



Universitat de Lleida

Weed management strategies based on cover crops, mulches and bioherbicides in Mediterranean vineyards

Carlos Cabrera Pérez

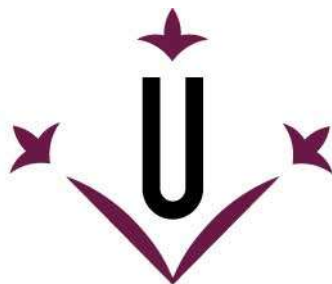
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Universitat de Lleida

TESI DOCTORAL

**Weed management strategies based on cover
crops, mulches and bioherbicides in
Mediterranean vineyards**

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Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
Programa de Doctorat en Ciència i Tecnologia Agrària i Alimentària

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2022

Presentación

La presente tesis doctoral se ha llevado a cabo bajo la dirección del profesor Dr. Jordi Recasens Guinjuan y el Dr. Aritz Royo Esnal en el seno del grupo de investigación consolidado de Malherbología y Ecología Vegetal (2017SGR283) del Departament d'Hortofruccultura, Botànica i Jardineria de l'Escola Tècnica Superior d'Enginyeria Agrària (ETSEA) de la Universitat de Lleida.

Para la realización de dicha tesis se obtuvo un contrato predoctoral en la convocatoria 2018 de la Universidad de Lleida (periodo diciembre 2018 a noviembre 2021) y de una prórroga de dicho contrato hasta abril de 2022 debido al Estado de Alarma decretado por el Covid-19.

El trabajo experimental se ha desarrollado como miembro del equipo de trabajo de los siguientes proyectos de investigación:

- MINECO (2017-2021). PROGRAMA ESTATAL I+D+I RETOS DE LA SOCIEDAD: Gestión de malas hierbas en cereales y viña mediante la integración de sistemas de manejo y herramientas de decisión. REF AGL2017-83325-C4-2-R.
- MINECO (2021-2024). PROGRAMA ESTATAL I+D+I RETOS DE LA SOCIEDAD: Integración de herramientas agronómicas y tecnológicas en la detección y manejo de malas hierbas. REF PID2020-113229RB-C42.
- ACCIÓ – GENERALITAT DE CATALUNYA (2018-2021). PROJECTES RIS3CAT COMUNITAT COTPA: “Low Input Sustainable Agriculture (LISA)”. Reducció de l'aplicació dels inputs agrícoles garantint la sostenibilitat econòmica i ambiental. REF 7.1.7. Agricultura de precisión. Tecnología facilitadora TIC.
- DARP (2018-2020) PROGRAMA DE DESENVOLUPAMENT RURAL. GRUPS OPERATIUS: MACMHER: Mètodes alternatius de control de males herbes en vinya ecològica. REF 16.01.01 PDR.
- AYUDAS A PROYECTOS DEL CENTRO PARA EL DESARROLLO TECNOLÓGICO INDUSTRIAL (CDTI) (2019-2022). Estudio del potencial alelopático asociado al control malherbológico en viña. Empresa beneficiaria: ALRASA-AGRARIA.

Agradecimientos

Después de cuatro años habiendo formado parte del Grupo de Malherbología y Ecología Vegetal de la Universitat de Lleida, puedo decir que les estoy enormemente agradecido por la oportunidad que me dieron de trabajar junto a ellos, primero como técnico del grupo y luego como doctorando. En especial, quiero agradecer a mis dos directores, Jordi Recasens y Aritz Royo, los cuales me han apoyado en todo momento a lo largo del doctorado. Me han animado a participar en congresos, cursos, a realizar una estancia en el extranjero, a participar en las reuniones con las empresas del sector y, en definitiva, a empaparme de experiencia y aprendizaje durante este camino. No puedo dejar de mencionar el trato humano por parte de todos los miembros del grupo, el cual ha sido inmejorable y es, sin duda, uno de los pilares de este grupo.

A las empresas Raventós-Codorníu S.A y Alrasa Agraria S.A, en especial a Jordi Civit, Agustí Torrelles, David Moliner y Aida Sala, por cedernos las parcelas para llevar a cabo los ensayos, facilitarnos maquinaria y personal y asesorarnos.

A Matteo Mota, por su hospitalidad al acogerme en su grupo de investigación en Nyon durante la estancia doctoral en Suiza.

A todas las personas que me llevo después de estos años viviendo en Lleida, compañeros y compañeras de piso, de máster y de doctorado, que luego se convirtieron en amigos y amigas.

A todas las personas que forman parte de mi vida en Barcelona, a las cuales esperaba con ganas para reencontrarme los fines de semana y vacaciones.

Finalmente, a mi familia. A mis padres por su incondicional apoyo en todo y a mi hermano, que es para mí un consejero de vida.

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Resum

L'actual emergència climàtica obliga a buscar estratègies més sostenibles per al maneig del sòl de la vinya. En aquest sentit, el control de les males herbes constitueix un veritable repte degut a la seva capacitat d'expansió i d'ocasionar pèrdues de rendiment. Entre les espècies més competitives i nocives que podem trobar destaquen *Cynodon dactylon* (L.) Pers. i *Conyza bonariensis* (L.) Cronquist. Tradicionalment, el laboreig ha estat el mètode més utilitzat per al maneig de les males herbes, tot i que el seu ús afavoreix el risc d'erosió, disminueix la matèria orgànica i comporta una alta petjada de carboni. Per altre banda, el continu ús d'herbicides de síntesis, ha comportat l'aparició de biotips de males herbes resistents a aquests i, a causa de la seva alta toxicitat, el seu ús es veu àmpliament qüestionat. La present tesi doctoral planteja diferents alternatives en el maneig de males herbes en vinya, tant al carrer com sota la línia. Els experiments duts a terme a aquest efecte, pretenen avaluar i aportar nova informació sobre la possibilitat d'establir, en el control de males herbes, cobertes vegetals, encoixinats orgànics o aplicar substàncies herbicides d'origen natural en un balanç sostenibilitat-producció.

La primera part d'aquesta tesi se centra en les cobertes vegetals. D'una banda, s'ha modelitzat amb èxit l'emergència de 18 espècies susceptibles de ser utilitzades com a cobertes vegetals en la vinya, on la incorporació del fotoperíode i la radiació solar han estat claus per a poder desenvolupar, per a cada espècie, un únic model útil per a tot l'any. Per altre banda, s'ha avaluat la utilitat de sembrar diferents espècies com a coberta vegetal durant el període hivernal per a després acabar la coberta amb el "roller-crimper". Aquesta tècnica ha resultat ser més útil que l'habitual picadora per a contenir una infestació de *C. dactylon*.

Pel que fa a la zona sota la línia de ceps, l'ús de compostos com el metabisulfit potàssic sol o barrejat amb àcid pelargònic, i la barreja d'àcid húmnic i fúlvic, han resultat ser eficaços per al control de *C. bonariensis*, si bé, aplicar-los en un estadi fenològic precoç de la mala herba resulta essencial per obtenir la seva màxima eficàcia.

Finalment, l'ús d'encoixinats orgànics amb palla de diferents espècies, amb estelles de pi o amb closca d'ametlla han estat eficaços per al control de males herbes sota la línia de la vinya mentre es mantenia una bona cobertura del sòl amb aquests. En aquest sentit, els dos últims encoixinats (pi i ametlla), s'han mostrat molt més persistents i eficaços en el temps, alhora que s'ha observat un increment en diferents paràmetres de vigor de la vinya, inclòs el rendiment, al comparar-se els dos encoixinats amb mètodes tradicionals com el laboreig mitjançant l'interceps o la segadora.

Resumen

La actual emergencia climática obliga a buscar estrategias más sostenibles para el manejo del suelo del viñedo. En este sentido, el control de las malas hierbas constituye un verdadero reto debido a su gran capacidad de expansión y de ocasionar pérdidas de rendimiento. Entre las especies más competitivas y nocivas que podemos encontrar destacan *Cynodon dactylon* (L.) Pers. y *Conyza bonariensis* (L.) Cronquist. Tradicionalmente, el laboreo ha sido el método más utilizado para manejo de las malas hierbas, aunque su uso favorece el riesgo de erosión, disminuye la materia orgánica y comporta una alta huella de carbono. Por otro lado, el continuo uso de herbicidas de síntesis, han llevado a la aparición de biotipos de malas hierbas resistentes a estos y, debido a su alta toxicidad, su uso se ve ampliamente cuestionado. La presente tesis doctoral plantea diferentes alternativas en el manejo de malas hierbas en viñedo, tanto en la calle como bajo la línea. Los experimentos llevados a cabo, pretenden evaluar y aportar nueva información acerca de la posibilidad de establecer, en el control de malas hierbas, cubiertas vegetales, acolchados orgánicos o aplicar sustancias herbicidas de origen natural dentro de un balance sostenibilidad-producción.

La primera parte de esta tesis se centra en las cubiertas vegetales. Por un lado, se ha modelizado la emergencia de 18 especies susceptibles de ser utilizadas como cubiertas vegetales en el viñedo, donde la incorporación del fotoperiodo y la radiación solar han sido claves para poder desarrollar, para cada especie, un único modelo útil para todo el año. A su vez, se ha evaluado la utilidad de sembrar diferentes especies como cubierta vegetal durante el invierno para luego terminar la cubierta con el “roller-crimper”. Esta técnica ha resultado ser más útil que el habitual pase de picadora para contener una infestación de *C. dactylon*.

En cuanto a la zona bajo la línea de cepas, el uso de compuestos como el metabisulfito potásico solo o mezclado con ácido pelargónico, y la mezcla de ácido húmico y fúlvico, han resultado ser eficaces en el control de *C. bonariensis*, si bien, aplicarlos en un estadio fenológico precoz de la mala hierba resulta esencial para obtener la máxima eficacia.

Finalmente, el uso de acolchado orgánicos con paja de diferentes especies, con astillas de pino o con cáscaras de almendra han sido eficaces para el control de malas hierbas bajo la línea del viñedo, mientras se mantenía una buena cobertura del suelo con estos. En este sentido, los dos últimos acolchados (pino y almendra) se han mostrado mucho más persistentes y eficaces en el tiempo, a la vez que se ha observado un incremento en diferentes parámetros de vigor de las vides, incluido el rendimiento, al compararse esos acolchados con métodos tradicionales como laboreo mediante intercepas o pases de segadora.

Summary

Climate emergency is forcing the search of more sustainable strategies for the soil management in vineyards. In this sense, the control of weeds is a real challenge due to their high expansion capacity and to caused yield losses. *Cynodon dactylon* (L.) Pers. and *Conyza bonariensis* (L.) Cronquist are among the most competitive and harmful weed species that can be found in vineyards. Traditionally, tillage has been the most widely used method for weed management, although its use increases the risk of erosion, decreases organic matter and leads to a high carbon footprint. On the other hand, the continuous use of synthetic herbicides has evolved herbicide-resistant biotypes and, due to their high toxicity, their use is widely questioned. This thesis dissertation proposes different alternatives for weed management in vineyards, both on the alleyways and in the under-vine zone. In order to manage weeds, the aim of the experiments carried out was to evaluate and provide new knowledge about the possibility of establishing cover crops, organic mulches or applying herbicide substances of natural origin in a sustainability-production balance.

The first part of this thesis focuses on cover crops. On one hand, the emergence of 18 species, susceptible to be used as cover crops in vineyards, was modelled. The inclusion of photoperiod and solar radiation was essential for developing a single successful model for each species, applicable throughout the year. On the other hand, the usefulness of sowing different species as cover crops during winter and terminating them with the roller-crimper was evaluated. The termination method with roller-crimper proved to be more successful than the usual shredding for containing *C. dactylon* infestations.

Regarding the under-vine zone, the use of alternative compounds such as potassium metabisulfite alone or mixed with pelargonic acid, and the mixture of humic and fulvic acid, showed to be effective for the control of *C. bonariensis*, although applying them in an early phenological stage of the weed is essential to obtain maximum effectiveness.

Finally, the use of organic mulches, such as straw of different species, chopped pine wood or almond shell, was effective for under-vine weed control as long as high soil cover was achieved with the mulches. In this sense, the last two mulches (pine and almond) showed to be much more persistent and effective over time, while an increase of several parameters of vine vigour, including yield, was observed when comparing these mulches with traditional methods like tillage through in-row tiller or mower passes.

CAPÍTULO 1

Introducción general y objetivos

Importancia del cultivo de la vid

El cultivo de la vid (*Vitis vinífera* L.) ha sido históricamente uno de los más importantes en la Península Ibérica. Los inicios de este cultivo en la parte más occidental del Mediterráneo se sitúan entre finales del s. IX e inicios del s VIII a. C. (Echevarría y Vera-Rodríguez, 2015). Actualmente, sigue siendo uno de los cultivos con más extensión e importancia en Europa, con aproximadamente 3,7 millones de hectáreas, siendo España (26 %), Francia (21 %) e Italia (19 %) los países con mayor superficie de viñedo (OIV, 2019). El sector vitivinícola en España ha mostrado una profunda evolución en el último siglo, marcada por la llegada de la Filoxera a finales del siglo XIX hasta el escenario actual marcado por una necesidad de adaptación a los actuales cambios en cuanto a la demanda de vino. La actividad vitivinícola (incluyendo la viticultura, la elaboración del vino y su comercialización), genera un Valor Añadido Bruto (VAB) total superior a los 23.700 millones de euros anuales (incluyendo los efectos indirecto e inducido), equivalentes al 2,2 % del VAB nacional. En particular, la contribución directa del sector se sitúa por encima de los 11.600 millones de euros. El vino mantiene un importante arraigo territorial, muy visible en las diferentes denominaciones de origen, siendo su entorno de procedencia un apreciado atributo del mismo. Además, su relación con la cultura y la sociedad es ineludible (OIVE, 2020).

Sin embargo, el escenario de la viticultura actual no es ajeno a importantes y singulares retos. En este sentido, los efectos del cambio climático introducen unas condiciones cada vez más adversas para el cultivo de la vid dado que la calidad y el volumen de vino que se podrán obtener en el futuro en España estarán condicionados por la realidad climática vigente. Todo ello obliga a una constante búsqueda de soluciones. Actualmente, el cambio climático, supone la mayor amenaza a la que se enfrenta el sector (Jones y Webb, 2010; Schultz y Jones, 2010) ya que el rendimiento de la vid depende en gran medida del estado hídrico de la planta (Miras-Avalos *et al.*, 2017). Debemos recordar que en España el 60 % de la superficie cultivada se encuentra en secano (MAPA, 2019), y este se caracteriza por una importante variación interanual como consecuencia de la fuerte dependencia de la pluviometría. La mayoría de los viñedos de Europa se encuentran en regiones con clima Mediterráneo, caracterizado por inviernos suaves y relativamente húmedos y veranos calurosos y secos que condicionan el desarrollo del cultivo y la composición de la uva, otorgándole unas propiedades organolépticas características. Sin embargo, estas regiones están clasificadas como áreas críticas frente al cambio climático (Giorgi, 2006) por lo que el impacto en el sector vitivinícola puede ser especialmente dañino.

Manejo de malas hierbas

Asociado al cultivo de la viña y a su manejo siempre han estado presentes las malas hierbas. Estas compiten por agua y nutrientes, llegando a mermar el crecimiento de las cepas y su producción, con lo que su presencia siempre se ha pretendido minimizar, bien sea para evitar tal competencia, porque dificultan las tareas agrícolas o bien por temas estéticos o culturales. El impacto generado por una mala gestión de las malas hierbas puede llegar a ser significativo. De hecho, las malas hierbas –o flora arvense- están reconocidas como la causa biótica con mayor potencial para ocasionar pérdidas de rendimiento en los cultivos, con un promedio estimado de un 34 % del rendimiento anual para los cultivos más extendidos a nivel mundial, valor muy superior a las pérdidas potenciales causadas por plagas (18 %), patógenos (16 %) o virus (3 %) (Oerke, 2006).

Tradicionalmente, el laboreo ha sido la técnica de manejo del suelo más empleada en los viñedos españoles. Consiste en trabajar el terreno en profundidad o en superficie para mantenerlo libre de malas hierbas, facilitar la incorporación de abonos y enmiendas, el enraizamiento profundo de las cepas, la infiltración del agua y la aireación de suelo (Ibañez, 2015). Aunque es un método eficaz para el manejo de las malas hierbas (Steenwerth y Guerra, 2012), son necesarias una gran cantidad de intervenciones a lo largo del año ya que el laboreo lleva a la superficie nuevas semillas que, combinado con el incremento de la mineralización del nitrógeno del suelo por el efecto de la aireación, acaba incrementado los flujos de emergencias de malas hierbas (Bàrberi, 2002). Por otro lado, el laboreo acarrea una serie de consecuencias negativas para el agroecosistema. La constante remoción del suelo incrementa el riesgo de erosión y la pérdida de suelo fértil, reduce el contenido de materia orgánica (Glover *et al.*, 2000, Smith *et al.* 2008), altera la población de microorganismos (Virto *et al.*, 2012), empobrece la estructura del suelo (Abad *et al.*, 2021a) y llega a crear una suela de labor. Además, la agresividad de esta técnica afecta negativamente a las cepas (Cerdan *et al.*, 2010, Prosdocimi *et al.*, 2016), sobre todo a las más jóvenes ya que reduce la presencia de raíces superficiales (Lanini *et al.*, 2011; Smart *et al.*, 2006). A su vez, el elevado consumo de combustible de este manejo genera el doble de huella de carbono que el de pesticidas o fertilizantes (Jradi *et al.*, 2018).

Otra técnica habitual de gestión de las malas hierbas es el uso de herbicidas. A mediados del s. XX, el progreso de los herbicidas de síntesis llevó a un incremento significativo de su uso, ya que eran fáciles de utilizar, de bajo coste y eficientes para controlar las malas hierbas. El éxito de esta tecnología queda reflejado en la cantidad de herbicidas que aún se aplican

anualmente en España. Durante el año 2020, 20.199 t de herbicidas fueron comercializadas, cifra que representa un incremento de un 4 % respecto al año anterior (MAPA, 2020) representando el 26,6 % de todos los fitosanitarios. Si nos fijamos a nivel mundial, su uso representa el 47,5 % de todos los fitosanitarios (De *et al.*, 2014). El uso masivo de herbicidas es el gran responsable de que en 2022 haya, globalmente, 266 especies de malas hierbas que han desarrollado algún tipo de resistencia a 21 de los 31 mecanismos de acción conocidos (Heap, 2022). Si a ello le sumamos la ausencia de nuevos mecanismos de acción herbicida descubiertos en los últimos 20 años (Peters y Streck, 2018), los problemas medioambientales que genera su masiva aplicación (Tourte *et al.*, 2008) y la mayor demanda social hacia una agricultura más sostenible (Harvey y Pilgram, 2011), ponen en cuestión su uso en un futuro próximo, sino ya actual (Heap y Duke, 2018).

Por otro lado, el cambio climático podría favorecer el crecimiento y desarrollo de malas hierbas, con impactos muy negativos sobre los cultivos (Peters *et al.*, 2014). El aumento de las concentraciones de CO₂ estimula el crecimiento de las plantas con metabolismo C₃, las cuales representan el 85 % de todas las especies vegetales, incluidas muchas malas hierbas (Ziska, 2003), a la vez que los cambios en las precipitaciones y las temperaturas pueden alterar la composición y competitividad de las plantas nativas e invasoras. Todo esto dibuja un escenario donde el control químico es cada vez más problemático (Ramesh *et al.*, 2017; Waryszak *et al.*, 2018). El incremento de la concentración de CO₂ y el cambio climático apuntan hacia una reducción de la eficacia de los herbicidas. Esta reducción estaría asociada a cambios medioambientales (temperatura, precipitación y viento) que influirían en la distribución del herbicida al aplicarlo (Ziska, 2016), así como en los efectos directos del CO₂ en la composición bioquímica de las plantas y en las posibles resistencias a los herbicidas (Refatti *et al.*, 2019), afectando, por tanto, a todos los aspectos biológicos de las malas hierbas, incluido su establecimiento, competitividad, distribución y manejo (Bradley *et al.*, 2016; Waryszak *et al.*, 2018). Se apunta (IPCC Secretariat, 2021) que la necesidad de control de malas hierbas se verá incrementada en un escenario de cambio climático, por lo que su vigilancia y monitoreo junto con estrategias eficaces para su control que minimicen el impacto medioambiental, serán esenciales y determinantes en un futuro inmediato.

Entre las diferentes especies de malas hierbas que podemos encontrar en un viñedo, algunas resultan especialmente difíciles de controlar a la vez que muy nocivas. El presente trabajo se centra en dos especies de malas hierbas que han alcanzado gran protagonismo en viñedos en nuestro país, y que presentan singularidades en su biología que obliga a planteamientos de programas de control específicos. Estas especies son *Cynodon dactylon* (L.)

Pers. y *Conyza bonariensis* (L.) Cronquist.

Cynodon dactylon es una gramínea plurianual vivaz de propagación preferentemente vegetativa a través de rizomas y estolones que mantienen las yemas latentes durante el invierno. Está considerada como una de las malas hierbas más nocivas en el mundo (Holm *et al.*, 1977), siendo, a su vez, tolerante a la salinidad y al estrés hídrico, atributos que le confieren una alta capacidad de adaptación a una gran variedad de suelos y climas (FAO, 2022). Además, *C. dactylon* presenta un periodo de desarrollo que comprende desde primavera hasta finales de otoño, por lo que, en climas mediterráneos, compite con la viña por los recursos del suelo, sobre todo por el agua durante los periodos secos. Al ser una gramínea de metabolismo C4, muestra una alta capacidad de desarrollo durante los periodos de alta temperatura y una tasa fotosintética muy alta. Este comportamiento dificulta la posible eficacia de los métodos de control químico. Por otro lado, el laboreo resulta de una eficacia desigual al favorecer la dispersión de rizomas y estolones (Abdullahi, 2002; Fernández, 2003).

Conyza bonariensis está considerada como una de las malas hierbas que mayor expansión ha mostrado a nivel global en las recientes décadas (Bajwa *et al.*, 2016), siendo particularmente importante en cultivos leñosos mediterráneos como frutales, olivar y viñedos, por lo que está catalogada en España como una de las especies introducidas más competitivas y nocivas (Zambrano-Navea *et al.*, 2013). Las aplicaciones de herbicidas bajo la línea del cultivo, y especialmente en campos con riego por goteo, han creado un nuevo escenario para la vegetación herbácea donde *C. bonariensis* se ha visto favorecida (Recasens *et al.*, 2020). Originaria de América del Sur, muestra una altísima fecundidad, llegando a producir alrededor de 100.000 semillas por planta (Wu and Walker, 2004). Además, las semillas pueden germinar a lo largo de todo el año dependiendo de las condiciones ambientales y generar diferentes cohortes, que al convivir al mismo tiempo muestran diferentes estadios fenológicos, hecho que dificulta su control. Cuando las plantas alcanzan el estadio de roseta disminuye de manera significativa su sensibilidad a los herbicidas, por lo que las aplicaciones suelen resultar de muy baja eficacia (Recasens *et al.*, 2020). Su expansión suele estar asociada a cambios en el manejo del suelo, de manera especial al pasar del laboreo intenso al mínimo laboreo o no laboreo (Storrie, 2007; Shrestha *et al.*, 2008). Este hecho, junto a la continua y exitosa dispersión de aquenios por el viento, garantiza la expansión de la población, pudiendo instalarse en diferentes tipos de sistemas y facilitar su germinación superficial. Por otro lado, esta y otras especies de *Conyza* han desarrollado resistencia a diferentes herbicidas en todo el mundo, particularmente a glifosato (Heap, 2022), herbicida ampliamente utilizado en diferentes cultivos leñosos en España, el cual ha ejercido una gran presión de selección sobre distintas especies de malas

hierbas. Se conocen, para *C. bonariensis*, casos de resistencia a glifosato en frutales en Aragón (Langa *et al.*, 2015).

Alternativas al manejo de malas hierbas para una viticultura más sostenible

En España hay actualmente 131.000 ha de viña gestionadas de manera ecológica con aumentos de superficie anuales muy destacados (MAPA, 2019) como reflejo de la actual tendencia del sector. Precisamente es en este tipo de sistemas donde la gestión de malas hierbas está considerada como el mayor problema de producción (Kloen and Daniels, 2000) puesto que se prescinde del uso de herbicidas y los métodos mecánicos no siempre son efectivos.

Uso de cubiertas vegetales

La implementación de una cubierta vegetal en las calles del viñedo constituye una alternativa al laboreo. Si bien su uso en España es aún muy minoritario -algo menor del 5 % de la superficie total de viñedo- muestra una clara tendencia al alza. El mínimo laboreo es aún la principal técnica de manejo del suelo utilizada, aproximadamente un 65,7 % de la superficie total del viñedo, seguido del laboreo tradicional (25,1 %) (MAPA, 2019).

La gestión del suelo mediante una cubierta vegetal presenta numerosas ventajas agronómicas y medioambientales con respecto a la técnica tradicional del laboreo (Ibáñez, 2015). Las principales ventajas se ven reflejadas en la mejora de las características del suelo (Steenwerth y Guerra, 2012; Abad *et al.*, 2021a). El incremento de la materia orgánica es evidente, ya sea mediante la siembra de gramíneas, leguminosas o con la propia flora espontánea (Mattii *et al.*, 2005). Como consecuencia, la estructura del suelo mejora y disminuye la erosión al permanecer el suelo cubierto (Virto *et al.*, 2012; Vrsic *et al.*, 2011). Este manejo resulta de interés en clima mediterráneo porque, ante las cada vez más habituales precipitaciones torrenciales que conllevan la pérdida de suelo fértil por escorrentía, las cubiertas mejoran la infiltración del agua. Además, las cubiertas vegetales permiten el paso temprano de maquinaria tras episodios de lluvia (Ovalle *et al.*, 2007). El incremento de la biodiversidad es también manifiesto, tanto en el propio bioma del suelo como en las poblaciones de artrópodos, pájaros y pequeños mamíferos (Abad *et al.*, 2021a). Mediante una buena gestión de la cubierta, se favorece el mantenimiento de un óptimo balance entre el desarrollo vegetativo de la cepa y el desarrollo de la uva a través del control de un posible exceso de vigor de los pámpanos (Abad *et al.*, 2021b), esto, desde el punto de vista fitosanitario, disminuye los riesgos de ataques de podredumbres a través de un mejor microclima de racimos (Valdés-Gómez *et al.*, 2008). Por otro lado, la cubierta vegetal puede llegar a ejercer presión sobre las malas hierbas favoreciendo

su control, ya sea mediante un efecto de competencia por recursos o por el efecto alelopático causado por los exudados de hojas y raíces durante el desarrollo de las cubiertas o durante su posterior descomposición (Farooq *et al.*, 2011; Sturm *et al.*, 2018).

No obstante, existen también desventajas ante la presencia de cubiertas vegetales. Entre ellas cabe destacar la competencia por agua y nutrientes con las plantas de vid y el mayor riesgo de heladas primaverales (Steenwerth and Guerra, 2012), por lo que es necesario planificar cuidadosamente para cada zona su incorporación en el viñedo. Si bien, a nivel general, las cubiertas vegetales se consideran como un elemento de calidad en el viñedo, donde los beneficios se sobreponen a las desventajas (Salazar y Melgarejo, 2005).

Como cubiertas vegetales, podemos considerar las cubiertas espontáneas, que serían 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 y pudiendo favorecerse la selección de unas u otras especies. En estas situaciones existe el riesgo que el manejo favorezca la presencia de especies que muestren más un comportamiento nocivo como especies infestantes que como elementos vegetales de la propia cubierta. Así, el mantenimiento de una cubierta espontánea gestionada mediante picadora, puede favorecer especies plurianuales poco deseadas, que se regeneran a partir de yemas basales como *Aster squamatus* (Spreng.) Hieron. o *Plantago lanceolata* L., o bien especies con una clara capacidad de expansión vegetativa mediante órganos subterráneos de propagación como *C. dactylon* (Valencia-Gredilla *et al.*, 2020).

Si la cubierta vegetal es sembrada, es importante que la especie o especies seleccionadas cumplan los requisitos para lograr el objetivo que se plantee (evitar la erosión, competir contra las malas hierbas). A la hora de seleccionar la especie a sembrar es necesario tener en cuenta, entre otros, varios atributos como la velocidad de establecimiento y la potencial biomasa. En este sentido, los modelos de emergencia pueden ser muy útiles porque permiten predecir el establecimiento de las especies seleccionadas (Cabrera-Pérez *et al.*, 2022). Los modelos de emergencia se han desarrollado con éxito en multitud de cultivos (Finch-Sauvage *et al.*, 1998; Guérif *et al.*, 2001; Porter and Rayner, 1984) y en especies de malas hierbas (Bajwa *et al.*, 2015; Royo-Esnal *et al.*, 2020; Sousa-Ortega *et al.*, 2020), pero son pocos los casos en los que se han desarrollado en especies con el propósito de su implementación como cubierta vegetal (Cabrera-Pérez *et al.*, 2022; Tribouillois *et al.*, 2018).

Una vez la cubierta vegetal se aproxima a su final de ciclo, el método más común para eliminarla es mediante el uso de picadora, a pero que trocea la cubierta dejando el residuo como acolchado y descomponiéndose rápidamente (Sims and Frederick, 1970; Bremer *et al.*, 1991). Recientemente, el uso del “roller-crimper” se está popularizando, sobre todo en cultivos

extensivos y hortícolas, ya que, al mantener la parte aérea de la planta conectada al suelo mediante las raíces, permite crear una capa uniforme y densa de acolchado que actúa, más eficazmente y durante más tiempo, como barrera física para evitar la proliferación de malas hierbas (Ackroyd *et al.*, 2019). El “roller-crimper” está formado por un rodillo muy pesado provisto de láminas acopladas a lo largo de este, el cual permite doblar y presionar cada planta por varios puntos a lo largo del tallo y así impedir su posterior levantamiento sin llegar a cortarla, lo que generará un progresivo y lento secado de la planta estando aun enraizada.

En cuanto a la zona bajo la línea de las cepas, se ha evaluado también la posibilidad de establecer una cubierta vegetal, si bien en esta zona existe más riesgo de que el rendimiento se vea afectado (Hickey *et al.*, 2016; Karl *et al.*, 2016). Esta estrategia puede ser una alternativa interesante en viñedos con riego o en zonas con alta pluviometría, donde la humedad no es un factor limitante (Abad *et al.*, 2020; Chou y Vanden Heuvel, 2018; Penfold *et al.*, 2018).

Uso de bioherbicidas bajo la línea de cepas

En párrafos anteriores se ha mencionado la dificultad, en viñedos convencionales, de controlar especies de malas hierbas con capacidad potencial de generar resistencias a herbicidas, tales como *C. bonariensis*, y sobre todo bajo la línea de cepas en sistemas de no laboreo. En estas situaciones, son pocas las alternativas disponibles hoy en día. Las recién mencionadas cubiertas vegetales, los acolchados o los bioherbicidas podrían ser algunas de ellas. Los bioherbicidas -definidos como sustancias de origen natural que tienen como objetivo reducir las poblaciones de malas hierbas sin dañar el medioambiente (Bailey, 2014)- son potenciales herramientas para incorporar en el manejo integrado de malas hierbas. Uno de los principales problemas de estos productos es la baja actividad herbicida en comparación con los de síntesis (Bordin *et al.*, 2021). Por ello los bioherbicidas son poco utilizados y pocos productos han conseguido tener una larga trayectoria en el mercado (Cordeau *et al.*, 2016). Sin embargo, cuando existen problemas concretos como resistencias a los herbicidas de síntesis, la necesidad de desarrollar nuevas tecnologías para el control de malas hierbas resulta necesaria y evidente (Bolda *et al.*, 2016). Si tenemos en cuenta que el desarrollo de productos de origen natural se ha centrado sobre todo en el control de plagas y enfermedades, queda un camino esperanzador para el desarrollo de productos dirigidos al control de malas hierbas (Seiber *et al.*, 2014). No obstante, los productos naturales se enfrentan a muchas trabas, sobre todo durante el proceso de registro debido a la falta de datos toxicológicos para su uso a escala comercial (Pavela y Benelli, 2016). Algunos productos como el ácido acético o el ácido pelargónico ya se

han usado en algunos cultivos para el control de malas hierbas, pero con eficacias muy variables según la especie diana: entre un 5 % y un 100 % en el primer caso (Webber *et al.*, 2018) y entre un 6 % y un 65 % en el segundo (Pline *et al.*, 2000), lo que pone de manifiesto su dificultad para un uso general. Cabe destacar que no hay ningún producto de origen natural autorizado para utilizar como herbicida en el cultivo ecológico de la vid.

Uso de acolchados bajo la línea de cepas

Si el uso de cubiertas vegetales es minoritario en España, el uso de acolchados en viñedos es aún más anecdótico, seguramente por varios factores como puede ser el coste de establecimiento, la falta de experiencia en su manejo y la falta de estudios que demuestren su efectividad. Un acolchado se define como cualquier material instalado sobre la superficie del suelo principalmente para evitar la presencia de malas hierbas, evitar la erosión, y preservar la humedad del suelo, entre otros propósitos. Los acolchados pueden ser orgánicos o inorgánicos, siendo los primeros en los que nos centraremos en esta tesis doctoral. El acolchado orgánico ha demostrado ser una práctica agronómica sostenible con efectos inhibidores en la emergencia de las malas hierbas por encima de los herbicidas o del laboreo (Steinmaus *et al.*, 2008). Los acolchados crean una barrera física que impide la penetración de la luz (Elmore *et al.*, 1998). Además, también tienen efectos alelopáticos a través de los compuestos liberados en el suelo que pueden reducir la emergencia de malas hierbas hasta un 80 % (Dhima *et al.*, 2006). Por lo tanto, el efecto positivo para el control de malas hierbas parece claro, si bien es cierto que un control eficaz va estrechamente relacionado con la duración del acolchado sobre el suelo y sobre el grosor instalado, influenciado por el tipo de material y su composición (Bremer *et al.*, 1991; Goh y Tutua, 2004; Teasdale *et al.*, 1991). Así, encontrar materiales adecuados, que sean duraderos y eficaces resulta clave para que los elevados costes iniciales sean amortizados en el tiempo.

Aparte del efecto sobre las malas hierbas, los acolchados orgánicos también pueden ser beneficiosos para el suelo. Al igual que las cubiertas vegetales, los acolchados protegen el suelo de la erosión, mantienen la humedad y mejoran la infiltración, ayudan a incrementar la materia orgánica y, en definitiva, la biodiversidad que puede albergar (Davies *et al.*, 2011; Varga y Májer, 2004; Steenwerth y Guerra, 2012). A estos beneficios se suman los que se pueden atribuir a las propias vides, ya que los acolchados, contrariamente a las cubiertas vegetales, no ejercen competencia alguna por recursos, y pueden, de hecho, mejorar el estado hídrico de estas al preservar mejor la humedad del suelo (Fraga and Santos, 2018). A su vez, las raíces de la vid

pueden proliferar más fácilmente por la superficie del suelo, mejorando la captación de nutrientes, con un efecto positivo en el rendimiento y composición de la uva (Mundy y Agnew, 2002; Linares-Torres *et al.*, 2018; Buesa *et al.*, 2021). Esto se traduce en un uso más eficiente del agua (López-Urrea *et al.*, 2020), clave para afrontar escenarios futuros en un contexto de emergencia climática (Fraga y Santos, 2018).

