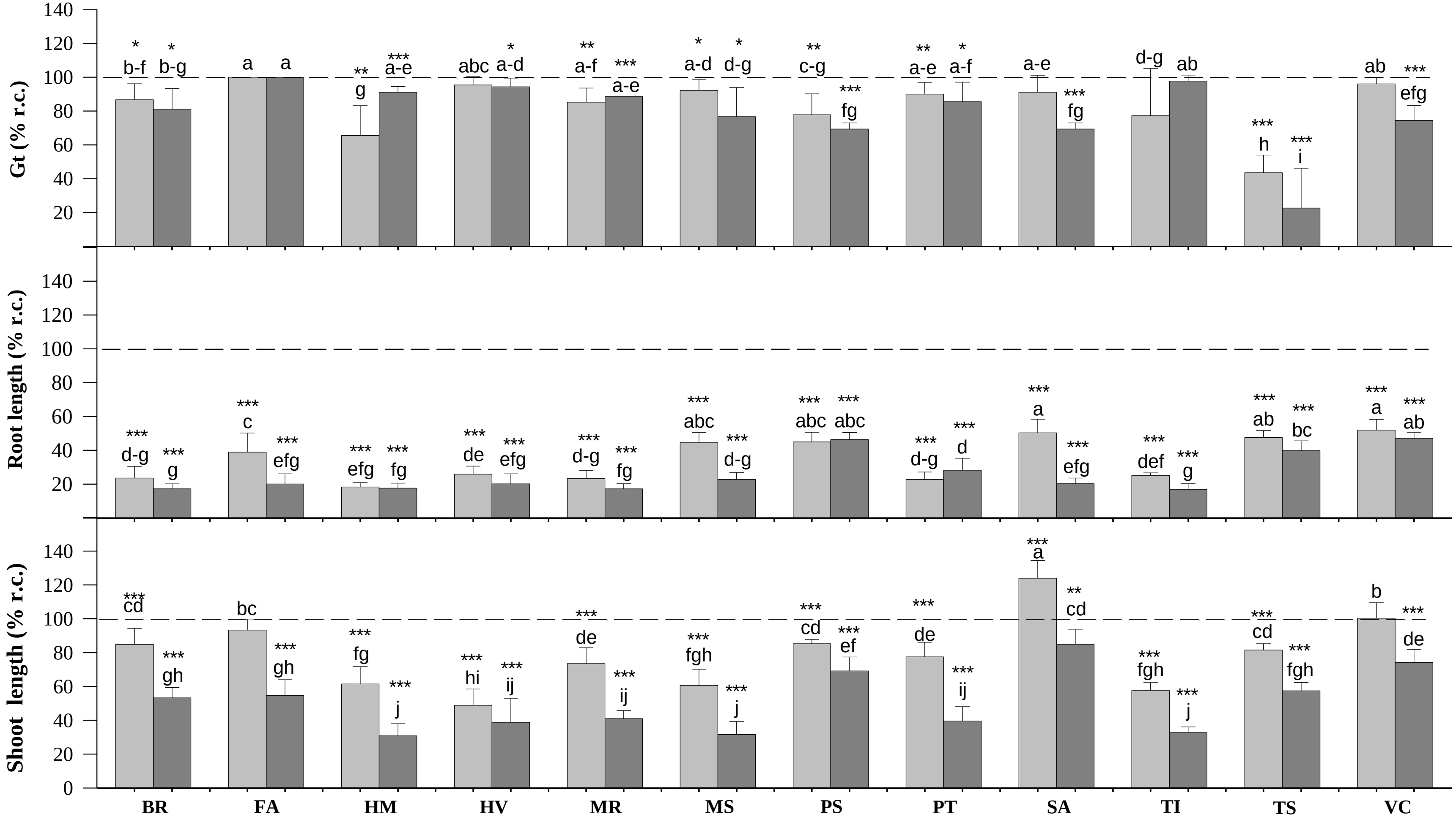

Figure 6. Effects of the aqueous extracts from the species assayed on the germination and growth of *Bassia scoparia* at the doses of 50% (33,3 g/L)■ and 100% (66,7 g/L)■. Gt: total percentage of germinated seeds; BR: *Bromus* mixture (*B. hordeaceus* and *B. rubens*); FA: *Festuca arundinacea*; HM: *Hordeum murinum*; HV: *Hordeum vulgare*; MR: *Medicago rugosa*; MS: *Medicago sativa*; PT: *Phacelia tanacetifolia*; SA: *Sinapis alba*; TI: *Trifolium incarnatum*; TS: *Trifolium subterraneum*; VC: *Vulpia ciliata*; PS: *Pinus sylvestris*. Mean values are represented as percentages relative to the control. Error bars represent standard deviation (SD). For each species, asterisks denote statistically significant differences between control (100%) and species at $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$ (independent samples t-test). Mean values labeled with distinct letters are significantly different at $P \leq 0.05$ (ANOVA or Kruskal-Wallis *H* test and post-hoc Waller-Duncan or Tamhane‘s T2 test).

Identification of phenolic compounds from the different cover crop species assayed

A total of 33 phenolic compounds (phenolic acids and flavonoids) were quantitatively and qualitatively identified by HPLC-DAD from the species studied, with a maximum of 13 compounds in the case of *H. vulgare* and a minimum of 6 compounds in the case of *Bromus* mixture and *P. tanacetifolia*. The total quantity of compounds was also very different among the species, from 33.53 µg.mL^{-1} in the case of *F. arundinacea* to 1571.77 µg.mL^{-1} in *S. alba* (Table 4).

The phenolic profile of *H. murinum* extract revealed the presence of 7 phenolic acids (protocatechuic, vanillic, *p*-Hydroxybenzoic, *p*-Hydroxybenzaldehyde, *p*-Coumaric, syringic and ferulic acid) and 5 flavonoids (apigenin and two derivatives `1´ and `2´, ellagic acid and luteolin derivative `5´) (Table 2). The most abundant compounds were apigenin derivative `2´ and `1´, with 187.84 µg.mL^{-1} and 150.18 µg.mL^{-1} respectively.

In *H. vulgare* aqueous extract, a total of 13 compounds were identified, 6 phenolic acids (protocatechuic, vanillic, *p*-Hydroxybenzoic, *p*-Coumaric, syringic and ferulic acid) and 7 flavonoids (luteolin derivative `1´, `2´, `3´ and `5´, apigenin and two apigenin derivatives `2´ and `3´) (Table 2). As in *H. murinum*, the most abundant were apigenin derivative `2´, with a 32.16% (54.60 µg.mL^{-1}).

Table 2. Phenolic acids and flavonoid compounds identified by HPLC-DAD from aqueous extracts of *Hordeum murinum*, *Hordeum vulgare***.** Data are expressed as µg of compound per mL of aqueous extract and percentage of each compound respect to the total.

		H. murinum		*H. vulgare*	
Compound	RT	µg.mL^{-1}	%	µg.mL^{-1}	%
Phenolic acids					
Protocatechuic acid	13.3	3.43	0.85	12.46	7.34
Vanillic acid	19.2	14.70	3.66	21.35	12.58
p-Hydroxybenzoic acid	22.3	7.22	1.80	9.25	5.45
p-Hydroxybenzaldehyde	22.9	0.25	0.06	-	-
p-Coumaric acid	33.0	1.36	0.34	1.23	0.73
Syringic acid	33.0	5.33	1.33	2.36	1.39
Ferulic acid	37.5	2.31	0.57	5.41	3.19
Flavonoids					
Apigenin derivative `1´	19.0	150.18	37.37	-	-
Luteolin derivative `1´	19.6	-	-	26.30	15.49
Luteolin derivative `2´	20.3	-	-	16.58	9.77
Apigenin derivative `2´	21.6	187.84	46.74	54.60	32.16
Luteolin derivative `3´	22.1	-	-	17.70	10.43
Ellagic acid	23.0	26.53	6.60	-	-
Taxifolin derivative	23.4	-	-	-	-
Apigenin derivative `3´	31.3	-	-	1.22	0.72
Luteolin derivative `4´	31.9	-	-	-	-
Luteolin derivative `5´	32.0	1.12	0.28	0.66	0.39
Apigenin	33.2	1.59	0.39	0.65	0.38
Total		401.84		169.77	

RT = retention time (min)

Six compounds were identified in *Bromus* mixture extracts, two phenolic acids (protocatechuic acid and chlorogenic/caffeic derivative) and 4 flavonoids (luteolin derivative `1´, `2´ and `4´, and apigenin derivative `2´). Chlorogenic/caffeic derivative `1´was the most abundant compound with 39.48% (Table 3).

In *V. ciliata* aqueous extract, nine compounds were identified, two phenolic acids (protocatechuic and vanillic acid) and 7 flavonoids (luteolin derivative `1´, `2´ `3´ and `5´, apigenin derivative `2´ and `3´, and taxifolin derivative) (Table 3). The most abundant compound was protocatechuic acid (23.97%, 16.60 µg.mL^{-1}).

Table 3. Phenolic acids and flavonoid compounds identified by HPLC-DAD from aqueous extracts of *Bromus* mixture and *Vulpia ciliata*. Data are expressed as µg of compound per mL of aqueous extract and percentage of each compound respect to the total.

		Bromus mix.		*V. ciliata*	
Compound	RT	µg.mL^{-1}	%	µg.mL^{-1}	%
Phenolic acids					
Protocatechuic acid	13.3	40.07	14.88	16.60	23.97
Vanillic acid	19.2	-	-	2.51	3.62
Chlorogenic/caffeic derivative	25.0	106.32	39.48	-	-
Flavonoids					
Apigenin derivative `1´	19.0	-	-	-	-
Luteolin derivative `1´	19.6	33.84	12.57	9.07	13.09
Luteolin derivative `2´	20.3	83.81	31.12	9.19	13.27
Apigenin derivative `2´	21.6	0.31	0.11	5.17	7.46
Luteolin derivative `3´	22.1	-	-	12.69	18.32
Ellagic acid	23.0	-	-	-	-
Taxifolin derivative	23.4	-	-	7.14	10.30
Apigenin derivative `3´	31.3	-	-	1.81	2.61
Luteolin derivative `4´	31.9	4.95	1.84	-	-
Luteolin derivative `5´	32.0	-	-	5.09	7.35
Total		269.30		69.26	

RT = retention time (min)

The phenolic profile of *F. arundinacea* revealed the presence of 6 phenolic acids (protocatechuic, vanillic, *p*-Hydroxybenzoic, *p*-Coumaric, syringic, and ferulic acid) and one flavonoid (taxifolin derivative). Protocatechuic acid was the most abundant compound of the seven identified in *F. arundinacea* aqueous extract (Table 4), with a value of 48.72%.

Nine compounds were identified in *S. alba,* 4 phenolic acids (vanillic, *p*-Hydroxybenzoic acid, *p*-Hydroxybenzaldehyde and *p*-Coumaric acid) and 5 flavonoids (ellagitannin `1´ and `2´, luteolin derivative `1´, ellagic acid and kaempferol derivative). (Table 4). With a 40.64%, luteolin derivative `1´ was the most abundant compound with a 26.28%.

Table 4. Phenolic acids and flavonoid compounds identified by HPLC-DAD in *Festuca arundinacea*, *Sinapis alba*. Data are expressed as µg of compound per mL of aqueous extract and percentage of each compound respect to the total.

		F. arundinacea		*S. alba*	
Compound	RT	µg.mL^{-1}	%	µg.mL^{-1}	%
Phenolic acids					
Protocatechuic acid	13.3	16.34	48.72	-	-
Vanillic acid	19.2	4.81	14.35	0.77	0.05
p-Hydroxybenzoic acid	22.3	2.49	7.44	11.62	0.74
Trans-cinnamic acid	22.5	-	-	-	-
p-Hydroxybenzaldehyde	22.9	-	-	0.65	0.04
Chlorogenic/caffeic derivative	25.0	-	-	-	-
Vanillin	30.0	-	-	-	-
p-Coumaric acid	33.0	2.81	8.39	0.89	0.06
Syringic acid	33.0	3.68	10.99	-	-
Ferulic acid	37.5	1.71	5.09	-	-
Flavonoids					
Ellagitannin `1´	17.4	-	-	239.62	15.25
Luteolin derivative `1´	19.6	-	-	638.75	40.64
Ellagitannin `2´	20.3	-	-	413.06	26.28
Ellagic acid	23.0	-	-	264.55	16.83
Taxifolin derivative	23.4	1.68	5.02	-	-
Kaempferol derivative	36.3	-	-	1.85	0.12
Total		33.53		1571.77	

RT = retention time (min)

In *P. tanacetifolia* only six compounds were identified, two phenolic acids (*p*-Hydroxybenzoic acid and chlorogenic/caffeic derivative) and four flavonoids (ellagitannin `1´ and `2´, eriodictyol and one luteolin derivative). Ellagitannin `1´was the most abundant compound, representing a 60.94% of the total compounds (Table 5).

Nine compounds were identified in *P. sylvestris* aqueous extracts, 8 phenolic acids (gallic, protocatechuic, vanillic, *p*-Hydroxybenzoic, trans-cinnamic, *p*-Coumaric and ferulic acid and vanillin) and one flavonoid (naringenin) (Table 5), the most abundant being vanillin with 52.57μg.mL^{-1}.

Table 5. Phenolic acids and flavonoid compounds identified by HPLC-DAD in *Phacelia tanatecifolia* and *Pinus sylvestris*. Data are expressed as μg of compound per mL of aqueous extract and percentage of each compound respect to the total.

		P. tanatecifolia		*P. sylvestris*	
Compound	RT	μg.mL^{-1}	%	μg.mL^{-1}	%
Phenolic acids					
Gallic acid	6.3	-	-	0.24	0.19
Protocatechuic acid	13.3	-	-	9.06	6.99
Vanillic acid	19.2	-	-	23.44	18.09
p-Hydroxybenzoic acid	22.3	28.29	7.45	18.83	14.53
Trans-cinnamic acid	22.5	-	-	6.35	4.90
p-Hydroxybenzaldehyde	22.9	-	-	-	-
Chlorogenic/caffeic derivative	25.0	98.31	25.87	-	-
Vanillin	30.0	-	-	52.57	40.58
p-Coumaric acid	33.0	-	-	11.15	8.61
Syringic acid	33.0	-	-	-	-
Ferulic acid	37.5	-	-	7.62	5.88
Flavonoids					
Ellagitannin `1´	17.4	231.59	60.94	-	-
Luteolin derivative `1´	19.6	-	-	-	-
Ellagitannin `2´	20.3	7.49	1.97	-	-
Naringenin	23.8	-	-	0.31	0.24
Eriodictyol	26.8	12.51	3.29	-	-
Luteolin derivative `4´	31.9	1.82	0.48	-	-
Total		380.01		129.56	

RT = retention time (min)

Both in *M. sativa* and *M. rugosa*, eight compounds were identified. In *M. sativa*, 4 of the compounds were phenolic acids (vanillic, *p*-Hydroxybenzoic*,* *p*-Coumaric and ferulic acid) and 4 flavonoids (luteolin and one derivative, kaempferol and apigenin). The most abundant compound was ferulic acid with a value of 49.57 µg.mL^{-1} (Table 6).

In *M. rugosa*, three phenolic acids were identified (vanillic, *p*-Coumaric and ferulic acid) and 5 flavonoids (ellagitannin `2´, luteolin derivative `3´ and `5´, ellagic acid and apigenin derivative `3´) (Table 4). Luteolin derivative `5´ was the most abundant compound in *M. rugosa* aqueous extract, representing a 27.06% of the total (Table 6).

Table 6. Phenolic acids and flavonoid compounds identified by HPLC-DAD in *Medicago sativa*, *Medicago rugosa*. Data are expressed as µg of compound per mL of aqueous extract and percentage of each compound respect to the total.

		M. sativa		*M. rugosa*	
Compound	RT	µg.mL^{-1}	%	µg.mL^{-1}	%
Phenolic acids					
Vanillic acid	19.2	2.81	1.68	4.57	2.13
p-Hydroxybenzoic acid	22.3	2.96	1.77	-	-
Caffeic acid	25.7	-	-	-	-
p-Coumaric acid	33.0	13.58	8.12	10.64	4.95
Syringic acid	33.0	-	-	-	-
Ferulic acid	37.5	49.57	29.64	19.53	9.10
Flavonoids					
Ellagitannin `2´	20.3	-	-	42.48	19.78
Apigenin derivative `2´	21.6	-	-	-	-
Luteolin derivative `3´	22.1	-	-	16.59	7.72
Ellagic acid	23.0	-	-	30.64	14.27
Luteolin	28.6	23.88	14.28	-	-
Apigenin derivative `3´	31.3	-	-	32.18	14.99
Luteolin derivative `4´	31.9	-	-	-	-
Luteolin derivative `5´	32.0	37.68	22.53	58.12	27.06
Kaempferol	32.5	4.34	2.60	-	-
Apigenin	33.2	32.41	19.38	-	-
Prunetin	33.7	-	-	-	-
Total		167.24		214.76	

RT = retention time (min)

The phenolic profile of *T. subterraneum* revealed the presence of 10 compounds, 6 phenolic acids (protocatechuic, vanillic, *p*-Hydroxybenzoic, caffeic, *p*-Coumaric and ferulic acid) and 4 flavonoids (taxifolin, naringenin, prunetin and one prunetin derivative) (Table 7). Prunetin derivative was the most abundant compound with a 27.859% of the total (Table 7).

In *T. incarnatum* aqueous extracts, nine compounds were identified, 4 phenolic compounds (protocatechuic, vanillic, *p*-Hydroxybenzoic, and syringic acid) and 5 flavonoids (luteolin derivative `1´, `2´ and `5´, and apigenin derivative `2´ and `3´ (Table 7). The most abundant compound was apigenin derivative `2´ with a 42.57%.

Table 7. Phenolic acids and flavonoid compounds identified by HPLC-DAD in *Trifolium subterraneum* and *Trifolium incarnatum*. Data are expressed as µg of compound per mL of aqueous extract and percentage of each compound respect to the total.

		T. subterraneum		*T. incarnatum*	
Compound	RT	µg.mL^{-1}	%	µg.mL^{-1}	%
<u>*Phenolic acids*</u>					
Protocatechuic acid	13.3	6.84	5.18	16.86	5.92
Vanillic acid	19.2	1.20	0.91	10.37	3.64
p-Hydroxybenzoic acid	22.3	3.41	2.58	9.08	3.19
Caffeic acid	25.7	29.59	22.39	-	-
p-Coumaric acid	33.0	5.03	3.81	-	-
Syringic acid	33.0	-	-	5.12	1.80
Ferulic acid	37.5	30.74	23.26	-	-
<u>*Flavonoids*</u>					
Taxifolin	17.1	7.93	6.00	-	-
Luteolin derivative `1´	19.6	-	-	40.79	14.33
Ellagitannin `2´	20.3	-	-	-	-
Apigenin derivative `2´	21.6	-	-	121.17	42.57
Luteolin derivative `3´	22.1	-	-	-	-
Ellagic acid	23.0	-	-	-	-
Naringenin	23.8	8.09	6.12	-	-
Prunetin derivative	25.7	36.81	27.85	-	-
Luteolin	28.6	-	-	-	-
Apigenin derivative `3´	31.3	-	-	12.96	4.55
Luteolin derivative `4´	31.9	-	-	61.00	21.43
Luteolin derivative `5´	32.0	-	-	7.27	2.55
Prunetin	33.7	2.51	1.90	-	-
Total		132.15		284.62	

RT = retention time (min)

Discussion

The aqueous extracts of the assayed species showed, in general, a great inhibitory effect on the germination and root elongation of *C. bonariensis* and *A. squamatus*, and a lower but evident effect on *B. scoparia*. On the contrary, shoot elongation of *B. scoparia* was more affected by the extracts than the other two target weed species. The phenolic acids and flavonoids identified by HPLC (both qualitatively and quantitatively) from the different plant aqueous extracts gave us an approximation of what could be released in the field, since water is the most natural solvent in nature. Our results highlighted the interest of implementing these species as a cover crop or mulch due to the phytotoxic effects observed from their aqueous extracts. The phytotoxicity of some of the assayed species had been previously reported by other authors, both for germination and early growth.

Our results agree with those of Fujii (2001), who performed in vitro bioassays to study the allelopathic effect of different species usually implemented as cover crops and observed different levels of radicle reduction of lettuce (*Lactuca sativa* L.) depending on the cover crop extracts: *M. rugosa* inhibited a 74%, followed by *M. sativa* and *T. incarnatum* (68 and 64% respectively). *Hordeum vulgare* reduced 62%, followed by *F. arundinacea* (55%), *Brassica alba* (*S. alba*) (53%) and *T. subterraneum* (30%). These results are in an intermediate point from our results that completely inhibited root elongation of *C. bonariensis* and *A. squamatus* but less so*, B. scoparia*. The allelopathic effect of some of these species had also been reported by other authors: Xuan et al. (2003) observed an allelopathic effect on rice exerted by *M. sativa*. In the case of *S. alba,* Rice et al. (2007) observed in a growth chamber experiment a phytotoxic effect on lettuce seed emergence, meanwhile Alcántara et al. (2011) observed that *S. alba* cover crop residues reduced weed infestation level in field experiments. Creamer et al. (1996) observed in field trials a *Solanum ptycanthum* Dun. emergence suppression by *T. incarnatum* and *H. vulgare* as cover crop residue. In addition, the allelopathic potential of *T. incarnatum*, as well as *Medicago lupulina* L. (similar species to *M. rugosa*) was observed by Price et al. (2008) in greenhouse trials. Also, *Ipomoea lacunosa* L. emergence and dry weight decreased after *T. incarnatum* and *T. subterraneum* residues incorporated into the soil (White et al., 1989; Lehman and Blum, 1997). The allelopathic potential of *H. vulgare* has also been reported by other authors (Baghestani et al., 1999; Bertholdsson et al., 2004, Chon and Kim, 2004) as well as its allelopathic potential in *Echinochloa crus-galli* (L.) P. Beauv., *Setaria verticillata* (L.) P. Beauv., *Veronica hederifolia* L. or *Papaver roheas* L. in a field experiment (Dhima et al., 2006, 2008).

Festuca arundinacea showed a high phytotoxic effect in all bioassays except for *B. scoparia* seed germination that was similar to the control. This cover crop species has been widely implemented in several vineyards due to its perennial habit that can control vine vigour and reduce erosion, among other advantages. Our results give evidence of another positive characteristic of the species that make it a very suitable cover crop (living mulch) in vineyard. The allelopathic potencial of *F. arundinacea* was also reported by Bertoldi et al. (2012) who observed 33% reduction of lettuce (*Lactuca sativa* L.) germination from in vitro bioassays and effective weed control in field conditions when incorporated aboveground *F. arundinacea* cv. \`Villegeoise´ biomass, inhibiting species such as *Anagallis* sp. or *Sinapis* sp.