El escenario aquí planteado acerca las alternativas en el manejo de malas hierbas en viñedo, tanto en la calle como bajo la línea, y constituye el reto principal de la presente tesis doctoral. Los experimentos llevados a cabo con este fin, pretenden evaluar y aportar nueva información acerca de la posibilidad de establecer, en el control de malas hierbas, cubiertas vegetales, acolchados orgánicos o aplicar sustancias herbicidas de origen natural en viñedos dentro de un balance entre sostenibilidad y producción.

Objetivos

El objetivo general de esta tesis doctoral titulada “Weed management strategies based on cover crops, mulches and bioherbicides in Mediterranean vineyards” se centra en evaluar el potencial que ofrecen diferentes técnicas alternativas de manejo del suelo de los viñedos para controlar las malas hierbas y estudiar su efecto sobre las vides.

Para alcanzar este objetivo general, se establecen los siguientes objetivos específicos:

1. Modelizar la emergencia de 18 especies susceptibles de ser utilizadas como cubiertas vegetales en viña para poder predecir su establecimiento.
 - 1.1. Elaborar diferentes modelos sujetos a los siguientes factores: temperatura, humedad y luz.
 - 1.2. Validar estos modelos con datos de otros estudios de zonas agroclimáticas distintas.
2. Evaluar dos métodos distintos de manejo de las cubiertas vegetales en viñedo sobre la presencia de *C. dactylon*.
 - 2.1. Evaluar, tras su siembra, el ritmo de establecimiento y la capacidad de cobertura del suelo de siete cubiertas vegetales.
 - 2.2. Estimar el efecto de las cubiertas sembradas sobre las especies de malas hierbas presentes en primavera.

- 2.3. Seguir la evolución del recubrimiento de *C. dactylon* en las distintas cubiertas vegetales en función de su finalización: mediante picadora o “roller-crimper”.
3. Evaluar el efecto herbicida de diferentes materias activas alternativas para el control de *C. bonariensis* bajo la línea de cepas.
 - 3.1. Elaborar las curvas dosis-respuesta de los compuestos probados sobre dos estadios fenológicos distintos de la mala hierba.
 - 3.2. Evaluar la eficacia de los distintos compuestos sobre el recubrimiento y biomasa de *C. bonariensis* en condiciones de campo durante tres campañas.
4. Seguir la evolución de distintos acolchados orgánicos (astilla de pino y cáscara de almendra) instalados bajo la línea de cepas.
 - 4.1. Comparar el efecto, sobre las malas hierbas, de distintos materiales usados como acolchado respecto al tradicional pase del intercepas.
 - 4.2. Comparar el efecto, sobre las malas hierbas, de dos grosores distintos de cada uno de los acolchados estudiados y compararlo con el pase del intercepas.
 - 4.3. Comparar el efecto de un acolchado de pino, como alternativa a la aplicación de glifosato, sobre la emergencia, recubrimiento y biomasa de *C. bonariensis*.
5. Evaluar el efecto de distintos acolchados orgánicos instalados bajo la línea de cepas sobre distintos parámetros de la vid y del suelo, respecto a los tradicionales pases de segadora o intercepas.
 - 5.1. Evaluar el recubrimiento de las malas hierbas en función de los distintos manejos realizados.
 - 5.2. Seguir la evolución de la temperatura del suelo a 7 cm de profundidad y analizar las propiedades de este en función de los manejos llevados a cabo.
 - 5.3. Estudiar el efecto de los cuatro manejos sobre el estado hídrico de las cepas, el crecimiento de pámpanos, el rendimiento, el peso de poda, el estado sanitario del cultivo y el estado nutricional de las hojas.
 - 5.4. Incorporar la tecnología LiDAR para estimar la evolución del dosel foliar de las vides en función de los distintos manejos realizados.

Metodología

Para alcanzar los distintos objetivos, se llevaron a cabo ensayos de campo y trabajos experimentales en invernadero y laboratorio. Con los resultados obtenidos se han elaborado cinco artículos que se desglosan en los siguientes capítulos:

Chapter 2: Modelización de la emergencia de 18 especies susceptibles de ser utilizadas como cubiertas vegetales en viña (Objetivo 1).

Para el Objetivo 1, se planteó un ensayo en los campos experimentales del campus ETSEA de la Universidad de Lleida (41° 37' 34.5" N 0° 35' 49.7" E ETRS89) donde se sembraron 100 semillas de las 18 especies estudiadas en macetas enterradas y en cuatro repeticiones. Se hicieron cuatro siembras distintas: en otoño de 2018 y de 2019 y en primavera de 2019 y de 2020 donde periódicamente se hicieron conteos de las nuevas emergencias. Se obtuvieron datos de distintas estaciones y años para poder elaborar de manera fiable modelos de emergencia. También se recopilaron los datos meteorológicos de la estación más cercana y se hizo una estimación de la temperatura y humedad del suelo mediante el programa “Soil Temperature and Moisture Model” (STM²) (Spokas y Forcella, 2009). Los resultados de este ensayo están publicados en: “*European Journal of Agronomy* 2022, 132, 126413” y recogidos en el *Chapter 2*.



Figura 1: Emergencias de *Camelina sativa* (L.) Crantz. (izquierda) y vista del ensayo recién instalado (derecha) en parcelas del campus de la ETSEA.

Chapter 3: Cubiertas vegetales gestionadas con “roller-crimper” para el manejo de Cynodon dactylon en las calles del viñedo (Objetivo 2).

Para el Objetivo 2 se planteó un ensayo durante dos campañas en una parcela de Raimat (41° 40' 22.5" N, 0° 29' 25.3" E ETRS89) con una alta infestación de *C. dactylon*. Se sembraron siete cubiertas vegetales: 1) *Avena strigosa* (L.) Schreb, 2) *Hordeum vulgare* (L.), 3) *Lolium multiflorum* Lam., 4) *Phacelia tanacetifolia* L., 5) *Sinapis alba* (L.) Rabenh. y 6) *xTriticosecale*

sp. en el mes de noviembre de 2019 y 2020, incluyendo también 7) una cubierta espontánea como testigo, para comparar el efecto de la picadora y del “roller crimper” sobre la posterior evolución de *C. dactylon*. Para hacer el seguimiento de la evolución tanto de la cubierta vegetal como de *C. dactylon*, cada una de las tres repeticiones de 50 m de largo se dividió en 5 zonas y en cada una de ellas se realizó la estimación porcentual de su recubrimiento. Los resultados están enviados a la revista “*Weed Research*” y recogidos en el *Chapter 3*.



Figura 2: Estado de la cubierta de *Lolium multiflorum* en abril de 2020 (izquierda) y aspecto de la cubierta de *Hordeum vulgare* una vez pasado el “roller-crimper” en mayo de 2020 (derecha).

Capítulo 4: Efecto herbicida de diferentes compuestos alternativos para el control de C. bonariensis bajo la línea de cepas (Objetivo 3).

Para el objetivo 3 se plantearon dos ensayos. Para realizar las curvas dosis-respuesta se recolectaron semillas de la población de *C. bonariensis* de un viñedo de Raimat, en Lleida (41° 39' 26.8" N, 0° 31' 10.3" E ETRS89), se hicieron germinar en bandejas con turba y se trasplantaron en pequeñas macetas para posteriormente aplicar los distintos productos estudiados a diferentes dosis y en dos estadios fenológicos distintos. Pasados 28 días desde la aplicación, se pesó la biomasa aérea de las plantas supervivientes de cada una de las macetas por separado y se elaboraron las curvas. Para el ensayo de eficacia, se aplicaron los productos en un viñedo comercial de Raimat con infestación de *C. bonariensis* bajo la línea de cepas. La parcela de estudio fue distinta para cada una de las tres campañas de seguimiento. Se estimó la cobertura de *C. bonariensis* antes y después de cada aplicación. Los resultados de estos ensayos están publicados en: “*Agronomy* 2022, 12, 960” y recogidos en el capítulo 4.



Figura 3: Evolución de las plántulas de *Conyza bonariensis* después de una aplicación de ácido pelargónico (izquierda) y efecto del mismo en campo (derecha).

Chapter 5: Acolchados orgánicos como alternativa al manejo de malas hierbas bajo la línea de cepas (Objetivo 4).

Para este objetivo, se plantearon tres experimentos distintos durante tres campañas consecutivas en diferentes parcelas de un viñedo de Raimat, en Lleida. En cada campo experimental se instalaron los acolchados al principio del ensayo, sin hacer ninguna otra intervención: 1) acolchado de paja de *Medicago sativa* L., 2) acolchado de paja de *Festuca arundinacea* (L.) Schreb, 3) acolchado de paja de *Hordeum vulgare* L., 4) acolchado de astillas de *Pinus sylvestris* L., para comparar el efecto sobre las malas hierbas respecto el pase de intercepas o la aplicación de herbicida. Se hizo un seguimiento del recubrimiento de las malas hierbas a lo largo de cada campaña para cada tratamiento y ensayo, dando un valor porcentual para cada una de las especies. Los resultados están enviados a la revista “*Agroecology and Sustainable Food Systems*” y recogidos en el *Chapter 5*.



Figura 4: Acolchado de paja de *Hordeum vulgare* (izquierda) y acolchado de astillas de pino (derecha).

Chapter 6: Estrategias alternativas al control de malas hierbas bajo la línea de cepas: efecto en el desarrollo de las vides (Objetivo 5).

En este experimento se estudió, en una parcela de un viñedo de Raimat, en Lleida (41°

39° 25' N, 0° 27' 1" E ETRS89) durante tres campañas, el efecto sobre las vides de cuatro estrategias de manejo de malas hierbas bajo la línea de cepas: 1) laboreo con intercepas, 2) mantenimiento con segadora, 3) acolchado de cáscara de almendra *Prunus dulcis* [Mill.] D.A. Webb, 4) acolchado de astillas de pino *Pinus sylvestris* L. Durante cada campaña y para cada uno de los manejos, se hizo el seguimiento del estado hídrico mediante medidas de potencial hídrico de peciolo con una cámara Scholander, se midieron los pámpanos periódicamente, así como el rendimiento antes de cosecha y el peso de poda al final de campaña. Las medidas se tomaron en nueve cepas de cada una de las tres repeticiones (filas) de cada tratamiento, configurando un total de 108 cepas de seguimiento. También se instalaron sensores de temperatura a 7 cm de profundidad en el suelo para cada tipo de manejo y, cada campaña, se escaneó la parcela dos veces con un sensor de infrarrojos con la tecnología LiDAR para la medición del dosel foliar. Los resultados están en revisión en la revista “*European Journal of Agronomy*” y recogidos en el *Chapter 6*.



Figura 5: Escaneo de la parcela con LiDAR (izquierda) y estado del acolchado de cáscara de almendra (derecha).

Discusión general y conclusiones

En el *Chapter 7* se realiza una discusión de los resultados obtenidos en los distintos capítulos con el fin de analizar conjuntamente las diferentes estrategias disponibles para el control de malas hierbas en viña. Finalmente, en el *Chapter 8* se enumeran las conclusiones que se han alcanzado en la presente tesis doctoral.

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CHAPTER 2

Emergence modelling of 18 species susceptible to be used as cover crops in Mediterranean vineyards

Published in: *European Journal of Agronomy* **2022**, 132, 126413

Emergence modelling of 18 species susceptible to be used as cover crops in Mediterranean semiarid vineyards

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Abstract

Cover Crops (CC) are increasingly appreciated in vineyards because they can provide ecosystems services, such as preventing soil erosion and compaction, increasing soil organic matter or, controlling weeds. Many species from different botanical families can be used depending on the final purpose of the CC, but their successful establishment in Mediterranean semiarid conditions of NE Spain can be challenging. Therefore, it is mandatory to understand and be able to predict the emergence patterns of the chosen species as their success is crucial to achieve a good soil cover. Different models based on thermal time (TT), hydrothermal time (HTT), photohydrothermal time (PhHTT) and photosolar hydrothermal time (PhSHTT) have already been used in crops and weeds for this purpose. In this paper, these four models have been developed for the 18 species susceptible of being CC, some of them being successfully validated with independent data from southern France. Results suggest that, although TT and HTT based models are accurate, their precision is improved when light is included ($R^2 > 0.9$). Models including light could be widespread used in some species as the successful validation with independent data demonstrates. These models considerably contribute to inter-row management in vineyards as decision support systems (DSS) tools to predict CC establishments.

Keywords: living mulch, soil temperature, thermal time, hydrothermal time, photohydrothermal time, photosolar hydrothermal time.

Introduction

Vineyards are among the most important perennial crops in Mediterranean climates (Prosdocimi *et al.*, 2016) with 964037 ha cultivated in Spain in 2020 (MAPA, 2020). Most of this cropped area is traditionally managed, which means the inter-row space is managed with tillage, leaving the soil bare most of the year. However, coverless (bare) soil exacerbate erosion, compaction and loss of soil structure, decreases soil organic matter, water-holding capacity and infiltration, while biological activity and soil chemical properties are negatively affected (Durán Zuazo *et al.*, 2006; Ibáñez-Pascual, 2014; Polge de Combret-Champart *et al.*, 2013; Smith *et al.*, 2008), especially in Mediterranean areas, due to their topographic, edaphic and climatic conditions. Soil degradation is combined with the social concern about the food-energy-environment trilemma with the demand to low input and more sustainable agroecosystems (Harvey and Pilgrim, 2011; Horlings and Marsden, 2011). Soil management techniques have undergone important changes over the last decades in other wine regions, from the maintenance of the soil completely free of non-crop plants to the maintenance of annual or perennial vegetation in the inter-rows (Dastgheib and Frampton, 2000; Pardini *et al.*, 2002). Cover crops (CC) are known to provide several ecosystem services that reduce the problems that tillage causes in the soil, like erosion or a decrease in water infiltration rates (Napoli *et al.*, 2017; Ruiz-Colmenero *et al.*, 2013) among other benefits. Cover crops are also used with varied success to regulate vine vegetative growth and vigour (Dry and Loveys, 1998; Hatch *et al.*, 2011), and improve vine health by reducing the risk of grey mould (Valdés-Gómez *et al.*, 2008). Besides, CC contribute to the control of competitive weeds (Valencia-Gredilla *et al.*, 2020) that cause severe competition during the first few years after planting in areas where vine root growth is limited due to shallow or compacted soil (Haviland *et al.*, 2019). Given this wide range of ecosystem functions of CC, it is important to know the emergence behaviour of the species selected for an optimal establishment and to achieve the benefits provided by CC.

Emergence models have been developed for many plant species, either to improve their establishment, especially in crops (Finch-Savage *et al.*, 1998; Guérif *et al.*, 2001; Porter and Rayner, 1984), or for their management and control, especially in weeds (Bajwa *et al.*, 2015; Royo-Esnal *et al.*, 2020; Sousa-Ortega *et al.*, 2020a). The objective of these models is to be able to describe the emergence based on environmental factors, mainly soil temperature (thermal time, TT) and a combination of TT and soil moisture (hydrothermal time, HTT) so that they can be used as tools for crop or weed management (Forcella *et al.*, 2000; Roman *et al.*, 2000). In some cases, light, as photoperiod and solar radiance, has also been considered,

resulting in new units (photohydrothermal time, PhHTT; photosolar hydrothermal time, PhSHTT), which give more accurate descriptions of the emergences. Temperature and moisture have worked well in models describing germination of species that show autumn and spring emergence flushes (Royo-Esnal *et al.* 2010a), so it is expected that it will also work well to model CC, which can be sown in these two seasons. Light, which greatly differs between autumn and spring, may also be an important factor to take into account and may determine the need for developing one model for both seasons or one for each season (Royo-Esnal *et al.*, 2015a, 2015b).

There are few works that have modelled the emergence of CC species, like that from Tribouillois *et al.* (2018), who underline the importance of soil moisture in the accuracy of these models. For this reason, in this work, the emergence of 18 species that are susceptible to be used as CC in Mediterranean vineyards is studied and their patterns subjected to the above-mentioned factors (temperature, moisture and light), with the aim to develop models that can contribute to the decision support systems (DSS) of CC management in vineyards under semiarid conditions of North-Eastern Spain.

Material and methods

Plant material

The 18 studied species were supplied by commercial seeds companies that produced them in 2018, most of them close to the experimental site (Table 1).

Table 1. Species used in the present experiment, their variety (if available), the weight of 1000 seeds, the supplier company and their production site (origin).

Species	Variety	1000 seed weight (g)	Company	Origin
<i>Avena strigosa</i> (L.) Schreb	Saia 6	21.28	Semillas Fitó S.A.	NE Spain
<i>Bromus catharticus</i> (L.) Vahl	Jeronimo	8.92	Semillas Batlle S.A.	NE Spain
<i>Camelina sativa</i> (L.) Crantz	GP204	1.19	Camelina Company S.A	NE Spain
<i>Fagopyrum esculentum</i> Moench	-	27.83	Semillas Silvestres S.L.	South Spain
<i>Festuca arundinacea</i> (L.) Schreb	Fawn	3.05	Semillas Fitó S.A.	NE Spain
<i>Festuca ovina</i> L.	Ridu	0.88	Rocalba S.A.	NE Spain
<i>Hordeum vulgare</i> L.	Meseta	44.7	Semillas Batlle S.A	NE Spain
<i>Lolium multiflorum</i> Lam.	Elirix	1.93	Rocalba S.A	NE Spain
<i>Lolium perenne</i> L.	Sun	1.81	Semillas Fitó S.A.	NE Spain
<i>Onobrychis viciifolia</i> Scop.	Arinda	21.31	Semillas Batlle S.A.	NE Spain
<i>Phacelia tanacetifolia</i> L.	Lilla	1.73	Rocalba S.A.	NE Spain
<i>Plantago coronopus</i> L.	-	0.27	Semillas Cantueso S.L	South Spain
<i>Plantago lanceolata</i> L.	-	1.19	Semillas Cantueso S.L	South Spain
<i>Sinapis alba</i> (L.) Rabenh.	Accent	5.66	Rocalba S.A.	NE Spain
<i>Trifolium incarnatum</i> L.	Contea	6.59	Rocalba S.L	NE Spain
<i>Triticum spelta</i> L.	Benedeto	53.67	Semillas Batlle S.A.	NE Spain
<i>Vicia sativa</i> L.	Libia	49.31	Semillas Fitó S.A.	NE Spain
<i>Vulpia myuros</i> (L.) C.C. Gmelin	-	0.94	Semillas Fitó S.A.	NE Spain

Experimental site

The experiment was carried out in autumn 2018 and 2019 and in spring 2019 and 2020 in the experimental fields of the University of Lleida, in North-Eastern Spain (41°37' N, 0°35' E ETRS89). Soil texture was 31.25 % sand, 38.01 % silt, 30.74 % clay, with pH of 8.4 and 0.95 % of organic matter. According to Köppen-Geiger classification, the climate is cold semiarid (*BSk*) with an average annual precipitation of 374 mm, and a mean temperature of 14.8°C (average min of 8.0 °C and average max of 22.4 °C).

Weather data

Daily rainfall (l/m^2), maximum and minimum air temperatures (°C) and solar radiance (MJ/m^2) were obtained from a meteorological station (Lleida - La Femosa) situated 5 km away from the experimental site. All weather data as well as daylight hours can be consulted in the agrometeorological service www.ruralcat.cat. The CC emergence period lasted less than two months in each sowing date, for this reason, in order to show the environmental conditions, the weather data that have been considered is that of the two months immediately following sowing in each season and year.

Experimental design

A pot experiment was carried out in a randomized complete block design with four replicates. 100 seeds/pot of the 18 species separately were sown and distributed between 0-2 cm depth in pots of 19 cm of diameter x 22 cm deep and 6237 cm^3 . Each pot was filled with soil and buried to simulate field conditions. The upper 4 cm soil layer was previously sterilized at 120 °C during 24 hours. Seeds were sown on 5 December 2018 (sowing date was delayed due to inclement weather), 7 March 2019, 16 October 2019 and 26 March 2020. Pots were not irrigated, so that emergence was conditioned mainly by weather conditions. Emergences were sampled every 2-3 days by destructive counts until no more were detected. Due to lack of emergences, data of *F. esculentum* and *P. coronopus* in autumn 2018 and spring 2020, respectively, were excluded for the analysis, because they could bias the interpretation of the results.

Models development

Data of the autumn and spring emergence periods of the two years were used for the models development. The first model was obtained from the cumulative soil thermal time (TT) (Gupta, 1985).

$$TT = \sum T_t - T_b \quad (1)$$

T is the daily average soil temperature at 1 cm depth and T_b is the base temperature for seedling emergence for each species. $TT = 0$ when $T < T_b$.

A second model was developed based on the equation described by Roman *et al.* (2000), where simulated TT and water potentials (hydrotime, HT) were used to estimate hydrothermal time (HTT):

$$HTT = \sum HT_t \times TT_t \quad (2)$$

where $HT = 1$ when $\psi > \psi_b$, otherwise $HT = 0$; and $TT = T - T_b$ when $T > T_b$, otherwise $TT = 0$. Water potential (ψ) is the daily average water potential in the soil layer at 4 cm depth; ψ_b is the base water potential (in MPa) for seedling emergence (García *et al.*, 2013; Martinson *et al.*, 2007). With these formulas, growing degree days are accumulated only when water potential and temperature conditions were higher than the base water potential and the base temperature. A soil depth of 1 cm was chosen for soil temperature because seeds were buried at that layer; 4 cm depth was chosen for water potential because seedlings must elongate radicles to a certain depth to absorb enough water for emergence (Royo-Esnal *et al.*, 2019). The TT and HTT were estimated using the Soil Temperature and Moisture Model (STM^2) (Spokas and Forcella, 2009). The sowing date each season was chosen as the starting point for TT and HTT accumulation; T_b for each species was obtained from literature (see Table 5); and ψ_b was determined by modifying the value until the highest accuracy (R^2) was obtained for the relationship between HTT and cumulative emergence of each species (Royo-Esnal *et al.*, 2010b).

In order to improve model accuracy and compensate for differences in emergence patterns at the four sowing dates, a third model was developed based on HTT corrected by proportional daylight hours and convert it into photohydrothermal time, $PhHTT$, considering daylight of 24 h = 1, 12 h = 0.5 and 0 h = 0 (Royo-Esnal *et al.*, 2015b). Thus, HTT was multiplied by its corresponding proportional day length as follows:

$$PhHTT = \sum (HTT_t \times D_t) \quad (3)$$

Where HTT_t is the hydrothermal time in day t , and D_t is the proportional day length in day t . Finally, daily solar radiance (SR) was also included in a fourth model, creating the photosolar hydrothermal time ($PhSHHT$) with the combination of $PhHTT$ with solar radiance (Royo-Esnal *et al.*, 2015b, 2019), estimated as follows:

$$PhSHHT = \sum \frac{HTT_t \times SR_t}{\ln(SR_t) \times 100} \times D_t \quad (4)$$

Where HTT_t is the hydrothermal time in day t , SR_t is the solar radiance in day t , and D_t is the proportional day length in day t . The functional relationship between cumulative emergence

and TT, HTT, PhHTT and PhSHTT was described by a three-parameter sigmoidal (log-logistic) model as follows:

$$y = \frac{a}{1 + e^{-\left(\frac{x-x_0}{b}\right)}} \quad (5)$$

Where y is the percentage of emergence, x is the time expressed as TT, HTT, PhHTT or PhSHTT, and a , b and x_0 , are empirically derived constants: a , is the maximum percentage of the recorded emergence, b is the rate of increase and x_0 is the TT, HTT, PhHTT or PhSHTT required to obtain 50% of maximum emergence. To make this sigmoidal model simpler, a was assumed to be 100% for each species in each season. Fitting of the three-parameter sigmoidal function for cumulative emergence was performed using SigmaPlot 12.0 (Sistat Software, Inc, San José, CA, USA). The accuracy of models was evaluated with R^2 and the root mean-square error (RMSE) values for each sowing date emergence observations and considering the mean RMSE of the four of them. RMSE is estimated with the following formula:

$$RMSE = \sqrt{1/n \sum_{i=1}^n (x_i - y_i)^2} \quad (6)$$

where x_i represents observed cumulative percentage seedling emergence, y_i is the predicted cumulative seedling emergence, and n is the number of observations (Mayer and Butler, 1993). RMSE provided a measurement of the typical difference between predicted and actual values in units of percentage seedlings emergence. Low RMSE values indicate that emergence model fit had been optimized.

Supplementary data is provided with the fitted models separated by seasons (autumn and spring) for the species that clearly had two differentiated emergence rhythms.

Emergence model validation

The models of seedling emergence were validated with data published by Tribouillois *et al.* (2018) with the observed emergence of *A. strigosa*, *L. multiflorum*, *P. tanacetifolia*, *S. alba*, *T. incarnatum* and *V. sativa* in an irrigated experimental field in Auzeville, south of France, (43° 31' N, 1° 34' E ETRS89) in August 2012. Weather data was collected from NASA POWER (<https://power.larc.nasa.gov/>) and hydrothermal time was estimated with soil temperature and moisture data from STM². Predicted emergences were superposed on observed seedling emergences, and the differences between them were assessed by root mean-square error predictor (RMSEP), which is calculated with the same formula as (6).

Statistical analysis

Differences in the total percentage of emergence among seasons (autumn vs spring) and between years (2018-2019 vs 2019-2020) were analysed with two-way ANOVA. If there was any interaction between factors, these were separated and the statistical program automatically

applied a Least Significant Difference (LSD) for the comparison between means. Significances were considered at $p < 0.05$ level. When normality and/or homogeneity of variance tests failed, Kruskal Wallis test was performed. These analyses were performed also with Sigmaplot 12.0.

Results

Weather data

The two years differed considerably in temperature and precipitation (Table 2). The 2018-2019 season was colder and much dryer than 2019-2020. Number of rainy days (considered > 0.2 mm) also differed between years and seasons (6 in autumn 2018, 12 in spring 2019, 12 in autumn 2019 and 20 in spring 2020) which is reflected in the soil water potential (Figure 1) as long wet or dry periods. Solar radiation was always higher in spring (19-16.3 MJ/m²) than in autumn (6-10.5 MJ/m²). For modelling purposes, such great natural variability in magnitudes of explanatory variables is highly desirable.

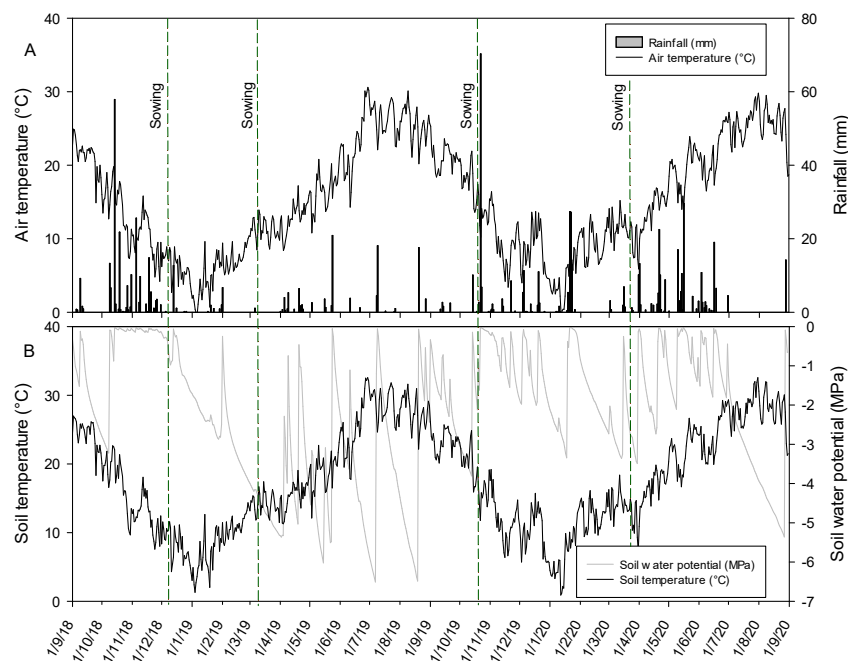


Figure 1. Mean daily air temperature and precipitation (A) and mean daily soil temperature and soil water potential at 1 cm and 4 cm depth, respectively (B), estimated with the STM² (Spokas and Forcella, 2009). Vertical dotted lines indicate sowing dates.

Table 2. Mean temperature (Tm), total precipitation (P), days of precipitation (Pd), and mean solar radiance (SR) of the two months following each sowing date in autumn and spring of the two years, and their corresponding historical average (in brackets) of the range 2007-2016.*

Year	Tm (°C)		P (mm)		Pd (days)		SR (MJ/m ²)	
	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr
2018-19	5.1	12.1	21.6	26	6	12	6	19
	(5.4)	(18.2)	(38.2)	(82)	(11.7)	(15.5)	(5.6)	(18)
2019-20	12.8	12.5	108.4	96	12	20	10.5	16.3
	(12.3)	(18.2)	(83.7)	(82)	(11)	(15.5)	(9.2)	(18)

*Data within the two months after the sowing date; December and January for autumn 2018-19, October and November for autumn 2019-20, March and April for spring of 2018-19 and 2019-20.

Seedling emergence

Species showed high variability in their emergence dynamics. Figure 2 shows the total seedling emergence and its variability within each species. Some species such as *T. incarnatum*, *P. tanacetifolia* and *P. lanceolata* presented high variability while others, like *F. esculentum* and *L. perenne*, had lower variability. Overall, considering all emergence data together, a gradient in total percentage of emergence was observed across the species, ranging from 25 % in *F. ovina* up to 70 % in *S. alba*.

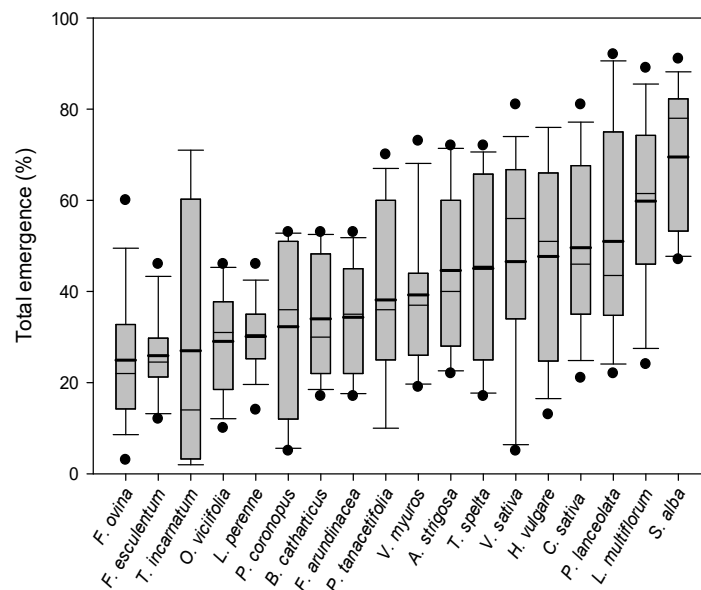


Figure. 2. Total emergence percentage for each species. Vertical bars represent standard errors (SE).

When total emergence percentage are separated by seasons (autumn or spring), the variability within each species is reduced (Figure 3). All species showed lower values of percentage of emergence in spring except *F. esculentum*, *P. tanacetifolia*, *A. strigosa*, *T. spelta* and *H. vulgare*. Furthermore, 9 of the 18 species analysed showed significant differences between autumn and spring, while 8 were different between years (Table 3). The highest variability was observed for *T. incarnatum*, ranging from 2.75 % in spring 2019-2020 up to 63 % in autumn 2018-2019.

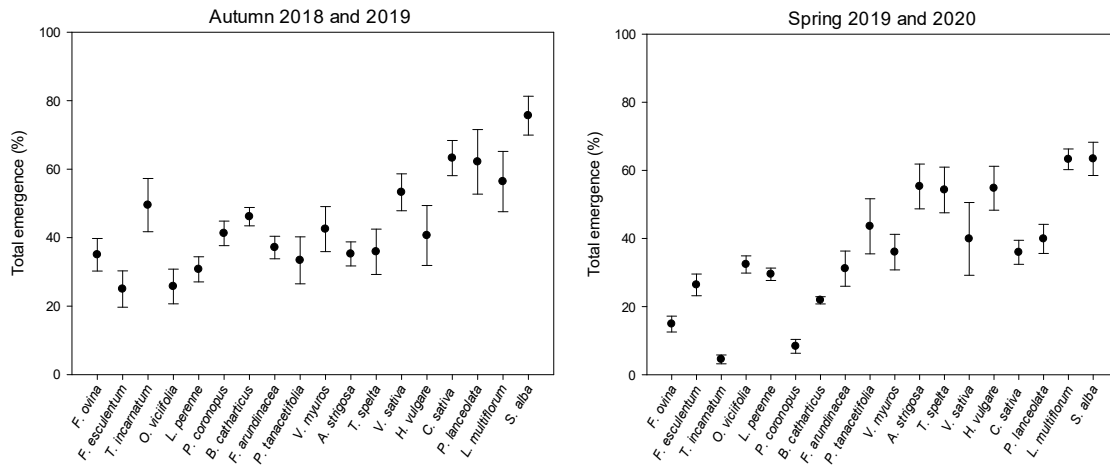


Figure. 3. Total emergence percentage for each species in autumn (left) and spring (right), pooling the data of the two years. Vertical bars represent standard error (SE).

Table 3. Percentages (%) of emergence achieved during the four sowing dates. Results of the ANOVA (or Kruskal Wallis) are also provided with the corresponding F value, significance (P), and interaction between factors (year and season).

Year	Season	<i>A. strigosa</i>	<i>B. catharticus</i>	<i>C. sativa</i>	<i>F. esculentum*</i>	<i>F. arundinacea</i>	<i>F. ovina</i>	<i>H. vulgare*</i>	<i>L. multiflorum</i>	<i>L. perenne</i>									
18-19	Autumn	39.50	46.50	65.25	-	39.25	38.25	19.75	68.25	24.25									
19-20	Autumn	31	45.67	61.25	25	35	31.75	61.50	44.50	37.25									
18-19	Spring	47.67	20.33	30.75	28.50	44.67	18	43	64.50	32.25									
19-20	Spring	61	23	41.125	24.25	21	15.50	66.50	62	26.75									
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year		0.115	0.741	0.086	0.776	0.253	0.624	0.371	0.557	10.790	0.007	0.626	0.446	25.230	<0.001	2.226	0.162	1.239	0.288
Season		7.181	0.021	60.77	<0.001	18.610	0.001	0.012	0.917	1.020	0.334	10.290	0.008	4.730	0.050	0.611	0.450	0.138	0.717
Year x Season		2.350	0.154	0.312	0.589	1.289	0.278	-	-	5.220	0.430	0.124	0.732	1.974	0.185	1.459	0.250	7.536	0.018

Year	Season	<i>O. viciifolia</i>	<i>P. tanacetifolia</i>	<i>P. coronopus</i>	<i>P. lanceolata</i>	<i>S. alba*</i>	<i>T. incarnatum</i>	<i>T. spelta*</i>	<i>V. sativa</i>	<i>V. myuros*</i>									
18-19	Autumn	13.50	29	45.75	37.75	82.50	63	23	54	48.50									
19-20	Autumn	38	37.75	36.75	86.50	68.75	36	48.75	52.50	36.50									
18-19	Spring	34.75	21.67	8.33	35.25	51	6.25	42.25	13.25	38.25									
19-20	Spring	30	60	-	44.50	75.75	2.75	66.25	66.50	33.75									
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year		8.577	0.013	8.314	0.015	2.226	0.174	37.400	<0.001	0.943	0.351	5.594	0.036	11.940	0.005	13.710	0.003	0.902	0.361
Season		3.860	0.073	0.834	0.381	32.980	<0.001	22.010	<0.001	4.676	0.051	48.710	<0.001	6.513	0.025	3.662	0.080	0.560	0.469
Year x Season		18.810	<0.001	3.282	0.097	-	-	17.340	0.001	11.550	0.005	3.321	0.093	0.015	0.905	15.340	0.002	0.186	0.674

*Non-parametric Kruskal Wallis test. Significant differences at $P < 0.05$.

Table 4. Emergence duration (days) during the four sowing dates. Results of the ANOVA (or Kruskal Wallis) are also provided with the corresponding F value, significance (P), and interaction between factors (year and season). Species are presented following the order of Figure 4

Year	Season	<i>A. strigosa</i>	<i>B. catharticus</i> *	<i>C. sativa</i>	<i>F. esculentum</i>	<i>F. arundinacea</i>	<i>F. ovina</i>	<i>H. vulgare</i> *	<i>L. multiflorum</i> *	<i>L. perenne</i>									
18-19	Autumn	33	33.50	40.88	-	40.50	43.75	31	35.25	35.50									
19-20	Autumn	24	58.67	32.75	11.25	60.75	65.25	22	47.50	63.25									
18-19	Spring	12.75	22.67	23	15.75	19.50	9.50	20.50	22.75	23.75									
19-20	Spring	6.67	19	8.63	14.50	16.67	12.50	8.67	17.25	14									
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year		6.186	0.031	4.416	0.062	4.689	0.051	0.0709	0.796	15.602	0.002	42.496	<0.001	7.325	0.020	0.764	0.399	7.707	0.017
Season		38.409	<0.001	29.111	<0.001	16.338	0.002	0.480	0.506	217.860	<0.001	535.860	<0.001	9.586	0.010	30.662	<0.001	88.507	<0.001
Year x Season		0.231	0.640	13.075	0.005	0.362	0.559	-	-	27.406	<0.001	24.230	<0.001	0.135	0.720	5.286	0.040	33.449	<0.001

Year	Season	<i>O. viciifolia</i>	<i>P. tanacetifolia</i> *	<i>P. coronopus</i> *	<i>P. lanceolata</i> *	<i>S. alba</i>	<i>T. incarnatum</i>	<i>T. spelta</i>	<i>V. sativa</i> *	<i>V. myuros</i> *									
18-19	Autumn	42.50	18	30.50	53	15.25	48.50	35.75	28.75	32.50									
19-20	Autumn	60.75	19	30.75	48.25	13	38.75	27.75	42.75	42									
18-19	Spring	19.25	7.75	7.67	16.33	18	9.50	18	13	17.25									
19-20	Spring	20.75	10.25	-	17.25	6.25	3	6	7.50	10.50									
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Year		6.181	0.029	0.164	0.693	0.0007	0.980	0.055	0.819	16.691	0.002	3.974	0.072	4.242	0.062	0.607	0.452	0.062	0.807
Season		63.396	<0.001	4.827	0.048	4.916	0.057	17.180	0.002	0.889	0.364	84.083	<0.001	16.547	0.002	21.854	<0.001	18.053	0.001
Year x Season		4.446	0.057	0.030	0.865	-	-	0.120	0.735	10.889	0.006	0.159	0.698	0.170	0.688	3.195	0.101	2.181	0.165

*Non-parametric Kruskal Wallis test. Significant differences at $P < 0.05$.

Emergence period

For most species, there were significant differences between the length of the emergence period in autumn and in spring (15 out of 18), but not between years (7 out of 18) (Table 4, Figure 4). While there was a range of emergence periods from less than 15 days up to more than 40 days in autumn, the emergence period of all species lasted less than 20 days in spring. This variation of autumn/spring emergence periods, though, was not the same for all studied species. While most of them showed significantly longer emergence periods in autumn, *S. alba* and *F. esculentum* did not, and their emergence periods were similar in both seasons (Table 4). Among those species showing autumn/spring differences for the emergence period, this varied from 25 to 55 days; almost one month of difference between the earliest and the latest species.

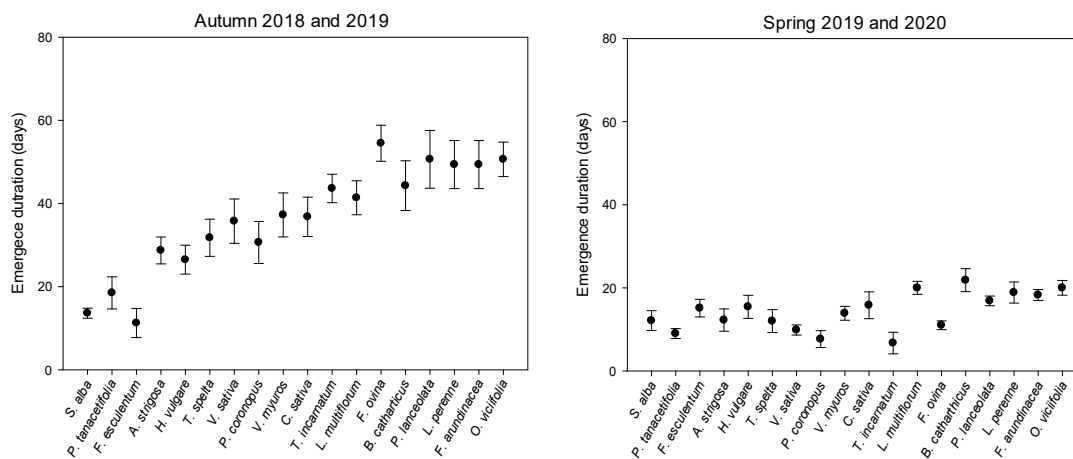


Figure 4. Emergence duration for each species in autumn (left) and spring (right), pooling the data of the two years. Vertical bars represent standard error (SE).

Development of emergence models

The emergence models were calculated using the four series of data (autumn 2018, spring 2019, autumn 2019 and spring 2020). All emergence patterns based in either factor (TT, HTT, PhHTT or PhSHTT) were significantly ($P < 0.01$) adjusted to sigmoidal log-logistic models (Table 5). According to literature, T_b varied from -0.7 °C (*C. sativa*) to 10.0 °C (*F. esculentum*), and the estimated Ψ_b ranged from -3.4 MPa to -1.4 MPa, which can indicate the degree of drought tolerance of each species at emergence. The graphical representations of developed models are shown in Figure 5, 6 and 7. In these graphs the observed difference between autumn and spring emergence periods can be observed based on the different thermal basis (TT, HTT, PhHTT or PhSHTT). In 17 of the 18 species, both PhHTT and/or PhSHTT based models achieved very good accuracy levels

($R^2 > 0.9$ and $RMSE < 15$). Based on (Royo-Esnal *et al.*, 2012) all models that considered light as day length alone or with solar radiation showed very good ($RMSE = 5-10$) or good ($RMSE = 10-15$) fit, and only in *P. lanceolata*, the accuracy was low ($RMSE = 16.3$). On the contrary, the best accuracy was obtained for *C. sativa*, with an R^2 of 0.98 and a $RMSE$ of 5.3.

Table 5. Parameters (a, x_0 and b) for the thermal time (TT), hydrothermal time (HTT), photohydrothermal time (PhHTT) and photosolar hydrothermal time (PhSHTT) based models. The R^2 , F value of the model and their significance (P) are also provided, as well as the estimated base water potential (Ψ_b), and the base temperature considered from literature (T_b).

Species	Model	a	x_0	b	R^2	F	P	Ψ_b (MPa)	T_b (°C)	Reference for T_b
<i>A. strigosa</i>	TT	100	135.94	19.32	0.76	254.08	<0.0001	-3.4	4.8	(Tribouillois <i>et al.</i> , 2016)
	HTT	135.94	19.32	0.76	254.08	<0.0001				
	PhHTT	61.07	4.79	0.90	746.22	<0.0001				
	PhSHTT	3.54	0.42	0.96	1711.70	<0.0001				
<i>B. catharticus</i>	TT	100	248.12	73.22	0.63	134.79	<0.0001	-2.1	5.8	(Lonati <i>et al.</i> , 2009)
	HTT	248.12	73.22	0.63	134.79	<0.0001				
	PhHTT	99.40	26.84	0.71	196.32	<0.0001				
	PhSHTT	4.75	0.81	0.90	745.54	<0.0001				
<i>C. sativa</i>	TT	100	199.59	27.98	0.93	1097.50	<0.0001	-3.2	-0.7	(Allen <i>et al.</i> , 2014)
	HTT	193.69	34.60	0.91	818.50	<0.0001				
	PhHTT	84.26	8.44	0.98	3483.90	<0.0001				
	PhSHTT	4.28	0.99	0.84	409.27	<0.0001				
<i>F. esculentum</i>	TT	100	75.34	23.54	0.75	146.09	<0.0001	-1.9	10	(Arduini <i>et al.</i> , 2016)
	HTT	57.67	14.02	0.83	240.94	<0.0001				
	PhHTT	28.24	5.64	0.86	311.32	<0.0001				
	PhSHTT	1.72	0.25	0.90	440.92	<0.0001				
<i>F. arundinacea</i>	TT	100	345.17	44.85	0.93	1093.39	<0.0001	-2.5	3.5	(Sharifiamina <i>et al.</i> , 2016)
	HTT	327.29	58.52	0.87	538.47	<0.0001				
	PhHTT	146.19	19.75	0.97	2745.46	<0.0001				
	PhSHTT	7.63	1.49	0.83	396.89	<0.0001				
<i>F. ovina</i>	TT	100	407.31	84.80	0.78	283.19	<0.0001	-2.1	1.1	(Lonati <i>et al.</i> , 2009)
	HTT	366.04	107.50	0.71	193.80	<0.0001				
	PhHTT	158.74	33.16	0.85	445.54	<0.0001				
	PhSHTT	7.75	1.09	0.95	1544.85	<0.0001				
<i>H. vulgare</i>	TT	100	172.05	26.34	0.81	347.35	<0.0001	-3.4	2.6	(Angus <i>et al.</i> , 1980)
	HTT	172.05	26.34	0.81	347.35	<0.0001				
	PhHTT	74.06	5.07	0.95	1447.86	<0.0001				
	PhSHTT	4.10	0.64	0.92	888.40	<0.0001				
<i>L. multiflorum</i>	TT	100	236.67	45.64	0.85	439.42	<0.0001	-3.2	1.5	(Moot <i>et al.</i> , 2000)
	HTT	231.57	44.94	0.84	409.47	<0.0001				
	PhHTT	105.54	18.36	0.92	865.89	<0.0001				
	PhSHTT	5.48	0.98	0.92	907.50	<0.0001				
<i>L. perenne</i>	TT	100	397.91	96.31	0.72	204.77	<0.0001	-2.3	1.9	(Moot <i>et al.</i> , 2000)
	HTT	372.88	117.70	0.65	147.25	<0.0001				
	PhHTT	162.92	36.78	0.80	323.86	<0.0001				
	PhSHTT	8.05	1.22	0.95	1397.25	<0.0001				
<i>O. viciifolia</i>	TT	100	292.34	76.61	0.85	453.27	<0.0001	-2.7	0	(Tribouillois <i>et al.</i> , 2016)
	HTT	272.55	73.47	0.82	360.60	<0.0001				
	PhHTT	126.33	31.39	0.91	776.31	<0.0001				
	PhSHTT	6.51	1.64	0.93	1006.39	<0.0001				
<i>P. tanacetifolia</i>	TT	100	151.90	22.68	0.83	393.72	<0.0001	-3.4	3.6	(Tribouillois <i>et al.</i> , 2016)
	HTT	151.90	22.68	0.83	393.72	<0.0001				
	PhHTT	66.11	5.78	0.96	1892.97	<0.0001				
	PhSHTT	3.50	0.54	0.91	755.48	<0.0001				
<i>P. coronopus</i>	TT	100	158.70	9.23	0.90	562.25	<0.0001	-1.4	4	(Payne <i>et al.</i> , 2018)
	HTT	158.70	9.23	0.90	562.25	<0.0001				
	PhHTT	73.26	6.34	0.95	1308.43	<0.0001				
	PhSHTT	3.82	0.73	0.86	392.02	<0.0001				
<i>P. lanceolata</i>	TT	100	206.23	79.26	0.58	107.74	<0.0001	-3.4	4	(Payne <i>et al.</i> , 2018)
	HTT	206.23	79.26	0.58	107.74	<0.0001				
	PhHTT	94.44	29.16	0.70	187.53	<0.0001				
	PhSHTT	4.72	0.90	0.87	537.78	<0.0001				
<i>S. alba</i>	TT	100	168.14	41.42	0.83	388.38	<0.0001	-3.4	1.2	(Tribouillois <i>et al.</i> , 2016)
	HTT	168.14	41.42	0.83	388.38	<0.0001				
	PhHTT	76.60	9.37	0.95	1541.81	<0.0001				
	PhSHTT	3.83	0.82	0.89	667.98	<0.0001				
<i>T. incarnatum</i>	TT	100	233.64	40.75	0.90	711.00	<0.0001	-2.3	-0.42	(Baxter <i>et al.</i> , 2019)
	HTT	217.82	28.56	0.89	665.77	<0.0001				
	PhHTT	100.89	19.79	0.92	908.85	<0.0001				
	PhSHTT	5.36	1.59	0.85	438.25	<0.0001				
<i>T. spelta</i>	TT	100	172.84	39.11	0.73	214.69	<0.0001	-3.4	2.6	(Angus <i>et al.</i> , 1980)
	HTT	172.84	39.11	0.73	214.69	<0.0001				
	PhHTT	75.20	8.03	0.88	556.44	<0.0001				
	PhSHTT	4.16	0.65	0.95	1410.78	<0.0001				
<i>V. myuros</i>	TT	100	335.95	78.96	0.83	379.71	<0.0001	-2.1	0.62	(Schermer <i>et al.</i> , 2017)
	HTT	271.31	64.26	0.80	318.54	<0.0001				
	PhHTT	124.57	23.17	0.91	769.40	<0.0001				
	PhSHTT	6.56	0.98	0.94	1170.89	<0.0001				
<i>V. sativa</i>	TT	100	248.91	56.96	0.78	284.95	<0.0001	-3.4	0	(Iannucci <i>et al.</i> , 2008)
	HTT	248.91	56.96	0.78	284.95	<0.0001				
	PhHTT	110.65	17.91	0.89	663.26	<0.0001				
	PhSHTT	5.66	0.75	0.94	1236.27	<0.0001				

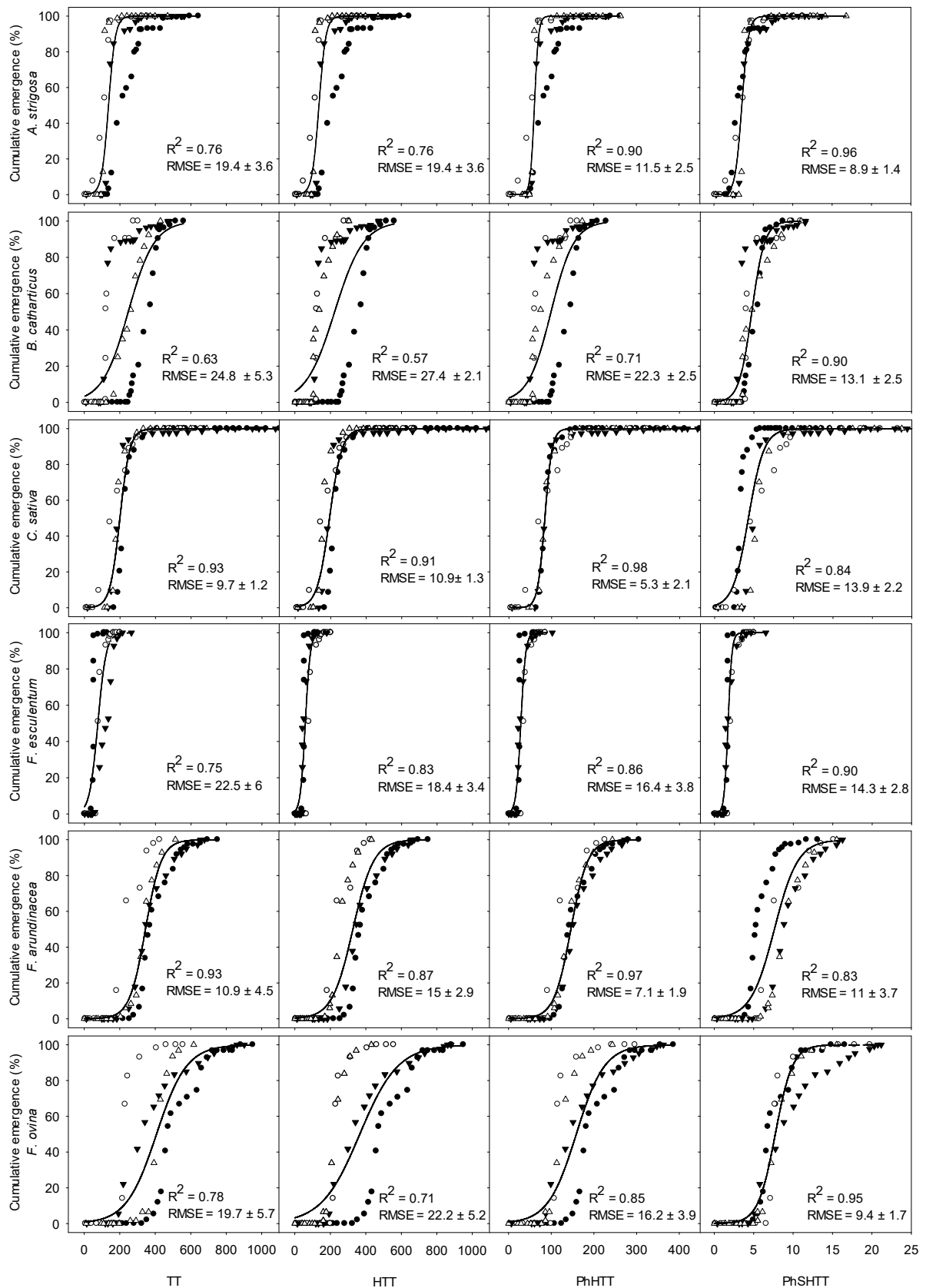


Figure 5. Cumulative percentage of emergence for each species sown in outdoor pots. Fitted emergences (black lines) are represented for each model based on TT, HTT, PhHTT and PhSHTT. Symbols represent different sowing dates: ●, autumn 2018; ○, spring 2019; ▼, autumn 2019; △, spring 2020. R^2 and RMSE (\pm SE) are also provided.

PhHTT

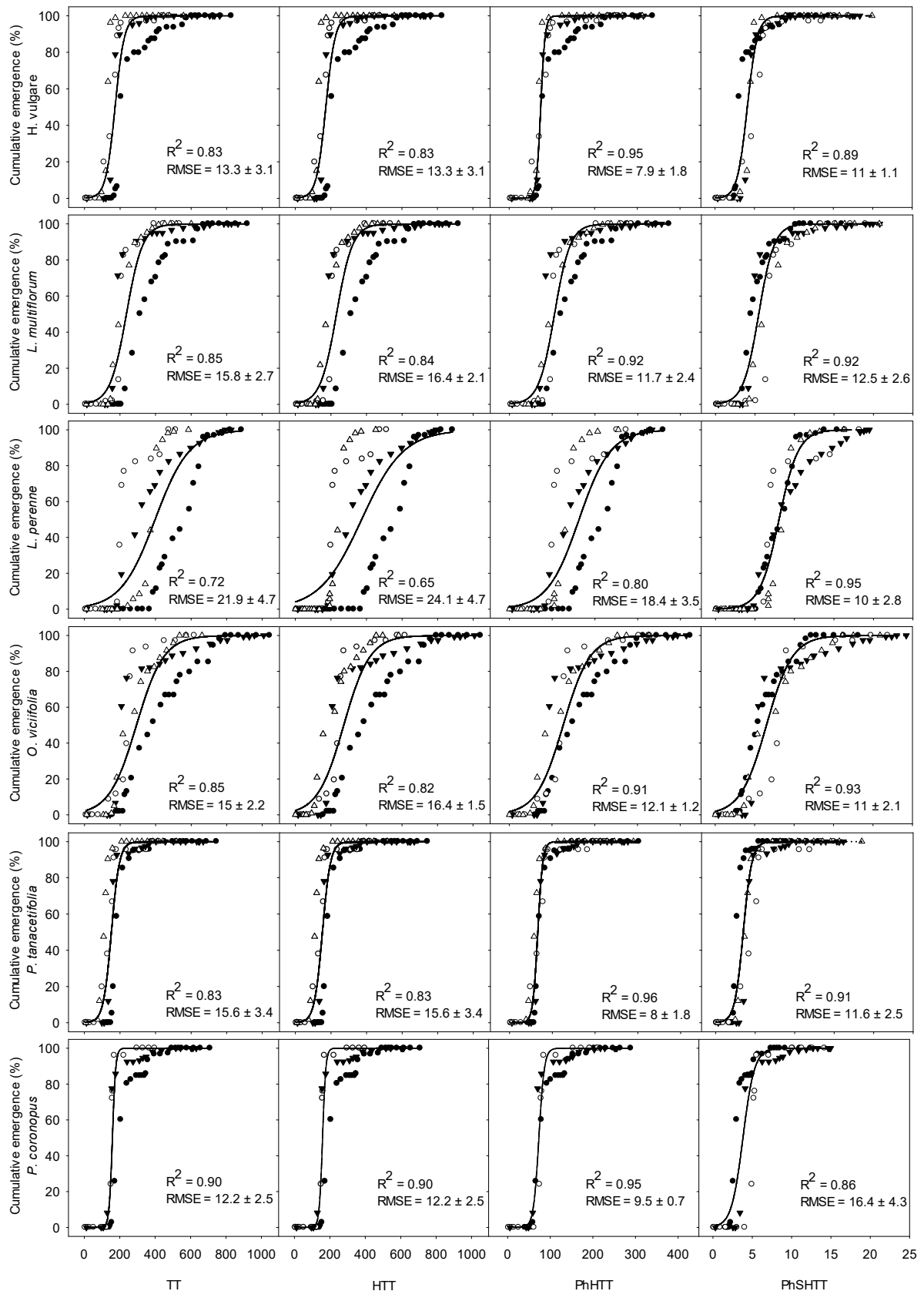


Figure 6. Cumulative percentage of emergence for each species sown in outdoor pots. Fitted emergences (black lines) are represented for each model based on TT, HTT, PhHTT and PhSHTT. Symbols represents different sowing dates: ●, autumn 2018; ○, spring 2019; ▼, autumn 2019; △, spring 2020. R^2 and RMSE (\pm SE) are also provided.

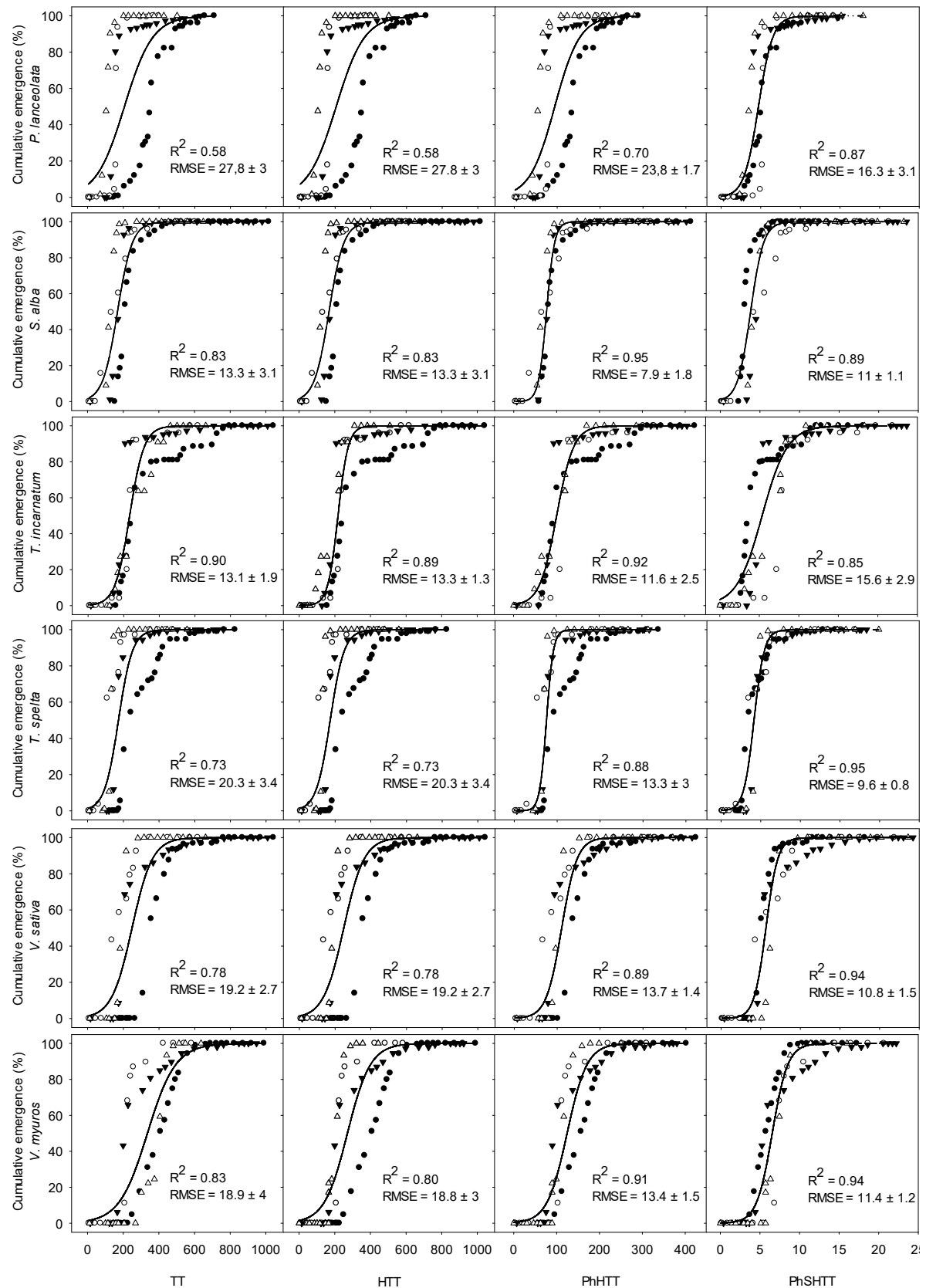


Figure 7. Cumulative percentage of emergence for each species sown in outdoor pots. Fitted emergences (black lines) are represented for each model based on TT, HTT, PhHTT and PhSHTT. Symbols represents different sowing dates: ●, autumn 2018; ○, spring 2019; ▼, autumn 2019; △, spring 2020. R^2 and RMSE (\pm SE) are also provided.

Validation of the models

The validation of the models was carried out for 6 species based on independent data from Tribouillois *et al.* (2018). Four of the 6 species analysed were successfully validated in, at least, one of the models developed. However, unequal results were found considering that for *L. multiflorum*, *T. incarnatum* and *V. sativa*, the best accuracy in the validation was achieved when light was included, either PhHTT or PhSHTT (best RMSEP of 8.6, 10.5 and 12.1, respectively), while for *S. alba*, the best accuracy was achieved with the less elaborated models (TT and HTT) with a RMSEP of 6.1 and 6.4, respectively. No model could be validated with the independent data for *A. strigosa* and *P. tanacetifolia* (RMSEP >35).

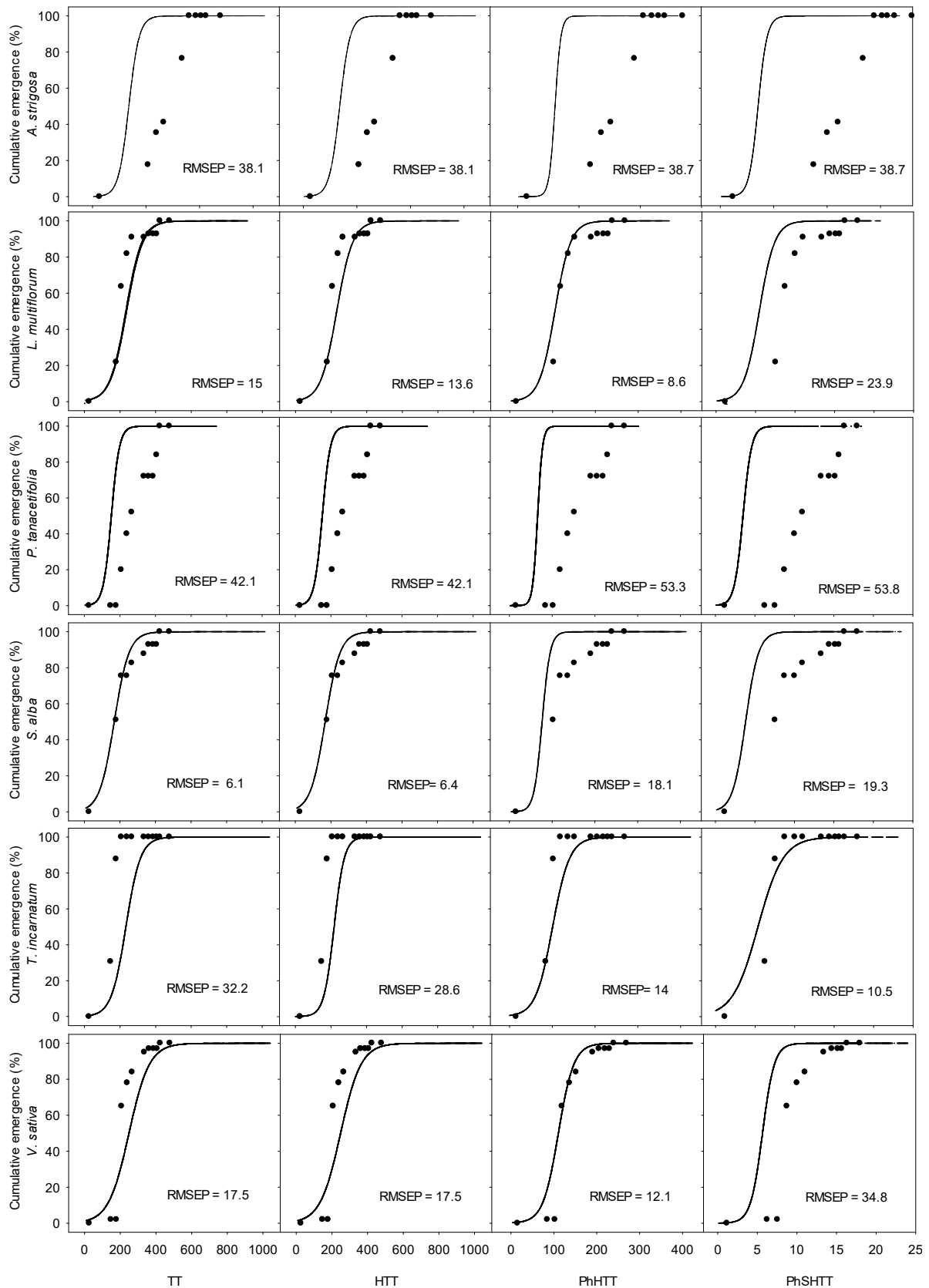


Figure 8. Validation of the models developed for *A. strigosa*, *P. tanacetifolia*, *S. alba*, *T. incarnatum* and *V. sativa* as a function of TT, HTT, PhHTT and PhSHTT. Black dots (●) represents observed data from Tribouillois *et al.* (2018); black lines represent predicted emergence by the corresponding model. RMSEP for each validation is also provided.

Discussion

Cover crops fill gaps in cropping systems that would otherwise be occupied by weeds (Liebman *et al.*, 2001; Baraibar *et al.*, 2021). To avoid bare soil where weeds could grow freely, a good establishment of the cover crop is needed, and this is a process conditioned, among others, by its emergence. Soil temperature, soil moisture, soil air quality and light quality are the main environmental factors affecting seedling emergence (Forcella *et al.*, 2000). Weather conditions in the two experimental years were very different, and they also differed quite a bit from the historical average (Table 2). Besides, the two sowing dates in autumn and in spring repeated two years provided a large variability in conditions, resulting in four emerging periods. So from an emergence-modelling perspective, this variability in all those factors contributes to strengthening the models and gives consistency to the results, especially when the majority of the species subjected to the independent data from the south of France (Tribouillois *et al.*, 2018) were successfully validated.

Emergence percentage

In general, the emergence of all species varied more between seasons within each year rather than between years. Some few exceptions were observed (i.e. *S. alba*). Most of the results in this sense are in accordance with the consulted literature. Grass species, such as *Festuca spp.* and *Lolium spp.*, emerged in the range 25 % to 65 %, similar or higher than those from Hill *et al.* (1985) at temperatures from 10 °C to 25 °C; *B. catharticus* showed higher emergence at colder temperatures (autumn) than at warmer temperatures (spring), as observed by Mollard and Insausti (2009), with higher germination rates at 15 °C (35 %) than at 20 °C (15 %). *Camelina sativa* was highly variable (8.6 % - 40.9 %), like for Urbaniak *et al.* (2008) or Royo-Esnaola *et al.* (2017), this variation in field emergence suggests that it is likely dependent on environmental conditions caused by the seed bed preparation (Urbaniak *et al.*, 2008). Also a high emergence variability was observed for *T. incarnatum* in the present study with values from < 5 % to > 50 % and similar to those observed by Brooker *et al.* (2020) (< 1 % to 32 %). The lack of seedling emergence of *F. esculentum* in autumn 2018 can be explained by its high base temperature (10 °C) (Arduini *et al.*, 2016) that might have prevented its seeds from germinating in December (mean temperatures < T_b). The lower emergences observed in the other grass species, *H. vulgare*, *T. spelta* and *A. strigosa* in 2018-2019, was a consequence of seed predation by birds. The lack of emergences of *P. coronopus*, the species with the smallest seeds, in spring 2020 might be explained by the formation of soil crusting in hot days after each

rainy period and the inability of its tiny seeds to go through it.

Emergence models

Most cover crop species considered in this study showed clear differences in the length of the emergence period between autumn and spring sowing dates. This fact, together with the variability of the emergence rates would impede the development of common emergence models for these two seasons, but the estimation of the thermal time (TT), adjusted by soil moisture (HTT), day length (PhHTT) and solar radiation (PhSHTT) significantly improved the overlap of emergence timings. The inclusion of light significantly improved the models for *Thlaspi arvense* (Royo-Esnal *et al.*, 2015a) and *Camelina microcarpa* (Royo-Esnal *et al.*, 2015b) compared to previous models from the same region. Our results showed that for *C. sativa*, *F. arundinacea*, *H. vulgare*, *P. coronopus*, *S. alba* and *T. incarnatum* the achieved accuracy was very good (RMSE = 5-10) or good (RMSE= 10-15), according to Royo-Esnal *et al.* (2012), in the four developed models. For the remaining species, TT and HTT based models were not accurate enough to predict the emergence. Nevertheless, the incorporation of day length, as well as solar radiation, significantly enhanced the accuracy of the model of the 18 species, either PhHTT or PhSHTT (Figure 5, 6 and 7) despite the variability in the weather conditions among sowing dates (Table 2), with R^2 values always > 0.9 and RMSE < 15 in, at least, one of the models (except for *P. lanceolata*, where the inclusion of light improved the model but with an $R^2 < 0.9$ and RMSE > 15). There is limited literature on the inclusion of light to develop emergence models, but it should be considered for species that show autumn and spring flushes, like in our results and in those of Royo-Esnal *et al.*, 2015a and 2015b. Our results show that, there is no need for developing separate models for autumn and spring flushes, as these can be integrated in one single model based on PhHTT or PhSHTT. Some examples of that are *A. strigosa*, *F. arundinacea*, *F. ovina* and *P. tanacetifolia*, where the overlap of the data was good for the TT and/or HTT based models between years but not between seasons. The inclusion of day length and/or solar radiation improved the overlap between years and seasons, enhancing the accuracy of the model (Figure 5, 6 and 7).