Some authors have studied the specie *P. tanacetifolia* for weed control: Tursun et al. (2018) observed in field experiments that *P. tanacetifolia* produced high amount of biomass and was the most effective cover crop to suppress weeds (almost 75%), but these results disagree with Schappert et al., (2019) who reported that this species can reach a high soil cover and biomass but weed control efficacy was lower than 40%.

Other Bromus species have been implemented as cover crops with good weed control results, such as *Bromus catharticus* Vahl cv. \`Samson´ (Ibañez et al., 2011) and the allelopathic potential of *Bromus porteri* (Coult.). Nash against *Taraxacum officinale* (Weber) ex Wigg., *Matricaria perforata* Mérat and *Hordeum jubatum* L. reported in greenhouse experiments by Serajchi et al. (2017).

The mulch of *Pinus* sp. has been widely implemented for weed suppression. In our study, *P. sylvestris* showed a strong phytotoxic effect, especially in *C. bonariensis* and *A. squamatus* germination. Similar to our results, Bulut and Demir (2007) and Aklıbaşında et al. (2017) reported bioassays where *P. sylvestris* leaves showed dose-dependent inhibitory effects on seed germination and growth of different grass species, and Bielinis et al. (2019) observed the same effect in *S. alba* cv. \`Borowska´.

Regarding the chemical analysis, the different species showed different chemical compositions. Apigenin derivative \`2´ is the most abundant compound in *H. murinum* and *T. incarnatum* extracts and the second in *Bromu*s mixture Apigenin and the other derivatives are found in *M. sativa, H. vulgare*, or *V. ciliata*, species that also had a strong inhibitory effect. Apigenin was reported in *M. sativa* by Zubair et al. (2017) and this crop showed an allelopathic effect on annual ryegrass (*Lolium rigidum*) germination. Also, *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids were identified in *M. sativa* extracts by Xuan et al. (2003).

Luteolin and five derivatives were also found in almost all species studied, with the exception of *F. arundinacea, P. silvestrys* and *T. subterraneum*. The potential of allelopathic effects of luteolin and the other two compounds that were also found in our analysis (ferulic and vanillic acid) were studied by Zhao et al. (2018) in *Mikania micrantha* H.B.K. This author found that germination energy and final germination rate were significantly reduced by the three compounds at different doses.

One compound found in high quantity in *S. alba and M. rugosa* was ellagic acid. This compound had been reported as a phytotoxic compound at the concentrations of 100 and 200 µg.mL^{-1} by significantly reducing *Arabidopsis thaliana* fresh weight and root elongation (Qin et al., 2006). In our study, ellagic acid was found at 264.55 µg.mL^{-1} in *S. alba*, and this species highly inhibited and reduced germination and root elongation of *A. squamatus* and *C. bonariensis*, as well as root elongation of *B. scoparia* at both doses and reduced germination of *B. scoparia* at 100% dose.

Other compounds found in our analysis, such as ferulic, *p*-hydroxybenzoic, *p*-coumaric, gallic acid and vanillin were reported to affect *Arabidopsis thaliana* germination and these compounds plus syringic, vanillic and protocatechuic also inhibited root elongation in the same species (Reigosa and Pazos-Malvino, 2007).

Ferulic acid was found in *H. murinum, H. vulgare, F. arundinacea, P. sylvestris*, *M sativa, M. rugosa*, and *T. incarnatum,* and caffeic acid in *M. rugosa*. This compound has been reported to inhibit germination and root elongation of different species: Pereira et al. (2018) identified ferulic and caffeic acid and, kaempferol in ethanolic extracts of *Canavalia ensiformis* L. aerial part, and observed a dose-dependent inhibitory effect on *Lactuca sativa, Digitaria insularis, Emilia coccinea* and *Portulaca oleracea* germination.

Ghimire et al. (2019a, 2019b) identified *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric and ferulic acids in extracts from ray (*Secale cereale*) and alfalfa (*M. sativa*) leaves. The extracts of these species inhibited callus growth of *Digitaria ciliaris,* *Chenopodium album, Amaranthus lividus, Portulaca oleracea,* and *Commelina communis.* The same author also tested these phenolic compounds in *Chenopodium album, Portulaca oleracea,* and *Pinellia ternata* and observed an inhibitory effect on callus growth. In our analysis, these compounds (with exception of syringic acid) were also found in *M. sativa* aqueous extracts.

Similar to our results, vanillic, *p*-hydroxybenzoic, *p*-coumaric, syringic and ferulic acid were identified in *H. vulgare* and its extract showed allelopathic potential on *Bromus diandrus* Roth. (Bouhaouel et al., 2019).

Nine compounds were identified in *V. ciliata*, and some of them had also been identified in *Vulpia myuros* extracts, showing also a phytotoxic effect on *Triticum aestivum* cv. `Vulcan´ (An et al., 2001).

In general, radicle growth was more affected than shoot growth. In fact, almost all of the cover crops showed significant stimulatory effects on hypocotyl growth of *C. bonariensis* compared to the control, especially when assayed at the lowest dose. It is known that low concentrations of allelopathic compounds can promote stimulatory growth effects (Reigosa and Pazos-Malvido, 2007). Xuan et al. (2003) observed that the acidic fraction of *M. sativa* in low concentrations promoted hypocotyl and radicle elongation of *Oryza sativa* L. compared to control or high concentrations.

The release of these phytotoxic compounds can be easily achieved in the field as in our study we have simulated natural compound extraction (water-soluble compounds), but its effect in the field can vary greatly due to the biotic and abiotic factors. Nevertheless, knowing which species are the most phytotoxic to the target weed species can help to choose the best cover crop or mulch according to weed presence. The next step would be to implement these species as a cover crop, living or dead mulch to study their weed suppression effect as well as their stability through the seasons.

Conclusions

Germination and root elongation of *C. bonariensis* and *A. squamatus* was almost completely inhibited by the 12 plant aqueous extracts. However, this inhibitory effect was not so successful on shoot elongation of *A. squamatus* and *B. scoparia*, and there was no inhibitory effect in the case of *C. bonariensis*. The qualitative and quantitative composition of the aqueous extracts compounds identified by HPLC-DAD revealed differences between species as well as the potential to be released in field conditions. The result of this study can help to choose the most suitable species to be implemented as a cover crop or mulch for weed control.

References

Aklıbaşında, M., Külekçi, E.A., Demir, M., Bulut, Y. (2017). The inhibiting effects of scots pine (*Pinus sylvestris*) on germination ability and growth of some culture ryegrass species. *J. Environ. Biol*. 38, 919-922.

Al-Alahmadi, M.J., Mohammad, K. (2007). Cardinal temperatures for germination of *Kochia scoparia* (L.). *J. Arid Environ.* 68, 308-314.

Alcántara, C., Pujadas, A., Saavedra, M. (2011). Management of *Sinapis alba* subsp. mairei winter cover crop residues for summer weed control in southern Spain. *Crop Prot*. 30, 1239-1244.

Álvarez-Iglesias, L., Puig, C.G., Revilla, P., Reigosa, M.J, Pedrol, N. (2018). Faba bean (*Vicia faba* L.) as green manure for field weed control in maize. *Weed Res.* 58, 437-449.

An, M., Pratley, J.E., Haig, T. (2001). Phytotoxicity of Vulpia Residues: IV. Dynamics of allelochemicals during decomposition of vulpia residues and their corresponding phytotoxicity. *J. Chem. Ecol.* 27, 395-409.

Baghestani, A., Lemieux, C., Leroux, G.D., Baziramakenga, R., Simard, R.R. (1999). Determination of allelochemicals in spring cereal cultivars of different competitiveness. *Weed Sci.* 47(5), 498-504.

Bajwa, A. A., Sadia, S., Ali, H. H., Jabran, K., Peerzada, A. M., Chauhan, B. S. (2016). Biology and management of two important *Conyza* weeds: a global review. *Environ. Sci. Pollut. Res.* 23, 24694-24710.

Bertholdsson, N.O. (2004). Variation in allelopathic activity over 100 years of barley selection and breeding. *Weed Res.* 44, 78–86.

Bertoldi, C., De Leo, M., Ercoli, L., Braca, A. (2012). Chemical profile of *Festuca arundinacea* extract showing allelochemical activity. *Chemoecology*, 22, 13–21.

Bettoni, J.C., Gardin, J.P.P., Feldberg, N.P., Dalbó, M.A. (2012). Potencial alelopático de plantas de cobertura verde de inverno sobre o crescimento do porta-enxerto VR 043-43. *Rev. Ceres*, 59, 136-141.

Bielinis, E., Kwiatkowski, J., Boiko, S. (2019). Identification of *Pinus sylvestris* clones with highest and lowest allelopathic potential. *Bal. For.* 25, 8.

Bouhaouel, I., Richard, G., Fauconnier, M.L., Ongena, M., Franzil, L., Gfeller, A., Slim Amara, H., du Jardin, P. (2019). Identification of barley (*Hordeum vulgare* L. subsp. vulgare) root exudates allelochemicals, their autoallelopathic activity and against *Bromus diandrus* Roth. germination. *Agronomy*, 9, 345.

Bulut, Y., Demir, M. (2007). The allelopathic effects of scots pine (*Pinus sylvestris* L.) leaf extracts on turf grass seed germination and seedling growth. *Asian J. Chem.* 19, 3169-3177.

Cerdan, O., Govers, G., Le Bissonnais, Y., Van Oost, K., Poesen, J., Saby, N., Gobin, A., Vacca, A., Quinton, J., Auerwald, K., et al. (2010). Rates and spatial variations of soil erosion in Europe: A study based on erosion plot data. *Geomorphology*, 122, 167-177.

Creamer, N.G., Bennett, M.A., Strinner, B.R., Cardina, J., Regnier, E.E. (1996). Mechanisms of weed suppression in cover crop-based production systems. *Hort. Sci.* 31, 410-413.

Chon, S.-U., Kim, Y.M. (2004). Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts. *J. Agron. Crop Sci.* 190, 145-150.

De Prado, R., Dominguez, C., Tena, M. (1989). Characterization of triazine- resistant biotypes of common lambsquarters (*Chenopodium album*), hairy fleabane (*Conyza bonariensis*) and yellow foxtail (*Setaria glauca*) found in Spain. *Weed Sci.* 37, 1-4.

Den Hollander, N.G., Bastiaans, L., Kropff, M.J. (2007). Clover as a cover crop for weed suppression in an intercropping design: I. Characteristics of several clover species. *Eur. J. Agron.* 26, 92–103.

Dhima, K.V., Vasilakoglou, I.B., Eleftherohorinos, I.G., Lithourgidis, A.S. (2006). Allelopathic potential of winter cereals and their cover crop mulch effect on grass weed suppression and corn development. *Crop Sci.* 46, 345-352.

Dhima, K., Vasilakoglou, I., Lithourgidis, A., Mecolari, E., Keco, R., Agolli, X., Eleftherohorinos, I. (2008). Phytotoxicity of 10 winter barley varieties and their competitive ability against common poppy and ivy-leaved speedwell. *Exp. Agric.* 44, 385-397.

Farooq, M., Jabran, K., Cheema, Z., Wahid, A., Siddique, K. (2011). The role of allelopathy in agricultural pest management. *Pest Manag. Sci.* 67, 493-506.

Friesen, L.F., Beckie, H.J., Warwick, S.I., Van Acker, R.C. (2009). The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. *Can. J. Plant Sci.* 89, 141-167.

Fujii, Y. (2001). Screening and future exploitation of allelopathic plants as alternative herbicides with special reference to Hairy Vetch. *J. Crop Prod.* 4, 257-275.

Gago, P., Cabaleiro, C., García, J. (2007). Preliminary study of the effect of soil management systems on the adventitious flora of a vineyard in northwestern Spain. *Crop Prot.* 26, 584-591.

Gallet, C. (1994). Allelopathic potential in bilberry-spruce forests: Influence of phenolic Compounds on spruce seedlings. *J. Chem. Ecol.* 20,1009-1024.

Garcia, L., Celette, F., Gary, C., Ripoche, A., Valdés-Gómez, H., Metay, A. (2018). Management of service crops for the provision of ecosystem services in vineyards: a review. *Agr. Ecosys. Environ.* 251, 158-170.

Ghimire, B.K., Yu, C.Y., Ghimire, B., Seong, E.S., Chung, I.M. (2019a). Allelopathic potential of phenolic compounds in *Secale Cereale* cultivars and its relationship with seeding density. *Appl. Sci.* 9, 3072.

Ghimire, B.K., Ghimire, B., Yu, C.Y., Chung, I.-M. (2019b). Allelopathic and autotoxic effects of *Medicago sativa*—derived allelochemicals. *Plants*, 8, 233.

Gómez, J., Llewellyn, C., Basch, G., Sutton, P., Dyson, J., Jones, C. (2011). The effects of cover crops and conventional tillage on soil and runoff loss in vineyards and olive groves in several Mediterranean countries. *Soil Use Manag.* 27, 502-514.

Heap, I. (2019). The International Survey of Herbicide Resistant weeds. Online. Internet. Tuesday, December 3, 2019. Available www.weedscience.org

Hussain, M.I., Gonzalez, L., Souto, X.C., Reigosa, M.J. (2011). Ecophysiological responses of three native herbs to phytotoxic potential of invasive *Acacia melanoxylon* R.Br. *Agroforest. Syst.* 83, 149-166.

Ibáñez, S. (2015). Mantenimiento del suelo en el viñedo mediante cubiertas vegetales. Ed. Gobierno de la Rioja. 2015, 167 p.

Ibánez, S., Perez, J. L., Peregrina, F., Garcia-Escudero, E. (2011). Utilización de cubiertas vegetales en viñedos de la D.O.Ca. Rioja (España). Bulletín de l'OIV. Vol. 84, 347-360.

Kempen, H. M., Graf, J. (1981). Weed seed production. Proceedings of the Western Society of Weed Science, 34, 78–81.

Kruidhof, H.M., van Dam, N. M,. Ritz, C., Lotz, L.A.P., Kropff, M.J., Bastiaans, L. (2014). Mechanical wounding under field conditions: A potential tool to increase the allelopathic inhibitory effect of cover crops on weeds? *Eur. J. Agron.* 52, 229-236.

Lehman, M.E., Blum, U. (1997). Cover crop debris effects on weed emergence as modified by environmental factors. *Allelopathy J.* 4, 69-88.

Mayer, A.M., Poljakoff-Mayber, A. (1963). The germination of seeds. Pergamon Press, Oxford. pp 244.

Morlat, R., Jacquet, A. (2003). Grapevine root system and soil characteristics in a vineyard maintained long-term with or without interrow sward. *Am. J. Enol. Viticult.* 54, 1-7.

Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., Drexler, D., Tóthmérész, B. (2015). Establishment of three cover crop mixtures in vineyards' *Sci. Hortic.* 197, 117-123.

Oerke, E. (2006). Crop losses to pests. *J. Agric. Sci.* 144, 31-43.

Osipitan, O.A. (2016). Relative ecological fitness of glyphosate-resistant Kochia from Western Kansas. Ph.D dissertation. Manhattan, KS: Kansas State University. Pp 14–114.

Osipitan, O., Dille, J., Bagavathiannan, M., & Knezevic, S. (2019). Modeling population dynamics of Kochia (*Bassia scoparia*) in response to diverse weed control options. *Weed Sci.* 67, 57-67.

Osuna, M. D., Fischer, A. J., de Prado, R. (2003). Herbicide resistance in *Aster squamatus* conferred by less sensitive form of acetolactate synthase. *Pest Manag. Sci.* 59, 1210-1216.

Pardo-Muras, M., Puig, C.G., López-Nogueira, A., Cavaleiro, C., Pedrol, N. (2018). On the bioherbicide potential of *Ulex europaeus* and *Cytisus scoparius*: Profiles of volatile organic compounds and their phytotoxic effects. *PLoS ONE,* 13(10).

Pereira, J.C., Paulino, C.L., Granja, B.D., Santana, A.E., Endres, L., Souza, R.C. (2018). Potencial alelopático e identificação dos metabólitos secundários em extratos de *Canavalia ensiformis* L. *Rev. Ceres*, 65, 243-252.

Price, A.J., Stoll, M.E., Bergtold, J.S., Arriaga, F.J., Balkcom, K.S., Kornecki T.S., Raper, R.L. (2008). Effect of cover crop extracts on cotton and radish radicle elongation. *Commun. Biometry Crop Sci.* 3, 60-66.

Prosdocimi, M., Cerdà, A., Tarolli, P. (2016). Soil water erosion on Mediterranean vineyards: A review. *Catena*, 141, 1–21.

Puig, C.G., Álvarez-Iglesias, L., Reigosa, M. J., Pedrol, N. (2013). *Eucalyptus globulus* leaves incorporated as green manure for weed control in maize. *Weed sci.* 61, 154-161.

Puig, C.G., Reigosa, M.J., Valentão, P., Andrade, P.B., Pedrol, N. (2018). Unravelling the bioherbicide potential of *Eucalyptus globulus* Labill: Biochemistry and effects of its aqueous extract. *PLoS ONE* 13(2): e0192872.

Qin, B., Perry, L.G., Broeckling, C.D., Du, J., Stermitz, F.R., Paschke, M.W., et al. (2006). Phytotoxic allelochemicals from roots and root exudates of leafy spurge (*Euphorbia esula* L.). *Plant Signal and Behav.* 1, 323-327.

Recasens, J., Valencia, F., Montull, J.M., Taberner, A. (2018). Malas hierbas problemáticas en viñedos con cubiertas vegetales y métodos químicos para su control. *Vida rural*, 448, 48-58.

Reigosa, M. J., Pazos-Malvido, E. (2007). Phytotoxic effects of 21 plant secondary metabolites on *Arabidopsis thaliana* germination and root growth. *J. Chem. Ecol.* 33, 1456-1466.

Rice, A., Johnson-Maynard, J., Thill, D., Morra, M. (2007). Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. *Renew. Agr. Food Syst.* 22, 204-212.

Šajna, N., Kaligarič, M., Ivajnšič, D. (2014). Reproduction biology of an alien invasive plant: a case of drought-tolerant *Aster squamatus* on the Northern Adriatic Seacoast, Slovenia. In: Rannow S., Neubert M. (eds) Managing Protected Areas in Central and Eastern Europe Under Climate Change. Advances in Global Change Research, vol 58. Springer, Dordrecht.

Savage, D., Borger, C. P., Renton, M. (2014). Orientation and speed of wind gusts causing abscission of wind-dispersed seeds influences dispersal distance. *Funct. Ecol.* 28, 973-981.

Schappert, A., Schumacher, M., Gerhards, R. (2019). Weed control ability of single sown cover crops compared to species mixtures. *Agronomy*, 9, 294.

Serajchi, M., Schellenberg, M.P., Lamb, E.G. (2017). The potential of seven native north American forage species to suppress weeds through allelopathy. *Can. J. Plant Sci.* 97, 881-890.

Souto, X.C., Bolaño, J.C., González, L., Santos, X.X. (2001). HPLC Techniques-Phenolics. In handbook of plant ecophysiology techniques. Springer Netherlands. pp. 251-282.

Sturm, D.J., Peteinatos, G., Gerhards, R. (2018). Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res*. 58, 331-337.

Tursun, N., Işık, D., Demir, Z., Jabran, K. (2018). Use of living, mowed, and soil-incorporated cover crops for weed control in apricot orchards. *Agronomy*, 8, 150.

Urbano, J., Borrego, A., Torres, V., Leon, J., Jimenez, C., Dinelli, G., Barnes, J. (2007). Glyphosate-resistant Hairy Fleabane (*Conyza bonariensis*) in Spain. *Weed Technol.* 21, 396-401.

Weston, L.A., Duke, S.O. (2003). Weed and crop allelopathy. *Crit. Rev. Plant Sci.* 22, 367-389.

White, R.H., Worsham, A.D., Blum, U. (1989). Allelopathic potential of legume debris and aqueous extracts. *Weed Sci.* 37, 674-679.

Wu, H., Walker, S., Rolling, M.J., Yuen, D.K., Robinson, G., Werth, J. (2007). Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist). *Weed Biol. Manag*. 7, 192-199.

Xuan, T.D., Tsuzuki, E., Terao, H., Matsuo, M., Khanh, T.D. (2003). Correlation between growth inhibitory exhibition and suspected allelochemicals (phenolic compounds) in the extract of alfalfa (*Medicago sativa* L.). *Plant Prod. Sci.* 6, 165-171.

Zhao, R., Hui, R., Ma, R. (2018). The effects of six allelochemicals on seed germination, seedling growth, and the Mitotic index of *Mikania micrantha* H.B.K. *Fresenius Environ. Bull.* 27. 54-67.

Zubair, H. M., Pratley, J. E., Sandral, G. A., Humphries, A. (2017). Allelopathic interference of alfalfa (*Medicago sativa* L.) genotypes to annual ryegrass (*Lolium rigidum*). *J. Plant Res. 130,* 647-658.

Chapter 6

General discussion and conclusions

General discussion

The present work provides new information about the management of some problematic weeds in vineyards through an innovative and organic tool, namely ground vegetation covers. Weed management is still the main concern of winegrowers when deciding the appropriate soil management practices in vineyards and they invest a lot of effort, time, and money on managing these plants. Weed management using conventional tools entails a series of disadvantages such as soil degradation, in the case of tilled fields, or the possible appearance of herbicide resistance after repeated herbicide applications, in no-till fields, among others. Therefore, more sustainable and environmentally-friendly soil management systems, focused on weed management, are desired (or needed).

Ground vegetation covers and weed management

Several studies support the advantages of implementing ground vegetation covers in vineyards (Ibáñez, 2015; Pardini et al., 2002) but, in Spain, this soil maintenance system is still scarce (MAPA, 2018). Both spontaneous vegetation covers and cover crops can suppress the development of noxious weeds through increasing competition (light, water, nutrients, space…) or by releasing allelochemicals (Bettoni et al., 2012; den Hollander et al., 2007; Farooq et al., 2011; Gago et al., 2007; Garcia et al., 2018; Miglécz et al., 2015; Sturm et al., 2018). The effect of the ground cover on the weed community and on the target weed species depends on the type of cover, its management, and on the characteristic of the weed species (Gago et al., 2007; Recasens et al., 2018) (Chapters 2 and 3). In this PhD thesis two target species have been chosen as representative of the most important weeds affecting vineyards: *Cynodon dactylon* (L.) Pers., as a type of a vivacious weed with perennial underground organs; and *Conyza bonariensis* (L.) Cronquist, as a reference for annual/biennial weeds.