The validation carried out with data from Tribouillois *et al.* (2018) was successful for the majority of the species tested, mainly when day length and/or solar radiation were included. The climate in Auzeville (southern France), with a stronger Atlantic influence, is very different from the one in north-eastern Spain, and the sowing date in Auzeville was carried out in summer (mid-august). Despite the differences in the sowing timing between localizations, the validation was successful in 4 of the 6 species tested, so that corroborate that temperature, moisture and

light are the main factors influencing emergence. Therefore, models including light are potentially useful to be applied in a widespread area. The validation was unsuccessful for only 2 of the 6 species tested (*P. tanacetifolia* and *A. strigosa*), both species with a large spectrum of emergence, which suggest that sensibility to light may differ within some species between Mediterranean and temperate climate as Bell *et al.* (1995) and Torra *et al.* (2016) conclude. In this sense, for some species may be necessary to develop independent models for each geo-climatic area, as suggested for *L. rigidum* by Sousa-Ortega *et al.* (2020b). On the other hand, *S. alba* was successfully validated with the models including only temperature and moisture, which suggest that these two factors are enough to predict accurately its emergence.

How light influences emergence models when seeds are buried is not clear. Direct radiation may inhibit the germination of some seeds, but low irradiances can promote it (Baskin and Baskin, 1998). Seeds were distributed between 0 and 2 cm when sowing, and light apparently penetrates only the top few millimetres of soil. However, light penetration depth depends on soil particle size, moisture content, and colour (Benvenuti, 1995; Baskin and Baskin, 1998), so a fraction of light may be able to cross the upper soil layer and would be detected by seeds depending on those factors. Another explanation of how seeds detect light is indirectly through soil temperature, light is correlated with temperature (van den Besselaar *et al.*, 2015) and daily temperature variation is detected by seeds in the soil (Ghersa *et al.*, 1992), so it could be that hourly temperature based emergence models could avoid the need of considering photoperiod as proposed by Royo-Esnal *et al.* (2015b). Hourly temperature is somehow affected by photoperiod itself, and is underlying in the hourly based HTT. Seeds may detect the period of time of the daily temperature fluctuation, so this would be another way of detecting photoperiod without receiving light directly.

Practical application

An interesting approach of this work is that PhHTT and PhSHTT based models are more accurate than those based on TT and HTT and could be applied to a wide range of species as confirmed by the fact that the obtained RMSE values were below 15 in, at least, one of the first two models for 17 out of 18 species from different botanical families. Models including light can be applied in autumn or spring indistinctly, at least, in the same region where they were developed, and for some species even in different geo-climatic regions. This confirms the usefulness of these models as DSS tools to predict the establishment of CC. This knowledge considerably contributes to inter-row management in vineyards, as species can be chosen depending on the final purpose of the CC (weed control, erosion avoidance, increase of organic

matter, control of vine vigour etc.). For example, quick emerging annual species, such as *S. alba*, *C. sativa* or *P. tanacetifolia*, and perennial ones, like *F. arundinacea* or *P. lanceolata* (Figure 5, 6 and 7), that differ in their emergence rhythm, could be combined in a single sowing date, so that the annual species can cover the soil very fast and reduce weed pressure, while the perennial one is emerging and growing underneath. By the time annual species reaches the end of its life cycle, the perennial could have developed a considerable coverage and continue its growth, and be maintained by mowing. Similarly, models could be used to choose the most appropriate species to quickly cover the soil if erosion is a problem or to match emergence time with vine vigour control. Also, an accurate combination of entomophilous species could provide new sources of pollen for beneficial fauna, which can significantly contribute to the functional flows of the vineyard agroecosystem.

Conclusion

Emergence models were successfully developed for different species with potential use as CC, either based on TT, HTT, PhHTT or PhSHTT. The validation of the models for four species out of six with independent data from the south of France reinforces the validity of the model's development method. The accuracy of the models was improved when light was included and, for some species, models could be used widely. These models considerably contribute to inter-row management in vineyards as decision support systems (DSS) tools to predict CC establishments.

Acknowledgements

This work has been supported by the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project AGL2017-83325-C4-2-R and also by the project LISA (Low Input in Sustainable Agriculture) financed by ACCIÓ-Generalitat de Catalunya inside the Community RIS3CAT (COTPA). The first author obtained a PhD grant from the University of Lleida (PhD grants). The third author obtained a Beatriu de Pinós post-doc grant from Generalitat de Catalunya. We would also like to acknowledge Maria Casamitjana and Irene de la Fuente for their help in the field work.

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CHAPTER 3

Cover crops terminated with roller-crimper to manage *Cynodon dactylon* in vineyards

Submitted to *Weed Research*, June 2022.

Cover crops terminated with roller-crimper to manage *Cynodon dactylon* in vineyards

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Abstract

Cover crops in vineyards have been recognized as a beneficial soil management technique with many advantages, including weed suppression. However, their use is still not commonplace and the effectiveness of the management could rely on the weed community, the cover crop species, and the termination method. The most common practice of cover crop termination in vineyards is shredding, despite the cover crop residues decompose rapidly and allow noxious species like *Cynodon dactylon* proliferate during summer and compete with the vines. Recently, the use of roller-crimper as an alternative method for killing cover crops has demonstrate to be effective in some cropping systems, but no studies have focused on its use in vineyard inter-rows. The objective of this study was to evaluate the effectiveness of seven cover crops 1) Spontaneous, 2) *Avena strigosa*, 3) *Hordeum vulgare*, 4) *Lolium multiflorum*, 5) *Phacelia tanacetifolia*, 6) *Sinapis alba*, 7) Triticale, combined with two different termination methods (shredding or roller-crimper) to manage *C. dactylon* during summer. Terminating cover crops with roller-crimper reduced the overall coverage of *C. dactylon* from 25 % to 12 % (September 2020) and from 30 % to 15 % (September 2021) when compared with shredding. The redundancy analysis (RDA) showed that the weed community composition was mainly influenced by the type of cover crop (spontaneous or sown), with a clear reduction of the species richness in the sown ones. Thus, the cover crop termination method is an important issue to be considered for the management of *C. dactylon* in vineyard inter-rows.

Keywords: Bermudagrass, shredding, weeds, sustainable viticulture, conservation agriculture, mulches.

Introduction

Grapes are one of the most important crops in the world, and almost one million hectares are cultivated in Spain (MAPA, 2019). In Mediterranean vineyards, weeds are commonly managed through tillage, leaving the soil bare most of the year, which increase soil erosion and negatively affects its physical, chemical and biological characteristics (Smith *et al.* 2008; Steenwerth and Guerra, 2012). Spontaneous or sown cover crops, as an alternative practice to tillage, are growing trends for inter-rows management in irrigated conditions or organic vine farming in Spain (MAPA, 2019; Gago *et al.*, 2007; Valencia *et al.*, 2016) and in other countries. Cover crops bring many benefits to the farm, such as avoiding erosion, improving soil structure, and enhancing nutrient and moisture availability, among other ecosystem services (Gómez *et al.*, 2011; Steenwerth and Guerra, 2012; Abad *et al.*, 2021a). This practice can also help maintain an optimal balance between vine vegetative growth and fruit development by controlling the excess grapevine shoot vigour through proper cover crop management (Hartwig *et al.*, 2002; Ripoché *et al.*, 2011; Abad *et al.*, 2021b), and can suppress weeds by exerting competition and by releasing allelopathic compounds, either when growing and/or during the residue degradation (Farooq *et al.*, 2011; Sturm *et al.*, 2018). However, managing certain weeds, as *Cynodon dactylon* (L.) Pers. (bermudagrass), can be challenging (Recasens *et al.*, 2019). *Cynodon dactylon* is a stoloniferous and rhizomatous perennial grass considered as one of the world's worst weeds (Holm *et al.*, 1977). It is tolerant to salinity and water stress, adapted to a wide range of soils and climates, and very difficult to eradicate (FAO, 2022). In addition, as a summer perennial grass, *C. dactylon* can compete with vines for soil resources, especially water in the Mediterranean climate characterized by severe summer droughts and scarce rainfall. On one hand, recurrent tillage could partially control *C. dactylon* in vineyards, but it is usually ineffective because several interventions are required and it contributes to the dispersal of rhizomes and stolons (Abdullahi, 2002; Fernandez, 2003). On the other hand, the shade provided by a cover crop can reduce the weed development and biomass (Guglielmini and Satorre, 2002). In this sense, Valencia-Gredilla *et al.* (2020) reported that the implementation of a *Hordeum vulgare* L. as a cover crop was more effective in reducing *C. dactylon* infestation than spontaneous vegetation cover managed with tillage, herbicide applications or shredding. However, the effect of other potential cover crop species for reducing *C. dactylon* in vineyards is still being studied.

The most common practice of cover crop termination in vineyards is shredding, which leaves a dead mulch composed of small particles that decompose very fast (Sims and Frederick,

1970; Bremer *et al.*, 1991), allowing *C. dactylon* to proliferate during the summer. Recently, the use of roller-crimper to terminate cover crops has increased as it permits reduce fuel and time compared to shredding, and because it creates a uniform and dense layer of cover crop residues on the soil surface, creating a physical barrier for weed emergence (Bavougian *et al.*, 2018). It has also been shown that the extracts of some cover crop species residues inhibit weed germination (Ciaccia *et al.*, 2015; Puig *et al.*, 2019). This residue layer limits the amount of light, heat, surface daily temperature range, and soil water content that can reach the soil and thus interferes with seed germination (Altieri *et al.*, 2011). Moreover, the residue can persist longer in the soil as it is still connected to the soil by the roots and deposited more uniformly in travel direction (Ackroyd *et al.*, 2019; Dorn *et al.*, 2013). But, weed germination and proliferation is strongly conditioned by mulch biomass, which quantity has been demonstrated to be more important than its type (Teasdale and Mholer, 2000), and specific seasonal weather conditions, which could also affect in the residue decomposition rate.

Studies in vineyard inter-rows managed with cover crops report impacts in the weed biomass and diversity (Baumgartner *et al.*, 2008). Valencia-Gredilla *et al.*, 2020 reported that weeds were more related with shredding or tilling rather than with a *H. vulgare* cover crop, and Lososová *et al.*, 2003 observed changes in the proportion of plant life forms in vineyards due to mulching, with a higher presence of hemicryptophytes when compared to tillage. Studies of the effect of roller-crimper management on weed species richness are scarce, and mainly focused in extensive crops (Halde *et al.*, 2015). Navarro-Miró *et al.*, 2019 concluded that the use of this management reduced weed species richness in seven trials across Europe. Moreover, to the best of our knowledge, the effect of roller-crimper in vineyard inter-rows has not been studied yet, and specifically if we focus on *C. dactylon* infestations. For that reason, a two-year experimental trial was established in a commercial vineyard in Raimat (northeast Spain), highly infested with this species, to compare different cover crop species and two termination methods (roller-crimper and shredding) for managing infestations of *C. dactylon*, as well as to analyse the weed flora composition associated to the established cover crops.

Material and methods

Study area

The experiment was conducted in a commercial wine grape vineyard of *Vitis vinifera* L. cv Pinot noir established in 2015 in Raimat (Lleida, NE Spain, 41° 40' 22.5" N, 0° 29' 25.3" E ETRS89) during two growing seasons (2019-2020 and 2020-2021). Vines were drip-irrigated and trained as double cordon with rows separated 2.4 m and vine spacing of 1.7 m. Soil texture

was 15.2 % sand, 52.7 % silt, 32.1 % clay, with a pH of 8 and 2.41 % of organic matter. The climatic classification of this area is cold semiarid (*BSk*) (Kottek *et al.*, 2006), with an average annual precipitation of 342 mm, and annual mean temperature of 14.1°C (average min of 8.1°C and average max of 20.7°C).

The traditional soil management in the farm consists of shredding the inter-rows spontaneous cover 3-4 times per season and mechanical cultivation in the under-vines area with an in-row tiller 3-4 times along the season.

Experimental design

Cover crops were sown by hand in different inter-rows of 2 m wide and 200 m long in an incomplete split-plot design with a row spatial adjustment where three factors and three replications were analysed. The main factor was assigned to the cover crop termination method: A) Shredding or B) Rolling/Crimping. Each method was performed on six alternate rows. The type of cover crop was the secondary factor: 1) Spontaneous, 2) *Avena strigosa*, 3) *Hordeum vulgare*, 4) *Lolium multiflorum*, 5) *Phacelia tanacetifolia*, 6) *Sinapis alba*, 7) × *Triticosecale* *sp.* (hereafter Triticale). Finally, differences between samplings (July and September) were also analysed. Each inter-row of 200 m was divided into four plots of 2 m x 50 m, each plot being sown with one cover crop species. Inter-row cover crops were sown in November each season at the corresponding dose for each species (Table 1). Seed-bed preparation was performed with a tractor mounted rotatory tiller before each sowing. Each season, cover crops were shredded (termination method A) when its life cycle finished in late June, and rolled (termination method B) when plants were in flower by early May. Neither irrigation nor fertilization was applied to the cover crop. During the study, in-row weeds were managed through mechanical cultivation by an in-row tiller.

Table 1. Species used in the present experiment, their variety, the supplier company, their production site (origin) and the dose.

Species	Variety	Company	Origin	Dose (Kg/ha)
<i>Avena strigosa</i> (L.) Schreb	Saia 6	Semillas Fitó S.A.	NE Spain	70
<i>Hordeum vulgare</i> L.	Meseta	Semillas Batlle S.A	NE Spain	150
<i>Lolium multiflorum</i> Lam.	Elirix	Rocalba S.A	NE Spain	40
<i>Phacelia tanacetifolia</i> L.	Lilla	Rocalba S.A.	NE Spain	10
<i>Sinapis alba</i> (L.) Rabenh.	Accent	Rocalba S.A.	NE Spain	20
× <i>Triticosecale</i> Wittm.	Misionero	Semillas Fitó S.A.	NE Spain	210

Cover crop and weed sampling

Each plot was divided in five subplots (sample units) of 2 m x 10 m where samplings were performed. Data analysed were the mean of the five subplots. In each subplot, cover crop

establishment was measured by seedling counting within a 0.33 m x 0.33 m quadrat. Afterwards, cover crop coverage was visually estimated as a percentage of the whole subplot. Each season in April, weed coverage was estimated species by species to analyse the influence of the cover crop species on the weed community composition. Finally, in July and September, *C. dactylon* coverage was visually monitored as a percentage of the whole subplot after cover crop was terminated by the shredding or rolling method.

Statistical analyses

Coverage percentage of *C. dactylon* was analysed with a three-way ANOVA, followed by the Tukey's honestly significant difference (HSD) ($\rho < 0.05$), considering termination method, cover crops and sampling dates as fixed factors. Since seed-bed preparation affected *C. dactylon* coverage and frequency (Valencia-Gredilla *et al.*, 2020), it was decided to analyse each year separately. The original data were previously square-root transformed if needed to achieve normality and homoscedasticity. Analyses and graphs were performed with JMP Pro 15 (SAS Institute 2010. SAS Campus Drive, Cary, NC27513, USA. SAS Institute Inc.) and SigmaPlot 12.0 (Systat Software, San José, CA, USA). The weed species composition between cover crops was analysed with a redundancy analysis (RDA), based on the gradient of the variability in the species-plot response data (2.7 and 2.5 SD units in 2020 and 2021 respectively), which showed a linear method to be the best option, and performed with CANOCO 5.0 (Microcomputer Power: Ithaca, NY, USA, 2012).

Results

Weather conditions

During cover crops' development (from November to May), mean temperature (T_m) in season 2019-2020 was higher (10.4 °C) than the historical average (9.3 °C), and similar in 2020-2021 (9.6 °C) (Figure 1). The same pattern was found during the development of *C. dactylon* (from May to September), when T_m in 2019-2020 season was higher (21.5 °C) than the historical average (21 °C), and similar in 2020-2021 (21.1 °C). Regarding precipitation, 2019-2020 was very wet during cover crops development (364 mm), while in 2020-2021 it decreased (237 mm) to similar levels to the historical average (218 mm). Precipitation during the life cycle of *C. dactylon* was slightly higher (155 mm and 161 mm, respectively for 2019-2020 and 2020-2021) than the historical average (120 mm).

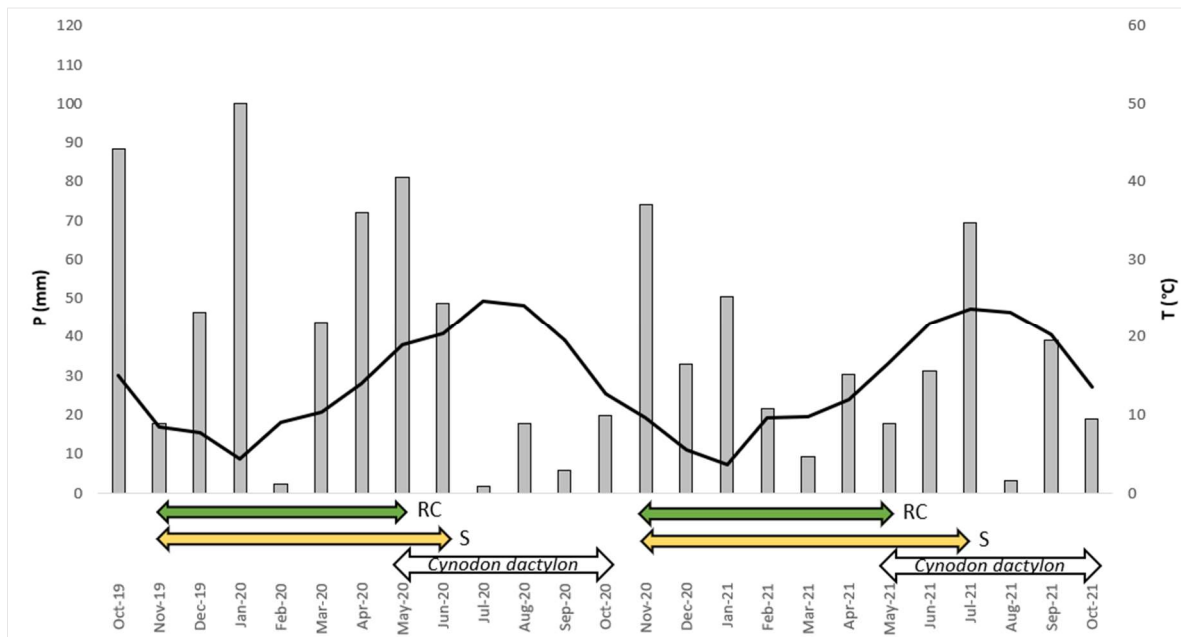


Figure 1: Weather conditions during the experimental period. Grey bars, total monthly precipitation (P); black line, mean monthly temperature (T). Arrows represent the cover crop and *Cynodon dactylon* growing period each year. RC: Roller-crimper; S: Shredding.

Cover crop development

In 2019-20, faster emergence rhythm was observed in *S. alba* and *P. tanacetifolia* than in grass species (Table 2), but by April 2020 maximum coverage of *S. alba* was < 50 %, while in the rest of the cover crops varied between 71 % (*A. strigosa*) and 94 % (*P. tanacetifolia*). In 2020-2021, the establishment of *P. tanacetifolia* failed, and its final coverage was very low (< 5 %). The highest coverage was obtained by *H. vulgare* (78 %), but all values from grass species decreased compared to 2019-2020, while it increased in *S. alba* (63 %).

Table 2: Establishment of cover crop species (plants/m²) and coverage evolution (%) during 2019-2020 and 2020-2021. ne: not estimated. Mean values ± standard errors of the mean.

2019-2020	Establishment (plants/m ²)				Cover crop soil coverage (%)			
	28/11/2019	09/12/2019	23/12/2019	10/01/2020	30/01/2020	25/02/2020	27/03/2020	14/04/2020
<i>Avena strigosa</i>	2.8 ± 1.8	269.1 ± 26.7	228.2 ± 92.1	210.6 ± 91.9	9.6 ± 1.7	23 ± 4	48.2 ± 6.1	70.8 ± 7.9
<i>Hordeum vulgare</i>	108.1 ± 36	263.9 ± 41.2	207.4 ± 44	200.6 ± 41.9	16.2 ± 4.4	37.8 ± 7.7	64.8 ± 5.9	89.8 ± 3.1
<i>Lolium multiflorum</i>	36.4 ± 18.9	732.2 ± 62	705.5 ± 179.2	n.e.	15 ± 1.6	40.7 ± 3	67 ± 3.4	93.3 ± 2.5
<i>Phacelia tanacetifolia</i>	339.6 ± 47.9	355.5 ± 32.5	248.9 ± 113.5	265.1 ± 51.3	31.2 ± 6.1	55.3 ± 5.5	77.1 ± 3.6	93.8 ± 3.2
<i>Sinapis alba</i>	170.1 ± 43.9	157.9 ± 50.5	92 ± 81.2	n.e.	2.8 ± 1.4	9 ± 5.8	21.3 ± 9.9	46.7 ± 12
Triticale	46.4 ± 20.3	360 ± 25.3	377.7 ± 102.8	n.e.	31.5 ± 9.8	45 ± 12.3	64.2 ± 9.5	83.7 ± 5.4
2020-2021	Establishment (plants/m ²)			Cover crop soil coverage (%)				
Cover crop	19/11/2020	02/12/2020	21/12/2020	28/12/2020	27/01/2021	04/03/2021	19/04/2021	
<i>Avena strigosa</i>	282.6 ± 67.7	300.4 ± 84.9	n.e.	17 ± 3.6	25.4 ± 5	46.5 ± 9.5	64.9 ± 7.2	
<i>Hordeum vulgare</i>	315.3 ± 31	n.e.	n.e.	30.1 ± 3	39.3 ± 5.3	62.5 ± 5.8	78.3 ± 5.3	
<i>Lolium multiflorum</i>	390.9 ± 85.8	466.6 ± 64.3	n.e.	11 ± 3	16.2 ± 5.6	32.2 ± 10.4	54.5 ± 6.5	
<i>Phacelia tanacetifolia</i>	n.e.	n.e.	6.9 ± 2.4	0.5 ± 0.1	1.1 ± 0.4	4.4 ± 1.8	ne	
<i>Sinapis alba</i>	350.1 ± 50.7	n.e.	n.e.	26.6 ± 6.9	36.6 ± 10.3	46.8 ± 10	63.1 ± 7.3	
Triticale	393.3 ± 17.3	n.e.	n.e.	24.3 ± 4.8	33.7 ± 7.7	38.6 ± 7.3	63.9 ± 2.8	

Weed flora response to cover crops

The final coverage of the spontaneous cover crop was of 71.7 % in 2020 and of 82.5 % in 2021, and it was formed by 8.0 and 10.2 species on average, respectively (Table 3). Regarding sown cover crops, the highest weed coverage was observed in 2020 in *S. alba* (32.0 %) and in *P. tanacetifolia* (78.3 %) in 2021, coinciding with the highest species richness, 8.2 and 9.7, respectively. Figure 2 shows high negative correlation between cover crop coverage (without the spontaneous cover crop) and weed coverage ($R^2 = 0.88$).

The RDA analyses showed a variance of 31.8 % in 2020 and 24.8 % in 2021 on the weed community composition. In the figure 3, the first axis slightly separates spontaneous cover crop from those that were sown (except with *P. tanacetifolia* in 2021 which failed to establish). *Diploaxis eruroides* (mainly), *Sonchus oleareous*, *Fumaria officinalis* and *Veronica polita* were the species most related with the spontaneous cover crop. No species was clearly related with sown cover crops (*C. dactylon* was not present in April yet).

Table 3: Weed coverage (%) and richness (number of species) in all cover crops in April 2020 and 2021. Mean values \pm standard errors of the mean.

Cover	April 2020		April 2021	
	Weed coverage (%)	Richness (number of species)	Weed coverage (%)	Richness (number of species)
Spontaneous	71.7 \pm 6.8	8.0 \pm 0.9	82.5 \pm 7	10.2 \pm 1.4
<i>Avena strigosa</i>	27.5 \pm 3.4	7.3 \pm 0.5	18.3 \pm 12.4	6.0 \pm 0.7
<i>Hordeum vulgare</i>	13.0 \pm 4.9	4.7 \pm 1.2	6.8 \pm 4.7	2.2 \pm 1.0
<i>Lolium multiflorum</i>	14.8 \pm 4.3	4.0 \pm 0.6	25.8 \pm 9.0	5.8 \pm 1.6
<i>Phacelia tanacetifolia</i>	3.0 \pm 0.9	2.2 \pm 0.5	78.3 \pm 5.3	9.7 \pm 1.4
<i>Sinapis alba</i>	32.0 \pm 10.9	8.2 \pm 1.3	26.8 \pm 9.4	5.8 \pm 0.9
<i>Triticale</i>	13.0 \pm 6.0	5.2 \pm 1.1	25.8 \pm 7.8	5.5 \pm 1.4

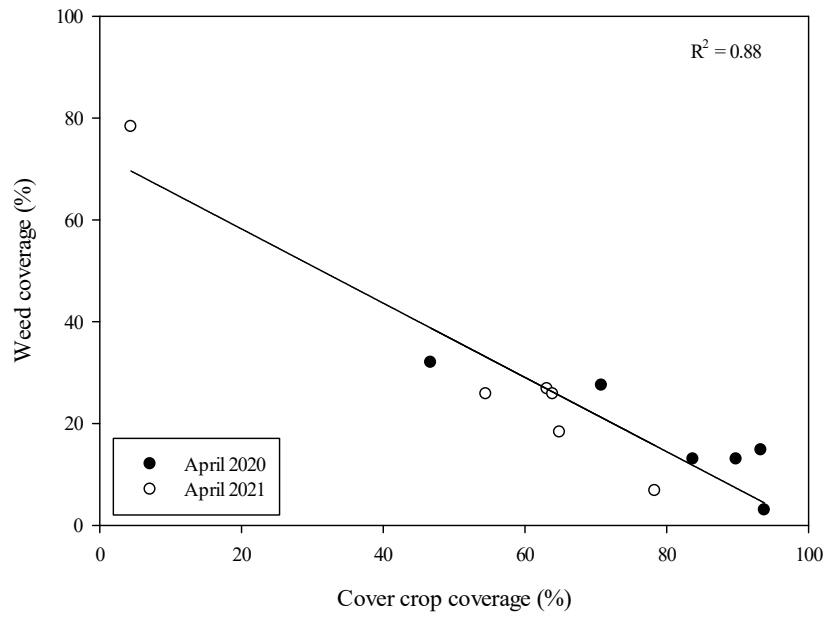


Figure 2: Weed coverage and cover crop coverage correlation: $f = 72.822 - 0.7294 * x$, $R^2 = 0.88$.

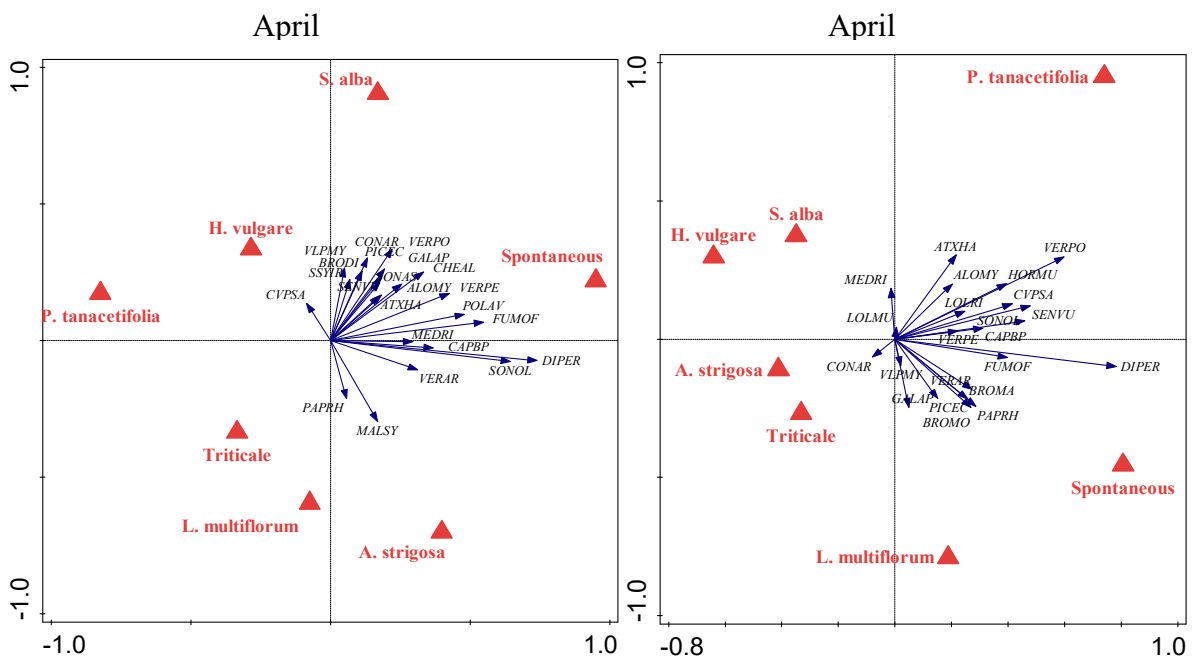


Figure 3: Redundancy analysis of species composition for 2020 (left) and 2021 (right). Red labels denote cover crop treatments. Arrows show the weed species present in the analysis. Weed species are abbreviated following EPPO Global Database (EPPO, 2022).

Cynodon dactylon response to management

In general, the coverage of *C. dactylon* was lower when the cover crop was terminated with roller-crimper than with shredding (Table 5, Figure 3). In 2020, a significant interaction between terminating method, cover crop, and sampling was observed (Table 4), which is detailed in Table 5, while in 2021, significant interaction was observed between terminating method and sampling. *Cynodon dactylon* coverage was always higher in September than in July, but these differences were greater within the shredded cover crops (Figure 3), where values increased from 14 % to 26 % in 2020 and from 11 % to 29 % in 2021. In contrast, within the cover crops managed with roller-crimper, the weed coverage increase was mitigated, from 10 % to 15 % in 2020 and from 8 % to 13 %. Despite the lack of influence of the type of cover crop, higher *C. dactylon* coverage was observed in the spontaneous cover crop, mainly with the shredding terminating method in 2020 (56 % in September).

Cover crops of *A. strigosa* and *L. multiflorum* terminated with roller-crimper were capable to maintain *C. dactylon* under low coverage (< 10 %) during both years. In 2020, significantly lower weed coverage was observed when the cover crop was terminated with roller-crimper than with shredding in the spontaneous and *A. strigosa* cover crop (in September and July), and in *P. tanacetifolia* in September.

Table 4. Three-way ANOVA of *C. dactylon* coverage. F and *p* values.

Source of variation	2020		2021	
	F value	Prob > F	F value	Prob > F
Block	0.7226	0.5325	0.1689	0.8494
Termination method (TM)	2.9878	0.163	16.421	0.0141*
Cover crop (CC)	4.6735	0.0073*	1.8834	0.1409
Sampling (S)	85.6559	<.0001*	89.7543	<.0001*
TM x CC	6.3377	0.0020*	0.7119	0.6448
TM x S	13.8695	0.0009*	13.7236	0.0009*
CC x S	2.112	0.0835	1.4313	0.238
TM x CC x S	3.4543	0.0111*	0.8415	0.5487

* Significant differences at $p < 0.05$.

Table 5. Coverage percentage (%) of *Cynodon dactylon* in July and September in each cover crop and termination method. Mean values \pm standard errors of the mean. Different letters denote significant differences at $p > 0.05$. Capital letters: differences between each termination method (shredding/roller-crimper) within each cover crop and sampling date; Lowercase letters: differences between sampling dates (July/September) within each cover crop and termination method.

<i>Cynodon dactylon</i> coverage (%)						
		2020		2021		
Treatment	Sampling date	Shredding	Roller-crimper	Sampling date	Shredding	Roller-crimper
Spontaneous	July	31.7 \pm 7.3 aA	13.9 \pm 4.6 aB	July	13.5 \pm 0.9	15.5 \pm 2.5
	Sep	55.6 \pm 1.5 bA	16.1 \pm 5.3 aB	Sep	30.1 \pm 0.7	22.4 \pm 4.8
<i>A. strigosa</i>	July	10.1 \pm 5.9 aA	4.0 \pm 1.7 aB	July	12.3 \pm 5.4	3.1 \pm 2.1
	Sep	25.6 \pm 12.1 bA	7.2 \pm 3.5 aB	Sep	23.3 \pm 11.1	8.4 \pm 4.8
<i>H. vulgare</i>	July	18.9 \pm 7.6 aA	20 \pm 5.0 aA	July	11.5 \pm 2.3	3.7 \pm 2.3
	Sep	35.9 \pm 13.7 bA	29.4 \pm 7.8 bA	Sep	44.3 \pm 16.8	11.6 \pm 3.6
<i>L. multiflorum</i>	July	7.2 \pm 3.4 aA	4.9 \pm 1.3 aA	July	4.6 \pm 0.8	3.0 \pm 0.8
	Sep	12.1 \pm 5.2 bA	10 \pm 3.0 bA	Sep	12.3 \pm 1.5	6.5 \pm 1.8
<i>P. tanacetifolia</i>	July	19.4 \pm 3.6 aA	6 \pm 3.7 aA	July	19.5 \pm 7.7	13.8 \pm 8.7
	Sep	34.4 \pm 5.5 bA	8.9 \pm 5.2 aB	Sep	36.3 \pm 23.6	18.8 \pm 10.9
<i>S. alba</i>	July	5.7 \pm 1.1 aA	19.4 \pm 5.3 aA	July	11.6 \pm 2.8	7.9 \pm 2.9
	Sep	11.7 \pm 0.8 bA	25.6 \pm 5.6 bA	Sep	35.0 \pm 2.3	11.1 \pm 3.9
Triticale	July	7.1 \pm 4.0 aA	7.6 \pm 4.2 aA	July	4.2 \pm 1.6	5.1 \pm 2.2
	Sep	9.6 \pm 5.6 aA	13.9 \pm 6.8 bA	Sep	19.3 \pm 8.5	7.1 \pm 3.2

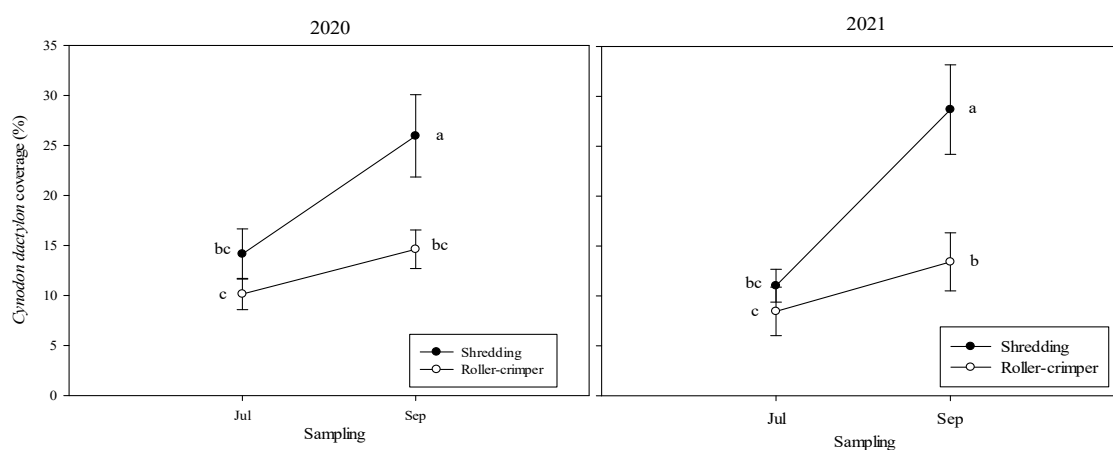


Figure 4: Overall coverage percentage (%) of *Cynodon dactylon* within each termination method (Shredding/roller-crimper) for each sampling date in 2020 (left) and 2021 (right). Different letters denote significant differences at $p < 0.05$ for the interaction of termination method and sampling date.

Discussion

In organic systems, the use of cover crops is considered a key strategy for managing weeds (Gallandt, 2014), as they are effective to suppress weed growth by competition (Gago *et al.*, 2007; Miglécz *et al.*, 2015). To avoid bare soil where weeds could grow freely, a good cover crop establishment is needed. In the present study, *S. alba* and *P. tanacetifolia* showed earliest and fastest emergence, followed by grass species. All the species studied showed similar results in a previous work (Cabrera-Pérez *et al.*, 2022). Nevertheless, *P. tanacetifolia* failed to establish in the second season, probably due to a combination of poor seedbed preparation and excessive seed burial.

The overall cover crop coverage was high in the first year, with values between 70 % and 94 %, (except for *S. alba*, < 50 %), positively influenced by an exceptional wet period (Figure 1). This situation favoured competition against weeds before terminating the cover crop, resulting in a negative correlation between cover crop coverage and weed coverage (Figure 2). The implementation of a cover crop reduces weed development and fecundity by competing for light and nutrients (Moonen and Barberi, 2004). Angelini *et al.* (1998) reported the weed-suppressive capacity of *Brassicaceae* cover crops, both by the physical effect of the cover crop itself and by the release of allelochemicals. Other authors also reported the weed-suppressive effect of grass species, whether physical, chemical, or the combination of both effects (Barberi, 2002; Smith *et al.*, 2001; Valencia-Gredilla *et al.*, 2020), this last option being supported by increasing number of literature (Brennan and Smith, 2005; Jabran, 2017). Regarding *C. dactylon* management, shredding a spontaneous cover crop was ineffective for controlling this weed, as coverage in September 2020 was 56 %. In fact, traditional and recurrent soil tillage in vineyards might be more effective against this weed, but also more aggressive for the soil (Phillips, 1993; Abdullahi, 2002). In the present work, only a single tillage was carried out each autumn, previous to the annual cover crops sowing, as the main objective of the cover crop implementation was to establish a more sustainable management (Abad *et al.*, 2021a). This soil disturbance, together with a spontaneous flora as cover crop, was not enough to control *C. dactylon* infestation. Nevertheless, the overall *C. dactylon* coverage with the other shredded cover crops was also high, with values always above 10 % and, reaching > 40 % in the case of *H. vulgare* (September 2021) (Table 5). Conversely, sown cover crops were effective for controlling winter weeds, as long as high cover crop coverage values were achieved (Table 3, Figure 2). These results can be explained by the fast

degradation of cover crops once they are shredded and divided in small particles in contact with the soil (Sims and Frederick, 1970; Bremer *et al.*, 1991), which let *C. dactylon* expand during summer. Valencia-Gredilla *et al.* (2020) also observed the lack of reduction of *C. dactylon* infestations when the spontaneous cover crop was shredded, although the weed's coverage was indeed reduced when tillage was combined with *H. vulgare* cover crop shredded in June. Perennial weeds with underground organs with high storage capacity (like *C. dactylon*) can be insensitive to cover crop residue, and their growth could even be stimulated (Mirsky *et al.* 2011). Thus, the mass, thickness, and persistence of the residue could be a key factor for these weed species to be controlled in a cover crop-based weed management system.

Overall, roller-crimper terminated cover crops improved the control of *C. dactylon* compared to the shredded ones (Figure 4). A cover crop managed with roller-crimper, even if it is spontaneous, creates a consistent mulch linked to the soil by the roots that last longer than shredded cover crop residue, and creates a physical barrier avoiding the weed proliferation. Shading has already been reported as being useful to reduce the biomass of *C. dactylon* (Guglielmini and Satorre, 2002). Low-intensity light, provided by the cover crop's canopy before *C. dactylon* starts its cycle in May, could have provoked the failure of the weed in the biomass allocation in rhizomes and stolons, hence in their development (Dong and De Kroon, 1994). This, combined with the subsequent rolling of cover crops, would delay *C. dactylon* development and would reduce its final coverage (Figure 3).

The different cover crop species and their structure conditioned the weed community composition (Figure 3). Most weed species were related with the spontaneous cover crop or with *P. tanacetifolia* (in 2021), as it could be treated as spontaneous due to its establishment failure. Sown cover crops prevented the establishment of a rich weed community assemblage, which could be explained by the dense canopy they provided, and that was useful for reducing the emergence of annual weeds during winter and early spring. Furthermore, the cover crops residues after shredding or rolling can also have allelopathic effects on the germination and growth of weeds, as it has been previously reported (Bertholdsson 2004; Bouhaouel *et al.*, 2019; Puig *et al.*, 2021).

Cover crop-based weed suppression is highly affected by the cover crop biomass (Mirsky *et al.*, 2013). The quantity of residues is more important than the type of residues, and the increase of mulch biomass on the soil surface exponentially decreases weed emergence (Teasdale and Mohler, 2000). In the present work, the local weather seems to play an important role. Raimat is located in one of the driest areas of Spain (Ebro Basin)

and the good establishment and development of the cover crops depend on the rainfall during the first months after sowing. Navarro-Miró *et al.* (2019) also concluded that the specific weather conditions of each year, together with the cover crops management, have a remarkable influence on weed density, species richness, and community composition in vegetable crops.

Cynodon dactylon is a particularly difficult to weed control in vineyards, either mechanically or chemically (Valencia-Gredilla *et al.*, 2020), especially if a sustainable approach is incorporated. Thus, a combination of different tools might be the best option for this purpose. In the current work, a single tillage for seedbed preparation followed by a winter-spring cover crop terminated with a roller-crimper in May is preferred rather than maintaining a spontaneous or sown cover crop shredded in June.

Conclusion

The assessed cover crops and terminating methods had an unequal effect on *Cynodon dactylon* coverage in the vineyard inter-rows. The terminating method had more influence in the final *C. dactylon* coverage than the cover crop species, being the roller-crimper the best option for this task. The spontaneous cover crop shredded in June was ineffective for controlling the weed. Hence, a sown cover crop terminated with the roller-crimper has shown to be the best option to manage *C. dactylon* in the conditions of the present work. The weed community composition was mainly influenced by the type of cover crop (sown/spontaneous), being the number of species lower in sowed cover crops than in the spontaneous cover.

Acknowledgements

This work has been supported by the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the projects AGL2017-83325-C4-2-R. The first author obtained a PhD grant from the University of Lleida (PhD grants). We would also like to acknowledge Maria Casamitjana and Bruna Català for their help in the field work, and the facilities and technical support supplied by the company Raventós-Codorníu SL to carry out the field trials in their vineyards.

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CHAPTER 4

Herbicidal effect of different alternative compounds to control *Conyza bonariensis* in vineyards

Published in: *Agronomy* **2022**, 12, 960

Herbicidal effect of different alternative compounds to control *Conyza bonariensis* in vineyards

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Abstract

Conyza bonariensis (L.) Cronquist is a widespread noxious weed with high fecundity, associated to no-till systems, such as vineyards and other perennial crops, in Mediterranean climates. Seeds germinate in staggered flushes, consequently there is a great variation in growth stage between individuals in the same field, and chemical control becomes challenging. Besides, *Conyza* species have evolved resistance to herbicides worldwide, particularly to glyphosate. Despite tillage is expected to provide weed-free fields, it negatively affects vineyards, causing erosion, loss of soil structure, reduction of organic matter or vine growth (shallow roots can be affected), among other effects. Fuel consumption of this management is also very high because recurrent interventions of in-row tiller are required. In this context, bioherbicides, defined as environmentally friendly natural substances intended to reduce weed populations, are a potential tool for integrated weed management (IWM). In this work, the herbicidal effect of six products is tested on a glyphosate-resistant *C. bonariensis* population present in commercial vineyards: T1, mixture of acetic acid 20 % and the fertilizer N32; T2, mixture of potassium metabisulfite and pelargonic acid 31%; T3, pelargonic acid 68 %; T4, humic-fulvic acid 80 %; T5, hydroxy phosphate complex; T6, potassium metabisulfite. The results showed high field efficacy for T1 and T4 (> 80 % biomass reduction). For the rest of the products, high efficacy was obtained only in dose-response greenhouse experiments. The present work demonstrates the potential of certain bioherbicide compounds to manage herbicide-resistant weed species, such as *C. bonariensis*. Therefore, bioherbicides could be successfully incorporated in vineyards for IWM.

Keywords: bioherbicides, no-till, conservation agriculture, sustainable weed management, organic viticulture.

Introduction

Conyza bonariensis (L.) Cronquist (hairy fleabane) is one of the most problematic weed species throughout the world (Bajwa *et al.*, 2016) and in Spain, it is considered one of the most competitive introduced noxious weeds (Zambrano-Navea *et al.*, 2013) that harms crops and leads to yield loss (Davis and Johnson, 2008; Urbano *et al.*, 2007), particularly under soil conservation management (Wu *et al.*, 2007). In fact, the increase of *C. bonariensis* prevalence has been associated to changes from conventional tillage to minimum tillage or no-till, as reducing soil disturbance favours seed germination and establishment success of this species (Storrie, 2007; Shrestha *et al.*, 2008). Apart from the adaptability to undisturbed crops, *Conyza* species have evolved resistances to herbicides worldwide (Heap, 2021), particularly to glyphosate, which has been widespread applied in Spain for weed control in citrus orchards, olive groves, grape vineyards, and others perennial and annual crops (González-Torralva *et al.*, 2010, 2014). Synthetic herbicides are important weed management tools in intensive cropping systems, but the numerous herbicide-resistant weed biotypes and environmental concerns provide limited lifespan to these chemical tools (Heap and Duke, 2018). This situation has been worsened with the lack of new herbicide modes of action discovery in the past few decades (Peters and Streck, 2018).

Conyza bonariensis has a high fecundity, producing over 100,000 non-dormant seeds per plant (Wu and Walker, 2004). Seeds germinate in staggered flushes throughout the year, depending on the environmental conditions, consequently there is a great variation in growth stage between individuals in the same field, and chemical control becomes challenging (Wu *et al.*, 2010), especially in perennial irrigated crops. For example, in Mediterranean vineyards, mainly those with dry or semiarid climates like in North-Eastern Spain, this weed can be established in high densities in the in-row area of the vine, competing for water and nutrients (Oerke, 2006). This competition is aggravated if glyphosate-resistant biotypes are present in a particular field, because these are more competitive against young vines than glyphosate-susceptible biotypes (Alcorta *et al.*, 2011).

Tillage is expected to provide weed-free fields, including *Conyza* species, in Mediterranean vineyards, but it negatively affects vines (Prosdocimi *et al.*, 2016), mainly damaging the young ones, in part, because tillage decreases the presence of grapevine roots in the topsoil (Smart *et al.*, 2006). Tillage also causes erosion and loss of soil

structure, and reduces organic matter content (Smith *et al.*, 2008). Furthermore, fuel consumption of this management doubles the carbon footprint of pesticides or fertilizers (Jradi *et al.*, 2018), because recurrent interventions of in-row tiller along the season are required to effectively manage weeds in vineyards.

Under this scenario, and considering new challenges related with economic, environmental and social concerns for more sustainable and environmentally friendly weed managements (Lechenet *et al.*, 2014), the finding of alternative weed control tools is mandatory, and especially for *C. bonariensis* in Mediterranean vineyards. Hence, bioherbicides, defined as substances of natural origin intended to reduce weed populations without damaging the environment (Bailey, 2014), are a potential tool for integrated weed management. One of the challenges still faced in bioherbicide production is the low herbicidal activity compared to the effects of chemical herbicides (Bordin *et al.*, 2021). Thus, Bioherbicides are currently underused, and few products have actually been launched on the market (Cordeau *et al.*, 2016). Nevertheless, development of new bioherbicides is compelling as these products are lagging far behind those for pests other than weeds (Seiber *et al.*, 2014). Furthermore, there are strong needs for any new weed management technology because of the rapid evolution and spread of herbicide resistances, and because weed management is the most difficult (and expensive) pest management problem in organic agriculture (Bolda *et al.*, 2016). Natural substances face several opponents since there are doubts regarding the registration processes of natural products due to the lack of relevant toxicological data for their use at commercial scale (Pavela and Benelli, 2016). Although these concerns might exist, there is evidence that most essential oils and their main compounds are not necessarily harmful to human health (Bakkali *et al.*, 2008). Such natural herbicides are sometimes less hazardous for environmental and human health in comparison to the commercial synthetic herbicides. Some commercial products, as acetic or pelargonic acid, have been already used as weed control agents. Acetic acid ($C_2H_4O_2$), sold as horticultural vinegar, is not persistent in either soil or water and has a low to medium oral toxicity to most biodiversity. However, it is highly corrosive and so may damage anything it comes in contact with. Pelargonic acid ($C_9H_{14}O_2$) is a saturated fatty acid naturally occurring as esters in the essential oil of *Pelargonium* spp. and can be derived from the tissues of various plant species. Toxicity tests on non-target organisms, such as birds, fish, and honeybees, revealed little or no toxicity (EFSA, 2013). To our knowledge, other products such as a hydroxy phosphate complex and humic-fulvic acid, widely used as organic fertilizers in many crops;

potassium metabisulfite, a preservative, antioxidant and bleaching agent in food, especially in acidic foods, such as wine; or N32, a synthetic fertilizer, have never been used as herbicides.

The aim of this study is to assess the mentioned products in order to identify alternative compounds to use as herbicides, which could be incorporated in weed management programs in vineyards, considering *C. bonariensis* as the main weed. In this study, the suppressive effect of six products on that weed is evaluated in comparison with an untreated control.

Material and methods

Conyza bonariensis population from vineyards located in Raimat, Lleida (NE Spain) was studied. The site was known to have histories of weed-control failures because of field manager complains about the impossibility to control *C. bonariensis* with glyphosate. Seeds were collected from a treated field with high *C. bonariensis* density and stored during summer 2018 as potentially herbicide-resistant population.

Characterisation of the herbicide resistance

In autumn 2018, a dose-response experiment was set with the Raimat population and with a sensitive (SP) population from Argentina, as it was very unlikely to find SP in the region. Seeds were sown in peat and after seven days, seedlings were transplanted to $7 \times 7 \times 8$ cm plastic pots filled with a mixture of silty loam soil 30 % (w/v), sand 20 % (w/v), and peat 50 % (w/v). Four seedlings were transplanted per pot. When populations reached BBCH 12-13 (Weiber *et al.*, 1998), Glyphosate 360 g a.i. L⁻¹ (Roundup; Bayer CropScience) was applied at 90, 180, 360 (1x), 720 and 1440 g a.i. ha⁻¹, with a precision bench sprayer delivering 200 L ha⁻¹ at a pressure of 215 kPa. Seven replicates (pots) were included for each population and dose. Pots were placed in a greenhouse at the University of Lleida (UdL), Spain, and watered regularly. Four weeks after treatments, the above ground part of the plants from each dose was harvested to measure the dry weight. Samples were oven dried at 65 °C for 48 h and weighted with a precision weighter (Mettler Toledo AB54-S). For the Raimat population, the results obtained on percentage of reduction of dry weight, respect to control, were 10 %, 30 %, 56 %, 65 % and 80 % at doses 0.25x, 0.5x, 1x, 2x and 4x, respectively (ED₅₀ = 1.057). On the contrary, the percentages of reduction in dry weight for the SC population were 60 % at 0.25x, 80 %

at 0.5x and 100 % at 1x, 2x and 4x ($ED_{50} = 0.176$), thereby confirming a resistance factor of 6 in the population from Raimat.

Bioherbicide field trials

A field trial was carried out from February to June in Raimat, Lleida (NE Spain) in an herbicide-managed commercial vineyard (Raventós-Codorníu S.L.). The field trial was repeated in three seasons (2019, 2020, 2021), but changing the location within the vineyard in each of them (Table 1). The climatic classification of this area is cold semiarid (BSk) (Kottek *et al.*, 2006), with an average annual precipitation of 342 mm, and annual mean temperature of 14.1 °C (average min of 8.1 °C and average max of 20.7 °C). Weather data were collected from a nearby meteorological station (<https://meteocat.cat>).

The trial locations were drip irrigated regularly throughout the growing season and vines were trained as bilateral cordons. The vineyard alleyways were maintained with a spontaneous cover crop shredded 2-3 times per season. The soil at this site was classified as a Petrocalcic Calcixerept; the specific field and vineyard characteristics are shown in Table 1, where three different previous levels of *C. bonariensis* infestation are indicated.

Table 1. Field characteristics by season. Vine variety, Caber: Cabernet Sauvignon, Chard: Chardonnay; Coordinates, Lat.: Latitude, Long.: Longitude; Vine spacing; Soil texture; pH, O.M.: Organic matter; Initial infestation level of *Conyza bonariensis*.

Season	Vine Variety	Coordinates ETRS89		Spacing (m)		Soil texture (%)			pH	O.M. (%)	Initial infestation
		Lat.	Long.	Between	Within	Sand	Silt	Clay			
2019	Caber	41°39'26.8"N	0°31'10.3"E	2.7	1.7	59.5	28.1	12.4	8.4	3.18	Low
2020	Caber	41°39'16.5"N	0°30'51.3"E	2.7	1.7	28.4	47.7	24.2	8.4	1.61	High
2021	Chard	41°40'42.9"N	0°27'51.0"E	3	1.5	27.9	38.9	33.2	8.2	2.32	Medium

Six treatments were studied combining different compounds (Table 2) to test their herbicidal effect on *C. bonariensis*: T1, mixture of acetic acid 20 % (BioEmpe-20, Bodegas Dinastia S.L.) and the fertilizer N32 (YaraVita LAST N, Yara Iberian S.A.) (70 and 30 % v/v respectively); T2, mixture of potassium metabisulfite (AGROVIN S.A.) and pelargonic acid 31% (Finalsan RTU, W. Neudorff GmbH KG); T3, pelargonic acid 68% (Kalina, Comercial Química Massó S.A.); T4, humic-fulvic acid 87 % (Herbiz, PRO&Garden); T5, hydroxy phosphate complex (Xekator, Aldamus Hispania, S.L.); T6, potassium metabisulfite (AGROVIN S.A.). The herbicidal effect of these compounds is by contact, for this reason, their effect is immediate (1-2 days).

Previous essays with T1 and T4 were carried out at the UdL to choose the best application dose (Montull *et al.*, 2019). For T2 and T6, these previous essays were

performed by the winery. For T3 and T5, the application doses were chosen according to the manufacturer recommendations.

Table 2. Compounds tested, application dose and application volume.

Treatment	Compounds	Application dose	Application volume (L/ha)
T1	Acetic Acid 20 % ⁽¹⁾ + N32 ⁽²⁾	⁽¹⁾ 122.5 L/ha ⁽²⁾ 52.5 L/ha	175
T2	Potassium metabisulfite ⁽¹⁾ + Pelargonic acid 31 % ⁽²⁾	⁽¹⁾ 70 kg/ha ⁽²⁾ 17.5 L/ha	500
T3	Pelargonic acid 68 %	16 L/ha	200
T4	Humic-Fulvic acid 87 %	35 L/ha	700
T5	Hydroxy phosphate complex	15 L/ha	150
T6	Potassium metabisulfite	60 kg/ha	250

Each year, a completely randomized design was established with six treatments and four replicates. In 2019 and 2020, the treatments were an untreated control, T1, T2, T3, T4 and T5. In 2021 T5 was excluded due to the low efficacy observed the previous years, and a new treatment (T6) proposed by the winery was added. Thus, treatments were an untreated control, T1, T2, T3, T4 and T6. The treated area was along the space within three vines (3.0 m or 3.4 m) always with width of 0.6 m. In 2019 and 2020, treatments were applied four times, between February and May (2019) and from March to May (2020). In 2021, treatments were applied three times, from April to May, when the growth stage of the plants were between BBCH 11-12 (first application) and BBCH 31-32 (last application). All plots were treated the same day in each application with a manual hand sprayer at mid-day in sunny days. A *C. bonariensis* assessment was made before each application to estimate the initial weed coverage, and another one, two days after treatment (DAT) to evaluate the herbicidal effect. In July 2020 and 2021, the above ground biomass of *C. bonariensis* plants from each plot was harvested, oven dried at 65 °C for 48 h, and dry weights were measured.

Dose-response experiment

Seeds of *C. bonariensis* from the Raimat population were sown in peat and, after seven days, seedlings were transplanted to 7 × 7 × 8 cm plastic pots filled with a mixture of silty loam soil 30 % (w/v), sand 20 % (w/v), and peat 50 % (w/v). Four seedlings were transplanted per pot, placed in a greenhouse at the UdL, and watered regularly. The experiment was carried out for T1, T2, T3, T4 and T6 at two different phenological stages (PS) of the weed: when seedlings achieved BBCH 12-13 and BBCH 14-15. Five replicates (pots) were included for each treatment, PS and dose. Pots were treated with the following doses: T1) 0, 21.9, 43.8, 87.5, 175 L/ha at BBCH 12-13 and 0, 43.8, 87.5,

175, 350 L/ha at BBCH 14-15; T2) 0 + 0; 8.75 + 2.19, 17.5 + 4.37, 35 + 8.75, 70 + 17.5, 140 + 35 kg/ha + L/ha, respectively for each compound of the mixture, at both BBCH 12-13 and BBCH 14-15; T3) 0, 4, 8, 16, 32 L/ha at BBCH 12-13 and BBCH 14-15; T4) 0, 1.1, 2.2, 4.4, 8.8, 17.6 L/ha at BBCH 12-13 and 0, 2.2, 4.4, 8.8, 17.6 L/ha at BBCH 14-15; T6) 0, 5, 10, 20, 40, 80, 160 kg/ha at BBCH 12-13 and 0, 20, 40, 60, 80, 100, 160 kg/ha at BBCH 14-15. Treatments were applied using a manual hand sprayer. Four weeks after treatments, the above ground part of the plants was harvested, oven dried and weighted as in point 2.1, to measure their dry weight.

Weather conditions

The highest observed mean temperature (T_m) during the potential emergence period of *C. bonariensis* in Raimat (grey arrows in Figure 1) was in 2020 (14.5 °C), followed by 2021 (14 °C), and it was lowest in 2019 (13.5 °C). 2020 and 2021 also resulted warmer than the historical average (13.5 °C). 2019 and 2021 were similar in terms of precipitation during the whole growing season, with 102 mm and 110 mm, respectively, and below the historical average (162 mm), while in 2020 the growth season was very wet (248 mm).

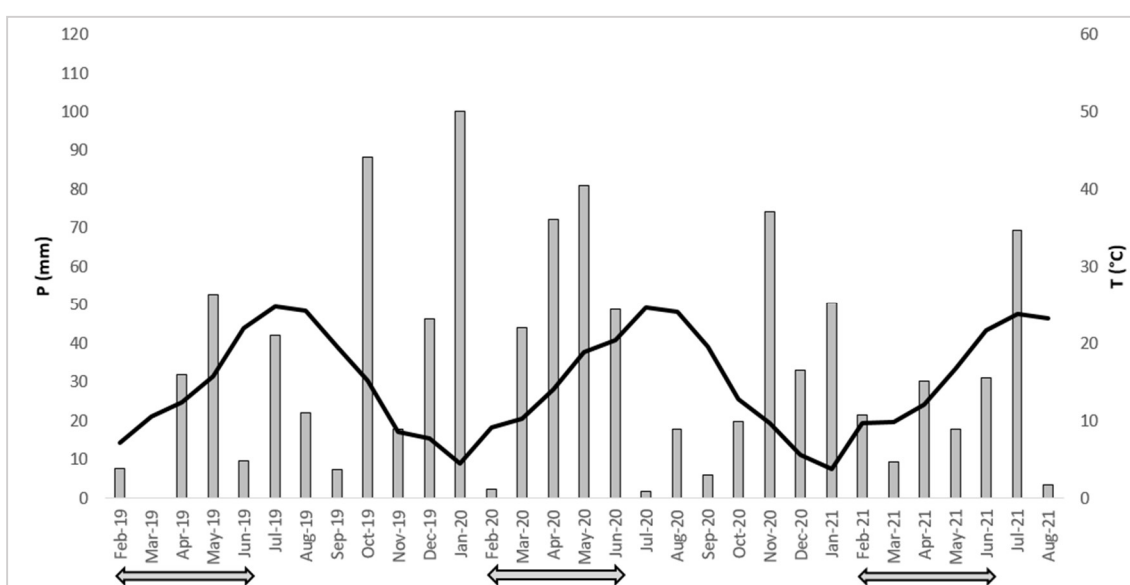


Figure 1. Weather conditions of the experiment period. Grey bars, total monthly precipitation (P); black line, mean monthly temperature (T_m). Arrows represent the growing season each year.

Statistical analyses

The field efficacy results of each treatment were expressed as cover reduction after Henderson-Tilton formula (Henderson and Tilton, 1995). After testing for normality

(Shapiro-Wilk) and homoscedasticity (Leven's test) requirements for parametric analysis, both coverage and above ground biomass data were subjected to a one-way ANOVA, followed by multiple comparisons of treatment effects with Tukey's HSD-test ($p < 0.05$). In the case of heteroscedasticity, the variance was analysed by Kruskal-Wallis H test. Data from dose-response experiments were analysed using a nonlinear regression model (1). The treatment curve was fitted with a four-parameter logistic function:

$$y = c + \frac{d-c}{1+(\frac{x}{EC_{50}})^b} \quad (1)$$

Where y is the response expressed as percentage of reduction with respect to the untreated control, c is the lower level of the curve, d is the upper level of the curve, b is the slope, EC_{50} indicates the concentration that causes 50 % growth reduction, and x is the treatment dose (independent variable). Data were analysed using JMP Pro 15 (SAS Institute 2010. SAS Campus Drive, Cary, NC27513, USA. SAS Institute Inc.) and SigmaPlot 12.0 (Sistat Software, Inc, San José, CA, USA).

Results

Field efficacy trials

Significant differences were found in all sampling dates between treatments (Table 3). Most of the compounds tested succeeded in decreasing the cover of *C. bonariensis* (Table 4), mainly in 2019, when lower overall cover of the weed was observed, and when the maximum values of the untreated plots occurred in June (10.8 %). All treatments significantly reduced *C. bonariensis* cover compared to the untreated control, except T5, which showed unsatisfactory efficacy in all application dates (between 13 % and 36 %). The efficacy in T1 and T2 was very high from April until June (> 85 %) and, in the case of T4, from February until June (> 90 %), while T3 always ranged between 52 % and 77 %. In contrast, in 2020 highest *C. bonariensis* cover were observed, coinciding with the wettest and hottest season, and the untreated plots showed 83 % weed cover (on average) by May 20. Again, the lower efficacy was observed in T5, which never exceed 7.5 %; in T1 and T3, the efficacy was also low (15 % and 56 %, respectively); while in T2 and T4, the efficacy was high and close to 80 % or 90 % in most sampling dates. This trend was repeated in 2021, but this year T6 was incorporated instead of T5, and efficacy varied between 64 % and 85 % depending on the application date; T1 and

T3 continued with low efficacy (11 % and 36%, respectively). The biomass measured (g/plot) after the last application date in 2021 supported the weed cover results, with the lowest values obtained by T2 (10.4), followed by T4 (45.6) and T6 (53.2), although significant differences were observed only for T2 with respect to T3 (244.0) and the untreated control (393.8), and for T4 and T6 compared to the untreated control (Table 5). In 2020, with higher *C. bonariensis* emergences and cover, no significant differences were found in biomass, although lower weight was also measured for T2 and T4.

Table 3. Significance of the one-way ANOVA or Kruskal-Wallis test. F/H and p values of each sampling date.

	Date	F/H	p
2019	February	4,185	0,014
	April	20,963	<0,001
	May	19,62	<0,001
	June	21,448	<0,001
2020	March	43,58	<0,001
	April	70,444	<0,001
	May	69,285	<0,001
	June	21,488	<0,001
2021	April	31,403	<0,001
	May	20,329	<0,001
	June	22,273	<0,001

Table 4. Treatments efficacy in each application date expressed as % after Henderson-Tilton formula. Mean values \pm standard errors of the mean. Different letters mean significant differences among treatments at $p < 0.05$. T1: acetic acid 20 % + N32, T2: potassium metabisulfite + pelargonic acid 31 %, T3: pelargonic acid 68 %, T4: humic-fulvic acid, T5: hydroxy phosphate complex, T6: potassium metabisulfite.

2019				2020				2021			
Applic. date / BBCH	Treat.	Pre-spray cover (%)	Cover reduction (%)	Applic. date / BBCH	Treat.	Pre-spray cover (%)	Cover reduction (%)	Applic. date / BBCH	Treat.	Pre-spray cover (%)	Cover reduction (%)
February 7	Control	0.6	0.0 \pm 0.0 b	March 11	Control	26	0.0 \pm 0.0 c	April 15	Control	5	0.0 \pm 0.0 b
BBCH 11-12	T1	0.5	64.3 \pm 20.6 ab	BBCH 11-12	T1	21	30.4 \pm 7.4 b	BBCH 11-12	T1	3	11.3 \pm 6.6 b
	T2	1	76.8 \pm 16.3 ab		T2	20	37.5 \pm 7.9 b		T2	4	89.6 \pm 3.6 a
	T3	2	76.8 \pm 14.1 ab		T3	19	15.4 \pm 3.1 b		T3	4	13.4 \pm 7.7 b
	T4	2.4	91.7 \pm 4.5 a		T4	21	66.9 \pm 7.3 a		T4	4	90.6 \pm 3.6 a
	T5	1.9	34.5 \pm 5.6 ab		T5	33	2.1 \pm 2.5 c		T6	4	74.6 \pm 7.8 a
April 16	Control	3	0.0 \pm 0.0 b	April 15	Control	51	0.0 \pm 0.0 d	May 11	Control	9	0.0 \pm 0.0 c
BBCH 11-15	T1	2	87.5 \pm 7.5 ab	BBCH 11-15	T1	34	56.4 \pm 12.6 b	BBCH 11-15	T1	8	28.3 \pm 6.4 b
	T2	2.3	100 \pm 0.0 a		T2	26	91.4 \pm 3.0 a		T2	1	60.0 \pm 10.0 ab
	T3	5.5	73.8 \pm 4.7 ab		T3	36	31.8 \pm 9.6 bc		T3	6	34.7 \pm 8.3 b
	T4	2	100 \pm 0.0 a		T4	32	85.6 \pm 6.1 a		T4	2	75.4 \pm 10.5 a
	T5	4	23.8 \pm 10.3 ab		T5	59	7.2 \pm 2.8 cd		T6	3	85.3 \pm 4.0 a
May 23	Control	7	0.0 \pm 0.0 b	May 7	Control	64	0.0 \pm 0.0 c	June 1	Control	23	0.0 \pm 0.0 d
BBCH 11-31	T1	1.9	98.3 \pm 1.7 a	BBCH 11-18	T1	38	39.7 \pm 13.0 b	BBCH 11-31	T1	12	10.7 \pm 4.3 cd
	T2	1.1	86.7 \pm 8.1 ab		T2	16	85.5 \pm 5.4 a		T2	2	87.1 \pm 5.3 a
	T3	9.5	53.2 \pm 8.1 ab		T3	34	26.5 \pm 6.9 bc		T3	11	36.3 \pm 15.8 bc
	T4	1.9	97.5 \pm 2.5 a		T4	26	78.5 \pm 7.2 a		T4	3	88.1 \pm 4.7 a
	T5	7.4	36.2 \pm 14.7 ab		T5	66	7.6 \pm 0.4 c		T6	5	64.4 \pm 8.9 ab
June 13	Control	10.8	0.0 \pm 0.0 c	May 20	Control	83	0.0 \pm 0.0 c				
BBCH 11-31	T1	0.4	92.5 \pm 7.5 a	BBCH 11-32	T1	49	24.7 \pm 12.0 abc				
	T2	0.3	90.0 \pm 5.8 a		T2	11	81.4 \pm 1.6 a				
	T3	3	52.1 \pm 12 b		T3	44	26.4 \pm 4.7 abc				
	T4	0.9	100 \pm 0.0 a		T4	26	69 \pm 8.5 ab				
	T5	4	13.1 \pm 9.4 c		T5	76	6.6 \pm 0.2 bc				

Table 5: Dry weight (g) biomass of *C. bonariensis* in July 2021. Mean values \pm standard errors of the mean. Different letters denote significant differences among treatments at $p < 0.05$. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%, T4: humic-fulvic acid, T5: hydroxy phosphate complex, T6: potassium metabisulfite.

Treatment	g/plot	
	2020	2021
Control	308.8 \pm 14.4	393.8 \pm 106.5 a
T1	271.8 \pm 10.0	161.9 \pm 65.5 abc
T2	216.9 \pm 18.9	10.4 \pm 5.2 c
T3	262.2 \pm 41.5	244.0 \pm 103.5 ab
T4	231.5 \pm 30.3	45.6 \pm 18.7 bc
T5	278.1 \pm 47.7	-
T6	-	53.2 \pm 23.6 bc

Dose-response curves

The equation parameters of the best fitted models, based on the coefficient of determination (r^2) and the EC_{50} values, are shown in Table 6 and represented in Figure 2. The obtained r^2 values were always above 0.8, and most above 0.9, which indicate the suitability of this function to describe growth response of *C. bonariensis* to different concentrations of the tested compounds. Biomass reduction was greatly influenced by the phenological stage of the treated plants, while 100 % of biomass reduction was achieved in BBCH 12-13 in almost all treatments, this value resulted more difficult to reach in BBCH 14-15. On the other hand, the EC_{50} value at least doubled that of BBCH 12-13 when the population of *C. bonariensis* was in BBCH 14-15.

Table 6. Parameters of dose-response curves represented in Figure 2. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%; T4: humic-fulvic acid, T6: potassium metabisulfite.