Effect of soil management and cover crops on the presence and widespread of Cynodon dactylon

Cynodon dactylon is one of the most noxious weed species worldwide (Holm et al., 1977), and is also problematic in vineyards (Recasens et al., 2018). The control of this weed can be achieved through correct ground cover management (Chapters 2 and 3). However, our results reveal that different interventions can increase or reduce the weed infestation level: in the short-term, a spontaneous vegetation cover exclusively managed by shredding, increased *C. dactylon* infestation. But this management combined with herbicide applications or tillage as well as the implantation of an *Hordeum vulgare* L. cover, decreased weed infestation level (Chapter 2). The result obtained by shredding a spontaneous vegetation cover was expected due to the morphological characteristics of *C. dactylon* because its vegetative growth in spring and summer through stolons and rhizomes that develop horizontally in the soil surface or underground prevents this species from being eliminated by only shredding. Besides, mechanical interventions such as shredding mainly destroy erect species and hardly affect species that develop horizontally in the soil surface such as *C. dactylon*. Therefore, shredding can actually create more favorable conditions for *C. dactylon,* with more light and less competition from other weed species.

If spontaneous vegetation is used as the ground cover and *C. dactylon* is the target weed species, a combination of shredding and herbicide applications can offer good control levels (Chapter 2). Glyphosate (probably the most used herbicide worldwide) can achieve different levels of effectiveness, depending on the weed phonological stage and infestation levels, timing and number of herbicide applications, dose rates, and on the combination with other tools (Abdullahi, 2002; Bryson and Wills, 1985; Farthing et al., 2018; Gómez de Barreda et al., 2017). But indiscriminate applications can also destroy the vegetation cover, and the surviving species, which generally are difficult-to-control weeds, are favored. To overcome this challenge, site-specific herbicide applications can reduce *C. dactylon* development while ensuring the viability of the vegetation cover. In this sense, de Castro et al. (2020) developed a procedure detection system that allows, in vineyard managed with cover crops, obtaining precise *C. dactylon* infestation maps, and thus, performing site-specific herbicide applications. This system may be used to localize *C. dactylon* patches also when the ground is covered by spontaneous vegetation.

As mentioned before, tillage and cover crops are suitable options to manage *C. dactylon* in vineyards, but results depend on both the weed infestation level and the cover type (Chapters 2 and 3). In our experiment, different tillage interventions were required to reduce the weed infestation level before cover crop seeding (Chapter 3). The effectiveness of tillage reducing *C. dactylon* agrees with other studies (Dalley et al., 2013; Phillips, 1993). Mechanical soil disturbance is successful in reducing *C. dactylon* coverage because it breaks rhizomes and stolons up into smaller fragments and brings them up to the soil surface where they dry and die (Guglielmini and Satorre, 2004; MAGRAMA, 2014). On the other hand, the mortality of the fragments is also increased when they are buried deeper into the soil compared to those that remain buried close to the soil surface, especially small fragments (Valencia-Gredilla et al., 2019). The weed control level is also affected by the timing of soil disturbance, *i.e.* autumn tillage reduces the survival of the rhizomes and stolons, compared to the winter tillage (Valencia-Gredilla et al., 2019). The main problem of tillage interventions is that the surviving fragments are dispersed through the field; they establish again and can form new plants. Thus, the combination of tillage with other tools to obtain long-term control is required. In this sense, the implementation of a vegetation cover that could efficiently compete against the surviving fragments is a successful option (Chapter 2 and 3).

Cynodon dactylon stays dormant below 10 ºC (McCarty and Miller, 2002), and changes its growth pattern under low light availability, forming orthotropic shoots, failing to develop rhizomes, and increasing stolons and their internodes elongation (Dong and de Kroon, 1994). Also, under low light and nutrients levels, stolons and rhizome branching intensities are reduced (Dong and de Kroon, 1994). For this reason, the vegetation cover should grow enough biomass to form a dense canopy to intercept light and compete strongly for other resources before *C. dactylon* sprouts. This objective can be achieved by letting a spontaneous ground vegetation cover grow, or by seeding a cover crop that grows during autumn and winter. Spontaneous vegetation with annual tillage and *H. vulgare* covers succeeded in reducing *C. dactylon* infestation levels (Chapter 2). The higher biomass provided by *H. vulgare*, and its coverage homogeneity increased its competitiveness over *C. dactylon*, compared to the spontaneous vegetation. The allelopathic characteristics of *H. vulgare* (Chapter 5) could also have contributed to this suppression. Focusing on weed control, potential cover crop species must show characteristics such as good growth rates, competitive ability, provide a high amount of biomass, and being allelopathic in addition to self-seeding capacity or being pluriannual in order to reduce costs of the annual seeding. The unfavorable results obtained by shredding a spontaneous cover and the good results obtained by the implantation of an *H. vulgare* cover (Chapter 2) encourages the search of other cover crop species to manage (or control) this weed. The implementation of different cover crops species focusing on weed control was tested in Chapter 3. The implantation of the pluriannual *Festuca arundinacea* L., which is a commonly used as cover crop in vineyards (Chou et al., 2019; Coniberti et al., 2018; Pardini et al., 2002), was able to stop and decrease *C. dactylon* development with greater success than the spontaneous vegetation cover assessed in Chapter 2, despite being both managed through shredding. This result could be attributed to the competition pressure (by light, water, nutrients, or space), and also by an allelopathic effect exerted by *F. arundinacea* (Chapter 5). Moreover, this cover crop can be managed by shredding without compromising its viability, which allows the cover crop to continue to compete against *C. dactylon* after being managed. Our results also show that when the ground is covered by spontaneous vegetation, its control and management may be more difficult because it depends on the specific characteristics of the weed community, and so, the implementation of a specific cover crop can make management easier.

The control of *C. dactylon* achieved by the different cover crops differed depending on the selected species and the initial weed infestation level (Chapter 3). In plots with initial *C. dactylon* presence, *F. arundinacea* and *H. vulgare* reduced its coverage and frequency, whereas the mixture of *Bromus* species (*Bromus rubens* L. and *B. hordeaceus* L.), *Vulpia ciliata* Dumort., and *Medicago rugosa* Desr. did not succeed. On the other hand, in the initially weed-free plots, low coverage levels of *C. dactylon* appeared after one year and a half, but values remained low in all cover crops treatments throughout the duration of the experiment. However, *C. dactylon* could not be eradicated in any case. These results demonstrate the ability of the cover crops to stop or reduce the *C. dactylon* infestation levels, but also the difficulty to get rid of this weed, suggesting that they need to be combined with other control methods to achieve complete control.

Effect of soil management and cover crops on the weed community

Different weed associations were identified in the different established managements (Chapters 2 and 3). The Redundancy Analysis of species composition showed that shredding (only shredding or combined with herbicide applications) promoted different associations compared to tillage (in both spontaneous vegetation and *H. vulgare* covers). None of the recorded species showed preference to the *H. vulgare* cover (according to the diagnostic species for individual management), which demonstrates the capacity of this cover crop to reduce also the presence of other weeds (Chapter 2). The Canonical Correspondence Analysis showed clear differences between the pluriannual species (*F. arundinacea*) and the annual ones (*H. vulgare, B. rubens* and *B. hordeaceus, Vulpia ciliata*, and *Medicago rugosa*) (Chapter 3). Within the annual cover crop species, a lower number of weed species was related to *H. vulgare* and to the mixture of *Bromus* spp. These results, presented in Chapter 3, highlight the role of the different cover crops and their management on the configuration of the weed associations.

Germination behavior of Conyza bonariensis

Among the weed species that appeared in the surveys, *Conyza bonariensis* showed no preference for any management (Chapter 2). This species is very problematic under the vine row, where management is usually mechanically or chemically performed, but is not problematic in the inter-rows, where the soil surface is tilled and/or covered by vegetation. Mulches can inhibit the presence of this species in the vine row (Valencia et al., 2018) but more detailed knowledge on its germination behavior (Chapter 4) and the potential allelopathic effect of this by cover crops or mulches (Chapter 5) is essential.

Conyza bonariensis is a noxious weed, originally from South America that is difficult to control in several crops, especially in no-till systems (Bajwa et al., 2016). This species is widely spread in Spain, being frequent in orchards, olives and vineyards (Jiménez-Díaz et al., 2019), and some biotypes have even evolved herbicide resistant (de Prado et al., 1989; Urbano et al., 2007; Langa et al., 2015). The different phenology and development of different populations across crops and regions require deep knowledge of key aspects of its biology. In this sense, a study of the germination behavior of different populations with distinct origins was carried out (Chapter 4). In general, the results show that the different populations behaved similarly and all of them showed highest germination percentages close to the estimated optimal temperature for germination (22ºC). On the other hand, at temperatures far away from the optimal, more seeds germinated under alternating temperatures compared to the equivalent constant temperatures of those intervals: 5/15 and 25/35ºC compared to 10 and 30ºC, respectively. These results could explain the behavior of the seeds at the soil surface, where temperature fluctuations are more pronounced (Ren et al., 2002). Temperature fluctuations can be mitigated by mulches, which tend to stabilize soil temperatures and can contribute to reduce *C. bonariensis* germination, in addition to act as a physical barrier and reduce light penetration (Teasdale and Mohler 1993, 2000). The germination response of the different populations varied depending on the climate of the region of origin of the seed (Chapter 4): seeds produced at the location with the coldest winter showed the highest germination percentage at the lowest assayed temperature (5ºC), and the lowest base temperature (4.9ºC). These differences between populations might impede the development of a common emergence model for *C. bonariensis*, but could contribute to readjust already developed site-specific germination models, like that created by Zambrano-Navea et al. (2013), and help decide the best timings for control methods.

Herbicide effects of aqueous extracts from plants implemented as a cover crop and/or mulch

Weed presence in the vine-row can also be avoided by the release of allelochemicals from mulches and cover crops (Farooq et al., 2011; Jabran et al., 2015), however, their chemical profile, as well as the allelopathic potential against some problematic weed species, remains unknown. This knowledge is necessary to evaluate the magnitude of the allelopathic potential in controlled conditions to select the most allelopathic cover crop before using at the farm level.

The potential herbicide effect of the released allelochemicals by different cover crop species was tested in *in-vitro* assays over *C. bonariensis*, *Aster squamatus* (Spreng.) Hieron and *Bassia scoparia* (L.) A.J. (Chapter 5). *A. squamatus* is a difficult-to-control species in vineyards because of its adaptation to mechanical shredding (Recasens et al., 2018), high fecundity, and airborne dispersion (Sajna et al., 2014) that allows it to easily colonize new fields. Moreover, this species has the capacity to develop herbicide-resistant biotypes (Osuna et al., 2003). *Bassia scoparia* combines a set of characteristics that make it an important weed in vineyards. Its leaves are difficult to wet and therefore, herbicide efficacy is reduced (Friesen et al., 2009) but also herbicide-resistant biotypes of this species have been found (Heap, 2020). It also has a high potential fecundity, an effective dispersion and, a rapid growth (Friesen et al., 2009; Osipitan 2016; Osipitan et al., 2019; Recasens et al., 2018).

In order to evaluate new approaches for the control of these weeds, the allelopathic potential and chemical profile of *F. arundinacea*, *H. vulgare, H. murinum* L.*, Medicago rugosa, M. sativa* L.*, Phacelia tanacetifolia* Benth.*, Sinapis alba* L.*, Trifolium incarnatum* L.*, T. subterraneum* L.*, Bromus* spp. *(B. hordeaceus* and *B.*

rubens), Vulpia ciliata and *Pinus sylvestris* L. bark, which are commonly used species as cover crops and mulches, were studied. Because water is the only extracting solvent present in field conditions, only water-soluble compounds (phenolic acids and flavonoids) were analyzed. The results of the study show that, in laboratory conditions, aqueous extracts of the twelve assayed species highly inhibited and/or reduced germination and radicle and hypocotyl growth of the three target weed species (Chapter 5). The effect was more noticeable in *C. bonariensis* and *A. squamatus*, which germination and root elongation were practically inhibited by all extracts and doses. In the case of *B. scoparia*, the germination was not highly affected, but root and shoot elongation was. HPLC-DAD analysis identified a total of 33 compounds (phenolic acids and flavonoids), both quantitatively and qualitatively. *Hordeum vulgare* was the species with the highest number of identified compounds, with 6 phenolic acids and 7 flavonoids, while *Bromus* mixture and *P. tanacetifolia* showed only six compounds, the lowest number. On the other hand, *F. arundinacea*, the best species controlling *C. dactylon* (Chapter 3), showed the lowest concentration of compounds (33.53 $\mu g.mL^{-1}$), contrary to *S. alba,* with 1571.77 $\mu g.mL^{-1}$. According to these promising results, the straw from these cover crop species can be shredded and placed under the vine row to manage noxious weeds. Potential limitations of this option could be the low availability of some those materials in the market (when they cannot be produced on the plot itself, as in the case of pine bark), their expensive price, and the method (machinery) for their application.

General comments

Overall, the results from the different chapters of this thesis introduce, provide and validate new information about weed management in vineyards through cover crops, both in field experiments and *in vitro* assays, and provide knowledge to improve the control of four of the most problematic weeds species in this crop. Cover crop sowing, management and species selection are key aspects to consider when using these management practices to manage *C. dactylon*, but also other noxious weeds. The new knowledge on weed germination behavior and on the weed response to different management strategies that is presented in this work will help design better integrated weed management practices in vineyards.

The results presented in this thesis constitute a unique contribution to the knowledge on the biology and control of problematic weeds in vineyards. These results constitute a starting point for new approaches for future studies, both regarding the biology of weeds and the optimization and search for new control methods. In this sense, the inclusion of tools such as the roller-crimper in the management of cover crops, the use of new organic materials derived from industrial by-products for their application as mulch, or the research of new phytotoxic components derived from species implemented as cover crops or material used as mulches, are promising future options for weed management in vineyards.

Conclusions

The conclusions of this thesis are:

Effect of different ground vegetation covers managements on Cynodon dactylon

1. Across the three seasons, the combination of shredding a spontaneous vegetation cover with herbicides reduced total weed coverage and weed frequency by 62.4% and 14.7%, respectively, while the combination with tillage reduced weed coverage by 82.6% and weed frequency by 52%. On the other hand, shredding interventions alone did not reduce *C. dactylon infestation* level.
2. *Hordeum vulgare* cover crop turned out to be the best management compared to any of the spontaneous vegetation cover managements reducing, after three seasons, weed coverage by 93.9%, and weed frequency by 73.3%.

Effect of different cover crops managements on Cynodon dactylon

3. Tillage carried out the first year to implement the cover corps reduced weed infestation level up to 60% for weed coverage and by 14.8% for weed frequency.
4. *Festuca arundinacea* and *H. vulgare* are the cover species with the highest *C. dactylon* reduction effect, after three seasons, on the initially infested plots: 38.8% and 21.3% reduction in weed coverage, and 38.7% and 46.4% reduction on weed frequency, respectively. The other three implemented covers (*Bromus* spp., *Vulpia ciliata* and *Medicago rugosa*) were not able to suppress the weed infestation in this period of time.

5. In those areas of the vineyard where there was no previous weed infestation, all cover crops behaved similarly, being able to contain the expansion of *C. dactylon* that reached these areas, and maintaining, after three seasons, low percentages of weed coverage, with final values ranging between 3.9% for the *Bromus* mixture and 6.9 % for *M. rugosa*.
6. The cover crops reduce and contain *C. dactylon* expansion, but they need to be combined with other control methods in order to eliminate the weed completely.

Effect of different ground vegetation covers management on other weeds

7. The Redundancy Analysis (RDA) clearly separated the weed community composition depending on whether tillage was performed or not. The weed community on spontaneous vegetation shredded and spontaneous vegetation shredded plus herbicide applications completely differed from the one where spontaneous vegetation and *H. vulgare* cover crop were tilled annually.
8. The analysis of the diagnostic species revealed that none of the recorded weed species showed preference to *H. vulgare* cover. The spontaneous cover shredded showed the highest number of related species (13), which was reduced when combined with herbicide applications (7) and tillage (6). Lastly, thirteen additional species, some of them being among the most problematic species (*Conyza bonariensis*, *Convolvulus arvensis* L. or *Malva sylvestris* L.), had no preference for any management, which shows that their presence was not influenced by the cover crop management.

Effect of different cover crops management on other weeds

9. The Canonical Correspondence Analysis (CCA), confirmed changes on the weed community depending on the species implemented as cover crops, with clear differences between the pluriannual *F. arundinacea* and the annual species.
10. The forward selection of explanatory variables revealed that the initial presence or absence of *C. dactylon* in the plots had no effect explaining the weed composition, so these changes in weed community can be attributed exclusively to the cover crops.

Germination behaviour of Conyza bonariensis

11. The different populations of *C. bonariensis* showed similar germination behavior, with the highest germination percentages close to the estimated optimal temperature (22ºC on average between the populations).
12. The alternating temperatures far away from the estimated optimum, positively affected total germination compared to the equivalent constant temperature (5/15ºC *vs* 10ºC or 25/35ºC *vs* 30 ºC).
13. The germination response of the *C. bonariensis* populations varied depending on the climate of the region of origin. The population from Lleida (the population from the coldest winter zone) had higher percentages of germination at the lowest assayed temperature (5ºC), and the lowest base temperature (4.9ºC) compared to the populations from Bahía Blanca, Badajoz, and Seville (6.9ºC, 8.4ºC and 8.9ºC, respectively).

Potential herbicide effect of aqueous extracts from cover crops and/or mulch

14. The aqueous extracts from the species *F. arundinacea, H. vulgare, H. murinum, M. rugosa, M. sativa, P. tanacetifolia, S. alba, T. incarnatum, T. subterraneum, Bromus* spp. *(B. hordeaceus* and *B. rubens), V. ciliata* and *P. sylvestris* showed a clear phytotoxic effect on the germination and early growth of *C. bonariensis, A. squamatus* and, *B. scoparia*.
15. *Conyza bonariensis* germination was significantly reduced by all of the aqueous extracts and 17 out of 24 solutions (12 aqueous extracts and 2 doses) completely inhibited its germination. Radicle growth was completely inhibited by 17 of the assayed solutions and only one (*P. sylvestris* at 50% dose) had not any inhibitory effect. Hypocotyls growth was only reduced by two extracts (*H. murinum* at 100% dose and *P. sylvestris* at 50% dose).
16. All extracts significantly reduced *A. squamatus* germination and at 100% dose, the germination was completely inhibited by all species with exception of *M. rugosa* (5% of final germination). Also, all aqueous extracts at both doses (50% and 100%), completely inhibited and significant reduced, the radicle and hypocotyl growth, respectively.
17. *Bassia scoparia* germination was affected by almost all the extracts but, in general, germination percentages remained high, over 65% of the control, except

for *Trifolium subterraneum* extracts (23% and 44% alt 50% and 100% doses, respectively). Lower values than 50% of the control were obtained for radicle growth. Hypocotyl growth was also reduced by almost all extracts.

18. A total of 33 compounds (phenolic acids and flavonoids) were identified by HPLC-DAD, considering all the species. In *H. vulgare* 13 compounds were identified, being the species with the highest number. *Sinapis alba* (with nine identified compounds) showed the highest compound concentration (1571.77 µg.mL^{-1}), followed by *H. murinum* and *P. tanacetifolia* (410 and 380 µg.mL^{-1} respectively).

References:

Abdullahi, A.E (2002). *Cynodon dactylon* control with tillage and glyphosate. *Crop Prot*. 21, 1093-1100.

Bajwa A.A., Sadia S., Ali H.H., Jabran K., Peerzada A.M., Chauhan B.S. (2016) Biology and management of two important *Conyza* weeds: a global review. *Environ. Sci. Pollut. Res*. 23, 24694–24710.

Bettoni, J.C., Gardin, J.P.P., Feldberg, N.P., Dalbó, M.A. (2012). Potencial alelopático de plantas de cobertura verde de inverno sobre o crescimento do porta-enxerto VR 043-43. *Rev. Ceres*, 59, 136-141.

Bryson, C., Wills, G (1985). Susceptibility of Bermudagrass (*Cynodon dactylon*) biotypes to several herbicides. *Weed Sci*. 33, 848-852.

Chou, M.Y., Vanden Heuvel, J. (2019). Annual under-vine cover crops mitigate vine vigor in a mature and vigorous cabernet franc vineyard. *Am. J. Enol. Vitic*. 70, 98-108.

Coniberti, A., Ferrari, V., Disegna, E., Garcia Petillo, M. (2018). Under-trellis cover crop and planting density to achieve vine balance in a humid climate. *Sci. Hortic*. 227, 65-74.

Dalley, C. D; Viator, R. P, Richard, E. P (2013). Integrated Management of Bermudagrass (*Cynodon dactylon*) in Sugarcane. *Weed Sci*., 61, 482-490.

de Castro, A., Peña-Barragán, J. M., Torres-Sánchez, J., Jiménez-Brenes, F., Valencia-Gredilla, F., Recasens, J., López-Granados, F. (2020). Mapping *Cynodon dactylon* infesting cover crops with an Automatic Decision Tree-OBIA procedure and UAV imagery for precision viticulture. *Remote Sens*. 12, 56.

de Prado, R., Dominguez, C., Tena, M. (1989). Characterization of triazine- resistant biotypes of common lambsquarters (*Chenopodium album*), hairy fleabane (*Conyza bonariensis*) and yellow foxtail (*Setaria glauca*) found in Spain. *Weed Sci.* 37, 1-4.

den Hollander, N.G., Bastiaans, L., Kropff, M.J. (2007). Clover as a cover crop for weed suppression in an intercropping design: I. Characteristics of several clover species. *Eur. J. Agron*. 26, 92–103.

Dong, M., De Kroon, H (1994). Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*. 70, 99–106.

Farooq, M., Jabran, K., Cheema, Z., Wahid, A., Siddique, K. (2011). The role of allelopathy in agricultural pest management. *Pest Manag. Sci.* 67, 493-506.

Farthing, T.S., Muir, J.P., Falk, A., Murray, D., (2018). Efficacy of seven invasive Bermudagrass removal strategies in three Texas ecoregions. *Ecol. Restor*. 36, 306–314. https://doi.org/10.3368/er.36.4.306.