Treatment	Compounds	BBCH	r^2	EC_{50}	slope (b)
T1	Acetic Acid 20% + N32	12-13	0.900	47.62 (L/ha)	1.44
		14-15	0.940	96.92 (L/ha)	1.45
T2	Potassium metabisulfite + Pelargonic acid 31%	12-13	0.909	10.06 + 2.52 (kg/ha + L/ha)	1.92
		14-15	0.887	21.02 + 5.26 kg/ha + L/ha)	1.15
T3	Pelargonic acid 68%	12-13	0.954	4.10 (L/ha)	3.25
		14-15	0.827	10.07 (L/ha)	2.2
T4	Humic-Fulvic acid	12-13	0.939	1.30 (L/ha)	1.25
		14-15	0.906	4.12 (L/ha)	2.01
T6	Potassium metabisulfite	12-13	0.892	17.84 (L/ha)	1.43
		14-15	0.812	37.32 (L/ha)	1.64

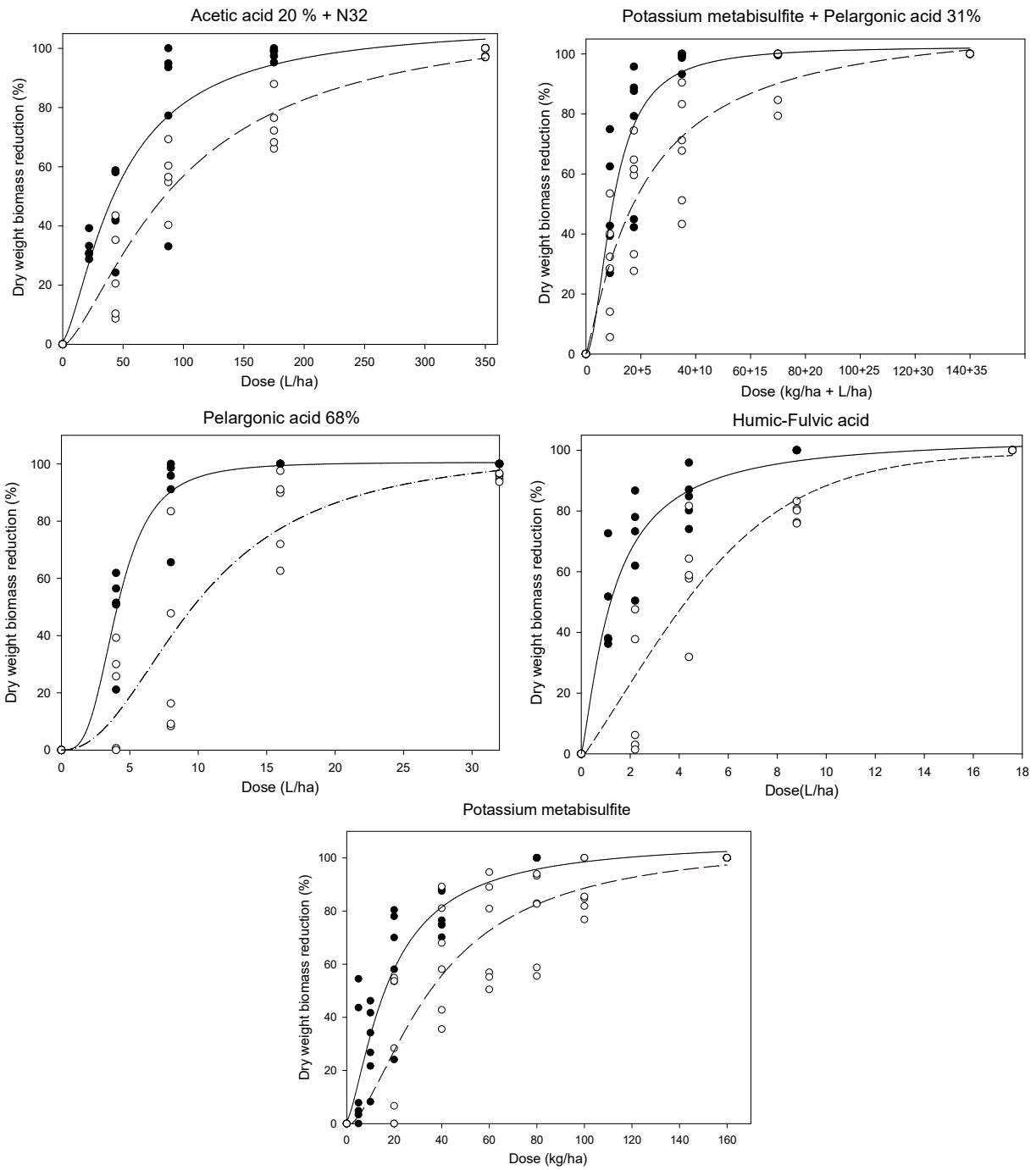


Figure 2. Dose-response curves of T1: acetic acid 20 % + N32, T2: potassium metabisulfite + pelargonic acid 31 %, T3: pelargonic acid 68 %; T4: humic-fulvic acid, T6: potassium metabisulfite. Values are presented as dry weight biomass reduction (%) of the no-treated control. Black points (●) and solid lines (—), BBCH 12-13; white points (○) and dashed lines (---), BBCH 14-15.

Discussion

The *C. bonariensis* populations from Raimat vineyards were found to be resistant to glyphosate with a resistance factor of 6. This species is known to easily evolve resistant biotypes as several cases are reported in the literature. For example, Travlos and Chachalis (Travlos and Chachalis, 2010) observed 4- to 7-fold resistance levels in *C. bonariensis* growing in Greek perennial crops, including vineyards. Similarly, Urbano *et al.* (2007) established a 7- to 10-fold resistance level in *C. bonariensis* collected from Spanish olive fields, and more recent studies have showed a 27-fold resistance level for this weed in South African vineyards (Okumu *et al.*, 2019). The cropping systems of the above-mentioned examples are similar to that of this study, and they share common features like a long and repeated history of glyphosate use, and lack of crop and herbicide rotation.

According to Bailey (2014), bioherbicides are products of natural origin that are useful for weed control, and that can be either living organisms or products derived from living organisms. All tested compounds fulfil that definition, except N32 (synthetic fertilizer) and potassium metabisulfite. Nevertheless, that last compound was tested because its use in winemaking is very common and, according to the International Chemical Safety Cards (ICSCs), the environmental effects of potassium metabisulfite has no significant effects, according to the current knowledge. T2, T4 and T6 stand out from the rest, as high field efficacy was observed along the application dates (Table 4), which was confirmed with the harvested above ground biomass in 2021 (Table 5), but not in 2020, where emergences were more abundant and constant until July, probably due to a higher initial presence of seeds with the combination of an extraordinary wet spring. Furthermore, differences in 2020 biomass between high-effective treatments (T2 and T4) and low-effective ones (T1, T3 and T5) could have been diminished because of intraspecific competition of *C. bonariensis* plants in the latter treatments, as higher weed cover was observed but with smaller plants. The efficacy of T1, T3 and T5 was unequal and not always enough to maintain low *C. bonariensis* cover. The lack of efficacy of T5 is likely due to a harmless effect on the plant rather than in the applied dose. Conversely, the burning effect of the acetic (T1) and pelargonic (T3) acids are highly effective in early rosette stages of this species; nonetheless, some individuals showed green growth regions in the centre of the rosette after applications, which eventually developed inflorescences and disseminated achenes in the field trials. Pline *et al.* (Pline *et al.*, 2000) observed a variation of pelargonic acid efficacy from only 6 % up to 65 %, depending on the annual

weed species and similar to Travlos *et al.* (2020), who re-reported an efficacy between < 20 % up to > 90 %. Webber III *et al.* (2014) observed good grass control (> 80 %) and fair (> 70 %) broadleaf control (without *Conyza* spp.), and Kanatas *et al.* (2021) attributed the low weed control efficacy of pelargonic acid in olive fields to the presence of *C. bonariensis*, which indicates tolerance of this species to this acid, similar to the observations in the present study. The presence of buds in the *C. bonariensis* taproot enables a rapid regrowth after clipping (Wu, 2007). Efficacy variations are also found in literature with acetic acid: Webber *et al.* (2018) observed an efficacy ranging from 4.5 % up to 100 %, depending on the weed species, when acetic acid at 20 % was applied at 187 L/ha. In the current study, acetic acid (T1) obtained good efficacy only in 2019, with low *C. bonariensis* cover (probably due to little emergences) and when all plants could be treated in an early phenological stage.

Similar to synthetic herbicides, the effect of the tested compounds can rely on dosage, the phenological stage of the target weed, and on the environmental conditions (Ghorbani *et al.*, 2006; Hallett, 2005). In fact, the most effective treatments, potassium metabisulfite (T2) and humic-fulvic acid (T4), obtained better results when applied in April, although in 2019 and 2020 there was a previous application in February and March, respectively, and according to dose-response results (Figure 2, Table 5), would be expected to be more effective, as *C. bonariensis* rosettes were smaller. This contradictory result can be explained by the weather conditions. In April 2019 and 2020, temperature was higher than in February 2019 and March 2020, which is known to improve herbicide efficacy (Steward *et al.*, 2009). Waltz *et al.* (2004) attributed this enhanced effect with higher temperatures to a change in the epicuticular wax that facilitates herbicidal effect. This statement would lead to think that the treatments' effect should improve during spring, but *C. bonariensis* plants that survived the firsts applications of low-effective treatments (T1, T3 and T5) were in an advanced phenological stage by May, so despite the high temperatures, the efficacy was lower, especially in 2020, when there was an abundant emergence of the weed and could hinder droplet contact with the leaves.

The herbicide's efficacy is clearly influenced by the phenological stage of *C. bonariensis* (Urbano *et al.*, 2007; Okumu *et al.*, 2019), with sensitivity or injury decreasing as the growth stage is advancing. This has been confirmed by the dose-response curves in all treatments (Figure 2). When plants grow from BBCH 12-13 to BBCH 14-15, the EC₅₀ in all treatments doubles up (Table 5). In the dose-response experiment, nearly 100 % of biomass reduction was achieved in BBCH 12-13 at some

dose in all treatments, compared to the untreated control, demonstrating the potential of these compounds to control *C. bonariensis*. Plants showed visible injury ranging from chlorosis, going through necrosis, to eventually complete the wilting of plants. In general, the observed injury symptoms increased with increasing compound concentrations. The same symptoms were observed in the field trials two days after treatments. But a long-term control for *C. bonariensis* is challenging because of its germination and emergence characteristics, with overlapped cohorts along the season. *Conyza* species can potentially germinate at any time throughout the year (Zambrano-Navea *et al.*, 2018), and irrigated crops like drop-irrigated vineyards ease this process. For this reason, contrasting reports about the main emergence season for *Conyza* spp. are found in the literature, which sometimes is considered as winter annuals (Wu, 2007) and some others as summer annuals (Davis and Johnson, 2008). Moreover, Valencia-Gredilla *et al.* (Valencia-Gredilla *et al.*, 2020) observed the highest germination percentage of *C. bonariensis* at 22 °C, but they also reported that the biotypes from the Lleida region had more germinated seeds at lower temperatures than biotypes from warmer regions. Thus, the application of a control method (either bioherbicide or synthetic chemical) in homogeneous phenological stage is extremely difficult, and explains differences found in efficacy between greenhouse experiments of dose-response curves and field efficacy trials.

Consequently, although the available bioherbicides are promising compounds for weed control, few have achieved long-term commercial success in the field (Cordeau *et al.*, 2016). According to our results, bioherbicides may unleash their potential when addressed to specific species in early phenological stages, rather than pretending a widespread use to many species. The increase of *C. bonariensis* prevalence forces to find alternatives for their control. In this sense, new herbicidal compounds may be incorporated as tools for integrated weed management (IWM). In fact, none of the individual techniques on their own can be expected to provide acceptable control levels, but when combined with other tools, successful results can be achieved (Zambrano-Navea *et al.*, 2016). So, the combination of different techniques such as cover crops, mulching and bioherbicides, could facilitate the decrease of *C. bonariensis* infestations in no-till viticulture. It is important to know that in the Mediterranean climate earlier cohorts of *C. bonariensis* contribute most to the following generation, therefore, they should be preferably targeted when designing control strategies (Zambrano-Navea *et al.*, 2018). Predicting the emergence of *C. bonariensis* with already developed models based on

climate parameters, like that from Zambrano-Navea *et al.* (2013), can also contribute to decision support system for an optimum application timing.

Although many naturally occurring materials, such as most of the tested compounds, have herbicide properties, there is controversy whether they should be allowed to be used in organic crop production systems (Webber *et al.*, 2012; Dayan *et al.*, 2009). Therefore, producers need to know the regulation policies that cover their organic, natural, or sustainable crop production. Finally, given the necessity to reduce carbon footprint caused by tillage, and lack of new modes of actions in synthetic herbicides, innovations on bioherbicides are much needed (Charudattan and Dinooor, 2000), so that these can be successfully incorporated in vineyards for IWM in the short-term.

Conclusion

To date, no studies have focused in the herbicide potential of alternative compounds specifically on *C. bonariensis* in commercial vineyards. The findings of the present study revealed that, despite most of the compounds tested were able to control the weed in the greenhouse dose-response experiment, only the potassium metabisulfite + pelargonic acid 31 % (T2), the humic-fulvic acid (T4) and the potassium metabisulfite (T6) obtained high field efficacy along the application dates and were able to maintain an acceptable *C. bonariensis* cover.

Acknowledgements

This work has been supported by the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project AGL2017-83325-C4-2-R and also by MACMHER “Grups operatius” promoted by Department of Agriculture of Catalan Government (DARP) in 2018. The first author obtained a PhD grant from the University of Lleida (PhD grants). We would also like to acknowledge Maria Casamitjana, Bruna Català and Irene Llobera for their help in the field work, and the facilities and technical support supplied by the company Raventós–Cordorníu to carry out the field trials in their vineyards in Raimat.

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CHAPTER 5

Organic mulches as an alternative to conventional under-vine weed management in Mediterranean irrigated vineyards

Submitted to *Agroecology and Sustainable Food Systems*, June 2022.

Organic mulches as an alternative to conventional under-vine weed management in Mediterranean irrigated vineyards

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Abstract

Vineyard growth and grape yield can be significantly reduced by weeds, especially when these are located in the under-vine zone. Traditional weed management consist in tillage or herbicide, the former associated to soil erosion and high fuel consumption and the latter to environmental and human health damage. In order to find alternative weed management methods, three field trials were carried out in Raimat (Lleida, NE Spain) with the aim to evaluate the suppressive effect of four mulches against weeds in. Treatments included (1) straw mulch of *Medicago sativa* L., (2) straw mulch of *Festuca arundinacea* (L.) Schreb, (3) straw mulch of *Hordeum vulgare* L., (4) chopped pine wood mulch of *Pinus sylvestris* L., (5) mechanical cultivation and (6) herbicide. Results showed that mulches were efficient to control weeds when compared with the two traditional methods, as long as the % of soil covered by mulches was high (> 75%). In this way, pine mulch stood out above straw mulches, as it achieved high soil cover during the three growing seasons, and avoided weed growth. This, together with the multiple benefits of mulches, make them a sustainable tool to be considered as an alternative to traditional under-vine weed management in vineyards.

Keywords: Sustainable viticulture, soil management, herbicide resistance, in-row tiller, conservation agriculture.

Introduction

Grapes are one of the most spread crops along the Mediterranean basin, and Spain has the largest vineyard area cultivated with 964,037 ha (MAPA, 2021a). Vineyard growth and grape yield can be significantly reduced by weeds (Oerke, 2006), mainly in young vines (Byrne and Howell, 1978), as weeds compete for water, nutrients and light (Hembree and Lanini, 2006). In most Spanish vineyards, weeds are traditionally managed through mechanical cultivation or herbicide application in the under-vine zone (MAPA, 2019), leaving the soil bare most of the year. The use of herbicides has been proven more effective than tillage in controlling vineyard weeds, being more cost effective and easier to use, which has justified its use in weed management (Steenwerth and Guerra, 2012). However, herbicides may cause many problems linked to environmental contamination and human health (Narayan *et al.*, 2017), high risk of toxicity, including vines (Tourte *et al.*, 2008), potential impact of drift from commonly used auxin herbicides on leaves and grapes (Haring *et al.*, 2022), and a reduction of root mycorrhization that alter nutrient composition in grapevine roots, leaves or grape juice (Zaller *et al.*, 2018). Besides, herbicides' success is limited depending on the weed species characteristics, the timing of the application and on weather conditions. Among the weeds that are difficult to control with herbicides in vineyards, *Conyza bonariensis* (L.) Cronquist stands out for its noxiousness (Recasens *et al.*, 2018, Bajwa *et al.*, 2016). It is a vigorous and competitive weed that can evolve into herbicide-resistant biotypes due to the continuous use of non-selective herbicides (i.e., glyphosate) (Urbano *et al.*, 2007). This weed can establish at high density in the under-vine zone, competing for water and nutrients, which can be aggravated if glyphosate-resistant biotypes are present, as these are more competitive against young vines than glyphosate-susceptible biotypes (Alcorta *et al.*, 2011).

The limited lifespan to chemical tools (Heap and Duke, 2018), combined with the social demand for more sustainable agroecosystems (Harvey and Pilgrim, 2011), are encouraging wine growers to rethink their farming management. In fact, there are already 131,000 ha of vineyards organically managed in Spain (MAPA, 2021b), where weed control is recognised as the foremost production-related problem (Kloen and Daniels, 2000). Mechanical weed management is expected to provide weed-free fields, but it can negatively affect vineyards (Cerdan *et al.*, 2010, Prosdocimi *et al.*, 2016), mainly damaging young vines, in part, because tillage decreases the presence of grapevine roots in the topsoil (Lanini *et al.*, 2011; Smart *et al.*, 2005). Tillage also leads to erosion and loss of soil structure (Abad *et al.*, 2021a), and reduces organic matter content (Glover *et al.*, 2000, Smith *et al.* 2008), altering the population of soil

microorganisms (Virto *et al.*, 2012). Furthermore, fuel consumption of this management doubles the carbon footprint of pesticides or fertilizers (Jradi *et al.*, 2018), because recurrent interventions of in-row tiller along the season are required to effectively manage weeds in vineyards as cultivation brings new seed to the surface, which combined to an enhance of soil nitrogen mineralization, leads to weed emergence flushes (Bàrberi 2002).

In addition, repeated use of any weeding method is likely to cause a shift in the weed flora to resistant or tolerant species. Integration different weed control techniques would help to avoid this, and may provide more effective or more economic control in the current crop (Bond *et al.*, 2001). But, finding environmentally friendly techniques to manage weeds while maintaining the grapevine performance and the soil quality is not easy, and mulching the under-vine zone can be a useful tool. Mulch is any bulk material placed on the soil surface to control weeds and/or preserve moisture. Organic mulching is a sustainable agronomic practice that has an inhibitory effect in the emergence of weeds, reducing the overall weed biomass even more than herbicides or cultivation (Steinmaus *et al.*, 2008) by creating a physical barrier for light and temperature interception (Elmore *et al.*, 1998). Furthermore, organic mulches can cause allelopathic effects due to the substances released into the soil that can reduce the weed emergence by 80 % (Dhima *et al.*, 2006). Organic mulches can also minimize water loss through evaporation (Davies *et al.*, 2011) that is enhanced with an increase in mulch thickness (Myburgh, 2013). Hence, soil water content and vine water status are improved (Buesa *et al.*, 2021), which can also be attributed to proliferation of fine roots provoked by mulches (Linares-Torres *et al.*, 2018). Consequently, mulches provide substantial water savings (López-Urrea *et al.*, 2020). Moreover, water soil infiltration can be also improved (Varga and Májer, 2004), as organic mulches increase the soil organic matter content and the soil biological activity (Thomson and Hoffman, 2007) with a positive effect for grapevine yield and must composition (Mundy and Agnew, 2002).

The aim of the present work is to identify alternative methods to the use of herbicides and mechanical cultivation, that can be incorporated in weed management programs in vineyards. In this study the weed suppressive effect of four mulches was evaluated: (1) straw mulch of *Medicago sativa* L., (2) straw mulch of *Festuca arundinacea* (L.) Schreb, (3) straw mulch of *Hordeum vulgare* L., (4) chopped pine wood mulch of *Pinus sylvestris* L., in comparison with mechanical cultivation and herbicide.

Material and methods

Experimental site

Three field trials were established in a commercial wine grape vineyard located in Raimat (Lleida, NE Spain); the first two trials (Trials 1 and 2) were carried out from 2017 to 2019, and located in an organically managed vineyard where the traditional under-row weed management consisted on soil cultivation with an in-row tiller with 3-4 interventions per season. Trial 3 was carried out from 2019 to 2021 in a conventional vineyard historically managed with 2-3 under-row herbicide applications per season. The field of Trial 3 had an important infestation of *C. bonariensis* in the under-vine zone ($45 \% \pm 5.2$ of soil covered in autumn 2018). All trials were drip irrigated regularly throughout the growing season, and vines were trained as bilateral cordon. A spontaneous cover crop in the inter-row was shredded 2-3 times per season in all trials. The specific field and vineyard characteristics are shown in Table 1. The climatic classification of this area is cold semiarid (BSk) (Kottek *et al.*, 2006), with an average annual precipitation of 342 mm, and annual mean temperature of 14.1 °C (average min of 8.1 °C and average max of 20.7 °C). Climate data were obtained from an automatic weather station belonging to the regional meteorological network, located close to the vineyard in Raïmat (www.meteocat.cat).

Table 1: Field trials characteristics. Vine variety; Vineyard establishment; Coordinates; Vine spacing; Soil texture; pH, O.M.: Organic matter.

Trial	Vine Variety	Vineyard establ.	Coordinates ETRS89		Spacing (m)		Soil texture (%)				
			Latitude	Longitude	Inter-row	In-row	Sand	Silt	Clay	pH	O.M.
1	Cab sauv.	2009	41°39'28.1"N	0°31'11.3"E	3.0	1.5	28.4	47.7	24.2	8.40	1.61
2			41°39'30.7"N	0°31'13.8"E							
3	Pinot noire	2010	41°40'28.8"N	0°28'00.0"E	2.8	1.5	27.9	38.9	33.2	8.18	2.32

Experimental design

In Trial 1, five treatments (four different mulches with no tillage and one tillage control) were established in the vineyard following a randomized complete block design with three replicates distributed in 15 rows of 40 m long: 1) straw mulch of *M. sativa*, 2) straw mulch of *F. arundinacea*, 3) straw mulch of *H. vulgare*, 4) chopped pine wood mulch of *P. sylvestris*, and 5) mechanical cultivation (Tillage). Mulches were applied along the under-row with 0.4 m wide and 10 cm thick. Experimental units were the average of 3 plots comprising 3 m in each row. In Trial 2, the same mulches as Trial 1 were studied but with two different thicknesses, 5 and 10 cm, and one tillage control. A total of nine treatments were established following a complete

randomized design distributed over 9 rows of 35 m long, with three 0.4 x 3 m replicates each. Three mechanical interventions were done in the control treatment (Tillage) in both trials on the first season, four during the second season and one in the third season, before the last sampling, always between February and September. Mulches were applied only at the beginning of the experiment, in March 2017.

In Trial 3, a complete randomized design was established over six rows with two treatments, 1) chopped pine wood mulch of *P. sylvestris* with no tillage, and 2) herbicide application, each replicated six times. Experimental units were 12 plots of 0.4 m wide x 6 m long. The herbicide applied was Glyphosate 360 g a.i. L⁻¹ (Roundup; Bayer CropScience), which was applied with a manual backpack sprayer (Matabi®) twice in 2019 (May and June) and in 2020 (March and May), and once in 2021 (March). The mulch was installed only at the beginning of the experiment, in January 2019, and was 15 cm thick. All vines within each trial were irrigated with the same amount of water and fertilized according to the standard practice of the farm. No further action (soil labour, herbicide application) was taken in the mulched plots.

Weed and mulch sampling

Weed cover was evaluated three to four times each year, except in 2019 in Trials 1 and 2, where only one sampling was performed at the beginning of the third season, in April. Total weed cover of each species was visually estimated as the percentage of the whole plot, in the case of Trials 2 and 3, or as the mean of the three subplots in the case of Trial 1. Samplings were done after each mechanical intervention in Trials 1 and 2. In Trial 3, samplings were done independently of herbicide application and total aerial biomass of *C. bonariensis* was collected from the whole plot in each treatment every year in September, oven-dried at 67°C for 72 hours, and weighted with a precision weight. Mulch persistence was also visually estimated every season as soil cover percentage.

Statistical analyses

Weed and mulch cover data of Trial 1 and 2 were subjected to one-way ANOVA for each sampling date, followed by multiple comparisons of treatment effects with Tukey's HSD-test ($p < 0.05$). When necessary, data were square root transformed to meet assumptions of ANOVA, normality (Shapiro-Wilk) and homoscedasticity (Leven's test). Data were back transformed for clarity in the results. To analyse total weed cover data and biomass of *C. bonariensis* in Trial 3,

Mann-Whitney Rank Sum Test was applied due to the impossibility to accomplish the assumptions of the ANOVA. Analyses and graphs were performed with JMP Pro 15 (SAS Institute 2010. SAS Campus Drive, Cary, NC27513, USA. SAS Institute Inc.) and SigmaPlot 12.0 (Systat Software, San José, CA, USA). In order to evaluate differences in weed species composition among the different managements, a redundancy analysis (RDA) was performed for Trials 1 and 2 with CANOCO 5.0 (Microcomputer Power: Ithaca, NY, USA, 2012).

Results

Weather conditions

The mean monthly temperature (T_m) was similar between years during the growing season (black arrows in Figure 1), with 17.9 °C for 2017, 17.1 °C for 2018, 17.0 °C for 2019, 17.6 °C for 2020 and 16.8 °C for 2021 and differed very little from the historical average (17.0 °C). On the other hand, 2017, 2018 and 2020 were the wettest years during the growing season, with 211 mm, 228 mm and 248 mm respectively, and above the historical mean (156 mm), while 2021 could be considered an average year with 158 mm, and 2019 was the driest with 136 mm of rain.

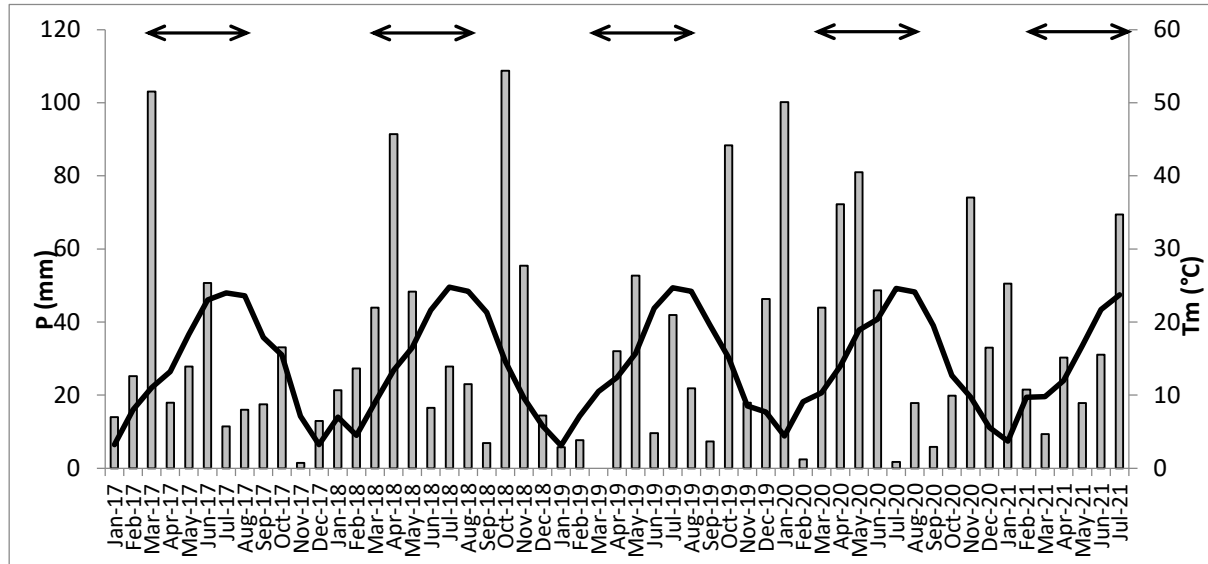


Figure 1: Weather conditions of the experiment period. Grey bars, total monthly precipitation (P); black line, mean monthly temperature (T_m). Arrows represent the growing season each year (from March to September).

Weed cover response to management and mulch persistence

The overall weed cover percentage for Trial 1 was very low during the 2017 season (Figure 2), with medium values < 5 % in all treatments and samplings. Winter-spring species (March-May 2017) were more abundant than summer species (July-September 2017). In March

2017 and 2018, there were significant differences between the tilled control (4.3 % and 11.2 % respectively) with respect to *M. sativa* (0.2 % and 3.4% respectively) and *H. vulgare* (0.2 % and 2.6% respectively) mulches. Conversely, from May 2018 until September 2018, the tilled control and *P. sylvestris* mulch maintained lower weed cover along the samplings (< 7 %) than straw mulches (*M. sativa*, *F. arundinacea* and *H. vulgare*), with significant differences between the two former ones with respect to *F. arundinacea* mulch in September 2018. In early 2019, weed presence increased, especially in *M. sativa* (70.3 %) and *F. arundinacea* (78.9 %) mulches. The lowest weed cover was observed in *P. sylvestris* mulch (10.3 %), significantly different from straw mulches, but not when compared with the tilled control (11.7 %). The persistence of the mulches (mulch cover) decreased from one year to the next, mainly in straw ones (Figure 3), with the fastest degradation observed between 2018 to 2019, from > 90 % in May 2018 for all treatments down to 2 % in *F. arundinacea* mulch, 15 % in *M. sativa* mulch, and 26 % in *H. vulgare* mulch, with significant differences between *Pinus sylvestris* mulch with respect to the others. In fact, *P. sylvestris* was the only mulch that remained almost unchanged, with over 95 % cover in 2019.

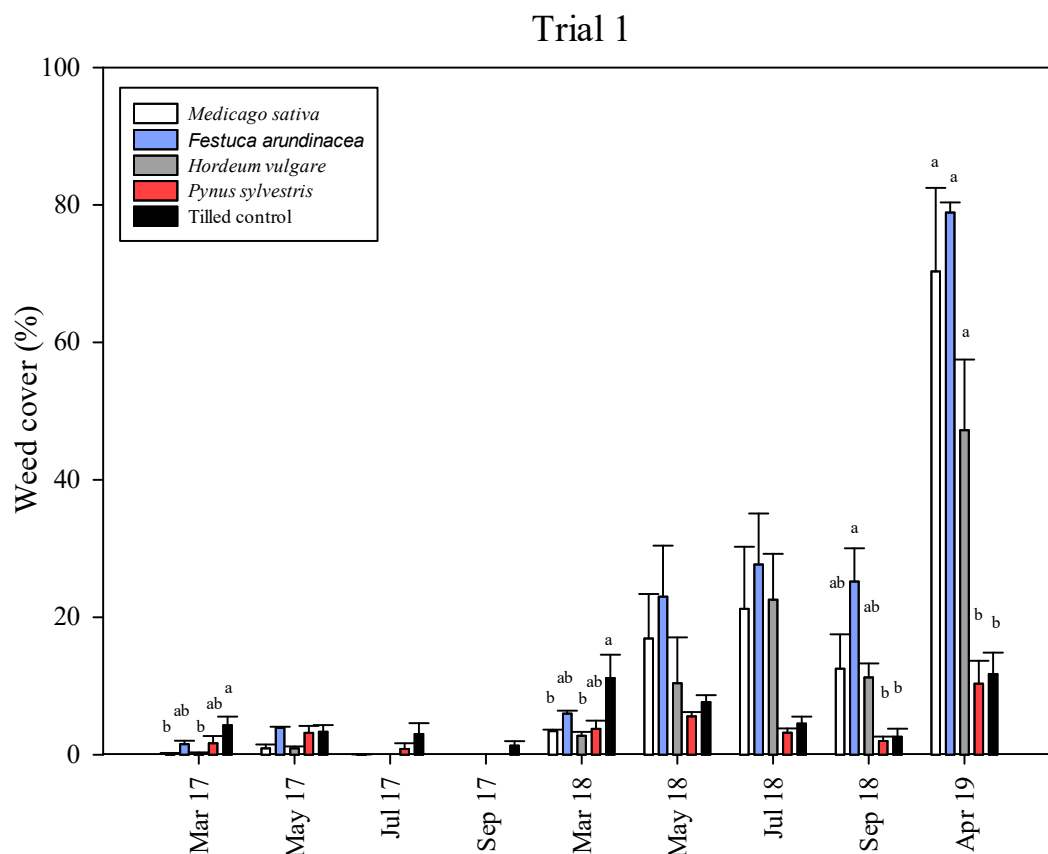


Figure 2: Weed cover (%) in each treatment along the three seasons (2017, 2018, 2019) of Trial 1. Vertical bars represent standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$.

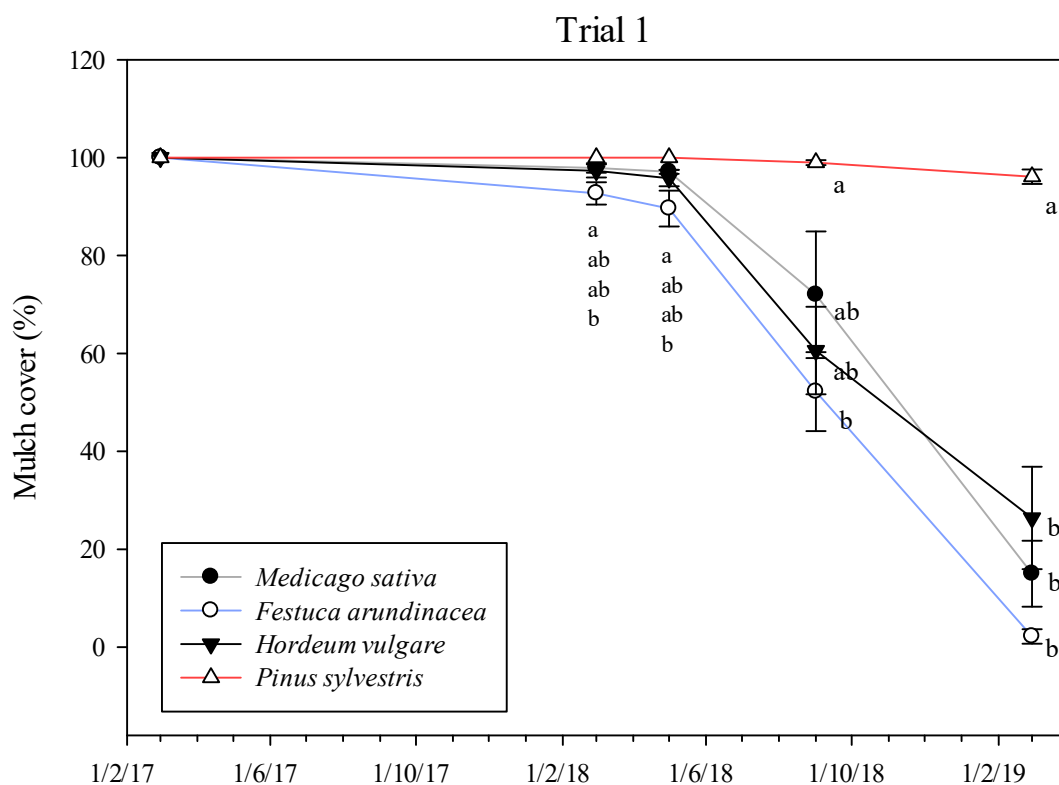


Figure 3: Mulch cover (%) of each mulch treatment along time in Trial 1. Vertical bars represent standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$.

In Trial 2, significant differences on total weed cover were observed in each sampling date (Figure 4). In 2017, the overall weed cover was $< 20\%$ in all treatments, with *H. vulgare* mulches showing the lowest cover values ($< 1\%$) at the two thicknesses considered, and significantly different with most of the other treatments. During 2018, only *P. sylvestris* and *H. vulgare* mulches, altogether with the tilled control, maintained low cover ($< 15\%$), improving significantly the effectiveness of *M. sativa* and *F. arundinacea* mulches. In April 2019, cover values of *P. sylvestris* mulches and the tilled control were still low ($< 12\%$) and differences increased with respect to straw mulches, where cover values varied from 57% up to 81% . Few significant differences were found between thicknesses within each mulch, only reflected in July 2017 and May 2018 in *M. sativa* mulch. The persistence of the mulches decreased similarly to those mulches in Trial 1, with cover values between 0% and 7% in the straw mulches in March 2019, and significantly lower than *P. sylvestris* mulch, with $> 80\%$ (Figure 5).