Friesen, L.F., Beckie, H.J., Warwick, S.I., Van Acker, R.C. (2009). The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. *Can. J. Plant Sci*. 89, 141-167.

Garcia, L., Celette, F., Gary, C., Ripoche, A., Valdés-gómez, H., Metay, A. (2018). Management of service crops for the provision of ecosystem services in vineyards: a review. *Agric Ecosyst Environ*. 251, 158-170.

Gago, P., Cabaleiro, C., García, J. (2007). Preliminary study of the effect of soil management systems on the adventitious flora of a vineyard in northwestern Spain. *Crop Prot*. 26, 584-591.

Gómez de Barreda, D.; Izquierdo, D.; de Luca, V. Evaluación del comportamiento de 20 materias activas herbicidas sobre la cespitosa "*Cynodon dactylon*" en diferentes épocas climáticas del año. XVI Congreso de la Sociedad Española de Malherbología. 25-27 October 2017, Pamplona-Iruña, España, 2017, 245-250.

Guglielmini, A.C., Satorre, E.H. (2004). The effect of non-inversion tillage and light availability on dispersal and spatial growth of *Cynodon dactylon*. *Weed Res*. 44, 366–374.

Heap, I.M. (2020). International Survey of Herbicide Resistant Weeds. Retrieved from: http://www.weedscience.org.

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P. (1977). The World's Worst Weeds. Distribution and Biology. University of Hawaii Press, Honolulu, USA, 1977.

Ibáñez, S. (2015). Mantenimiento del suelo en el viñedo mediante cubiertas vegetales. Ed. Gobierno de la Rioja. 2015, 167 p.

Jabran, K., Mahajan, G., Sardana, V., Chauhan, B. (2015). Allelopathy for weed control in agricultural systems. *Crop Prot*. 72. 57-65.

Jiménez Díaz, R., Castillo, P., García-Arenal, F., García Marí, F., Montesinos, E., Recasens, J. (2019). Enfermedades, plagas y malas hierbas que constituyen problemas de carácter estratégico en sectores productivos y masas forestales claves para la economía española. En: Libro Blanco de la Sanidad Vegetal en

España. Eds: R. Jiménez Díaz y M. M. López González. Ed. UCOPress. Ed Universidad de Córdoba. Córdoba. Pgs: 317-386. ISBN: 978-84-9927-455-3

Langa, R., Aibar, J., Cirujeda, A., Mari, A.I., León, M., Pardo, G. (2015). Estudio de la resistencia de *Conyza* spp. al herbicida glifosato en Aragón. p. 99-105. En: XV Congreso de la Sociedad Espanola de Malherbologia. Sevilla.

MAGRAMA (2014). Guía de gestión integrada de plagas. Uva de transformación. Ministerio de Agricultura, Alimentación y Medio Ambiente, España, Madrid, 2014.

MAPA (2018). Encuesta sobre superficies y rendimientos de cultivos (ESYRCE). Resultados 2018. Ministerio de Agricultura, Pesca y Alimentación. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/boletin2018_tcm30-504212.pdf

McCarty, L.B., Miller, G. (2002). Managing bermudagrass turf. E. Ann Arbor Press. Chelsea, MI, 2002 pp. 67-86.

Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., Drexler, D., Tóthmérész, B. (2015). Establishment of three cover crop mixtures in vineyards' *Sci. Hortic*. 197, 117-123.

Osipitan, O.A. (2016) Relative Ecological Fitness of Glyphosate-Resistant *Kochia* from Western Kansas. Ph.D dissertation. Manhattan, KS: Kansas State University. Pp 14–114.

Osipitan, O., Dille, J., Bagavathiannan, M., Knezevic, S. (2019). Modeling population dynamics of *Kochia* (*Bassia scoparia*) in response to diverse weed control options. *Weed Sci*. 67, 57-67.

Osuna, M. D., Fischer, A. J., de Prado, R. (2003). Herbicide resistance in *Aster squamatus* conferred by less sensitive form of acetolactate synthase. *Pest Manag. Sci*. 59, 1210-1216.

Pardini, A., Faiello, C., Longhi, F. (2002). Cover crop species and their management in vineyards and olive groves. *Adv. Hortic. Sci.* 16, 225-234.

Phillips M.C. (1993). Use of tillage to control *Cynodon dactylon* under small-scale farming conditions. *Crop Prot*. 12, 267-272.

Recasens, J., Valencia, F., Montull, J.M., Taberner, A (2018). Malas hierbas problemáticas en viñedos con cubiertas vegetales y métodos químicos para su control. *Vida Rural*, 444, 48-58.

Ren J, Tao L, Liu XM (2002) Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. *Journal of Arid Environments*, 51, 603-611.

Šajna, N., Kaligarič, M., Ivajnšič, D. (2014). Reproduction Biology of an Alien Invasive Plant: A Case of Drought-Tolerant *Aster squamatus* on the Northern Adriatic Seacoast, Slovenia. In: Rannow S., Neubert M. (eds) Managing Protected Areas in Central and Eastern Europe Under Climate Change. Advances in Global Change Research, vol 58. Springer, Dordrecht.

Sturm, D.J., Peteinatos, G., Gerhards, R. (2018). Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res*. 58, 331-337.

Teasdale, J.R., Mohler, C.L. (1993). Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. *Agron. J*. 85, 673–680.

Teasdale, J.R., Mohler, C.L. (2000). The quantitative relation-ship between weed emergence and the physical properties of mulches. *Weed Sci*. 48, 385-392.

Urbano, J., Borrego, A., Torres, V., Leon, J., Jimenez, C., Dinelli, G., Barnes, J. (2007). Glyphosate-resistant Hairy Fleabane (*Conyza bonariensis*) in Spain. *Weed Technol.* 21, 396-401.

Valencia, F., Mas, N., Arbonés, A., Cabrera, C., Recasens, J (2018). Uso de acolchados orgánicos y bioherbicidas en el control de malas hierbas en viña ecológica. Actas III Jornadas del Grupo de Viticultura, Palma: 177-181.

Valencia-Gredilla, F, Recasens, J, Royo-Esnal, A (2019). Supervivencia de rizomas y estolones de *Cynodon dactylon* en función de su estado vegetativo, tamaño y profundidad de enterramiento. Actas XVII Congreso de la Sociedad Española de Malherbología, Vigo: 249-253.

Zambrano-Navea C., Bastida F., Gonzalez-Andujar J.L. (2013) A hydrothermal seedling emergence model for *Conyza bonariensis*. *Weed Res.* 53, 213–220.

Appendix

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RESEARCH ARTICLE

Germination behaviour of *Conyza bonariensis* to constant and alternating temperatures across different populations

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Abstract

Conyza bonariensis is one of the most problematic weed species throughout the world. It is considered highly noxious due to its interference with human activities, and especially the competition it poses with economically important crops. This research investigated the temperature requirements for seed germination of four populations of *C. bonariensis* with distinct origin and the influence of daily alternating temperatures. For this, a set of germination tests were performed in growth chambers to explore the effect of constant and alternating temperatures. Seeds of the four populations (from Lleida, Badajoz and Seville, Spain and Bahía Blanca, Argentina) were maintained at constant temperatures ranging from 5 to 35°C. The final germination and cardinal temperatures (base, optimum and maximum) of each population were obtained. We also tested the influence of daily alternating temperatures on final germination. To do so, seeds were exposed to two temperature regimes: 5/15, 10/20, 15/25, 20/30 and 25/35°C night/day temperature (intervals increasing 5°C, with constant oscillation of 10°C) and to 18/22, 16/24, 14/26, 12/28 and 10/30°C night/day temperature (intervals with average of 20°C, but increasing the oscillation in 4°C between intervals). In general, all populations behaved similarly, with the highest germination percentages occurring in the optimum temperature range (between 21.7°C and 22.3°C) for both constant and alternating temperatures. In general, climatic origin affected germination response, where seeds obtained from the coldest origin exhibited the highest germination percentage at the lowest temperature assayed. In addition, we observed that the alternating temperatures can positively affect total germination, especially in oscillations that were further from the average optimum temperature (20°C), with high germination percentage for the oscillations of 15/25, 20/30, 18/22, 16/24, 14/26, 12/28 and 10/30°C in all populations. The cardinal temperatures obtained were significantly different across the populations. These results provide information that will facilitate a better understanding of the behaviour of *Conyza* and improve current field emergence models.

KEYWORDS
cardinal temperatures, germination response, hairy fleabane, weed biology, weed management

1 | INTRODUCTION

Weed management is one of the most important issues in crop production, especially in conservation cropping systems. Some weeds are difficult to control due to their specific biological characteristics and the lack of information about them.

Conyza bonariensis (L.) Cronquist (hairy fleabane, flaxleaf fleabane), is one of the most problematic weed species throughout the world (Bajwa et al., 2016), appearing in more than 40 crops in 70 countries (Holm, Doll, Holm, Pancho, & Herberger, 1997). In Spain, it is one of the most competitive introduced noxious weeds (Zambrano-Navea, Bastida, & Gonzalez-Andujar, 2013) that harms crops and leads to yield loss (Davis & Johnson, 2008; Trezzi et al., 2013, 2015; Urbano et al., 2007). *Conyza bonariensis* is an annual or short-lived perennial weed native to South America (Thebaud & Abbott, 1995; Wu et al., 2007). Its invasive behaviour is due to high plant fecundity that varies from 85,000 (Dauer, Mortensen, & Vangessel, 2007) to 375,500 seeds per plant (Kempen & Graf, 1981). Moreover, the anemochorous dispersion of the seeds permits their long-distance spread and establishment in new fields (Savage, Borger, & Renton, 2014).

Conyza bonariensis is difficult to control in minimum tillage and conservation cropping systems (Somervaille & McLennan, 2003; Wicks, Felton, Murison, & Martin, 2000), but is easily controlled with tillage (Brown & Whitwell, 1988). Herbicide control depends on the growth stage of the plants (Shrestha, Hembree, & Wright, 2008) and if the population presents herbicide resistance, given that *C. bonariensis* has been cited as evolving resistance to herbicides with different action sites (EPSP synthase inhibitors, PSI Electron Diverter, ALS inhibitor, etc.) in several countries (Argentina, Australia, United States or Spain), in addition to evolving multiple resistance (PSI Electron Diverter and EPSP synthase inhibitors; Heap, 2019).

Conyza is photoblastic, emerging from the upper layers of the soil surface (0–2 cm) with limited persistence, as it has very low dormancy levels and the viability of the ungerminated seeds is severely lost in the first year (Wu et al., 2007). Annual weed species survival is highly dependent on seedling emergence and recruitment (Forcella, Benech-Arnold, Sánchez, & Ghersa, 2000). Thus, it is important to know both timing and magnitude of seedling emergence in the field in order to implement successful control measures for weeds (García, Recasens, Forcella, Torra, & Royo-Esnal, 2013; Royo-Esnal, García, Torra, Forcella, & Recasens, 2015). In this respect, Zambrano-Navea et al. (2013) modelled the emergence of *C. bonariensis* and developed a cohort-based stochastic model of the population dynamics (Zambrano-Navea, Bastida, & Gonzalez-Andujar, 2016). However, studying the germination response of more populations and at more temperatures and intervals would expand upon these existing models. *Conyza bonariensis* biology is well understood, but additional information regarding germination temperature thresholds is required to implement integrated management control measures. An added complexity is that the variation of threshold parameters between populations can be significant due to local adaptations (Bajwa et al., 2016; Tozzi et al., 2014). For example, in *C. bonariensis*, Wu et al. (2007) established cardinal temperatures of 4.2°C (base temperature, T_b), 20°C (optimum temperature, T_o) and 35°C (ceiling temperature, T_c), while Zambrano-Navea et al. (2013) cited a T_b of 10.6°C.

This research investigated thermal requirements for seed germination of four different *C. bonariensis* populations collected from contrasting environments. The final aim was to compare the total germination percentage of each population at constant temperatures and at different alternating temperatures. An additional objective was to compare the cardinal temperatures (T_b, T_o, T_c) of each population.

2 | MATERIALS AND METHODS

2.1 | Plant material collection

Conyza bonariensis seeds were harvested at maturity in September 2016 in Spain and in November 2017 in Argentina. Seeds from Spain were collected from three different habitats: a vineyard in Lleida (41.658010, 0.523766), a garden in Seville (37.352824, −5.933194) and an olive orchard in Badajoz (38.702537, −5.573246). The population from Argentina belonged to a garden in Bahía Blanca (−38.695394, −62.253302). The four locations have specific climatic conditions (Table 1). According with Torra, Royo-Esnal, and Recasens (2016), seeds were collected from different plants throughout the field, were air-dried under laboratory conditions for 1 week and dry stored in the dark in paper bags at 4°C until the beginning of the experiment.

2.2 | Experimental design

Three germination tests were established at different temperature conditions and repeated twice: the first one at constant temperatures, the second at constant day/night temperature oscillations (T_{osc}) and different mean temperatures (T_m), and the third one at a different day/night T_{osc} but with the same T_m. All the experiments were performed at the Departamento de Agronomía, Universidad Nacional del Sur and CONICET (Bahía Blanca, Argentina). In all three tests, batches of 30 seeds were sown on 9-cm Petri dishes lined with a No. 1 filter paper layer wetted with distilled water. Four replicates per population and temperature were used following a completely randomised design. Germinated seeds were counted on a daily basis until 21 days or until no further germination occurred during 5 consecutive days.

2.2.1 | Germination test at constant temperatures (Test 1)

Seeds from each population were incubated, in November 2017, at constant temperatures of 5, 10, 15, 20, 25, 30 and 35°C with a 12/12 day/night photoperiod. Temperatures were maintained at ±0.2°C and monitored with digital temperature data loggers (Thermochron Ibuttons, Model DS1921G-F50; Maxim Integrated Products, Inc., San Jose, CA).

TABLE 1 Climatic characterisation of the origin locality of each population

Climatic characterisation							
Location	Macro Bioclimate	Ombrotype	Thermotype	Bioclimate	T_{mean}	T_{warm}	T_{cold}
Lleida	Mediterranean	Xeric	Mesomediterranean	Continental	15.0	25.2	5.5
Badajoz	Mediterranean	Xeric	Mesomediterranean	Oceanic	17.1	26.1	8.6
Seville	Mediterranean	Xeric	Thermomediterranean	Oceanic	19.2	28.2	10.9
Bahía Blanca	Temperate	Xeric	Mesotemperate	Oceanic	15.3	23.6	7.5

Notes: T_{Mean}, annual mean temperature; T_{Warm}, mean temperature of the warmest month; T_{Cold}: mean temperature of the coldest month. Temperature means calculated for the period 1983–2010 for the Spanish locations and 1981–2010 for the Argentinian.

2.2.2 | Germination test at alternating temperatures (variable T_m/constant T_{osc}; Test 2)

In this assessment, seeds from each population were incubated at different alternating temperatures with 10°C of T_{osc} 5/15, 10/20, 15/25, 20/30 and 25/35°C night/day temperatures using an Electronic Gradient Plate Germinator (SECELEC, CCT-Bahía Blanca, CONICET). Seeds were placed inside independent germination chambers with automatic temperature control (±0.1°C).

2.2.3 | Germination test at alternating temperatures (constant T_m/variable T_{osc}; Test 3)

In this test, T_m was maintained constant (20°C) while T_{osc} was varied. The T_m considered was the optimum temperature (T_o) obtained by Wu et al. (2007). Seeds were then placed at constant 20°C (in a growth chamber) and at 18/22, 16/24, 14/26, 12/28 and 10/30°C night/day alternating temperatures. Thus, incubation temperature amplitudes (T_{osc}) were 0, 4, 8, 12, 16 and 20°C.

For the different tests, a seed was considered germinated when the radicle had extended more than 1 mm beyond the seed coat (Steinmaus, Prather, & Holt, 2000; Wu et al., 2007). Germinated seeds were removed from the dish once counted. Seed viability at the end of the germination tests was assessed by counting the number of germinated seeds after incubation at 20°C (Wu et al., 2007) under a 12-hr photoperiod for 5 days.

2.3 | Statistical analysis

Total germination percentages between populations and incubation temperatures as well as cardinal temperatures were subjected to analysis of variance (ANOVA). The SED and LSD are provided.

2.3.1 | Estimation of cardinal temperatures (base temperature, T_b, optimum temperature, T_o and ceiling temperature, T_c)

In order to estimate the cardinal temperatures for each population, a three-parameter logistic function was first fitted to the cumulative emergence of each replication of each population at each constant temperature (Equation (1)).

$$Y = \frac{a}{1 + e^{\left(\frac{-(x-d_{50})}{b}\right)}} \tag{1}$$

where Y is the germination percentage, a is the maximum germination percentage, d_{50} is the time in days to achieve 50% of germination and b is the germination rate at d_{50}.

2.3.2 | Estimation of the optimum temperature (T_o)

Once d_{50} was defined, its inverse value ($1/d_{50}$) where represented in a figure and a three-parameter Lorentzian function was fitted, equally, to each replicate of each population (Equation (2)).

$$Y = \frac{a}{1 + \left(\frac{x-d_{50}}{b}\right)^2} \tag{2}$$

where Y is $1/d_{50}$ value at each temperature x, a is the maximum $1/d_{50}$ value, x_0 is the temperature at which the highest value of $1/d_{50}$ is obtained, and coincides with the centre of the peak and the optimum temperature at the same time; and parameter b is the mean width of the peak.

2.3.3 | T_b and T_c estimation

Once T_o was defined, suboptimal temperatures were used to obtain T_b and supraoptimal temperatures were used to obtain T_c, and regression lines were fitted, respectively, to each (Equation (2); Guillemin et al., 2013; Torra et al., 2016).

$$Y = ax + b \tag{3}$$

where Y is the $1/d_{50}$ value at each temperature x, a is the slope and b is a constant value. The point where the regression lines intercept the X-axis, estimated with the mathematical approach of the regression line, was considered as the T_b and T_c, respectively, for each population (Holt & Orcutt, 1996; Steinmaus et al., 2000; Wu et al., 2007), assuming there are no intra-population variations.

All statistical analyses were performed with JMP Pro 14 software (SAS Institute Inc., 2010, Cary, NC) and all linear and non-linear regression analysis were conducted with SigmaPlot 11.0 (Systat Software, San Jose, CA).

3 | RESULTS

At constant temperatures, the highest germination percentages for all populations were obtained between 15 and 25°C (Table 2, Test 1), being maximum at 20°C. Seeds from Seville population had significantly different germination response comparing to the rest of the populations. A decrease in the germination percentage was observed at much lower (10 and 5°C) or higher (30°C) constant temperatures (Table 2). Each population was affected similarly by the gradient of temperatures assessed, but the observed germination percentage was different depending on the biotype. Statistical differences were found between populations and incubation temperatures at all constant temperatures ($p < .001$; Test 1, Table 2). At constant 5°C, the population from Lleida showed >25% of germination, value significantly higher than those observed in the population from Badajoz (1.3%), Seville (2.9%) and Bahía Blanca (9.6%). At constant 10°C, the populations from Lleida and Bahía Blanca showed significantly higher germination percentages (82.1 and 86.3%, respectively) than the population from Seville (48.8%) and Badajoz (55.4%). At 20°C, the population from Seville showed the lowest germination percentage (81.3%) significantly different from the rest of the populations, with values between 99.2% for Bahía Blanca and 96.3% for Badajoz population. On the other hand, the population from Badajoz showed the highest germination percentage (25.0%) at constant 30°C, with values significantly different from the rest of the populations. Finally, no germination was observed at 35°C in any of the populations.

With respect to Test 2, the seed exposure to a constant T_{osc} and at different T_m significantly affected the germination percentage. Statistical differences were found between populations and incubation temperatures at all constant temperatures ($p < .001$) except for 20–30°C ($p < .397$). Higher germination percentages were observed at 20–30°C for populations from Lleida and Seville, with germination

TABLE 2 Total percentage of germination for the *Conyza bonariensis* populations at each experiment

	T (°C)	Lleida	Badajoz	Seville	Bahía Blanca	SED	LSD	d.f.
Test 1	5	26.7	1.3	2.9	9.6	4.93	9.81	31
	10	82.1	55.4	48.8	86.3	8.98	18.39	31
	15	92.5	86.3	74.6	97.1	6.48	13.27	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	25	93.3	90.0	77.9	99.2	3.35	6.87	31
	30	15.8	25.0	7.1	5.4	3.78	7.75	31
	35	0.0	0.0	0.0	0.0	–	–	–
	SED	5.80	5.44	5.09	3.78			
	LSD	11.60	10.92	10.23	7.58			
	d.f.	55	55	55	55			
Test 2	5–15	90.0	94.2	64.6	82.1	5.84	11.96	31
	10–20	92.5	83.3	58.3	89.2	5.90	12.09	31
	15–25	97.1	87.9	76.7	98.3	4.55	9.32	31
	20–30	98.3	93.3	95.0	94.6	2.99	6.12	31
	25–35	21.3	45.0	19.6	10.0	5.07	10.38	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	SED	2.98	4.77	6.49	3.94			
	LSD	6.01	9.63	13.10	7.96			
	d.f.	47	47	47	47			
Test 3	18–22	98.3	93.8	83.3	94.6	3.74	7.65	31
	16–24	97.1	90.8	71.7	94.6	5.10	10.45	31
	14–26	95.0	94.2	85.0	99.2	4.14	8.49	31
	12–28	99.1	93.3	79.6	100.0	4.09	8.38	31
	10–30	93.8	95.4	75.8	92.5	5.12	10.48	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	SED	2.76	3.79	6.45	3.11			
	LSD	5.57	7.64	13.01	6.28			
	d.f.	47	47	47	47			

Notes: Test 1, constant temperatures; Test 2, alternating temperatures (variable mean/constant oscillation); Test 3, alternating temperatures (constant mean/variable oscillation).
Abbreviations: d.f., degrees of freedom associated with LSDs and SEDs; LSD, least significant difference between two means at $p = .05$; SED, standard error of the difference between two means.

percentages higher than 93% for all of them, at 5–15°C for Badajoz and at 15–25°C for Bahía Blanca (Test 2, Table 2). At 10–20°C and at 5–15°C, the population from Seville showed lower germination percentages (58.3 and 64.6%, respectively) than the rest of the populations, which always showed values above 82%. At 15–25°C, the population from Bahía Blanca showed a 98.3%, significantly different to those observed in population from Seville (76.7%) and Badajoz (87.9%) but not with Lleida (97.1%). At 25–35°C, the population from Badajoz showed the highest germination percentage (45.0%), which was significantly higher than the populations from Lleida (21.3%), Seville (19.6%) and Bahía Blanca (10.0%).