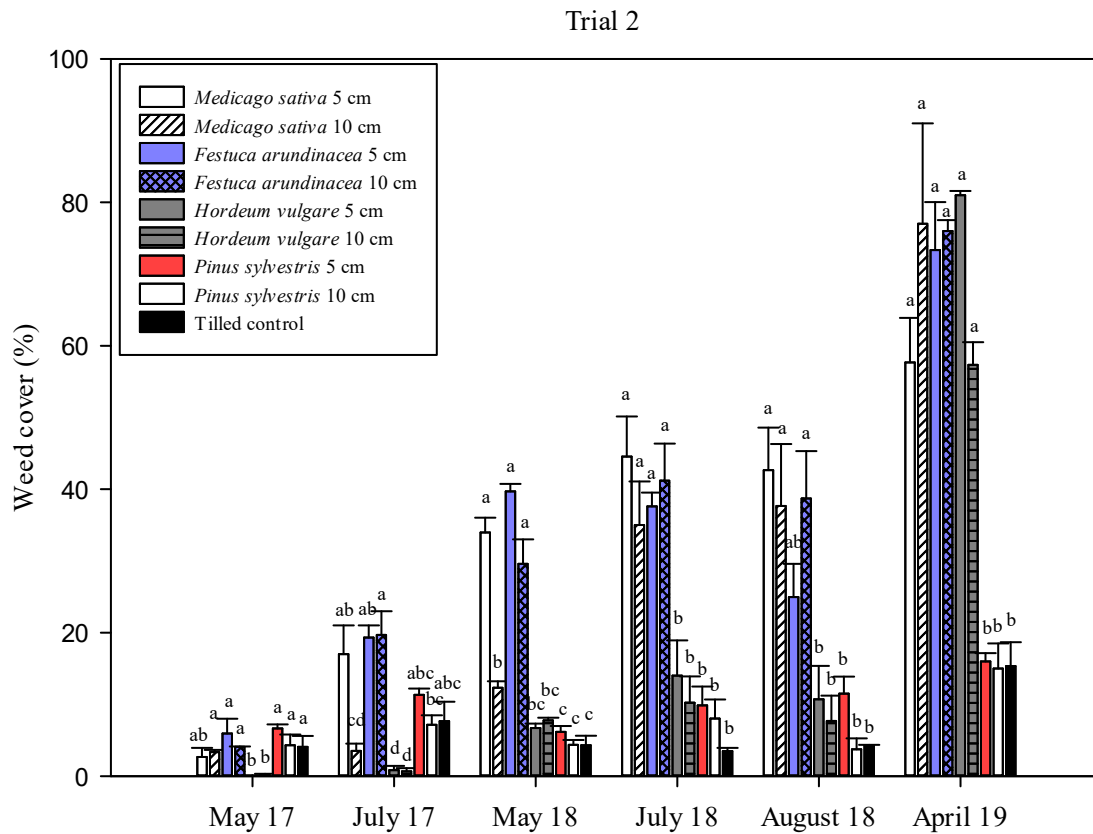


Figure 4: Trial 2, weed cover (%) in each treatment along the three seasons (2017, 2018 and 2019). Vertical bars represent standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$.

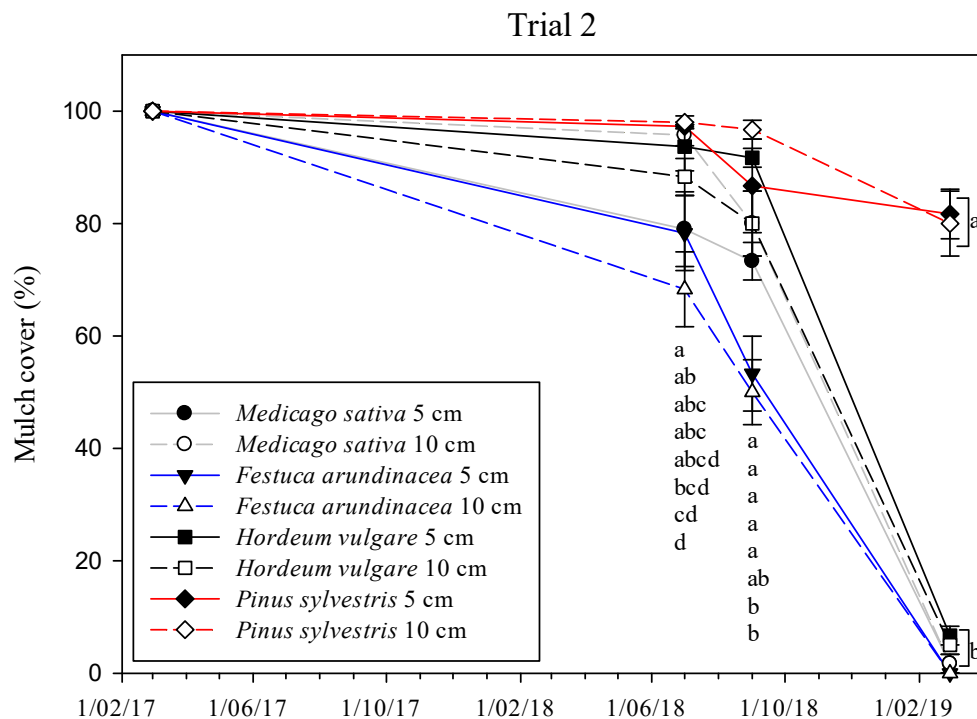


Figure 5: Mulch cover (%) of each mulch treatment along time in Trial 2. Vertical bars represent standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$.

In Trial 3, weed cover values were significantly higher in the herbicide control than in *P. sylvestris* mulch (Figure 6) from May 2019 to March 2021, and again in July 2021. During the mentioned period, weed cover in the herbicide control was above 40 % in many samplings, while by the end of 2021 season, it decreased to < 10 %. Conversely, weed cover in *P. sylvestris* mulch was below 4 % in 2019, 2020 and 2021 along the 5 samplings of each year. *Pinus sylvestris* mulch covered the totality of the soil (100 %) along the three seasons and, although the thickness of the mulch decreased over time, it always remained above 10 cm. The predominant species in the herbicide control was *C. bonariensis* (representing 60-85 % of total weed cover in summer), except for the last year. Winter grass species (*Hordeum murinum* L. and *Bromus rubens* L.) were also important (representing 70-90 % of total weed cover in winter months) but only until glyphosate was applied. In *P. sylvestris* mulch, *H. murinum* and *B. rubens* were predominant in the winter-spring months and *Convolvulus arvensis* L. during summer months, but always with cover values < 4 %.

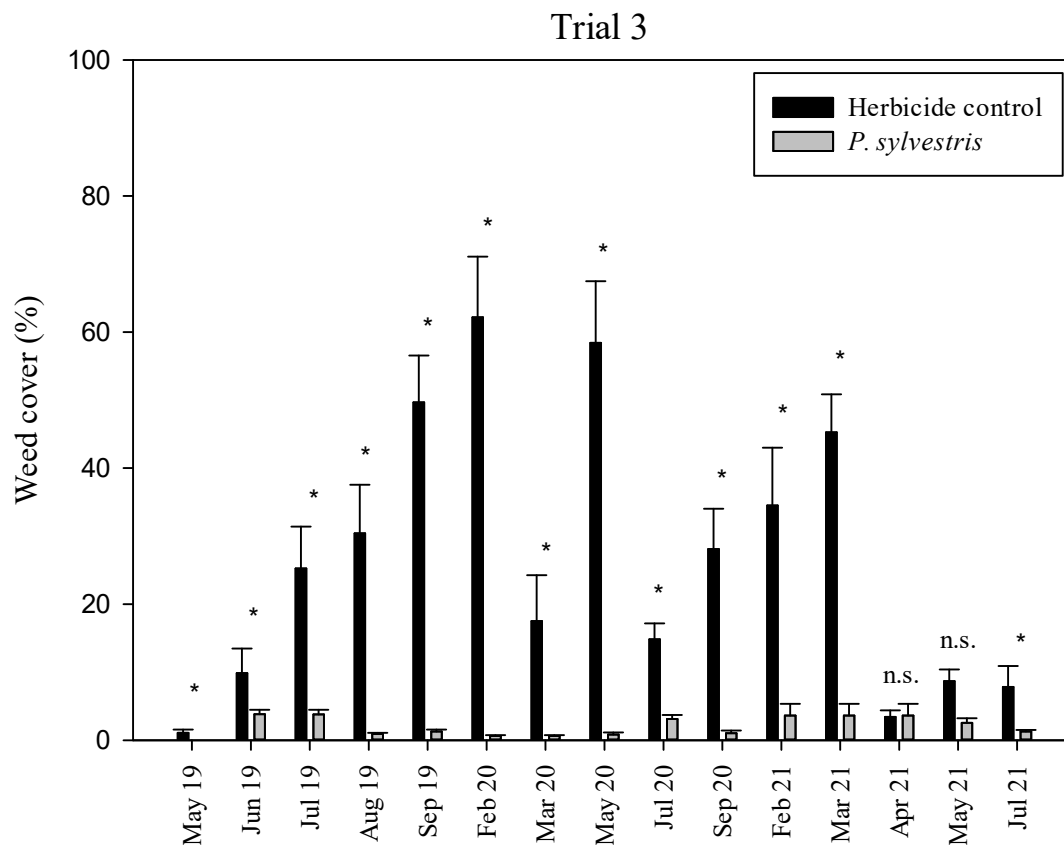


Figure 6: Trial 3, weed cover (%) in each treatment along the three seasons (2019, 2020 and 2021). Vertical bars represent standard errors of the mean. *: significant differences among treatments at $p < 0.05$; n.s.: no significant.

Conyza bonariensis was the predominant weed in the plots treated with herbicide. The presence of this weed decreased over time, which was reflected in decreasing values of biomass,

from 854 g/plot in 2019 to 89 g/plot in 2020 and 1.8 g/plot in 2021 (Table 2). The presence of this weed in *P. sylvestris* mulch was extremely low, hence, significantly lower biomass values were obtained compared to the herbicide control in 2019 and 2020. Despite there were no significant differences in 2021, no *C. bonariensis* plants were found in *P. sylvestris* mulch.

Table 2: Total biomass (g/plot) of each treatment along the three seasons (2019, 2020 and 2021). Mean values \pm standard errors of the mean.

Trial 3	Dry weight Biomass of <i>C. bonariensis</i> (g/plot)		
	2019	2020	2021
<i>Pinus sylvestris</i> mulch	0 \pm 0	0.7 \pm 0.5	0 \pm 0
Herbicide control	854.45 \pm 119.6	89.03 \pm 21.5	1.79 \pm 0.6
Mann-Whitney Rank Sum Test ^a	$p = 0.002^*$	$p = 0.002^*$	$p = 0.065$

*Significance at $p < 0.05$.

Weed and mulch cover correlation

Mulch cover was negatively related with weed cover by a linear function ($p < 0.01$; $R^2 = 0.80$ and 0.71 , respectively for Trials 1 and 2) (Figure 7) In Trial 2, mulch thicknesses were not considered for the graphical representation, and treatments are shown by mulch type.

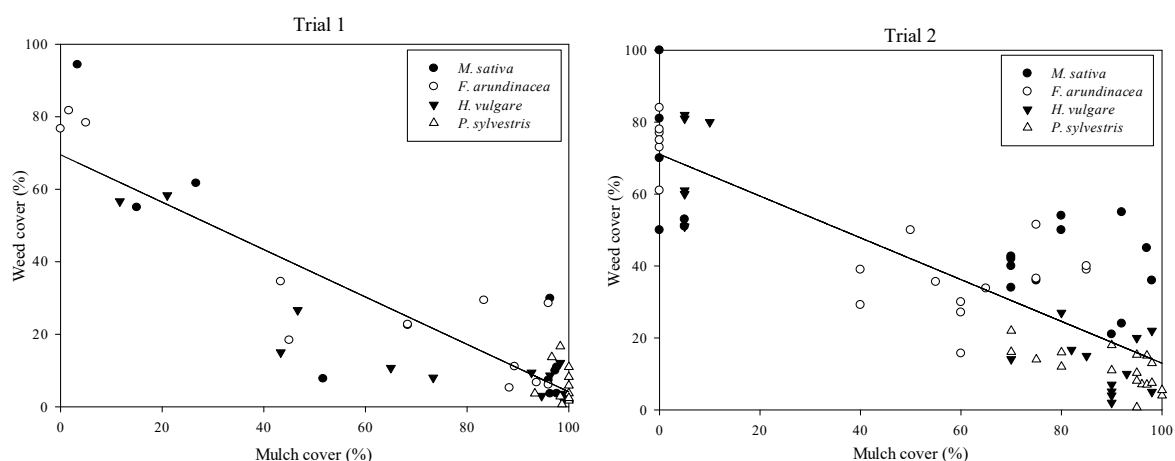


Figure 7: Weed cover and soil mulched correlation in Trial 1 and 2. In Trial 2, thicknesses are not differentiated to facilitate interpretation. Trial 1: $f = 69.5177 - 0.6538 * x$, $R^2 = 0.80$; Trial 2: $f = 71.0591 - 0.5807 * x$, $R^2 = 0.80$.

Weed flora response to management

The species composition in all trials was the typical in vineyards of NE Spain. In July 2018, 17 and 23 weed species were found, respectively in Trials 1 and 2 (Figure 8). Permutation test showed significant variation among treatments in Trial 1 (pseudo-F = 3.4; $p < 0.011$) and Trial 2 (pseudo-F = 2.9; $p < 0.001$). The RDA analysis explained a variance of 57.7 % (49.0 % and 4.7 % by the first and second axis) on the weed community composition for Trial 1, where the analysis clearly separated the tilled control and *P. sylvestris* mulch from *M. sativa* and *F.*

arundinacea mulches. These last mulches favoured species such as *Aster squamatus* (Spreng.) Hieron., *Sonchus oleraceus* L. and *Solanum nigrum* L. among others. *Diplotaxis erucoides* (L.) DC and *Lactuca serriola* L. had some affinity for the tilled control and *P. sylvestris* mulch, while no species could be clearly related to *H. vulgare* mulch. In Trial 2, the RDA analysis explained a variance of 55.9 % (36.2 % and 10.8 % by the first and second axis), and the same pattern as in Trial 1 was observed, with the tilled control and *P. sylvestris* mulch separated from *M. sativa* and *F. arundinacea* mulches, these last two being very related with *S. oleraceus*, *Chenopodium album* L. and *S. nigrum*. No species could be clearly related to the tilled control, *P. sylvestris* or *H. vulgare* mulches, and some problematic perennial species found in vineyards, such as *Cynodon dactylon* (L.) Persoon or *C. arvensis*, did not show any preferred treatment.

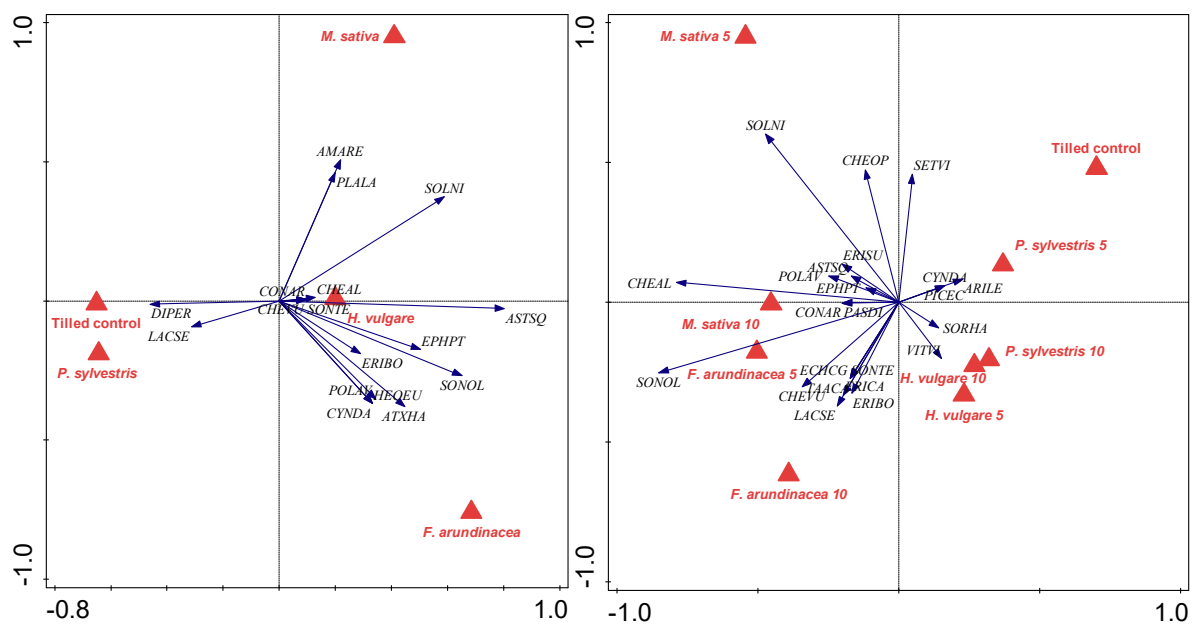


Figure 8: Redundancy analysis of species composition for Trial 1 (left) and Trial 2 (right). Red labels denote treatments. Arrows show the weed species present in the analysis. Weed species are abbreviated following EPPO Global Database (EPPO, 2022).

Discussion

Mulching has proved to be an effective strategy to control weeds in the under-vine zone. Organic mulches are known to suppress weed growth through light exclusion by creating a physical barrier (Teasdale & Mohler, 1993), and through the release of allelochemicals (White *et al.*, 1989; Moonen & Barberi, 2006) that may inhibit the germination of some weeds. In Trials 1 and 2, straw mulches could maintain low rates of weeds the first season, (Figures 2 and 4), but an important increase of weed cover was observed during the second year, except in *H. vulgare* mulch, where this increase was observed the third year. *Pinus sylvestris* mulch

maintained low percentages of weed cover along the three growing seasons, being the last values of weed cover in 2019 10.3 % in Trial 1 and 15 % and 16 % in Trial 2 for the 10 cm and 5 cm thickness, respectively. On the other hand, tillage was effective in maintaining an acceptable level of weed cover (< 15 %) provided three or four number of mechanical interventions were performed (in 2017 and 2018), and after the first tillage in April 2019, (Figures 2 and 4). However, high weed cover percentages (30-50 %) proceeded each tillage event (data not shown), which implies high competition for resources.

One of the most problematic weed species is *C. bonariensis* (Bajwa *et al.*, 2016) which is very competitive against crops; nevertheless, it is easily controlled with tillage (Brown and Whitwell, 1988). In fact, in Trial 1 and Trial 2, the presence of this species was minimized, either with tillage or with mulches, but it is difficult to control with chemical tools (Wicks *et al.*, 2000), especially when the population presents herbicide resistance biotypes, as it happens in several countries (Heap, 2020), Spain among them (Urbano *et al.*, 2007). In Trial 3, *P. sylvestris* mulch was an effective alternative in a context of glyphosate-resistant weeds like *C. bonariensis*. Glyphosate controlled the winter-spring grass weeds but was unable to control *C. bonariensis*, which eventually developed inflorescences and disseminate achenes. In *P. sylvestris* mulch, only testimonial *C. bonariensis* plants were counted in the transition zone (at the edge of the established mulch, 20 cm from the center of the under-vine zone). The total weed cover in that mulch never exceeded 5 % along the three seasons, which clearly indicates the efficacy of this mulch to prevent the presence of weeds. Plant residues are known to decrease the germination of *C. bonariensis*, like with sorghum straw (Loura *et al.*, 2020). The physical barrier caused by the thickness of the applied mulch, might be the main reason. On one hand, the emergence of *C. bonariensis* is known to decrease with increased burial depth, and no seedling is able to emerge deeper than 2 cm (Wu *et al.* 2007). On the other hand, the absence of light provoked by the mulch also decreases the germination of weeds (Benvenuti and Macchia, 1995).

The main weed species observed in the mulching plots was *C. arvensis*, which is a vivacious species adapted to many weed managements, difficult to control with straw or bark mulch, as Tebeau *et al.* (2017) observed in their study comparing straw mulches, living mulches and tillage. Anyhow this last method (tillage) also favours the presence of *C. arvensis*, as Abad *et al.* (2020) reported. Conversely, Ormeño-Núñez *et al.* (2008) observed an 82 % reduction of *Cynodon dactylon* dry matter (another problematic rhizomatous weed species) in *Secale cereale* mulch, compared to chemical plus mechanical control; while Valencia-Gredilla *et al.* (2020) observed that inter-row tilling and *H. vulgare* cover crop mulched in autumn was effective in

maintaining low levels of *C. dactylon* in the inter row zone. In our study, *C. dactylon* did not show any preference for mulch or tillage treatments (Figure 8), nevertheless, this species was capable of overcoming the physical barrier of mulches and developed on top of them.

Weed cover in spring and early summer was slightly higher than in late summer in all trials, probably due to the presence of annual winter-spring species (e.g., *D. erucoides*, *S. oleraceus* or *H. murinum*), that finish their life cycles before August. On the other hand, nitrophilous species, such as *A. retroflexus*, *C. album* or *S. nigrum* were related to *M. sativa* mulch (Figure 8), which could be associated with an increase of soil nitrate, as Teasdale and Mohler (2000) observed for *T. incarnatum* residue. In these sense, Gallagher and Cardina (1998) also observed that nitrate could increase the germination of *A. retroflexus* seeds.

The durability of the mulch is a key factor to achieve high weed control efficacies (Figure 7), and it is highly related to mulch thickness. Bartley *et al.* (2017), based on a one-year pot experiment, suggested 5 cm as the minimum thickness for a mulch, but they did not find differences between mulches of 5 cm and 10 cm. On the contrary, Lanini *et al.* (2011) concluded that organic mulches need to be at least 10 cm thick to block light and be effective. The persistence of mulches, at the two thicknesses considered in Trial 2, was similar over time, but the presence of weeds was more abundant in the 5 cm than in the 10 cm thick mulches in most of the samplings. Based on the mulch and weed cover regressions applied in Trials 1 and 2 (Figure 7), 75% to 90% mulch cover would be required to obtain 80% of weed cover suppression, lower than the 97% of mulch cover predicted by Teasdale *et al.* (1991). The thickness of organic mulches usually declines by 60% during the first year, depending on the material (Lanini *et al.*, 2011), so most mulches need to be reapplied every two to three years. In the present study, *P. sylvestris* mulch maintained at least 80% of the soil covered for two years in Trials 1 and 2 (Figure 3 and 5), and the totality of the soil (100%) after three years in Trial 3, which clearly indicates the better performance of this mulch over the other tested. The higher thickness of *P. sylvestris* mulch applied in Trial 3 (15 cm) might explain differences in mulch persistence between trials, together with the fact that, in Trials 1 and 2, the rows followed the slope of the ground, which was 3-4% compared to Trial 3, and may have contributed to mulch losses after rainy periods. The increase of the weeds' presence in the straw mulches in the last season in Trials 1 and 2 (Figures 2 and 4) can be explained by the fast degradation of the straw in comparison to the chopped pine wood of *P. sylvestris*. The large number of small particles present in the straw mulches result in more space of contact with the soil, which can lead to an early decomposition (Bremer *et al.*, 1991). This is supported by Sims and Frederick (1970), who found a linear relationship between early decomposition and potential surface of

straw in contact with the soil. The composition of mulches is another key factor that explains their persistence. Contrary to straw mulches, which are mainly composed of cellulose, chopped pine wood has a higher presence of lignin which favors lower rates of decomposition (Goh and Tutua, 2004). Thus, straw mulches need to be reapplied every year, like it would be the case of *M. sativa* and *F. arundinacea* mulches in the present work, or every two years, in the case of *H. vulgare* mulch. Another handicap of straw mulches is that they may have seeds incorporated, depending on their precedence.

Despite the low weed cover in *P. sylvestris* mulch in all three trials, a slight increase of weed cover was observed over time, but much less than in the tilled control when interventions were not frequent. There are many reports that support this, e.g., Steinamus *et al.* (2008) observed higher weed control efficacy of mulching in comparison to tillage in vineyards, Fredrikson *et al.* (2011) observed lower weed cover in their mulch treatment (an annual cover crop mix of cereal rye and *T. incarnatum* incorporated as a mulching in vineyards) when compared with mechanical cultivation, and DeVetter *et al.* (2015) also obtained better weed control with straw and a living mulch of *Festuca rubra* L. Pennlawn than with cultivation or herbicides. The effectiveness of the in-row tiller depends on the frequency of annual interventions, and three to four along the growing season are deemed necessary to maintain weed cover at low levels. It is important to highlight the lack of need of any intervention after the implementation of mulches, with the corresponding fuel saving.

Vineyards with organic mulch tend to suffer less thermal and water stress (Fraga and Santos, 2018) as water losses through soil evaporation are minimized and soil water holding capacity is increased in the long term due to higher soil organic matter content (Morlat and Chaussod, 2008). A shift from traditional tillage to a mulching strategy combined with no till in vineyards, avoids soil compaction in the soil layers below the depth reached by the in-row tiller. Conversely, it may increase soil compaction in the upper topsoil layers in the short term and, hence a deterioration in soil hydrophysical properties, as Buesa *et al.*, 2021 observed when they compared both strategies in an historically tilled vineyard. In these situations, an under-vine cover crop strategy can be a useful tool, as some species can compete well against undesired weed species (Abad *et al.*, 2020) while improving soil organic matter, soil aggregate stability, and water infiltration (Abad *et al.*, 2021a; García-Díaz *et al.*, 2018), while compaction is avoided by cover crop roots (Colugnati *et al.*, 2004). Even so, maintaining a sward under vines could lead to lower vigor and yield in some contexts, especially in rainfed Mediterranean vineyards, while mulching generally increase them (Steenwerth and Guerra, 2012; Fourie, 2011; Abad *et al.*, 2021b).

Thus, mulches become a promising alternative for weed control in vineyards, being the most effective method when compared with chemical or mechanical ones, mainly if mulch persistence is beyond three years, like in *P. sylvestris* mulch, justifying the initial cost of specialized equipment for spreading organic material (Manzone *et al.*, 2020; Steenwerth and Guerra, 2012). Despite the above-mentioned benefits of mulching, they can be expensive and messy (Ross, 2010). Hence, under-vine mulches may unleash their potential when addressed to specific fields with specific problems, i.e., herbicide-resistant biotypes, high erosion risk, among others, rather than pretending their widespread use in vineyards.

Conclusion

Mulches control weeds successfully when mulch coverage is maintained above 75 %. Depending on the nature of the mulch, the persistence is unequal. Chopped pine wood mulch of *P. sylvestris* stands out above straw ones as it achieved high soil cover during, at least, three growing seasons, avoiding weed growth. This, together with the multiple benefits of mulches, makes them a sustainable tool to incorporate in weed management programs in vineyards.

Acknowledgements

This work has been supported by the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the project AGL2017-83325-C4-2-R and also by MACMHER “Grups operatius” promoted by Department of Agriculture of Catalan Government (DARP) in 2018. The first author obtained a PhD grant from the University of Lleida (PhD grants). We would also like to acknowledge Bàrbara Baraibar for her advice, Maria Casamitjana, Neus Mas and Amadeo Arbonés for their help in the field work, and the facilities and technical support supplied by the company Raventós–Cordorníu to carry out the field trials in their vineyards.

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CHAPTER 6

Under-vine weed management strategies in Mediterranean irrigated vineyards: impact on agronomic performance

Submitted to *European Journal of Agronomy*, June 2022.

Under-vine weed management strategies in Mediterranean irrigated vineyards: impact on agronomic performance

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Abstract

One of the main challenges for organic vineyards is weed management. Weeds tend to compete for water and nutrients, and can cause large reductions in yields. Traditional under-vine weed management in organic vineyards consists on mechanical cultivation along the season, which is associated to soil and young vine root damages, and to high fuel consumption. Thus, sustainable alternatives need to be found. Cover crops are becoming common in the last decades due to their multiple benefits in agroecosystems. Nevertheless, under-vine cover crop implementation in Mediterranean vineyards is limited as this competes for resources (water and nutrients), reducing the yield, vegetative development, and grape size of vines. The use of organic mulches could overcome all these problems, while benefitting vine performance. In the present work, the response of vines, soil and weeds to mulching was evaluated. An experiment was carried out in Raimat, Lleida (Catalonia, NE Spain) in a commercial vineyard from 2019 to 2021, and the following treatments applied: 1) mechanical cultivation with an in-row tiller; 2) mowing a permanent spontaneous cover with an in-row mower; 3) almond shell mulch, and 4) chopped pine wood mulch. Results showed lower weed cover along the three seasons in mulched treatments, as well as higher yield, better vine water status, and greater vegetative development from traditional measurement. The latter was confirmed by and analysed with further detail with measurements acquired with a mobile terrestrial laser scanner (MTLS) based on light detection and ranging (LiDAR) sensors. Besides, petiole nutrient status was worse in vines with mowed cover. Organic mulches improved vine performance and weed control, so these results allow to optimize water use efficiency in the Mediterranean basin with scarce water resources. Mulching can be considered as a useful alternative strategy in viticulture.

Keywords: Sustainable viticulture, soil management, LiDAR, in-row tiller, conservation agriculture, organic mulches.

Introduction

Vineyards are common crops in many regions along the Mediterranean basin, representing an important economic activity, especially in Spain, where almost one million hectares are cultivated (MAPA, 2020). Vineyard performance can be significantly conditioned by weeds (Oerke, 2006) as these compete with the crop for water and nutrients (Hembree and Lanini, 2006). Worldwide, tillage is the most traditional soil management technique for controlling weeds in organic vineyards (Steenwerth and Guerra, 2012). Despite its effectiveness, tillage causes erosion and loss of soil structure, reduces organic matter (OM) content (Glover *et al.*, 2000; Smith *et al.*, 2008), which alters the population of soil microorganisms (Virto *et al.*, 2012), and decreases the presence of grapevine roots in the upper soil layers (Smart *et al.*, 2006), damaging young vines. Moreover, the carbon footprint of tillage is double of that of pesticides or fertilizers (Jradi *et al.*, 2018). In the last decades, cover crops have become a common practice for soil management, and an alternative to the traditional tillage. Cover crops provide numerous benefits, they avoid soil erosion, improve soil structure and water infiltration, and increase soil biological diversity, among others (Hatwing and Ammon, 2012; Morlat and Jacquet, 2003; Fourie 2010, Abad *et al.*, 2021a). Nevertheless, cover crop maintenance in the under-vine zone in Mediterranean vineyards is limited as this competes for resources (nutrients and water), and can reduce yield, vegetative growth and grape size (Ingles *et al.*, 2005; Tesic *et al.*, 2007; Celette *et al.*, 2008, 2009 Abad *et al.*, 2021b). The implementation of mulches in the under-vine zone can contribute to overcome all these concerns. A mulch is a groundcover made of any bulk material placed on the soil surface for weed control, soil amelioration, improvement of canopy microclimate and/or minimization of water loss (Ferrara *et al.*, 2012). Organic mulches, which are usually used as a by product, also modify soil water reserves, increase soil OM and improve water infiltration, getting better water use efficiency (Pinamonti, 1998), hence vine water status is ameliorated (Buesa *et al.*, 2021), which will become crucial to adapt viticulture to the current climate change scenario.

Future projections hint at a general increase in temperatures and a decrease in water availability, and the Mediterranean region will suffer extremely stressful conditions for plant growth, including grape crops (Toh and Végvári, 2016; Fraga *et al.*, 2018). In addition, grapevine yield are strongly decreased by water or nutrient deficit (Keller, 2005), so optimizing water management in vineyards, and the nutrients input and output balances within the farm system are essential subjects to enhance sustainability in viticulture (Quemada and Gabriel, 2016). Fraga *et al.* (2018) suggested that a combination of adaptation measures might be required to

keep vineyard yields under Mediterranean conditions, and mulching may be considered a strong candidate.

Soil management practices affect the grapevines vegetative growth (Steenwerth and Guerra, 2012), which can be estimated by direct measurements in the canopy (primary shoot length, pruning weight, total leaf area, etc.) even though these are costly and time consuming. Indirect measurements using mobile terrestrial laser scanner (MTLS) based on light detection and ranging (LiDAR) sensors were obtained to complement direct traditional measurements. Ground-based LiDAR has been used to detect and characterize vegetation since late 80s (Walklate, 1989; Wangler *et al.*, 1992). More recently, some works have presented MTLS designs and methodologies to obtain 3D point clouds of vineyards (Rosell-Polo *et al.*, 2009; Llorens *et al.*, 2011). Some other works present methods to improve information extraction out of them (Arnó *et al.*, 2013; Arnó *et al.*, 2015; Del-Moral-Martínez *et al.*, 2016; Siebers *et al.*, 2018; Moreno *et al.*, 2020). No publications were found using MTLS to compare cover crops or mulches effect on crop development. The objective of the current study was to assess the effects on vine water and nutritional status, vine vegetative growth and yield, soil properties, and weed control of two organic mulches installed in the under-vine zone (almond shell and chooped pine wood) under no-tillage, compared to that of tillage, and permanent spontaneous vegetation cover maintained by mowing.

Material and methods

Site description

The experiment was carried out during three consecutive seasons (2019-2021) in a commercial wine grape vineyard located in Raimat (41° 39' 25" N, 0° 27' 1" E ETRS89, elevation 300 m, Lleida, Catalonia, Spain). The vineyard was planted in 2010 with *Vitis vinifera* (L.) cv. Chardonnay at a spacing of 3 m by 1.5 m and organically managed since then. Shoots were vertically trellised with a pair of steel catch wires. The traditional weed management consisted on a permanent spontaneous vegetation cover mowed 2-3 times per season in both the under-vine zone and the alleyways. Vines were drip irrigated and trained as bilateral cordons. The soil at this site was classified as a Petrocalcic Calcixercept and the texture was 27.8 % sand, 41.1 % silt, 31.1 % clay, with pH 8.18 and 3.03 % of OM. The climate classification is cold semiarid (*BSk*) (Kottek *et al.*, 2006) with an average annual precipitation of 342 mm, and annual mean temperature of 14.1 °C (average minimum 8.1 °C and average maximum 20.7 °C). Weather data were obtained from an automatic meteorological station

belonging to the regional meteorological network, located close to the vineyard in Raimat. (Meteocat).

Experimental design

The experimental design was a randomized complete block with four treatments replicated three times. Twelve vine rows, each between 35 m and 60 m x 0.6 m, were treated as experimental units (plots). A buffer vine row separated each replicate on either side of the vine row. Observations were made on nine grapevines per row. Each row received one of the following weed management treatments in all its length and width: 1) mechanical cultivation with an in-row tiller (hereafter Tiller); 2) mowing a permanent spontaneous vegetation cover with an in-row mower (hereafter Mowing); 3) almond (*Prunus dulcis* [Mill.] D.A. Webb) shell mulch (hereafter Almond mulch); and 4) chopped pine wood (*Pinus sylvestris* L.) (hereafter Pine mulch). Cultivation (Tiller) was performed in May, June and July 2019; February, May and June 2020; March, May and June 2021. Mowing was performed in June and July 2019; May and June 2020; March, May and June 2021. Both mulches were applied mechanically only once, at the beginning of the experiment in January 2019, and they were 15 cm thick. The alleyways were sown with *Festuca arundinacea* Schreb as cover crop in January 2019 and shredded 2-3 times per season. All vines were irrigated with the same amount of water and fertilized uniformly according to the standard practice of the farm.

Soil measurements

Soil temperature was monitored during the three seasons with the RT-1 Soil Temperature Sensor (METER Group, Pullman, WA, USA) at 7 cm depth in each treatment of the central block, and these data were recorded and saved by the Data Logger Em5b (METER Group, Pullman, WA, USA). Soil samples were collected in each replicate from the under-vine area in July 2021. Samples were collected from the upper 15 cm soil layer, as treatments were expected to show the greatest effect over time in the topsoil.