In the case of Test 3, where a same T_m and different T_{osc} was assessed, no significant differences in germination percentage were observed inside each population ($p = .327$ for Lleida, $p = .780$ for Badajoz, $p = .334$ for Seville and $p = .09$ for Bahía Blanca), but there were differences between the different T_{osc} considered ($p < .001$; Test 3, Table 2). At all temperatures assayed, significantly lower germination percentages were observed between populations from Seville (with percentages between 71.7 and 85.0%) and the rest of the populations.

The effect of temperature did not only affect the final germination percentage, but also the germination timing and rate (Figure 1). The cumulate germination of all populations at all temperatures successfully fitted to log–logistic function, except for those temperatures at which germination was too low or null (Table 3). At the lowest and highest constant temperatures, higher values were estimated for parameter x_o, indicating a delay in germination (Table 3; Figure 1). This behaviour is similar in all the populations. In general, the germination rate, identified as parameter *b* (Table 3) was faster between 15 and 25°C, compared to at 5, 10 and 30°C, except for the population from Badajoz. Parameter b could not be significantly fitted ($p < .05$) for populations from Lleida and Bahía Blanca at 20 and 25°C, due to the fast germination rate, though the log–logistic function was significantly fitted (Table 3).

3.1 | Estimation of T_b, T_o and T_c

Lorentzian function was significantly adjusted to the $1/d_{50}$ values (Figure 2) for every population with high accuracy ($R^2 > .86$). The optimal temperature (T_o) for populations from Lleida and Seville was established at 21.7°C, whereas these values were higher for Bahía Blanca and Badajoz: 22.2 and 22.3°C, respectively (Table 4).

Regression lines were successfully fitted for the estimation of the T_b and T_c, with R^2 values ranging between .90 and .99 (Figure 3) in seven of the eight cases, and being $R^2 = .81$ for the T_c of the Badajoz population. The lowest T_b (4.9°C) was estimated for the population

TABLE 3 Values of parameters for the log-logistic models obtained from the cumulative germination data for each population assessed at every constant temperature

Population	*T* (°C)	*a*	*b*	x_0	*F*	*p*
Lleida	5	26.9957	1.2307	16.6264	3,702.9921	<.0001
	10	80.9581	0.7598	9.7044	7,866.2978	<.0001
	15	91.4461	0.6621	2.5887	366.9728	<.0001
	20	98.3114	0.0724*	1.7998*	502,481.464	<.0001
	25	93.0921	0.0911*	1.7858*	13,097.5736	<.0001
	30	15.4061	2.4003	4.7235	165.6618	<.0001
Badajoz	5	1.7422*	1.2499*	19.5611	135.2574	<.0001
	10	53.6136	0.9767	11.4118	1,659.4462	<.0001
	15	85.6338	1.2452	3.9909	264.9829	<.0001
	20	96.1187	0.1165*	1.8273	28,831.9755	<.0001
	25	89.1273	0.2082	1.8518	918.8929	<.0001
	30	24.2102	2.8306	4.6569	98.0223	<.0001
Seville	5	2.6267	0.2815*	17.0325	1,638.7769	<.0001
	10	48.6567	1.2155	12.6461	2,207.0097	<.0001
	15	74.5380	1.1501	5.0842	2,116.4081	<.0001
	20	80.2973	0.2188*	1.8141	463.9906	<.0001
	25	77.5177	0.4436	2.7171	2,614.6267	<.0001
	30	7.1728	2.9543	5.2191	143.8073	<.0001
Bahía Blanca	5	13.5797	2.0785	19.1641	1,058.6897	<.0001
	10	84.7909	0.8799	10.6539	4,965.3598	<.0001
	15	96.5026	0.8747	3.4091	459.5015	<.0001
	20	99.1229	0.1018*	1.8307	129,376.9258	<.0001
	25	98.9696	0.1173*	1.8312	19,924.4810	<.0001
	30	5.6035	2.4995	9.5902	415.4343	<.0001

*Parameters not fitted ($p < .05$) without incidence in the log–logistic function.

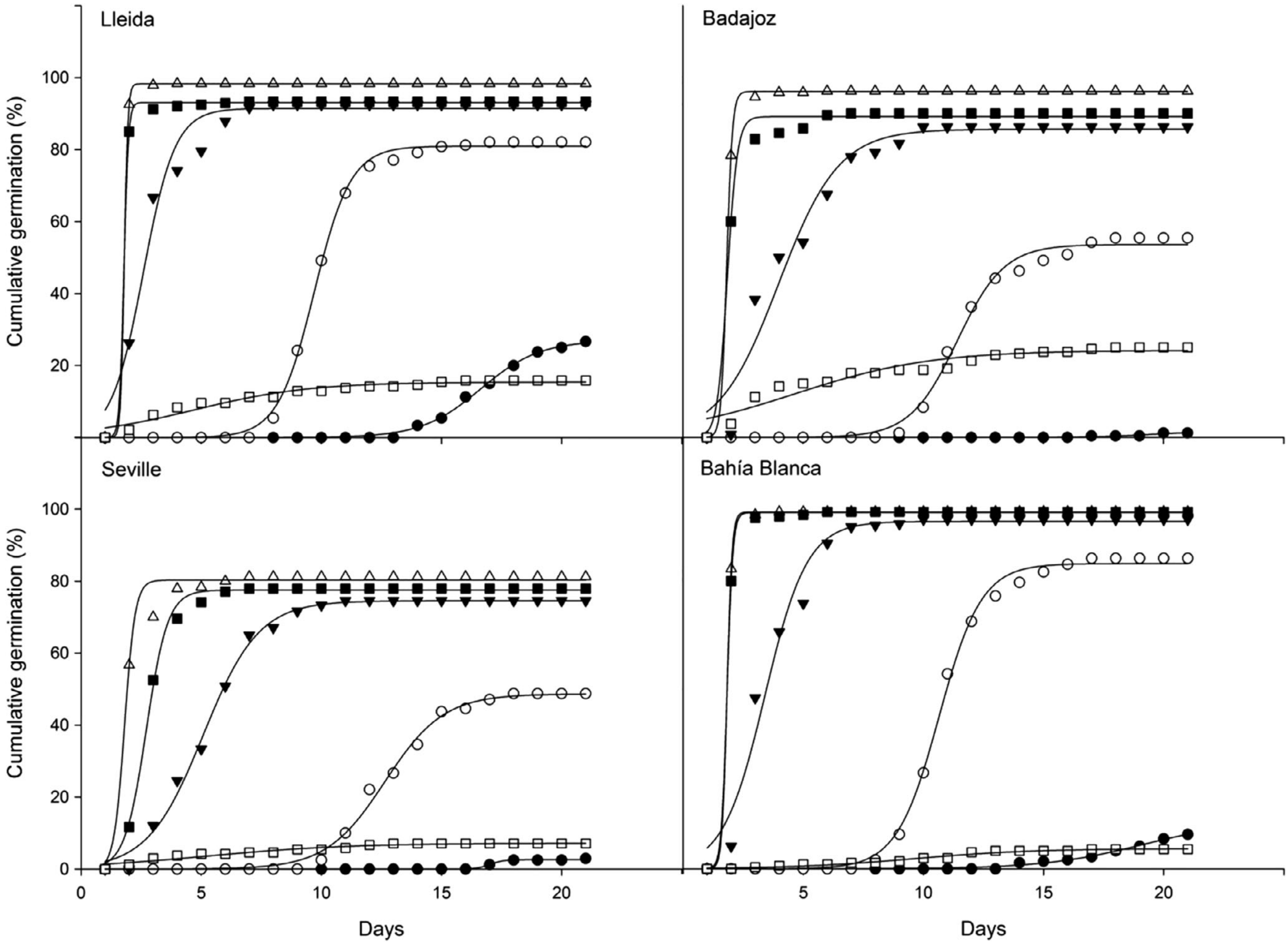

FIGURE 1 Cumulative germination percentage (%) of *Conyza bonariensis* at constant temperatures of 5°C (●), 10°C (○), 15°C (▼), 20°C (△), 25°C (■), 30°C (□) across the different populations

TABLE 4 Estimated base temperatures (T_b), optimal temperature (T_o) and ceiling temperatures (T_c), for each population of *Conyza bonariensis* assessed

	Lleida	Badajoz	Sevilla	Bahía Blanca	SED	LSD	d.f.
T_b	4.9	8.4	8.9	6.9	0.45	0.92	31
T_o	21.7	22.3	21.7	22.2	0.31	0.64	31
T_c	32.3	34.0	31.7	31.5	1.05	2.17	27

Note: Mean values are presented in °C.
Abbreviations: d.f., degrees of freedom associated with LSDs and SEDs; LSD, least significant difference between two means at p = .05; SED, standard error of the difference between two means.

from Lleida (Table 4), while the highest values (8.9 and 8.4°C) were obtained for populations from Seville and Badajoz, respectively. An intermediate T_b value (6.9°C) was observed in the Bahía Blanca population (Table 4). The lowest T_c value was also obtained from the Bahía Blanca population (31.5°C), followed by that from Seville (31.7°C) and Lleida (32.3°C). Finally, the highest value was observed in the Badajoz population (34.0°C). Statistical differences were found between populations (p < .001) for T_b but not for T_o and T_c (p < .246 and p < .103, respectively).

4 | DISCUSSION

All populations showed similar germination behaviour. Germination percentage was highest near the optimum temperature and there were significant differences in the final germination percentages, which appeared mainly at lower temperatures. These differences could be explained, in part, by the climate of the original localities, but also by a possible maternal effect. The environmental conditions under which the mother plant produced the seeds, and also the

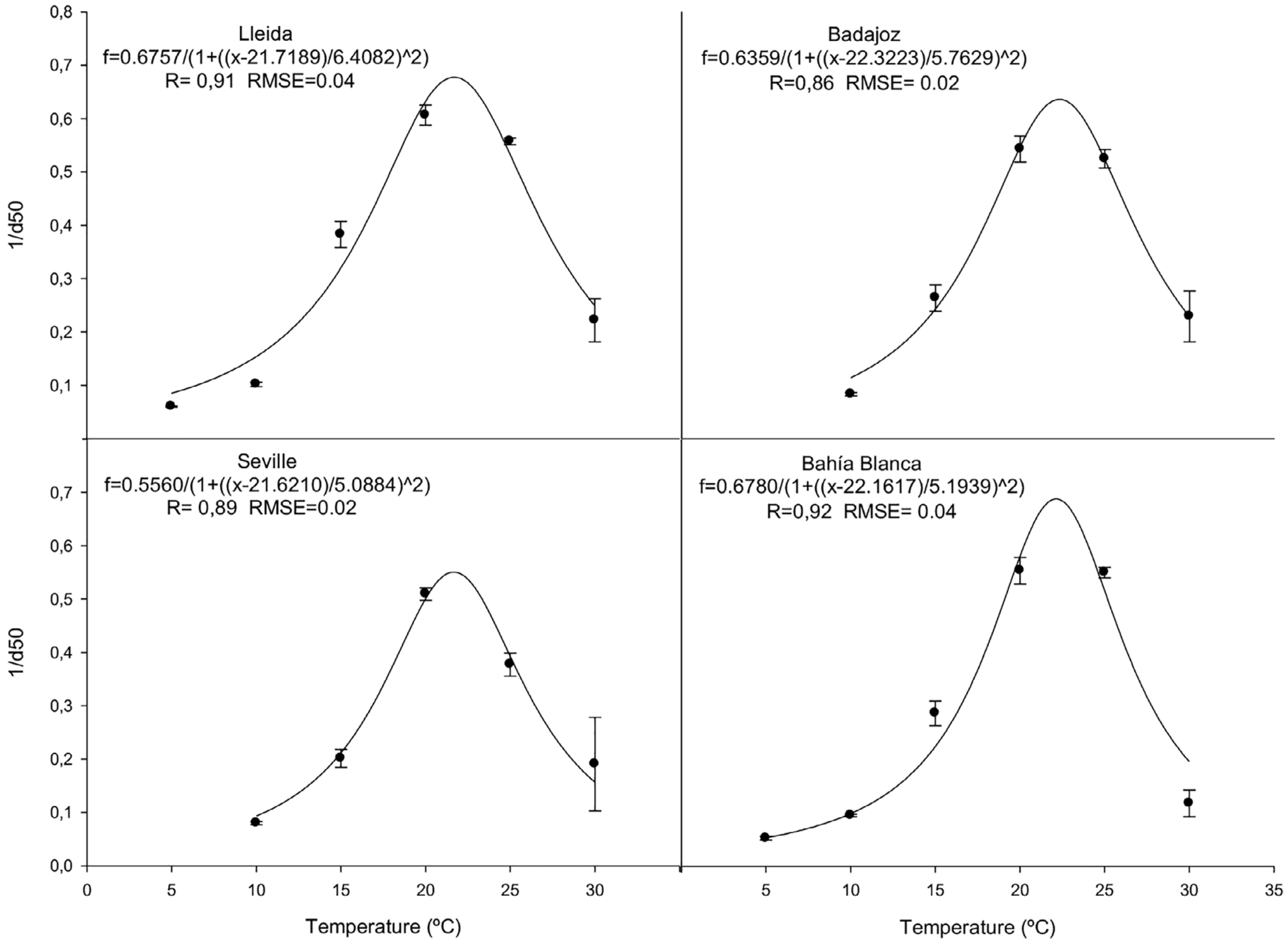

FIGURE 2 Lorentzian functions adjusted to the $1/d_{50}$ values obtained at each temperature by each population assessed. R^2 of the functions and RMSE are provided

position of the seed in the plant can impact seed germination. Likewise, water deficit, the age of the plant, the day length, the parental photo-thermal environment, light quality, altitude and temperature are known, among other factors, to affect germinability (and dormancy in some cases) in other species (Gutterman, 2000; Menegat, Milberg, Nilsson, Andersson, & Vico, 2018).

4.1 | Effect of temperature on germination

For all populations, the maximum germination percentage was reached near 20°C. The estimation of the optimal temperature (T_o; 21.7–22.3°C) allowed for little distinction between populations (Table 4). When the temperatures moved away from the optimal, the final germination percentage decreased. This decrease was faster for supra-optimal than for sub-optimal temperatures (Table 2, Test 1). These results are similar to those found by Hardegree (2006) for various gramineous species.

The alternating temperatures did not have a significant effect when the mean temperature was close to the optimum (20°C). Similar trend was observed by Ottavini, Pannacci, Onofri, Tei, and Jensen (2019) with *C. canadensis*, who did not observe significant differences between constant and alternating temperatures of 15, 20 and 25°C on average. Moving away from the optimal, the alternating temperatures (Table 2, Test 2) favoured the germination of the seeds compared to the corresponding constant mean temperature (Test 1, Table 2). For example, at 10°C, an oscillation of 10°C (5–15°C) increased the total germination percentage in Lleida, Badajoz and Seville, in between 8% and up to 39%. The Bahía Blanca population was apparently the only one not affected by the oscillation at such low temperature. Similarly, at constant temperatures of 30°C, germination percentage was, on average, 13.3%; whereas at oscillation, the germination percentage was 24% at 25–35°C. This increasing germination percentage is also observed by Vidal, Kalsing, Goulart, Lamego, and Christoffoleti (2007) when comparing constant 25°C to 20–30°C, which increased from less than 50% up to nearly 85%. Differences could be explained by alternating temperatures, which enhance germination in photoblastic species (Roeder, Ferraz, & Hölscher, 2013) such as *C. bonariensis*. Moreover, temperature changes are more pronounced at the soil surface where *C. bonariensis*, a very small-seeded species, germinates better. Furthermore, the germination of this

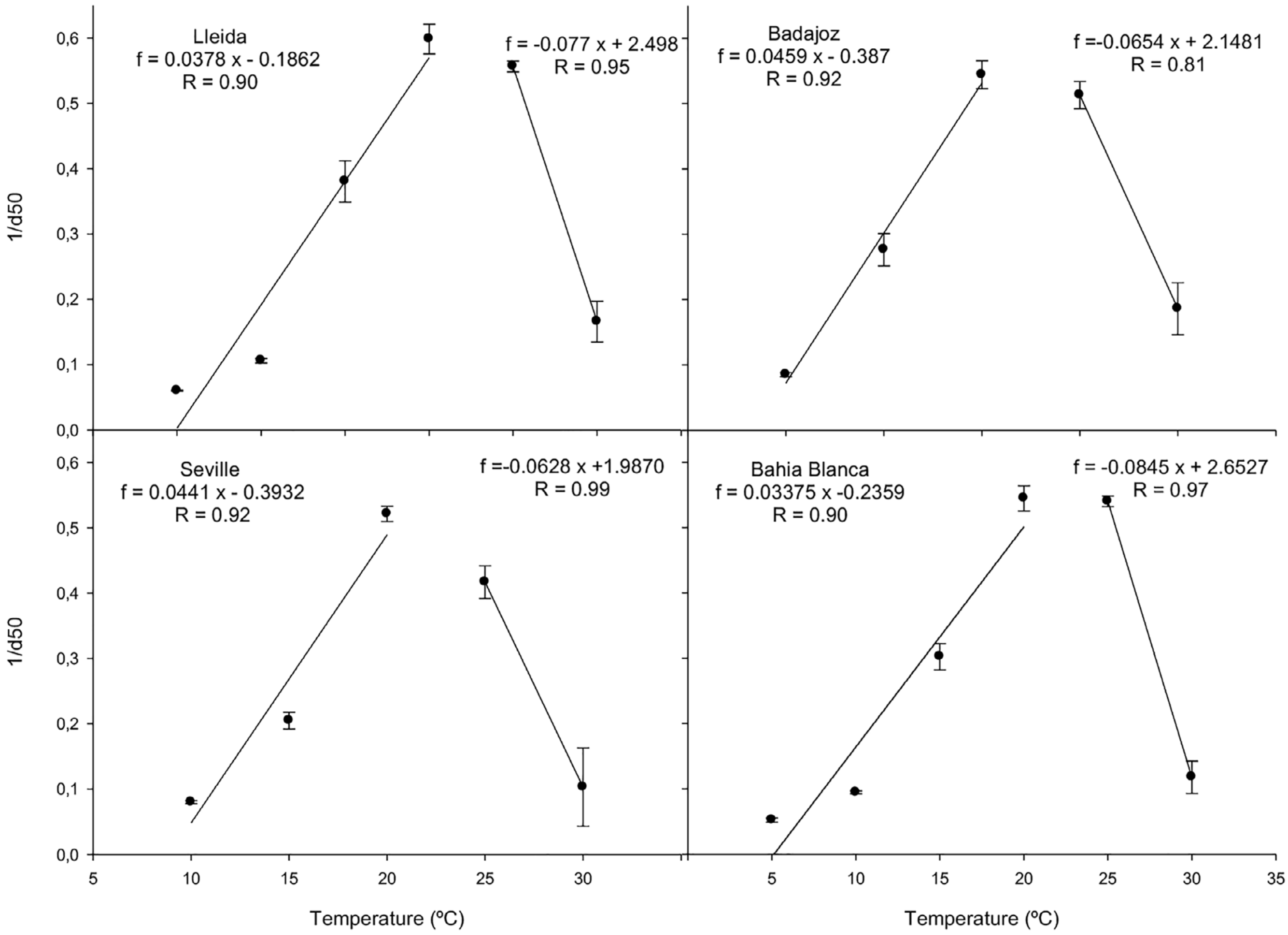

FIGURE 3 Estimation of the base temperatures (T_b) on the left and ceiling temperatures (T_c) on the right for each of the *Conyza bonariensis* populations. Regression lines are presented, together with the lineal functions for each T_b and T_c, followed by the R^2

species is null at depths deeper than 2 cm (Wu et al., 2007), similar to *C. canadensis* which germination is reduced to 0% between 0.5 and 1 cm (Ottavini et al., 2019).

In the intervals with constant mean temperatures and variable oscillation (Table 2, Test 3), there are no differences between the intervals and with the constant 20°C, with similar percentage germination with the intervals with variable mean and constant oscillation when the mean is close to 20°C.

The differences observed between germination percentages at constant and alternating temperatures could correspond to depth- and gap-sensing mechanism: temperature oscillations are more pronounced at or near to the soil surface and the amplitude of these fluctuations decreased with burial depth (Ren, Tao, & Liu, 2002). This could be an adaptation that staggers the germination with the changing temperatures throughout the seasons (Vidal et al., 2007).

4.2 | Effect of the origin of the population on germination

Differences in germination percentages are accentuated between populations (Table 2). The population from Lleida, which is a comparatively colder location (Table 1), showed more germination (26.7%) at the lowest temperature assayed (5°C). The populations from warmer climates had lower germination percentage at this temperature (1.3 and 2.9% for Badajoz and Seville, respectively). These differences are not so clear when comparing the populations at 30°C, wherein germination percentages from Badajoz and Seville populations showed statistical differences of 25.0 and 7.1%, respectively.

Under the 10°C oscillation treatment (Table 2, Test 2), excluding the higher one (25/35°C), there were a high germination percentages and significant differences between the intervals across the populations. Similarly, there were high germination percentages for seeds subjected to temperature oscillations that were 20°C on average (Table 2, Test 3).

Higher germination percentages with 10°C oscillation occurred between 15/25°C and 20/30°C in all populations, except for that of Badajoz which had the highest germination percentages at 5/15°C. Except for Seville population, these results are not in accordance with those from Travlos and Chachalis (2013), who found differences between 15/25°C and 20/30°C in populations of *C. bonariensis* from Greece. On the other hand, the lack of differences between 10/20°C and 15/25°C intervals in populations from Lleida and Badajoz agrees with other authors finding that there is less variation for these temperature ranges in climatically closer biotypes. Despite this, the Spanish populations from Lleida and Badajoz seem to be more adapted to colder winters (exhibiting more winter–summer oscillation) than those from Greece, as at 5/15°C the Spanish ones obtained over 90% germination, while the Greek ones did not exceed 35%.

Our results also agree with those from Karlsson and Milberg (2007), for populations from Ethiopia, Mexico and Morocco, for the 10/20, 15/25 and 20/30°C intervals, but not for the 5/15°C, where Lleida, Badajoz and Bahía Blanca populations obtained higher germination percentages (82.1–94.6%) than in previous studies (below 75%). The low germination percentages obtained by Karlsson and Milberg (2007) at 5/15°C could be explained by the local climatic conditions of these populations, which are classified as Tropical pluviseasonal (Mexico), Tropical xeric (Ethiopia) and Mediterranean xeric-oceanic (Morocco; Rivas-Martinez & Rivas-Saenz, 1996). Population differences could be due to the adaptation to the climatic characteristics of each original site where the seeds were produced (Clements & DiTommaso, 2011), as reported for *C. canadensis* (Tozzi et al., 2014).

4.3 | Germination patterns and threshold values

The germination patterns of all population at all constant temperatures were, in general, successfully fitted to a log-logistic sigmoidal function (Table 3, Figure 1). The lack of this adjustment in some cases (Table 3) could be partially explained by the exceedingly high or low germination rates of the populations: 20 and 25°C for Lleida and Bahía Blanca and 5 and 20°C for Badajoz and Seville.

The calculation of the x_0 with the log–logistic model allowed the estimation of the threshold values of T_b and T_c (Figure 3), while T_o was estimated with the Lorentzian model applied to the total germination percentages (Figure 2). Our results for the population from Lleida agree with Wu et al. (2007), which estimated that *C. bonariensis* can germinate between 4.2 and 30°C, even if the T_o is 20°C. The T_b of one of our populations (Lleida) is close to that from Wu et al. (2007), and the values are in accordance with the germination response explained above: T_b in Lleida shows the lowest value (4.9°C) followed by Bahía Blanca (6.9°C), which agrees with their local climatic origin. In accordance with this, the T_b in Seville (8.9°C) and Badajoz (8.4°C) are higher than in Lleida and Bahía Blanca, and not considerably different from that estimated by Zambrano-Navea et al. (2013) (10.6°C). The differences in the T_b from Lleida and Bahía Blanca (4.9 and 6.9°C) compared to the T_b obtained by Zambrano-Navea et al. (2013) can be explained by the variations in the experimental design, as these authors used constant 15°C as the lowest temperature, while we also experimented with 10 and 5°C, which obtained 48.8 and 2.9% germination, respectively. The idea that *C. bonariensis* is a summer weed could have led to the thought that its T_b was similar to other summer weeds, such as *Amaranthus retroflexus* which is estimated to be between 10.0 and 12.9°C (Loddo, Ghaderi-Far, Rastegar, & Masin, 2018), or *C. canadensis*, which is between 8 and 14°C, depending on the population (Tozzi et al., 2014).

The estimated ceiling temperature (T_c) varied from 31.5 to 34.0°C, which agrees with the lack of germinated seeds at a constant 35°C in any population. These results also agree, in part, with those from Yamashita and Guimaraes (2011), who only obtained 6% germination at a constant 35°C and 1% germination at 40°C. Similar to the tendency in the other threshold values, the lowest T_o was obtained in the population from Lleida, but also in Seville (21.7°C), while the highest one was obtained in the population from Badajoz (22.3°C). These T_o values, which could, in general, be established at about 22°C (±0.3°C), differ from that selected from the literature (Wu et al., 2007) to set the Test 2 and Test 3 of the experiment, and could be considered for future experiments.

4.4 | Implications for developing emergence models

The emergence model for *C. bonariensis* from Zambrano-Navea et al. (2013) has been proven to be valuable in several sites. This model was developed and validated with populations from the South of Spain, and has less relevance for other climatic biotypes and regions since the germination behaviour and the threshold values of other populations are different. The genetic variation of the *Conyza* species (Ren, Li, & Ding, 2010) could explain, in part, these results. Even if *C. bonariensis* has the ability to spread and disperse its seeds long distances (Savage et al., 2014), which could diminish the variations between populations, these differences are still important according to the results of the present and past work (Karlsson & Milberg, 2007). In addition to genetic origin, the maternal effect is another factor which could have enhanced differences between local populations.

In our study, there were four degrees (°C) of difference in T_b between the seeds from different origins, thereby impeding the development of a common model. In order to develop a model that could be widely applied, the next step is to test differences in the base parameters and germination behaviour of populations coming from different geographical sites, but belonging to the same climatic biotype. If there are not any differences between them, a more precise model could be developed or the current one created by Zambrano-Navea et al. (2013) could be readjusted to the populations of a certain climatic area.

5 | CONCLUSION

The germination percentage of *C. bonariensis* was higher when close to the optimal temperature obtained (22°C), both for constant and alternating temperatures. In the intervals with same T_{osc} and different T_m, some obtained higher germination percentages than at constant temperature. In the intervals with different T_{osc} but the same T_m, there are high germination percentages for all temperatures, without significant differences from the constant temperature. The different populations responded to the tests according to the apparent influence of their climatic origin. The biotype adapted to the coldest winter site (Lleida) had more germinated seeds at lower temperatures, while the biotypes adapted to warmer climatic sites (Badajoz and Seville) were more acutely affected by a temperature decrease. The differences in the threshold values for the cardinal parameters, as well as the in the germination behaviour of the different climatic biotypes prevents us from developing a common germination/emergence model. Thus, there is further need for the investigation to achieve the goal of obtaining accurate models for each climatic region.

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CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest.

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REFERENCES

Bajwa, A. A., Sadia, S., Ali, H. H., Jabran, K., Peerzada, A. M., & Chauhan, B. S. (2016). Biology and management of two important *Conyza* weeds: a global review. *Environmental Science and Pollution Research*, *23*, 24694–24710.

Brown, S. M., & Whitwell, T. (1988). Influence of tillage on horseweed (*Conyza canadensis*). *Weed Technology*, *2*, 269–270.

Clements, D. R., & DiTommaso, A. (2011). Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? *Weed Research*, *51*, 227–240.

Dauer, J. T., Mortensen, D. A., & Vangessel, M. J. (2007). Temporal and spatial dynamics of long-distance *Conyza canadensis* seed dispersal. *Journal of Applied Ecology*, *44*, 105–114.

Davis, V. M., & Johnson, W. G. (2008). Glyphosate-resistant horseweed (*Conyza canadensis*) emergence, survival, and fecundity in no-till soybean. *Weed Science*, *56*, 231–236.

Forcella, F., Benech-Arnold, R. L., Sánchez, R., & Ghersa, C. M. (2000). Modeling seedling emergence. *Field Crops Research*, *67*, 123–139.

García, A., Recasens, J., Forcella, F., Torra, J., & Royo-Esnal, A. (2013). Hydrothermal Emergence Model for Ripgut Brome (*Bromus diandrus*). *Weed Science*, *61*, 146–153.

Guillemin, J. P., Gardarin, A., Granger, S., Reibel, C., Munier-Jolain, N., & Colbach, N. (2013). Assessing potential germination period of weeds with base temperatures and base water potentials. *Weed Research*, *53*, 76–87.

Gutterman, Y. (2000). Maternal Effects on Seeds During Development. In M. Fenner (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities* (pp. 59–84). New York, NY: CABI.

Hardegree, S. P. (2006). Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Annals of Botany*, *97*, 1115–1125.

Heap, I.M. (2019). *International Survey of Herbicide Resistant Weeds*. Retrieved from: http://www.weedscience.org.

Holm, L., Doll, J., Holm, E., Pancho, J., & Herberger, J. (1997). *World Weeds, Natural Histories and Distribution* (pp. 226–235). New York, NY: Wiley.

Holt, J. S., & Orcutt, D. R. (1996). Temperature thresholds for bud sprouting in perennial weeds and seed germination in cotton. *Weed Science*, *44*, 523–533.

Karlsson, L. M., & Milberg, P. (2007). Comparing after-ripening response and germination requirements of *Conyza canadensis* and *Conyza bonariensis* (Asteraceae) through logistic functions. *Weed Research*, *47*, 433–441.

Kempen, H. M., & Graf, J. (1981). Weed seed production. *Proceedings of the Western Society of Weed Science*, *34*, 78–81.

Loddo, D., Ghaderi-Far, F., Rastegar, Z., & Masin, R. (2018). Base temperatures for germination of selected weed species in Iran. *Plant Protection Science*, *54*, 60–66.

Menegat, A., Milberg, P., Nilsson, A., Andersson, L., & Vico, G. (2018). Soil water potential and temperature sum during reproductive growth control seed dormancy in *Alopecurus myosuroides* Huds. *Ecology and Evolution*, *8*, 7186–7194.

Ren, M.-X., Li, X.-Q., & Ding, J.-Q. (2010). Genetic variation and spread pattern of invasive *Conyza sumatrensis* around China's Three Gorges Dam. *Acta Oecologica*, *36*, 599–603.

Ottavini, D., Pannacci, E., Onofri, A., Tei, F., & Jensen, P. K. (2019). Effects of light, temperature, and soil depth on the germination and emergence of *Conyza canadensis* (L.) Cronq. *Agronomy*, *9*, 533.

Ren, J., Tao, L., & Liu, X. M. (2002). Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. *Journal of Arid Environments*, *51*, 603–611.

Rivas-Martinez, S., & Rivas-Saenz, S. (1996–2018). *Worldwide Bioclimatic Classification System*. Spain: Phytosociological Research Centre.

Roeder, M., Ferraz, I. D. K., & Hölscher, D. (2013). Seed and Germination Characteristics of 20 Amazonian Liana Species. *Plants*, *2*, 1–15.

Royo-Esnal, A., García, A., Torra, J., Forcella, F., & Recasens, J. (2015). Describing *Polygonum aviculare* emergence in different tillage systems. *Weed Research*, *55*, 387–395.

Savage, D., Borger, C. P., & Renton, M. (2014). Orientation and speed of wind gusts causing abscission of wind-dispersed seeds influences dispersal distance. *Functional Ecology*, *28*, 973–981.

Shrestha, A., Hembree, K., & Wright, S. (2008). *Biology and Management of Horseweed and Hairy Fleabane in California. Publication No. 8314*. Fresno, CA: University of California, Division of Agricultural and Natural Resources.

Somervaille, A., & McLennan, B. (2003). *The 2nd Fallow Weed Management Guide*. Toowoomba, Australia: Conservation Farmers.

Steinmaus, S. J., Prather, T. S., & Holt, J. S. (2000). Estimation of base temperatures for nine weed species. *Journal of Experimental Botany*, *51*, 275–286.

Thebaud, C., & Abbott, R. (1995). Characterization of invasive *Conyza* Species (Asteraceae) in Europe: quantitative trait and isozyme analysis. *American Journal of Botany*, *82*, 360–368.

Torra, J., Royo-Esnal, A., & Recasens, J. (2016). Temperature and light requirements for germination and emergence of three arable papaveraceae species. *Weed Science*, *64*, 248–260.

Tozzi, E., Beckie, H., Weiss, R., Gonzalez-Andujar, J. L., Storkey, J., Cici, S. Z. H., & Acker, R. C. (2014). Seed germination response to temperature for a range of international populations of *Conyza canadensis*. *Weed Research*, *54*, 178–185.

Travlos, I. S., & Chachalis, D. (2013). Relative competitiveness of glyphosate-resistant and glyphosate-susceptible populations of hairy fleabane, *Conyza bonariensis*. *Journal of Pest Science*, *86*, 345–351.

Trezzi, M. M., Balbinot Jr, A. A., Benin, G., Debastiani, F., Patel, F., & Miotto Jr, E. (2013). Competitive ability of soybean cultivars with horseweed (*Conyza bonariensis*). *Planta Daninha*, *31*, 543–550.

Trezzi, M. M., Vidal, R. A., Patel, F., Miotto, E., Debastiani, F., Balbinot, A. A., & Mosquen, R. (2015). Impact of *Conyza bonariensis* density and establishment period on soyabean grain yield, yield components and economic threshold. *Weed Research*, *55*, 34–41.

Urbano, J. M., Borrego, A., Torres, V., Leon, J. M., Jimenez, C., Dinelli, G., & Barnes, J. (2007). Glyphosate-resistant hairy fleabane (*Conyza bonariensis*) in Spain. *Weed Technology*, *21*, 396–401.

Vidal, R. A., Kalsing, A., Goulart, I., Lamego, F., & Christoffoleti, P. (2007). Impact of temperature, light and seed depth on emergence and germination of *Conyza bonariensis* e *Conyza canadensis* resistant to glyphosate. *Planta Daninha*, *25*, 309–315.

Wicks, G. A., Felton, W. L., Murison, R. D., & Martin, R. J. (2000). Changes in fallow weed species in continuous wheat in northern New SouthWales, 1981–90. *Animal Production Science*, *40*, 831–842.

Wu, H., Walker, S., Rolling, M. J., Yuen, D. K., Robinson, G., & Werth, J. (2007). Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* (L.) Cronquist). *Weed Biology and Management*, *7*, 192–199.

Yamashita, O., & Guimaraes, S. (2011). Germination of *Conyza canadensis* and *Conyza bonariensis* seeds under different conditions of temperature and light. *Planta Daninha*, *29*, 333–342.

Zambrano-Navea, C., Bastida, F., & Gonzalez-Andujar, J. L. (2013). A hydrothermal seedling emergence model for *Conyza bonariensis*. *Weed Research*, *53*, 213–220.

Zambrano-Navea, C., Bastida, F., & Gonzalez-Andujar, J. L. (2016). A cohort-based stochastic model of the population dynamic and long-term management of *Conyza bonariensis* in fruiting tree crops. *Crop Protection*, *80*, 15–20.

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agronomy

Article

Different Ground Vegetation Cover Management Systems to Manage *Cynodon dactylon* in an Irrigated Vineyard

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Abstract: Ground cover management in vineyards in Spain is focused on minimizing soil erosion and compaction. Such practices have influenced the weed community structure in the inter-rows, contributing to the spread of the high noxious weed *Cynodon dactylon* (L.) Pers. This fact highlights the need for further investigation of the interaction between ground cover practices and weed control techniques. In this study, the effect of four different ground cover managements (M) in the inter-rows on *C. dactylon* population dynamics (changes in coverage and frequency) was assessed over three seasons (2015–2017): (M1) a no-till spontaneous vegetation ground cover managed by shredding; (M2) a no-till spontaneous vegetation ground cover managed by shredding plus herbicide application, (M3) tilled soil and spontaneous vegetation growing; and (M4) tilled soil and a barley cover crop seeded (*Hordeum vulgare* L.). *Cynodon dactylon* and the other weeds responded differently to the various weed control methods. After three seasons, the barley cover crop was the most efficient management system to control *C. dactylon* and other weeds. Final soil cover in barley cover crop and tilled soil with spontaneous vegetation were 0.5% and 1.1%, respectively, compared to 3.7% and 7.7% obtained by spontaneous vegetation shredded with and without herbicide application, respectively. In addition, total weed frequency varied from 9.7% for barley cover crop to 45.8% for spontaneous vegetation only shredded. Weed community composition changed due to the pressure exerted by each management and the adaptive strategy of the different species. This study highlights the importance of knowledge of how vegetation management influences weed flora to improve the sustainability of wine grape production systems.

Keywords: integrated weed management; Cabernet Sauvignon; *Vitis vinifera*; barley

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1. Introduction

Grapes are one of the most important and profitable crops in the world. Wine production has increased 17% from 2017 to 2018, with 292 million hL (global wine production in 2018), and the global vineyard cover is about 7.4 million hectares in 2018 [1]. However, grape production can be reduced due to the presence of weeds, which negatively affect the productivity of crops [2] by competing for water and nutrients. Weed control in vineyards has been usually carried out by tillage or herbicide applications; however, the establishment of ground covers has gained interest and has been implemented for different reasons [3,4]. In general, vineyard soil management involves different procedures focused on organic matter improvement, water availability, biodiversity enhancement, reduction of soil erosion, vine vigor, or weed control [5]. However, tillage and herbicide applications have been changing over time for different reasons. On one hand, leaving the soil surface without any ground cover increases the erosion risk, with vineyards being one of the most affected crops [6,7]. On the other hand, the use of herbicides as the main tool for the control of weeds not only contaminates the environment, but it can also negatively affect vines by reducing root mycorrhization or altering nutrient composition in grapevine roots, leaves, or grape juice [8]. The reiterative use of herbicide increases the risk of evolving weed herbicide resistance, many of which appear in vineyards, such as *C. bonariensis*, *Lolium rigidum*, or *Amaranthus retroflexus*, among others [9]. In addition, environmentally friendly agronomic techniques are desired because wine consumers and producers are more aware of the environmental impacts of wine and consumers have a positive image of organic cultivation methods [10,11].

Ground covers are alternative soil managements to mechanical cultivation or herbicide applications. Spontaneous ground covers or sowed cover crops are sustainable floor management techniques that offer a variety of agronomic and environmental advantages, i.e., reduction of soil erosion and water runoff or increasing of organic matter, nutrient availability, or benefiting soil biota [12–14]. Moreover, weed management practices that promote higher plant diversity and density have shown to favor arthropod weed seed predators, promoting beneficial entomofauna and contributing to weed growth suppression [15–18]. Cover crops can compete with weeds for resources, but they can also release allelochemicals that interfere with their growth [19,20], which can be implemented to control difficult to manage weed species, which is the case of *Cynodon dactylon* L. (Pers).

Cynodon dactylon is a stoloniferous and rhizomatous perennial grass considered as one of the world's worst weeds [21]. Its water stress tolerance makes it a very efficient species in water and nutrient use, and its vegetative structures contribute to the colonization of new areas, especially when soil is disturbed [22–24]. The base temperature (in soil) for bud sprouting is 10 °C [25], below which the weed becomes dormant and control measures are less effective. *Cynodon dactylon* (variety "princess 77") had been reported not to be controlled after a single glyphosate (N-(phosphonomethyl) glycine) application (36% at 4 L ha^{-1}), although different phytotoxic effects were observed [26], but different applications can reduce weed biomass and groundcover in more than 99% during the warm season with glyphosate (48.7% at 9 L ha ha^{-1}) [27]. Tillage can be performed to control *C. dactylon*, but this tool could be ineffective because, besides possibly requiring several interventions, it contributes to the fragmentation and dispersal of its rhizomes and stolons, and from them, new plants can be formed [28,29]. On the contrary, shading can reduce *C. dactylon* infestations due to the lack of tolerance to the shade of this weed [23,25].

When the establishment and management of the ground vegetation cover are focused on *C. dactylon* control, it should bring a dense canopy to cover this shade-intolerant species. In addition, because the ground cover management can influence weed flora composition [15], it is essential to select the most appropriate management (type of cover and the combination with other tools such as herbicide applications or tillage) to avoid the proliferation of other problematic weeds in vineyards, such as *C. bonariensis* or *Bassia scoparia* [30].

Due to the different efficacy of tillage or herbicide applications over *C. dactylon*, its integration with a ground cover could improve the control of this noxious weed. Based on the hypothesis that the presence of a vegetation cover exerts a competitive effect against *C. dactylon*, the objective of this work is to study the effect of such cover, either natural (spontaneous) or implemented (barley, as a representative cover crop), in combination with tools as shredding, tillage, and herbicide applications. Since some weeds can benefit from particular control methods against a specific weed, a second objective was considered to study the effect of the different managements on the weed community composition.

2. Materials and Methods

2.1. Study Area

The experiment was conducted in a commercial wine grape vineyard in Raimat (Lleida, NE Spain) from 2015 to 2017. The climate is semi-arid Mediterranean with an annual rainfall of 342 mm and a mean annual temperature of 15 °C [31]. The vineyard was established in May 1988 with Cabernet Sauvignon (*Vitis vinifera* L.). Rows were separated by 3 m and vine spacing of 1.5 m. Vines were trained as bilateral cordons and were drip irrigation.

The traditional soil management in the farm consists of shredding the inter-rows spontaneous flora 2–3 times per season and 4–5 herbicide intra-row applications across the season. The applied herbicides were glyphosate (36% at 3.5 L ha^{-1}), twice in winter and once in summer, plus a mixture of glyphosate (36% at 3.5 L ha^{-1}) and oxyfluorfen (24% at 3 L ha^{-1}), once in spring and once in summer. Herbicide applications were carried out with a tractor-mounted crop-sprayer with two fan-type nozzles. Initially, there was an important infestation, both intra- and inter-row of *C. dactylon*, and that was estimated between 4% and 10% at the beginning of the experiment.

2.2. Experimental Design

A completely randomized design was carried out, with four different soil management systems (treatments) placed in different inter-rows of 100 m long by 2 m wide (Table 1) with four replications.

Table 1. Timing of agricultural practices in the inter-rows for each management.

	2015			**2016**			**2017**		
	SPR	**SUM**	**AUT**	**SPR**	**SUM**	**AUT**	**SPR**	**SUM**	**AUT**
M1	Sd	Sd	Sd	Sd	Sd	Sd	Sd	Sd	Sd
M2	Sd + H	Sd	Sd + H	Sd + H	Sd	Sd + H	Sd + H	Sd	Sd + H
M3	Sd + Sb + T	Sd	Sd + T		Sd	Sd + T		Sd	Sd
M4	Sd + Sb + T	Sd	Sd + T + So		Sd	Sd + T + So		Sd	Sd

Abbreviations: SPR: Spring, SUM: Summer, AUT: Autumn, Sd: shredding, H: Herbicide, Sb: Subsoiling, T: Tilling, So: Sowing, M1: a no-till spontaneous vegetation ground cover managed by shredding, M2: a no-till spontaneous vegetation ground cover managed by shredding plus herbicide application, M3: tilled soil and spontaneous vegetation growing, and M4: tilled soil and a barley cover crop seeded (*Hordeum vulgare* L.).

- Management 1 (M1): a no-till spontaneous vegetation ground cover managed by shredding. The cover was shredded three times per season (May, July, and September). Shredding was performed when the spontaneous vegetation reached a height that could interfere with the vine growth. The exact shredding dates varied as the accumulated biomass of this spontaneous vegetation varied with the climatic conditions each season.
- Management 2 (M2): a no-till spontaneous vegetation ground cover managed by shredding plus regular herbicide application focused on *C. dactylon* (glyphosate 36% at 3.5 L ha^{-1}). Each season the ground cover was shredded three times as in M1, and two herbicide applications were performed, one in May, when the weed was actively growing and other in November, when the plant stopped its development after the mean temperature dropped under 10 °C. Applications were focused in the areas where *C. dactylon* was present. Herbicide was applied with a tractor-mounted crop-sprayer with five low drift nozzles (HARDI LD–110, ILEMO HARDI S.A.U., Lleida, Spain) when the weed was actively growing and more susceptible to the treatments.
- Management 3 (M3): inter-row tilling. In April 2015, the ground cover was shredded and the soil was sub-soiled and tilled. The spontaneous cover was shredded in July and October and tilled in October. In 2016, the cover was shredded twice (June and October) and tilled once (October). In 2017, the ground cover was also shredded twice (June and October) but not tilled, because the trial ended. Tillage was performed with a chisel plow at a soil depth of 20 cm.

- Management 4 (M4): inter-row tilling and barley cover crop (*Hordeum vulgare* L.) sowed in autumn. Management activities were similar to M3, but barley var. Meseta was sowed as cover crop at a rate of 150 kg ha^{-1} in November 2015 and 2016. This variety was selected for its good coverage, with low height and high tillering capacity [32]. Barley was shredded when its life cycle finished in June 2016 and 2017, providing a mulch of straw. Seed-bed preparation was performed with a chisel plow at a soil depth of 20 cm, and barley was sown with a vineyard seeder. No fertilization was applied.

2.3. Weed Sampling

Cynodon dactylon coverage was monitored in sixteen 2 × 10 m^2 plots (four for each management) situated in the center of the inter-rows, covering the area occupied by the ground cover. To carry out the surveys, each plot was subdivided into 80 subplots of 0.5 × 0.5 m. The weed coverage of each subplot and weed frequency were recorded. For the weed coverage, the mean of the 80 subplots was considered for each repetition, while for the frequency, it was just the percentage of subplots with the presence of *C. dactylon*. Samplings were performed in March (previous to the management implementation) and July 2015, February, May, and September 2016, and February, June, and September 2017. In order to understand the effect of each management as a whole, the final change on the percentage of coverage and frequencies were analyzed. For each management, these final changes are the percentage of increase or decrease observed at the end of the experiment (September 2017) for the corresponding variable considering their respective initial value (March 2015).

With the aim to reflect the spatial and temporal dynamics of *C. dactylon* in the experiment, one plot of each ground management was represented using the graph builder of JMP Pro 14 software with the option "contour: shows regions of data density" (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, USA. SAS Institute, Inc.).

At the end of the experiment, weed surveys were performed in the plots in April of 2017.

2.4. Statistical Analyses

Cynodon dactylon percentages of coverage and frequencies between managements and sampling dates, and their final change, were compared by several one-way ANOVAs and Tukey's honestly significant difference (HSD) ($p < 0.05$). Since field operations (till versus no till, herbicide versus no herbicide, and so on) affected *C. dactylon* coverage and frequency, it was decided to compare these variables separately for each management by date, and between managements for each date, discarding a two-way ANOVA. The original data were log or square-root transformed if needed to achieve normality and homoscedasticity. In the case of heteroscedasticity, data were subjected to the Kruskal–Wallis H test and post-hoc Dunn's test at $p \leq 0.05$. Analyses and graphs were performed with JMP Pro 14 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, USA. SAS Institute, Inc.) and SigmaPlot 11.0 (Systat Software, San Jose, CA, USA). In order to evaluate differences in weed species composition among the four management systems, based on the gradient of the variability in the species-plot response data (2.8 SD units), which showed a linear method to be the best option, a redundancy analysis (RDA) was performed. The response data were first log-transformed and centered by species, and a Monte Carlo permutation test was conducted in all constrained axes In addition, as a supplement of the RDA analysis, an analysis of the diagnostic species for each management system was performed, using the phi (ϕ) coefficient of association, which is independent of the number of samples, and it is little affected by the relative size of the sample unit [33]. Both analyses were performed with CANOCO 5.0 [34].

3. Results

3.1. Climatic Conditions

Average monthly temperatures were similar during the period when *C. dactylon* was actively growing (temperatures above 10 °C), but precipitations differed (Figure 1). Temperatures averaged 12–14 °C between March and May (spring); 23–24 °C between June and August (summer); 14–15 °C between September and November (autumn); and 5–7 °C between December and February (winter). Total spring precipitation was lower in 2015 (40 mm) than 2016 and 2017 (133 and 149 mm, respectively). During summer, 2015 presented the highest precipitation (90 mm) followed by 2017 (78 mm), and being lowest in 2016 (12 mm). In autumn, 87 mm were registered in 2015 and 107 mm was registered in 2016. It must be pointed out that autumn rains were concentrated in November, falling 64.5 mm and 72.0 mm, respectively in 2015 and in 2016. Winter precipitation in 2015–2016 doubled that from 2016–2017 (92 mm versus 46 mm) (Figure 1).

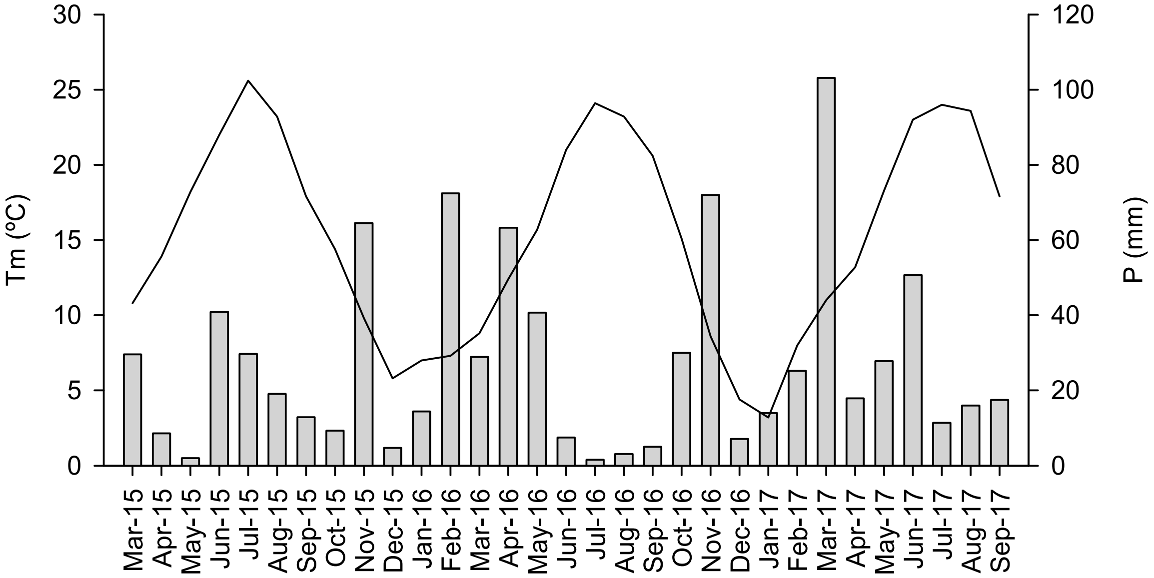

Figure 1. Climatic conditions for the experiment period (March 2015 to September 2017). The gray bars show total precipitation (P), and the black line indicates the mean monthly temperature (Tm) in each month [31].

3.2. Cynodon Dactylon Response to Management

Infestation levels of *C. dactylon* varied among managements and sampling dates, both in percentages of soil coverage and frequency (Tables 2 and 3; Figure 2).

Table 2. Soil covered (%) by *C. dactylon* in each management across the different sampling dates. Mean values of soil covered ± standard errors of the mean. Manag., management; Mar., March; Jun., June; Jul., July; Feb., February; Sep., September; 15, 2015; 16, 2016; 17, 2017.

Manag.	Mar. 15	Jul. 15	Feb. 16	May 16	Sep. 16	Feb. 17	Jun. 17	Sep. 17	Final Change
M1	4.2 ± 0.9 Aa	5.5 ± 0.7 Aa	2.9 ± 0.7 Aa	7.4 ± 1.6 Aa	5.9 ± 0.8 Aa	4.2 ± 1.4 Aa	6.9 ± 1.6 Aa	7.7 ± 2.1 Aa	82.5 ± 82.6 a
M2	9.7 ± 2.5 Aa	3.2 ± 1.2 Aa	1.8 ± 1.1 Aab	1.3 ± 0.5 Aab	4.9 ± 2.4 Aab	4.3 ± 2.1 Aa	3.7 ± 1.2 Aa	3.7 ± 1.1 Aa	−62.4 ± 9.6 ab
M3	6.6 ± 3.2 Aa	0.5 ± 0.1 Ab	0.1 ± 0.1 Aab	0.6 ± 0.2 Aab	0.6 ± 0.4 Ab	0.4 ± 0.2 Aab	1.2 ± 0.6 Aab	1.1 ± 0.8 Aab	−82.6 ± 11.1 b
M4	7.4 ± 1.3 Aa	0.4 ± 0.1 BCb	0.0 ± 0.0 Cb	0.1 ± 0.0 BCb	0.6 ± 0.2 Bb	0.1 ± 0.0 BCb	0.8 ± 0.3 Bb	0.5 ± 0.1 BCb	−93.9 ± 2.3 b

Different letters represent significant differences at $p < 0.05$; capital letters: differences among sampling dates; lowercase letters: differences among managements.

Table 3. Frequency (%) of *C. dactylon* in each management across the different dates. Mean values of number of subplots with presence ± standard errors of the mean. Manag., management; Mar., March; Jun., June; Jul., July; Feb., February; Sep., September; 15, 2015; 16, 2016; 17, 2017.

Manag.	Mar. 15	Jul. 15	Feb. 16	May 16	Sep. 16	Feb. 17	Jun. 17	Sep. 17	Final Change
M1	24.2 ± 3.6 Aa	37.1 ± 8.6 Aab	18.8 ± 3.3 Aa	38.3 ± 7.9 Aa	37.9 ± 7.9 Aa	24.6 ± 4.6 Aa	40.9 ± 6.7 Aa	45.8 ± 9.9 Aa	89.7 ± 23.4 a
M2	42.8 ± 11.5 Aa	46.3 ± 10.4 Aa	12.9 ± 5.2 Aab	23.8 ± 10.5 Aab	34.1 ± 9.2 Aab	28.5 ± 10.7 Aa	33.8 ± 11.2 Aa	36.6 ± 9.5 Aab	−14.6 ± 17.6 ab
M3	31.9 ± 16.4 Aa	10.9 ± 3.1 Ab	2.5 ± 1.4 Abc	13.8 ± 3.8 Aab	9.1 ± 4.8 Ab	14.1 ± 6.0 Aa	13.8 ± 4.6 Aa	15.3 ± 7.3 Aab	−52.0 ± 16.7 b
M4	36.3 ± 5.7 Aa	7.8 ± 0.6 Bb	0.6 ± 0.6 Cc	3.1 ± 1.7 BCb	9.7 ± 0.6 Bab	3.8 ± 1.5 BCa	12.8 ± 3.6 Ba	9.7 ± 3.0 Bb	−73.3 ± 12.6 b

Percentage calculated as the number of the 80 subunits of each plot with the presence of *C. dactylon*. Different letters in the same column mean significant differences at $p < 0.005$; capital letters, differences among initial and final dates; lowercase letters, differences among managements.

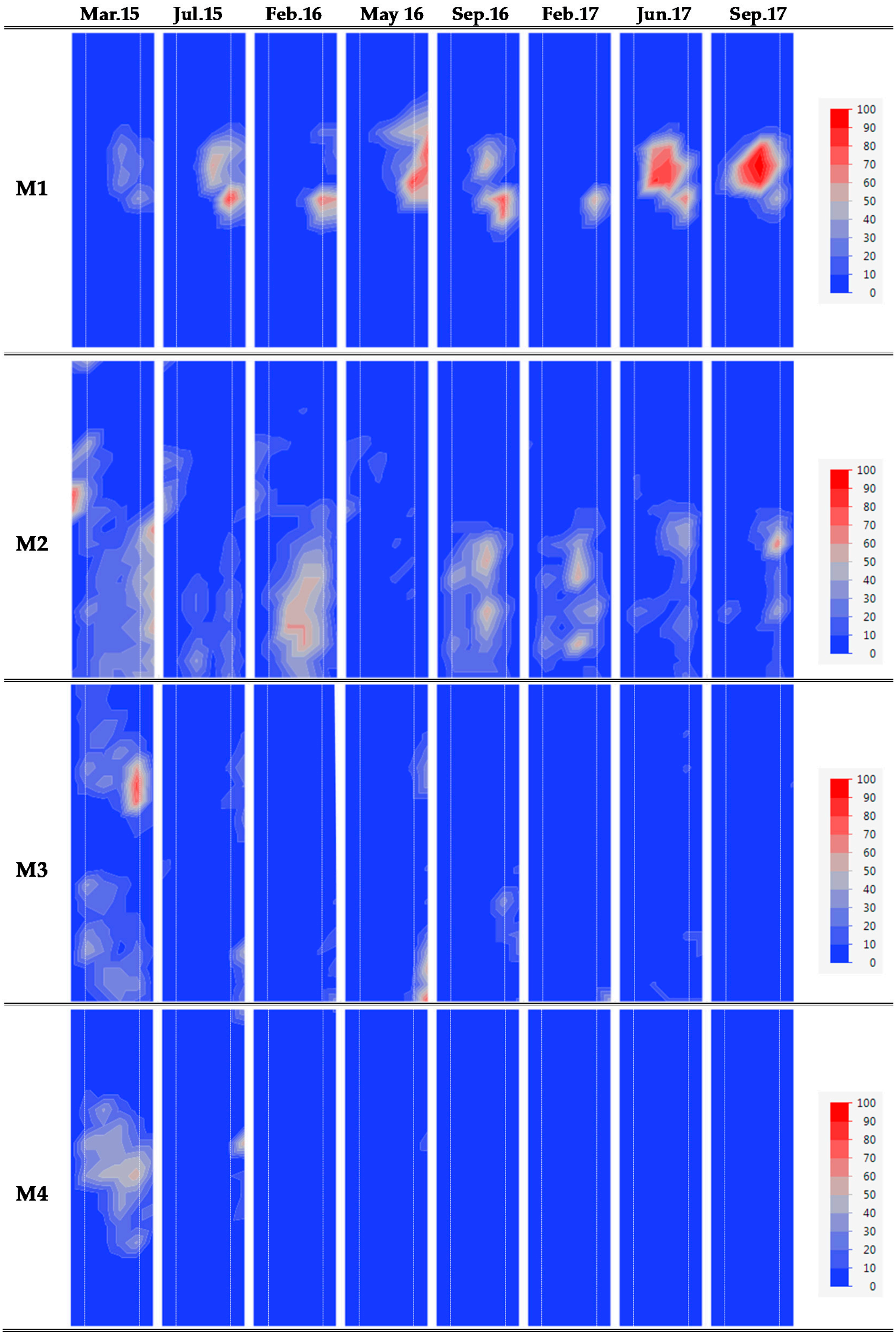

Figure 2. Soil coverage variation of *Cynodon dactylon* of a selected plot for each management across the different sampling dates. Each plot is divided with two white lines in each lateral to differentiate the central part (the inter-rows), where the management is completely done from the borders (the intra-rows), and where management is not complete and also mixed with intra-rows herbicide applications.

Barley cover crop (M4) significantly reduced the *C. dactylon* coverage from 7.4% to 0.5%, which results in a 93.9% reduction (Table 2). The spontaneous tilled cover also obtained a high reduction effect, with an initial 6.6% of coverage that decreased until 1.1% at the last sampling date (82.2% of coverage reduction). The spontaneous cover crop plus herbicide application management (M2) obtained a 62.4% of reduction (coverage values that varied from 9.7% to 3.7%). In the case of M1, no-till spontaneous vegetation ground cover was managed by shredding, and the initial weed coverage value (4.2%) increased until 7.7%, which results in an 82.5% of increase, a value that is significantly different to those obtained by the managements with soil disturbance (M3 and M4) (Table 2).

Managements affected weed coverage differently across the three seasons. Before management implementation, there were no significant differences (March 2015) among the plots selected for the four management systems. In July 2015, after tillage for M3 and M4, significant differences were observed between M1 and M2 (>3.2%) and M3 and M4 (≤0.5%). In February 2016, there were statistical differences between M1 (2.9%) and M4 (0%), which were maintained in May 2016 (7.4% and 0.1%, respectively for M1 and M4). One year after the barley cover crop establishment (September 2016), M3 and M4 maintained significantly lower infestation levels (0.6%) than M1 (5.9%) but not M2 (4.9%). These differences were maintained until June 2017, but in September 2017, M4 showed the lowest *C. dactylon* coverage (0.5%), which was statistically similar to M3 (1.1%), and different from M2 (3.7%) and M1 (7.7%).

Regarding the percentage of *C. dactylon* frequency, it particularly decreased in those managements with soil disturbance (M3 and M4) (Table 3). The comparison between the first and the last sampling dates along the three seasons showed a decrease of the presence of *C. dactylon* in M2 (14.6% frequency reduction), M3 (52%), and M4, which varied from 36.3% to 9.75%, showing the only significant decrease (73.3%). In no-till spontaneous vegetation ground cover managed by shredding (M1), the presence of *C. dactylon* increased by 89.7%.

Before management implementation (March 2015), the frequency of *C. dactylon* was similar among the plots selected. In July 2015, after tillage for M3 and M4, the weed frequency was highly reduced for M3 and M4, with low values that were maintained until the last sampling date. At the end of the experiment (September 2017), shredding (M1) and shredding plus herbicide applications (M2) showed the highest percentages of frequency (45.8% and 36.6% respectively), followed by M3 (15.3%). The lowest value was obtained by barley cover crop management, with 9.7%.

3.3. Weed Flora Response to Management

Excluding *C. dactylon*, a total of 39 weed species were recorded in the weed flora surveys, with 31 species in M1, 14 in M2, 23 in M3, and 6 in M4. The RDA analyses showed a variance of 46.6% on the weed community composition after three years of management (Figure 3). The first axis clearly separates those managements based on shredding from those based on soil disturbance, leading to the selection of different weed communities. Shredding (M1) favored the dominance of perennial species, such as *Trifolium repens*, *Crepis vesicaria*, or *Convolvulus arvensis*. *Bromus rubens* and *Crepis sancta*, among others, were related to M2, where shredding and herbicide applications were performed; while soil disturbance (M3 and M4) selected, in general, annual species, such as *Papaver rhoeas*, *Cerastium glomeratum*, *B. scoparia*, or *Diplotaxis erucoides* (Figure 3).

The species diagnostic for individual management showed that some problematic species for crop yield, such as *C. bonariensis*, *C. arvensis*, or *Malva sylvestris* had not any preferred group. None of the species showed preference to M4 (Barley cover crop) (Table 4).

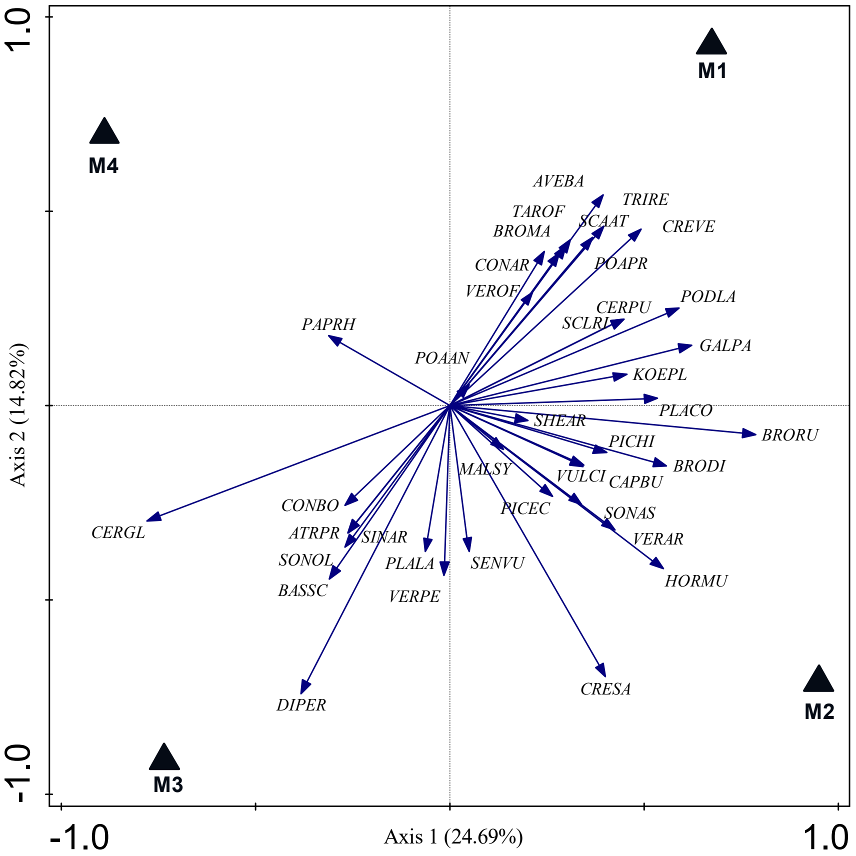

Figure 3. Redundancy analysis of species composition. Black labels denote managements abbreviated. Arrows showed the weed species present in the analysis. Weed species abbreviations are listed in Table 4.

Table 4. Plant species analyzed in Figure 2 and diagnostic species for individual management classes. Abbr: Abbreviation; PG: Preferred Group (management).

Abbr.	Name	PG	Abbr.	Name	PG
ATRPR	*Atriplex prostrata* Boucher ex DC.	M3	PICHI	*Picris hieracioides* Sibth. & Sm.	M2
AVEBA	*Avena barbata* Pott ex Link	M1	PLACO	*Plantago coronopus* L.	-
BASSC	*Bassia scoparia* (L.) A.J.Scott	M3	PLALA	*Plantago lanceolata* L.	-
BRODI	*Bromus diandrus* Roth	M2	POAAN	*Poa annua* L.	-
BROMA	*Bromus madritensis* L.	M1	POAPR	*Poa pratensis* L.	M1
BRORU	*Bromus rubens* L.	M2	PODLA	*Podospermum laciniatum* (L.) DC.	M1
CAPBU	*Capsella bursa-pastoris.* (L.) Medik	-	SCAAT	*Scabiosa atropurpurea* L.	M1
CERGL	*Cerastium glomeratum* Thuill.	M3	SCLRI	*Scleropoa rigida* (L.) Griseb.	M1
CERPU	*Cerastium pumilum* Curtis	M1	SENVU	*Senecio vulgaris* L.	-
CONAR	*Convolvulus arvensis* L.	-	SHEAR	*Sherardia arvensis* L.	-
CONBO	*Conyza bonariensis* (L.) Cronquist	-	SINAR	*Sinapis arvensis* L.	M3
CRESA	*Crepis sancta* (L.) Bornm.	-	SONAS	*Sonchus asper* (L.) Hill	M2
CREVE	*Crepis vesicaria* L.	M1	SONOL	*Sonchus oleraceus* L.	M3
DIPER	*Diplotaxis erucoides* (L.) DC.	M3	TAROF	*Taraxacum officinale* Weber	M1
GALPA	*Galium parisiense* L.	M2	TRIRE	*Trifolium repens* L.	M1
HORMU	*Hordeum murinum* L.	-	VEROF	*Verbena officinalis* L.	M1
KOEPL	*Koeleria phleoides* (Vill.) Pers.	M1	VERAR	*Veronica arvensis* L.	M2
MALSY	*Malva sylvestris* L.	-	VERPE	*Veronica persica* Poir.	M2
PAPRH	*Papaver rhoeas* L.	-	VULCI	*Vulpia ciliata* Dumort.	-
PICEC	*Picris echioides* L.	M1			

4. Discussion

The coverage and frequency of *C. dactylon* varied depending on the management system. Shredding solely (M1) was not only ineffective for controlling this weed, but also increased weed infestation. However, the other strategies (herbicide, tillage, or sowing a barley cover crop) partially controlled *C. dactylon*. Nevertheless, significant fluctuations of the weed coverage and frequency were observed over time (Tables 2 and 3).

The inconsistent effect of shredded spontaneous ground cover (M1) can be explained by *C. dactylon*'s characteristics and by the removal of other weeds that compete with *C. dactylon*. On one hand, shredding cannot disturb the underground rhizomes and the ground stolons that spread horizontally to the soil surface. On the other hand, this management decreases the presence of other species (whether beneficial or not for vineyard) that could generate more shading, especially in spring and summer. As a consequence, light could reach more efficiently the soil surface, benefiting horizontal growing species, such as *C. dactylon* [23].

The combination of shredding and herbicide applications (M2) slightly reduced *C. dactylon* coverage (62.4%), but not its frequency (14.6% of reduction). As described before, shredding alone increased weed infestation, and some authors had reported different weed control levels after glyphosate applications: Abdullahi [28] reported a relatively good control (60%) of this species by glyphosate application at 2.16 kg ha^{-1} in summer (when the weed is actively growing), its effect remaining for up to 16 weeks, and although Gómez de Barreda et al. [26] observed some phytotoxic effects, it did not control *C. dactylon* (var "princess 77") after a single application (36% at 4 L ha^{-1}). Bryson and Wills [35] showed variations in the effectiveness of glyphosate application between rates (1.12 and 2.24 kg ha^{-1}) across 17 different *C. dactylon* biotypes and observed that 13 weeks after application, the highest rate maintained 70% of control. These results are in accordance with our results and highlight the need to combine herbicide applications with other strategies or to perform more herbicide applications. Farthing et al. [27] observed 72.1% of *C. dactylon* canopy cover reduction with a single glyphosate application during the warm season (48.7% at 9 L ha ha^{-1}) and biomass and groundcover reduction of more than 98.6% after multiple applications (with or without previous shredding). Mau-Crimmins [36] also reported the effectiveness of glyphosate on *C. dactylon* removal but that herbicide applications increased other noxious weeds species.

Several factors can affect the herbicide efficacy; for example, the timing of herbicide application is crucial because the weed becomes dormant below 10 °C [25] and herbicide applications are not so effective. In the present study, glyphosate caused phytotoxic effects on *C. dactylon*, but the weed recovered and the infestation level was unaffected. This result could, again, be explained by the death of the rest of the spontaneous cover species, which reduced the competition against *C. dactylon*. On the other hand, in sugarcane, Campbell [37] reported that glyphosate applications without tillage, at rates between 1.14 and 2.88 kg ha^{-1}, obtained more than 80% of control 20 weeks after treatment, but when combined with a previous tillage, the control was reduced down to 60%, even at 2.88 kg ha^{-1}.

Tillage is known to be effective in reducing weed infestations level, as it happened in M3 and M4, where its coverage was reduced below 1% after three seasons. This result agrees with the recommendations of Spanish Ministry of Agriculture (MAGRAMA) [38], suggesting soil tillage so that rhizomes and stolons are fragmented as small as possible, while remaining within or close to the soil surface.

Guglielmini and Satorre [24] reported that mechanical cultivation cut and spread the structures of the weed, and although these fragments are then dispersed, only a small proportion of them (which are in contact with the soil) were able to survive and establish. In our case, the fragments that survived tillage also had to face the spontaneous vegetation competition. On the other hand, Dalley et al. [39] reported in sugarcane that conventional tillage (four cultivations of row sides each spring) and reduced tillage (two cultivations) significantly reduced *C. dactylon* coverage compared to no-till (no cultivation), with mean ground cover values of 60%, 70%, and 90%, respectively. These results agree with Phillips [40] in sorghum crop, who observed that treatments with winter and spring double-ploughing reduced

C. dactylon regrowth by 60% and that double spring ploughing reduced additional 33% compared to a single pass.

The management where tillage was combined with barley cover crop (M4) was the most effective in reducing the coverage and frequency (93.9% and 73.3% respectively) of *C. dactylon*. In this situation, an additive effect seems to be created by seedbed preparation (tillage) with a competitive pressure exerted by barley. At the end of the experiment, there were no significant differences between M3 and M4, but the different percentages, 1.1% and 0.5% respectively (very low in both cases) can be explained by the higher and more homogeneous biomass production obtained with barley than with the spontaneous vegetation. Moreover, barley is a winter cereal that produces a dense shading canopy when *C. dactylon* begins its growing activity, creating more unfavorable conditions for weed development. The influence of the shade provided by the cover crop could be a crucial factor in the weed management. This factor has previously been reported to reduce *C. dactylon* infestation [23].

Dong and De Kroon [41] studied the influence of light and nutrients availability in *C. dactylon* development in terms of morphology plasticity and biomass allocation. They observed that under low light and nutrients level, stolon and rhizome branching was reduced, which can be explained by the influence of barley when competing for light, nutrients, and other resources and consequently reducing weed infestation levels.

Shade treatments have been reported to obtain plants that produced leaves twice as long as the unshaded ones [41]. These aspects could explain part of our results. Barley provides shadow to *C. dactylon* during some moths, and during this time, the weed can change its growth pattern, producing more orthotropic buds instead of lateral stolons [41], which can make *C. dactylon* more susceptible to shredding. Dong and De Kroon [41] also reported that plants under low-intensity light, which could have been achieved in our experiment by the vegetation cover canopy, failed developing rhizomes, and stolons biomass allocation was slightly lower. Other cover crop species have been shown to affect *C. dactylon* development, as reported by Farthing et al. [27], who observed that shredding *C. dactylon* and overseeding *Vicia villosa* reduced weed biomass by 50%, although canopy cover was not decreased.

In addition to the shade and competition exerted by the cover crop, the effect on weed suppression can be explained by allelochemicals releasing and by a physical mulch effect once the cover crop is shredded. Ormeño-Núñez et al. [42] reported that rye (*Secale cereale* L.) mulch on the vine rows reduced 82% of *C. dactylon* dry matter compared to chemical plus mechanical control. The weed suppression effect by different species implemented as mulch or cover crop has been studied for other authors in vineyards: DeVetter et al. [43] obtained better weed control with straw and living mulch of *Festuca rubra* L. Pennlawn than with cultivation and herbicides. Steinmaus et al. [44] also obtained good results in weed control by different mulched cover crops. The bioassay study revealed several weeds' germination and growth reduction, including *Echinochloa crus-galli* (L.) P.Beauv. and *Setaria verticillata* (L.) P.Beauv., by extracts of barley and other cereals usually implemented as cover crops in Mediterranean areas [45], while this suppressive effect of barley (and rye) cover crops has also been observed on *Solanum ptychanthum* Dun. and *Setaria glauca* (L.) P.Beauv. in the United States [46].

The effect of the different cover crops species to suppress weeds development described in that studies are in agreement, in part, with our results: barley cover crop (M4) has been the management with the lowest number of registered weed species (6 versus 32, 15 and 29 for M1, M2, and M3, respectively. This result is also in accordance with Gago et al. [15], who observed a higher number of weed species in unsown plots than in those sown with cereals. Barley cover crop (M4) and spontaneous ground cover plus herbicide applications (M2) were related with lower species richness than shredding (M1) or tilling a spontaneous cover crop (M3). High amounts of hemicryptophyte species, such as *Taraxacum officinale* or *Trifolium repens*, were related to shredding (M1), as described in Figure 3, showing that the weed community was really influenced by the different three-year managements. The amount of species related to M1 is also found in orchards and can be explained because shredding can favor seedling recruitment, the survival, and the reproductive ability of the species [47,48]. In addition, the diagnostic species for individual management classes demonstrated that none of the recorded species showed any preference for barley cover crop (M4), which reinforces the implementation of this cover crop as the most appropriate management for weed control. The lack of preference to any group by some of the most problematic weeds (*C. bonariensis*, *C. avernsis*, or *M. sylvestris*) indicates that none of these species are favored by any management and that their implementation is not discouraged in the presence of these species. Focusing on *B. scoparia*, which is a very problematic species, the diagnostic species showed M3 as a preferred group. In this case, this result could be explained because *B. scoparia* is a very fast growing species that can overcome the cover in few days if the cover is not competitive and vigorous enough. Therefore, in the presence of this species, M3 is not recommended or more shredding could be required in order to stop its growth.

Among all managements, only shredding a spontaneous ground cover (M1) has been ineffective, but its combination with other tools, such as herbicide applications or tilling, increased the control of the *C. dactylon*, although the best management was the implementation of a barley cover crop. In this study, the cover crop has been limited to the inter-row, but in some vineyards, the cover crop is implemented intra-row, with an effective weed control with cover crops species such as *Trifolium fragiferum* L. [49]. The installation and management of the cover crop under the vines can be crucial for the management of *C. dactylon*, where this weed frequently gets shelter. Further installation of the cover crop in intra-row areas would reduce herbicide applications and inter-vine interventions, providing easier soil management.

Considering cover crops are non-chemical alternatives for weed management, our results enhance their implementation in a vineyard, which is in accordance with the Directive 2009/128/EC [50] where Integrated Pest Management is promoted.

5. Conclusions

Cover crop implementation can be a useful tool in weed management. In the vineyards that soil maintenance is carried out through vegetation ground covers, it is important to know the weed species that are present to carry out a proper management of the field. If *C. dactylon* is present, repeated use of the shredder is discouraged, because it not only does not control this weed, but also encourages its development and expansion. For this species, this work demonstrates that the best control strategy is to perform a mechanical intervention that disturbs the soil, such as tillage, combined with the implementation of a vegetation cover. The species selected for this purpose must meet several characteristics, such as rapid implementation and generate a large amount of biomass so that enough shade is provided before *C. dactylon* organs become active in spring. In this sense, despite the good results with the spontaneous cover, the species composition determines the amount of produced biomass, and this is uncertain. Therefore, sowing an already known species after tillage is the most appealing strategy. On the other hand, the quick effect of the implemented managements on the species composition has been demonstrated. For this reason, a previous study of the weed species present in the field is advisable to consider those that may become problematic in the near future.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

hL	Hectoliter
Feb	February
Jun	June
Aug	August
Sep	September
Nov	November
Dec	December
vs.	*versus*

References

1. OIV (Organisation Internationale de la Vigne et du Vin). Statistical Report on World Vitiviniculture. April 2019. Available online: http://www.oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf (accessed on 27 September 2019).
2. Oerke, E. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31–43. [CrossRef]
3. Pardini, A.; Faiello, C.; Longhi, F. Cover crop species and their management in vineyards and olive groves. *Adv. Hortic. Sci.* **2002**, *16*, 225–234.
4. García, L.; Celette, F.; Gary, C.; Ripoche, A.; Valdés-Gómez, H.; Metay, A. Management of service crops for the provision of ecosystem services in vineyards: A review. *Agric. Ecosyst. Environ.* **2018**, *251*, 158–170. [CrossRef]
5. Guerra, B.; Steenwerth, K. Influence of floor management technique on grapevine growth, disease pressure, and juice and wine composition: A review. *Am. J. Enol. Vitic.* **2012**, *63*, 149–164. [CrossRef]
6. Cerdan, O.; Govers, G.; Le Bissonnais, Y.; Van Oost, K.; Poesen, J.; Saby, N.; Gobin, A.; Vacca, A.; Quinton, J.; Auerwald, K.; et al. Rates and spatial variations of soil erosion in Europe: A study based on erosion plot data. *Geomorphology* **2010**, *122*, 167–177. [CrossRef]
7. Prosdocimi, M.; Cerdà, A.; Tarolli, P. Soil water erosion on Mediterranean vineyards: A review. *Catena* **2016**, *141*, 1–21. [CrossRef]
8. Zaller, J.G.; Cantelmo, C.; Santos, G.M.; Muther, S.; Gruber, E.; Pallua, P.; Mandl, K.; Friedrich, B.Q.; Hofstetter, I.; Schmuckenschlager, B.; et al. Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 23215–23226. [CrossRef]
9. Heap, I. The International Survey of Herbicide Resistant Weeds. Available online: www.weedscience.org (accessed on 8 January 2020).
10. Viers, J.H.; Williams, J.N.; Nicholas, K.A.; Barbosa, O.; Kotzé, I.; Spence, L.; Leanne, B.W.; Merenlender, A.; Reynolds, M. Vinecology: Pairing wine with nature. *Conserv. Lett.* **2013**, *6*, 287–299. [CrossRef]
11. Stolz, H.; Schmid, O. Organic Viticulture and Wine-Making: Development of Environment and Consumer Friendly Technologies for Organic Wine Quality Improvement and Scientifically Based Legislative Framework. Deliverable: D 2.7 Public Report about First Round Qualitative Consumer Research and Market Needs. 2007. Available online: https://orgprints.org/10608/1/stolz-schmid-2007-ORWINE_d2.7_consumers_research.pdf (accessed on 27 September 2019).
12. Le Bissonnais, Y.; Lecomte, V.; Cerdan, O. Grass strip effects on runoffand soil loss. *Agronomie* **2004**, *24*, 129–136. [CrossRef]
13. Steenwerth, K.; Belina, K.M. Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. *Appl. Soil Ecol.* **2008**, *40*, 359–369. [CrossRef]
14. Vukicevich, E.; Lowery, T.; Bowen, P.; Úrbez-Torres, J.R.; Hart, M. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agron. Sustain. Dev.* **2016**, *36*, 48. [CrossRef]
15. Gago, P.; Cabaleiro, C.; García, J. Preliminary study of the effect of soil management systems on the adventitious flora of a vineyard in northwestern Spain. *Crop Prot.* **2007**, *26*, 584–591. [CrossRef]
16. Sanguankeo, P.; León, R. Weed management practices determine plant and arthropod diversity and seed predation in vineyards. *Weed Res.* **2011**, *51*, 404–412. [CrossRef]
17. Miglécz, T.; Valkó, O.; Török, P.; Deák, B.; Kelemen, A.; Donkó, Á.; Drexler, D.; Tóthmérész, B. Establishment of three cover crop mixtures in vineyards. *Sci. Hortic.* **2015**, *197*, 117–123. [CrossRef]

18. Sáenz-Romo, M.G.; Veas-Bernal, A.; Martínez-García, H.; Campos-Herrera, R.; Ibáñez-Pascual, S.; Martínez-Villar, E.; Pérez-Moreno, I.; Marco-Mancebón, V.S. Ground cover management in a Mediterranean vineyard: Impact on insect abundance and diversity. *Agric. Ecosyst. Environ.* **2019**, *283*, 106571. [CrossRef]
19. Farooq, M.; Jabran, K.; Cheema, Z.; Wahid, A.; Siddique, K. The role of allelopathy in agricultural pest management. *Pest. Manag. Sci.* **2011**, *67*, 493–506. [CrossRef]
20. Sturm, D.J.; Peteinatos, G.; Gerhards, R. Contribution of allelopathic effects to the overall weed suppression by different cover crops. *Weed Res.* **2018**, *58*, 331–337. [CrossRef]
21. Holm, L.G.; Plucknett, D.L.; Pancho, J.V.; Herberger, J.P. The World's Worst Weeds. In *Distribution and Biology*; University of Hawaii Press: Honolulu, HI, USA, 1977.
22. Kim, C.; Lemke, C.; Paterson, A.H. Functional dissection of drought- responsive gene expression patterns in *Cynodon dactylon* L. *Plant Mol. Biol.* **2009**, *70*, 1–16. [CrossRef]
23. Guglielmini, A.C.; Satorre, E.H. Shading effects on spatial growth and biomass partitioning of *Cynodon dactylon*. *Weed Res.* **2002**, *42*, 123–134. [CrossRef]
24. Guglielmini, A.C.; Satorre, E.H. The effect of non-inversion tillage and light availability on dispersal and spatial growth of *Cynodon dactylon*. *Weed Res.* **2004**, *44*, 366–374. [CrossRef]
25. McCarty, L.B.; Miller, G. *Managing Bermudagrass Turf. E.*; Ann Arbor Press: Chelsea, MI, USA, 2002; pp. 67–86.
26. Gómez de Barreda, D.; Izquierdo, D.; de Luca, V. Evaluación del comportamiento de 20 materias activas herbicidas sobre la cespitosa "*Cynodon dactylon*" en diferentes épocas climáticas del año. In Proceedings of the XVI Congreso de la Sociedad Española de Malherbología, Pamplona-Iruña, España, 25–27 October 2017; pp. 245–250.
27. Farthing, T.S.; Muir, J.P.; Falk, A.; Murray, D. Efficacy of seven invasive Bermudagrass removal strategies in three Texas ecoregions. *Ecol. Restor.* **2018**, *36*, 306–314. [CrossRef]
28. Abdullahi, A.E. *Cynodon dactylon* control with tillage and glyphosate. *Crop Prot.* **2002**, *21*, 1093–1100. [CrossRef]
29. Fernandez, O.N. Establishment of *Cynodon dactylon* from stolon and rhizome fragments. *Weed Res.* **2003**, *43*, 130–138. [CrossRef]
30. Recasens, J.; Valencia, F.; Montull, J.M.; Taberner, A. Malas hierbas problemáticas en viñedos con cubiertas vegetales y métodos químicos para su control. *Vida Rural* **2018**, *444*, 48–58.
31. Agencia Estatal de Meteorología (AEMET). Ministerio para la Transición Ecológica. Gobierno de España. Available online: www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos (accessed on 2 March 2020).
32. Ibáñez, S. *Mantenimiento del Suelo en el Viñedo Mediante Cubiertas Vegetales*; Consejería de Agricultura, Ganadería y Medio Ambiente: Gobierno de La Rioja, Logroño, Spain, 2015; p. 167.
33. Chytrý, M.; Tichý, L.; Holt, J.; Botta-Dukát, Z. Determination of Diagnostic Species with Statistical Fidelity Measures. *J. Veg. Sci.* **2002**, *13*, 79–90. [CrossRef]
34. Ter Braak, C.F.; Smilauer, P. *Canoco Reference Manual and User's Guide: Software for Ordination, Version 5.0*; Microcomputer Power: Ithaca, NY, USA, 2012.
35. Bryson, C.; Wills, G. Susceptibility of Bermudagrass (*Cynodon dactylon*) Biotypes to Several Herbicides. *Weed Sci.* **1985**, *33*, 848–852. [CrossRef]
36. Mau-Crimmins, T.M. Effects of removing *Cynodon dactylon* from a recently abandoned agricultural field. *Weed Res.* **2007**, *47*, 212–221. [CrossRef]
37. Campbell, P.L. Efficacy of glyphosate, alternative post-emergence herbicides and tillage for control of *Cynodon dactylon*. *S. Afr. J. Plant Soil* **2008**, *25*, 220–228. [CrossRef]
38. Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA). *Guía de Gestión Integrada de Plagas. Uva de Transformación. Ministerio de Agricultura*; Alimentación y Medio Ambiente: Madrid, Spain, 2014.
39. Dalley, C.D.; Viator, R.P.; Richard, E.P. Integrated Management of Bermudagrass (*Cynodon dactylon*) in Sugarcane. *Weed Sci.* **2013**, *61*, 482–490. [CrossRef]
40. Phillips, M.C. Use of tillage to control *Cynodon dactylon* under small-scale farming conditions. *Crop Prot.* **1993**, *12*, 267–272. [CrossRef]
41. Dong, M.; De Kroon, H. Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos* **1994**, *70*, 99–106. [CrossRef]

42. Ormeño-Núñez, J.; Pino-Rojas, G.; Garfe-Vergara, F. Inhibition of yellow nutsedge (*Cyperus esculentus* L.) and bermudagrass (*Cynodon dactylon* (L.) Pers.) by a mulch derived from rye (*Secale cereale* L.) in grapevines. *Chil. J. Agric. Res.* **2008**, *68*, 238–247.
43. DeVetter, L.W.; Dilley, C.A.; Nonnecke, G.R. Mulches reduce weeds, maintain yield, and promote soil quality in a continental-climate vineyard. *Am. J. Enol. Viticult.* **2015**, *66*, 54–64. [CrossRef]
44. Steinmaus, S.; Elmore, C.L.; Smith, R.J.; Donaldson, D.; Weber, E.A.; Roncoroni, J.A.; Miller, P.R.M. Mulched cover crops as an alternative to conventional weed management systems in vineyards. *Weed Res.* **2008**, *48*, 273–281. [CrossRef]
45. Dhima, K.V.; Vasilakoglou, I.B.; Eleftherohorinos, I.G.; Lithourgidis, A.S. Allelopathic Potential of Winter Cereals and Their Cover Crop Mulch Effect on Grass Weed Suppression and Corn Development. *Crop Sci.* **2006**, *46*, 345–352. [CrossRef]
46. Creamer, N.G.; Bennett, M.A.; Stinner, B.R.; Cardina, J.; Regnier, E.E. Mechanisms of Weed Suppression in Cover Crop-based Production Systems. *HortScience* **1996**, *31*, 410–413. [CrossRef]
47. Mas, M.T.; Poggio, S.L.; Verdú, A.M.C. Weed community structure of mandarin orchards under conventional and integrated management in northern Spain. *Agric. Ecosyst. Environ.* **2007**, *119*, 305–310. [CrossRef]
48. Juárez-Escario, A.; Conesa, J.A.; Solé-Senan, X.O. Management as a driver of functional patterns and alien species prominence in weed communities of irrigated orchards in Mediterranean areas. *Agric. Ecosyst. Environ.* **2017**, *249*, 247–255.
49. Abad, F.J.; Cibriáin, F.; Santesteban, L.G.; Marín, D.; Sagüés, A. Manejo del suelo bajo la línea de cultivo de viñedo sin empleo de herbicida ni laboreo. In Proceedings of the III Jornadas del Grupo de Viticultura, Universitat de les Illes Balears, Palma, Illes Balears, Spain, 28–29 November 2018; pp. 164–167.
50. European Union. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009. Establishing a Framework for Community Action to Achieve the Sustainable Use of Pesticides. *Off. J. Eur. Union* **2009**, *L309*, 71–86. Available online: https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:en:PDF (accessed on 5 September 2019).