Weed sampling

Weed surveys were performed in May, June (twice), July and August 2019; in March, April, May, June, July and August 2020; and in March, April, May (twice), June and July 2021. The total weed cover was visually estimated in nine subplots of 3 m x 0.6 m under the vines as percentage of the whole subplot. Mulch persistence was also visually estimated at the end of each growing season (autumn 2019, 2020 and 2021) as soil cover percentage. A final survey was performed in March 2022.

Vine performance measurements

Primary shoot length (PSL) was measured every two weeks in the same shoots from May until harvest, and the considered data was an average length of three different shoots per vine. Mid-day stem water potential (SWP) was measured each season every two weeks from May until harvest, using a pressure chamber; at each sampling date, one undamaged, fully expanded leaf on the centre of each of the nine vines was introduced in an aluminium bag at least 45 min before sampling to stop evapotranspiration. Measures were taken immediately after removing leaves from the vines. Grapes were harvested and weighted to determine the yield (kg/vine) in August every season. Vine vigour was also measured by prune weight (PW), which was weighted every autumn. For each variable, nine vines in each treatment and block were considered, except for primary shoot length, which was measured only in the central block.

In parallel, a mobile terrestrial laser scanner (MTLS) was used to indirectly estimate the vigour/development of the vines to assess the effect of the different treatments. The scans were performed twice every season. The first scans were performed in June or beginning of July (depending on the season) at BBCH 77-79 and the second scans were performed at the end of July or beginning of August (depending on the season), at full vegetation to monitor the total canopy development before harvest (BBCH 85-89). The MTLS used in 2019 was a self-developed MTLS based on a UTM-30LX-EW LiDAR sensor (Hokuyo, Osaka, Japan). The methodology was similar to that described in Escolà *et al.* (2017). In 2020 and 2021 the MTLS was a commercial backpack system BMS3D-4CAM (Viametris, Louverné, France) which mounts two LiDAR VLP-16 sensors (Velodyne, San Jose, CA, USA), a multi-constellation GNSS receiver, an inertial measurement unit and 4 RGB cameras. The MTLS was carried by a person traveling on an electric all-terrain vehicle (eATV). The eATV travelled along all alleyways in the plot at around 10 km/h. Subsequently, a 3D point cloud of the plot was created and self-developed algorithms were applied to extract geometric and structural parameters of the canopy. The geometric parameters used to assess canopy development were canopy height, width and cross-sectional area, the latter defined as the area occupied by biomass in a plane perpendicular to the longitudinal axis of the row. The structural parameter was canopy porosity, defined as the ratio of laser beams trespassing the canopy out of the total emitted. Each parameter was computed every 10 cm along each row, according to their longitudinal axis (Figure 1). Thus, each row had a set of geometric and structural parameters which were compared to those of the other rows to assess the effect of the different treatments.

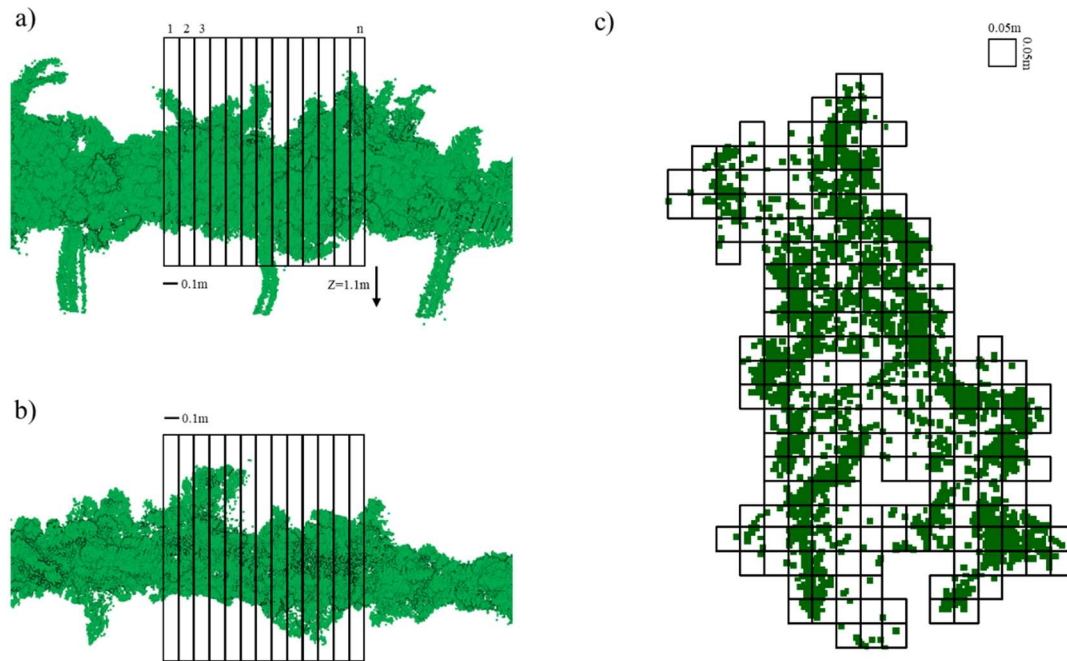


Figure 1. Graphical representation of the process of crop parameters extraction. a) Side view of the LiDAR point cloud, in green, with the vertical prism of 0.1 m width used to extract canopy parameters along the row b) Top view of the row and c) representation of a cross-sectional view of a 10 cm long section of the LiDAR-derived points, in dark green, contained in a prism used for the calculation of the cross-sectional area as occupied surface using a regular 5 cm x 5 cm grid.

Finally, vine nutritional status was measured by elemental petiole analysis; fifty petioles from nodes opposite to inflorescences or clusters were collected along the rows for each treatment in July 2021 for essential nutrient content (NC) analysis. Before harvest, in August 2021, pest and disease incidence and severity were assessed in five vines for each treatment and replicate.

Statistical analysis

Data were subjected to one-way ANOVA, followed by multiple comparisons of treatment effects using Tukey's HSD-test ($p < 0.05$). When necessary, data were square root transformed to meet assumptions of ANOVA, normality (Shapiro-Wilk) and homoscedasticity (Leven's test). Data were back transformed for clarity in the results. Analyses were performed using JMP Pro 15 (SAS Institute 2010. SAS Campus Drive, Cary, NC27513, USA. SAS Institute Inc.).

Results

Weather conditions

The highest mean temperature (T_m) during the growing season (grey arrows in Figure 2) was in 2020 (18.7 °C), followed by 2019 (18.2 °C) and the lowest in 2021 (17.9 °C), but all three seasons were similar to the historical average (18.1 °C). Nevertheless, in June 2019 temperature reached 40.6 °C, the highest temperature ever recorded in Raimat. On the other hand, in March and April 2021, and coinciding with the bud break, the minimum temperatures dropped below 0 °C for several days. 2019 and 2021 were similar in terms of precipitation during the growing season, with 158 mm and 161 mm, respectively, slightly below the historical average (176 mm), while in 2020 the growing season was very wet (265 mm).

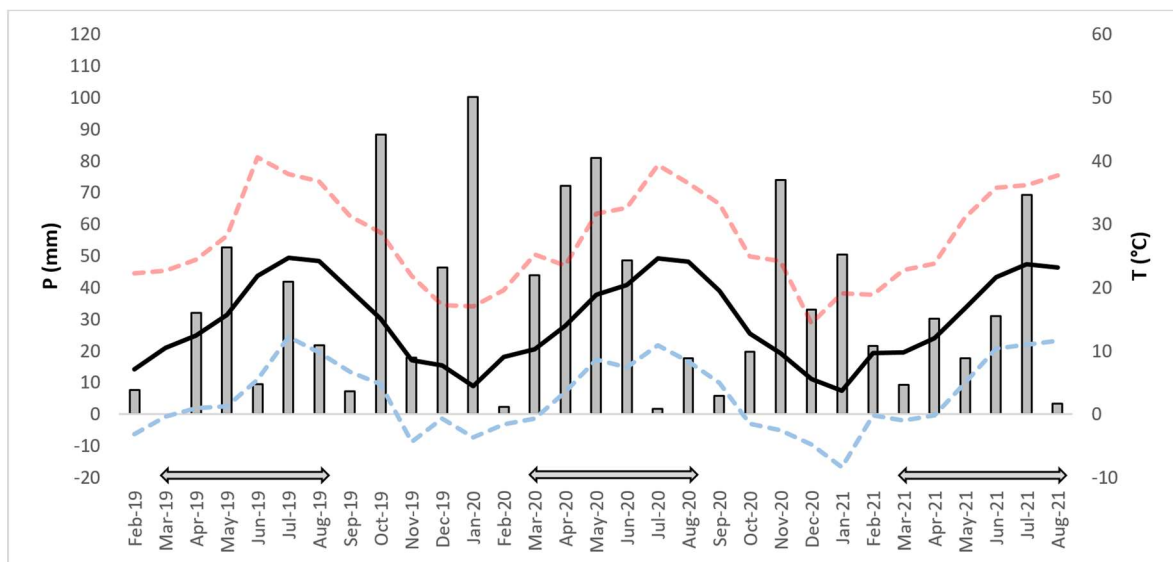


Figure 2: Weather conditions of the experiment period. Grey bars, total monthly precipitation (P); black line, mean monthly temperature (T_m); blue line, absolute minimum monthly temperature (T_n); red line, absolute maximum monthly temperature (T_x). Arrows represent the growing season each year (from March to August).

Soil temperature

The maximum monthly soil temperature at 7 cm depth was always lower in mulched treatments, especially during summer months (Figure 3). In summer 2019, maximum soil temperature in Tiller and Mowing reached 32 °C and 33 °C respectively, while in Almond and Pine mulches reached 25.5 °C and 27 °C, respectively. In summer 2020 and 2021, the tendency was similar, but the maximum temperature never exceeded 30 °C in any treatment, and Almond mulch did not reach even 25 °C in 2019 and 2020.

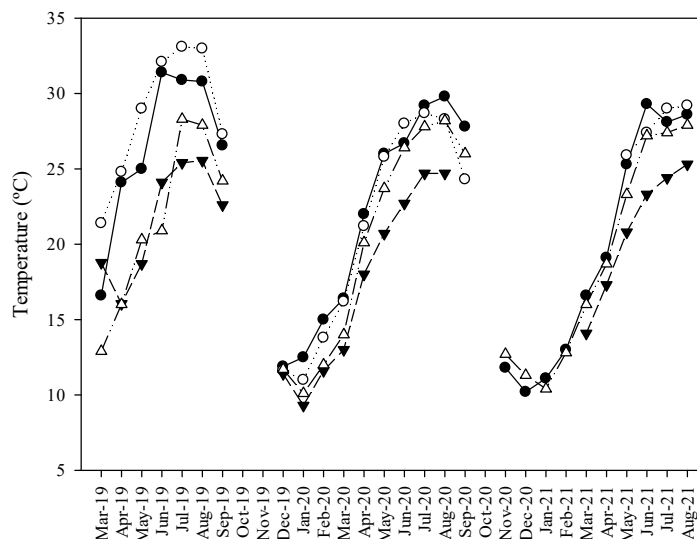


Figure 3. Absolute maximum monthly soil temperature at 7 cm depth. Missing data was due to damage caused by wild fauna or machinery. ●, Tiller; ○, Mowing; ▼, Almond mulch; △, Pine mulch.

Soil properties

After three seasons, there were few treatment differences in soil composition at 0-to-15-cm soil depth (Table 1). The pH was significantly less basic in Tiller (8.03) than in Almond mulch (8.47). Soil apparent electrical conductivity (EC_a) and N- NO_3 was higher in Tiller (0.37 dS/m and 82.67 mg/kg DM respectively) than in the other treatments (Mowing, 0.26 dS/m and 15.7 mg/kg DM; Almond mulch, 0.23 dS/m and 2.9 mg/kg DM; Pine mulch, 0.21 dS/m and 6.23 mg/kg DM, respectively).

Table 1. Soil analysis of main components and properties in each treatment at the experiment in July 2021. Mean values. Different letters in columns mean significant differences among treatments at $p < 0.05$. ns: no significant among treatments; *: significant differences among treatments.

Treatment	pH	Soil properties								
		dS/m	Values in % DM		Values in mg/kg DM					
		EC_a	C	OM	N- NO_3	P	K	Ca	Mg	Na
Tiller	8.03 b	0.37a	1.71	2.95	82.7 a	46.1	600.7	7114.3	304.3	39.0
Mowing	8.13 ab	0.2 b	1.77	3.05	15.7 b	37.8	527.7	6899.3	289.7	33.3
Almond mulch	8.47 a	0.2 b	1.78	3.06	2.9 b	34.3	930.7	6805.3	301.7	39.0
Pine mulch	8.30 ab	0.2 b	2.05	3.53	6.2 b	31.8	541.7	6795.3	319.3	36.0
	*	*	ns	ns	*	ns	ns	ns	ns	ns

Weed cover response to management

Weed cover was affected differently by each treatment depending on the sampling date (interaction $p < 0.05$) (Table 2). Weed cover was always higher in Mowing than in the other treatments along the years and samplings, with values commonly above 60 % and close to or higher than 90 % in late spring months. Tiller maintained low weed cover (< 15 %) only

immediately after the in-row tiller interventions, but quick weed cover increase took place during the following weeks, with values sometimes close to 40 % or 50 %. On the contrary, Almond and Pine mulches were capable to maintain low weed cover along the three years, especially the former, where values never exceeded 3 %. Almost complete weed control (0 % weed cover) was provided by mulched treatments in 2019, and weed cover increased slightly during 2020 and 2021, but remained always below 10 %. In March 2022, weed presence in both mulches was still punctual, with values < 4 %. In these two treatments, soil remained completely covered (100 %) by the mulch until March 2022. Mulches decreased only in thickness along the years, but this was always over 10 cm.

Table 2. Weed cover (%) in each treatment along the three seasons (2019, 2020 and 2021). Mean values \pm standard errors of the mean. Different letters in columns mean significant differences among treatments at $p < 0.05$.

	Weed cover (%)					
	06/05/2019	12/06/2019	26/06/2019	18/07/2019	26/08/2019	
2019						
Tiller	7.1 \pm 3.7 ab	7.2 \pm 4.4 b	27.4 \pm 3.8 b	19.2 \pm 9.3 ab	23.3 \pm 11.2 ab	
Mowing	14.3 \pm 4.8 a	64.1 \pm 6.2 a	64.1 \pm 6.2 a	36.6 \pm 8.1 a	48.3 \pm 9.8 a	
Almond mulch	0.1 \pm 0.1 b	0 \pm 0 b	0 \pm 0 c	0 \pm 0 b	0 \pm 0 b	
Pine mulch	0.6 \pm 0.4 b	0 \pm 0 b	0 \pm 0 c	0 \pm 0 b	0 \pm 0 b	
2020	18/03/2020	29/04/2020	12/05/2020	10/06/2020	07/07/2020	01/08/2020
Tiller	7.3 \pm 3.2 b	37.3 \pm 3.3 b	8.9 \pm 4.2 b	29.4 \pm 6.6 b	5.6 \pm 2.2 a	25.7 \pm 6 a
Mowing	57.6 \pm 7.7 a	84.9 \pm 3.2 a	92.3 \pm 1.3 a	82.0 \pm 3.8 a	9.7 \pm 2.2 a	30.5 \pm 5.3 a
Almond mulch	0.3 \pm 0.2 b	2.3 \pm 0.9 c	2.3 \pm 0.9 b	1.5 \pm 0.7 c	1.5 \pm 0.7 a	0 \pm 0 b
Pine mulch	3.4 \pm 2.6 b	7.3 \pm 2.9 c	7.3 \pm 2.3 b	7.2 \pm 5.6 c	7.2 \pm 5.6 a	0.6 \pm 0.6 b
2021	24/03/2021	30/03/2021	19/05/2021	29/05/2021	18/06/2021	07/07/2021
Tiller	47.6 \pm 5.2 b	15.7 \pm 2.4 b	49.4 \pm 5.7 b	8.3 \pm 2.8 b	51.9 \pm 13.8 b	14.5 \pm 1.1 b
Mowing	86.1 \pm 2.9 a	66.1 \pm 2.7 a	90.0 \pm 5.4 a	45.1 \pm 6.4 a	95.6 \pm 2.1 a	28.3 \pm 1.5 a
Almond mulch	2.3 \pm 1 c	2.3 \pm 1 c	0.4 \pm 0.3 c	0.4 \pm 0.3 b	2.1 \pm 0.2 c	2.1 \pm 0.2 c
Pine mulch	9.7 \pm 3.9 c	9.7 \pm 3.9 bc	9.2 \pm 8.8 c	9.2 \pm 8.8 b	8.5 \pm 4.9 c	8.5 \pm 4.9 bc
17/03/2022:	Tiller: 33.1 \pm 4.4(b), Mowing: 68.7 \pm 3.8(a), Almond mulch: 1 \pm 0.2(c), Pine mulch: 3.8 \pm 0.7(c)					

Vegetative growth

In 2019 and 2020, final PSL can be separated in two groups (Figure 4). Mulched treatments showed longer PSL than in Tiller and Mowing, while these values were similar within each group (between 137 cm and 172 cm in Almond and Pine mulches; and between 92 cm and 130 cm in Tiller and Mowing). Conversely, in 2021 the growth rhythm could not be separated in the same two groups, as Almond mulch obtained the longest PSL value (178 cm), followed by Pine mulch and Tiller (158 cm both) and Mowing (139 cm).

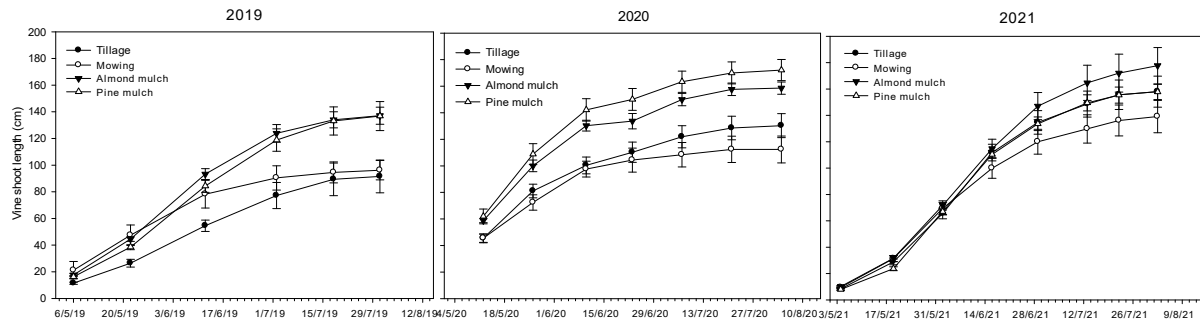


Figure 4. Mean primary shoot length (PSL) (cm) of block 2. Vertical bars represent \pm standard error.

Regarding the mobile terrestrial laser scanner (MTLS) results, the average cross sections every 10 cm along the rows under different treatments are displayed in Table 3. Most times, the rows presenting largest development correspond to those with Pine and Almond mulches, respectively. In 2019, the development of the rows with Pine mulch is even larger than those with Almond mulch. However, there are no significant differences among those two treatments in the 2020 and 2021 scans. The rows under the Tiller treatment are significantly more developed than those under Mowing (except for August 2019), placing the Tiller strategy right between mulches and Mowing. In Figure 5, vegetative growth can be observed expressed as canopy cross-sectional area averages. It is to be noted the large differences between crop development stages in 2019.

Table 3. Canopy cross-sectional area (m²) in each treatment along the three seasons (2019, 2020 and 2021). Mean values \pm standard errors of the mean. Different letters in columns per year mean significant differences among treatments at $p < 0.05$.

Canopy cross-sectional area (m ²)		
2019	03/07/2019	01/08/2019
Tiller	0.307 \pm 0.002 c	0.454 \pm 0.003 c
Mowing	0.286 \pm 0.002 d	0.446 \pm 0.003 c
Almond mulch	0.326 \pm 0.002 b	0.508 \pm 0.003 b
Pine mulch	0.338 \pm 0.002 a	0.524 \pm 0.003 a
2020	17/06/2020	31/07/2020
Tiller	0.448 \pm 0.004 b	0.459 \pm 0.004 b
Mowing	0.424 \pm 0.003 c	0.445 \pm 0.003 c
Almond mulch	0.494 \pm 0.004 a	0.509 \pm 0.004 a
Pine mulch	0.483 \pm 0.003 a	0.519 \pm 0.003 a
2021	28/06/2021	28/07/2021
Tiller	0.490 \pm 0.004 b	0.475 \pm 0.004 b
Mowing	0.451 \pm 0.004 c	0.452 \pm 0.004 c
Almond mulch	0.551 \pm 0.004 a	0.530 \pm 0.004 a
Pine mulch	0.561 \pm 0.004 a	0.539 \pm 0.004 a

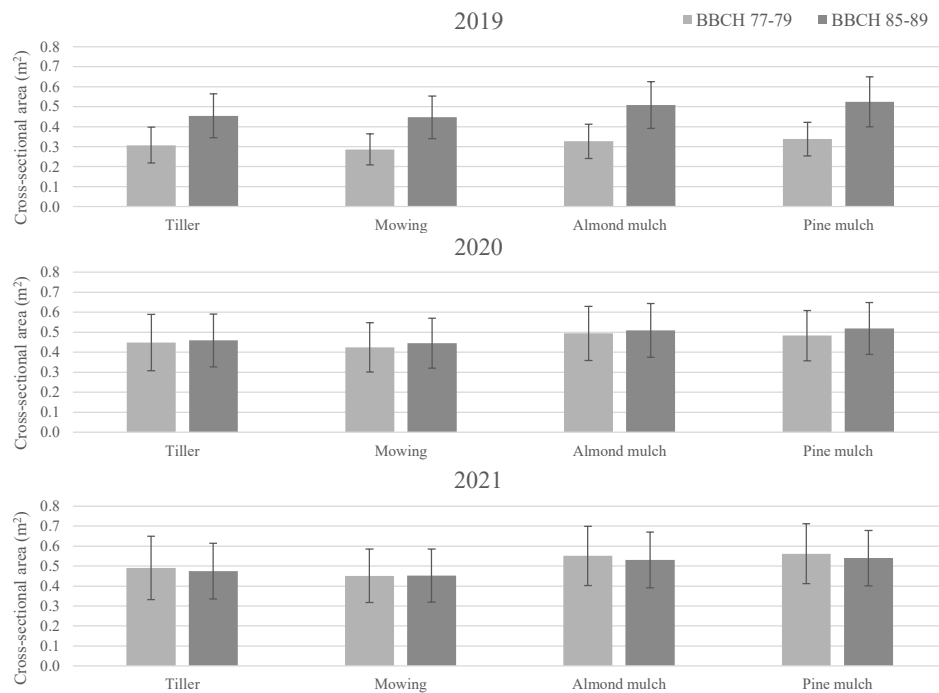


Figure 5. Bar plot of average cross-sectional areas measured with MTLs for each year, treatment and stage. Stage 1, BBCH 77-79 (light grey) corresponds to the end of June beginning of July and stage 2, BBCH 85-89 (dark grey) corresponds to the end of July and beginning of August. Vertical bars in each column represent \pm standard deviation of the mean.

Vine water potential

Two groups of SWP can be differentiated (Figure 6), independently of the year and sampling. The first one is formed by Tiller and Mowing, where SWP values were lower than those of the second group, formed by mulched treatments. No differences were found between Tiller and Mowing or between Almond and Pine mulches, except in June 2020, when Almond mulch obtained higher values than Pine mulch. Similar SWP values were obtained within each group and season along the sampling dates, being 2019 the season with the lower overall SWP values, mainly in July for Tiller and Mowing, coinciding with the maximum air temperature ever registered in Raimat (40.6 °C).

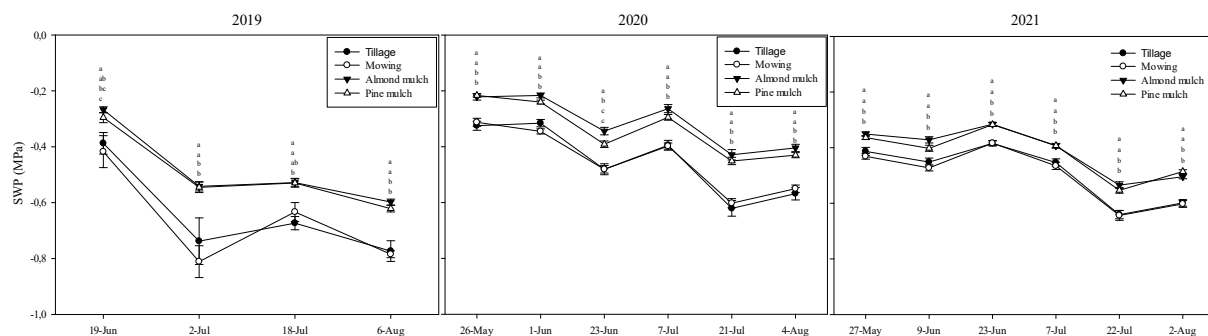


Figure 6: Stem water potential (SWP) measured at midday. Vertical bars represent SE; different letters mean significant differences among treatments at $p < 0.05$.

Grape yield

Vines in mulched treatments produced greater yields than Tiller and Mowing every year (Table 4), but differences between these two groups were significant only in 2020. In 2019, differences resulted significant only between Pine mulch and Tiller, while grape yield resulted similar in all treatments in 2021. The general overall yield was lower in 2020 and 2021 than in 2019.

Table 4: Yield per vine (kg of grapes/vine) in each treatment harvested in August 2019, 2020 and 2021. Mean values \pm standard errors of the mean. F value and *p* value are supplied by year; different letters in columns mean significant differences among treatments within each year at $p < 0.05$.

Treatment	yield (kg of grapes/vine)					
	2019		2020		2021	
Tiller	2.86 \pm 0.56 b		1.74 \pm 0.22 b		1.97 \pm 0.23 a	
Mowing	3.06 \pm 0.30 ab		1.72 \pm 0.20 b		1.84 \pm 0.16 a	
Almond mulch	3.78 \pm 0.40 ab		3.02 \pm 0.19 a		2.09 \pm 0.20 a	
Pine mulch	4.60 \pm 0.51 a		2.76 \pm 0.27 a		2.04 \pm 0.18 a	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Block	0.614	0.5495	3.7843	0.0262	5.553	0.0052
Treatment	3.7114	0.0252	11.8053	<0.0001	0.442	0.7235

Vine pruning weight

Almond and Pine mulches showed, in the three studied seasons, significantly higher PW values (always > 0.90 kg/vine) than those obtained in Tiller and Mowing (always < 0.75 kg/vine), with no significant differences within each group (Table 5).

Table 5: Pruning weight (PW) per vine (kg/vine) in each treatment pruned in November 2019, 2020 and 2021. Mean values \pm standard errors of the mean. F value and *p* value are supplied by year; different letters mean significant differences among treatments within each year at $p < 0.05$.

Treatment	PW (kg/vine)					
	2019		2020		2021	
Tiller	0.54 \pm 0.03 b		0.73 \pm 0.05 b		0.74 \pm 0.06 b	
Mowing	0.49 \pm 0.06 b		0.67 \pm 0.04 b		0.67 \pm 0.06 b	
Almond mulch	0.90 \pm 0.06 a		0.95 \pm 0.04 a		1.17 \pm 0.05 a	
Pine mulch	0.97 \pm 0.06 a		0.97 \pm 0.06 a		1.05 \pm 0.07 a	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Block	4.5198	0.0216	0.695	0.5016	10.0724	0.0001
Treatment	22.8382	<0.0001	11.2556	<0.0001	17.7219	<0.0001

Petiole nutrient content

Analysis of NC in petioles indicates that there was some effect of under-vine floor managements on vine nutrient uptake in July 2021 (Table 6). N, Ca and B were significantly

higher in Tiller than in Mowing, and similar to mulches (except for B with Pine). K and P were higher in mulches, but differences resulted significant with respect to the mechanical treatments only for P in Almond and for K in Mowing. No further nutrient differences were found between treatments.

Table 6: Analysis of principal components of petioles in each treatment at the end of the experiment in July 2021. DM, dry matter. Mean values are provided. Different letters in columns mean significant differences among treatments at $p < 0.05$. ns: no significant among treatments; *: significant differences among treatments.

Treatment	Petiole values												
	Weight (g)	Values in % DM					Values in mg/kg DM						
		N	P	K	Ca	Mg	Fe	Zn	Cu	Mn	B	Na	Mo
Tiller	0.754	0.64 a	0.12 b	2.25 ab	2.43 a	0.76	33.7	32.3	26.7	80.0	38.3 a	386.3	0.06
Mowing	0.701	0.52 b	0.12 b	1.89 b	2.13 b	0.69	31.7	31.3	26.3	71.3	35.0 c	386.7	0.06
Almond mulch	0.826	0.59 ab	0.30 a	2.80 a	2.33 ab	0.64	37.0	37.0	29.3	50.3	38.0 ab	367.7	0.06
Pine mulch	0.859	0.58 ab	0.21 ab	2.25 ab	2.26 ab	0.68	35.3	36.3	28.3	57.7	36.3 bc	357.7	0.06
	ns	*	*	*	*	ns	ns	ns	ns	ns	*	ns	ns

Sanitary status

Adults of *Scaphoideus titanus* (Ball) were the most counted pest in August 2021. Treatments did not affect to any pest, as their presence was similar in all treatments (Table 7).

Table 7: Pest inventory in each treatment at the end of the experiment in August 2021 before harvest. ns: no significant differences among treatments at $p < 0.05$.

Treat.	Sanitary status on August 2021						Botrytis (%)	Botrytis severity (%)
	Individuals/vine							
	Mites	<i>Parthenolecani -um corni</i>	<i>Planococcus ficus</i>	Adult <i>Scaphoideus titanus</i>	Nymph <i>Scaphoideus titanus</i>			
Tiller	0.4	0.3	0.0	3.0	0.5	0.7	0.9	
Mowing	0.1	0.2	0.0	2.8	0.5	0.0	0.0	
Almond mulch	0.0	0.3	0.1	2.9	0.3	1.1	2.0	
Pine mulch	0.0	0.1	0.0	2.9	0.3	0.1	0.7	
	ns	ns	ns	ns	ns	ns	ns	

Discussion

The aim of this three-year study was to evaluate the effect of different under-vine weed management strategies on vine performance. Both mulches were very effective in the control of weeds, and in the last survey of 2021, weed cover values were under 10 % in Pine mulch and under 3 % in Almond mulch (Table 2). Tiller also maintained low weed cover (< 10 %) after in-row tiller interventions, but these values increased during the following weeks up to 40 % to 50 %, which implies a high competition for resources. Similar results were obtained by Cabrera-Pérez *et al.* (unpublished results) who observed better under-vine weed control in mulches compared to mechanical practices, which needed at least four in-row tiller interventions to maintain weed cover at low levels during the crop cycle, and a huge fuel consumption (Jradi *et al.*, 2018). In Mowing, the spontaneous flora covered most of the surface along each year, and the recurrent cuts with the in-row mower only reduced their coverage punctually. The chosen mulches, almond shell and chooped pine wood, are rich in lignin and decompose slowly (Goh and Tutua, 2004), allowing high mulch durability, which is a key factor for weed control efficacy. These mulches need to be at least 10 cm thick to block light and be effective (Lanini *et al.* 2011). In the present work a thickness of 15 cm was chosen to guarantee success after

three seasons. In March 2022, 100 % of the soil was still covered by mulches and their thickness was over 10 cm.

Mulched treatments prevent from the increase of soil temperature in the topsoil, showing a capacity to buffer it (Mundy & Agnew, 2002), while its increase in Tiller and Mowing is greater (Figure 3). Mulches have a cooling effect on the soil, especially those that reduce evaporation and shade the soil surface from solar radiation (Ham *et al.*, 1993), like organic mulches. On the other side, a sward cover, like in Mowing, reduces soil moisture content and increases soil moisture tension (Tescic *et al.*, 2007). This fact was reflected in the mid-day stem water potential results. Although the values were not indicative of water stress, commonly defined as mid-day stem water potentials values below -1.3 MPa to -1.6 MPa (Lovisololo *et al.*, 2010), two treatment groups were clearly differentiated (Figure 6), the first included mulches and showed 0.1-0.2 MPa higher SWP values than the second, formed by Mowing and Tiller. Thus, mulches improved vine water status. These results are in accordance with Lopez-Urrea *et al.* (2020), who found increased SWP with mulch treatments, and with Buesa *et al.* (2021), who observed a 13 % vine water status improvement when mulch was applied to irrigated vineyards. Conversely, Abad *et al.* (2020) observed lower SWP when under-vine cover crop was used, and a reduction in pruning weight. The lack of differences between Tiller and Mowing could be explained by the fact that three in-row tiller interventions along the season do not guarantee fully weed control and it would be necessary a higher recurrence. This, combined with the deeper penetration of grapevine roots when groundcover is present under the vines (Smart *et al.*, 2006), as in Mowing, could likely attenuate differences in SWP between Tiller and Mowing. On the contrary, the improvement on vine water status in mulched treatments could be also due to the proliferation of fine roots in the superficial soil layers (Gaiotti *et al.*, 2017; Morlat *et al.*, 2008; Linares Torres *et al.*, 2018).

The overall PSL was increased season by season (Figure 4). Weather conditions of each growing season explain these results: 2020 was the wettest of the three years of the experiment, and when the highest SWP values were measured. This contributed to vine vegetative growth. In 2021, below 0 °C temperatures were registered on April 7th, 13th and 18th, once bud break already started, so the firsts grape cluster-shoots resulted damaged and vines tended to a rather vegetative growth. Even so, vegetative growth was clearly favored by mulches, mainly in 2019 and 2020, when two differentiated rhythms of growth were observed (Figure 4), and confirmed by the two groups observed in the

PW data (Table 5). Mulches obtained higher PW than Tiller and Mowing each season, regardless of the weather conditions. Previous research showed increased vine vegetative growth when employing mulches (Gaiotti *et al.*, 2017; Pinamonti, 1998; Agnew *et al.*, 2013), and Hostetler *et al.* (2007) attributed these results to their weed suppression capacity. MTLs LiDAR-derived measurements are consistent with those obtained by traditional methods. Moreover, scanning the entire plot allows more representative data to be obtained at a very high spatial resolution (10 cm). The results show that vines with mulches are significantly more developed than those under Tiller and Mowing. Furthermore, the accuracy and the resolution of the LiDAR-derived data provides further detail and highlighted differences between mulches in 2019, although they were not found in 2020 nor in 2021. Those results demonstrated MTLs may be an interesting alternative to traditional measurements to assess the effect of cover crops and mulches on crop development.

Mulched treatments obtained greater yields each season (significant in 2019 and 2020) compared to those of the mechanical managements (Table 4), confirming the observations made, in this sense, by Hostetler *et al.*, 2007; Steenwerth and Guerra, 2012 and Nguyen *et al.*, 2013. Yield increase can be attributed to the better weed control achieved by mulches each season. Similar conclusions were suggested by Fourie (2011) when mulches and other methods were compared. Furthermore, in Mediterranean conditions, Mirás-Avalos *et al.* (2017) suggest that vine water status is a major determinant for grape yield so, differences in SWP between mulches and mechanical treatments would contribute to explain yield results. The lower overall production in 2020 than in 2019 can be explained by the extraordinary mildew (*Plasmopara viticola*) attack observed that season (INCAVI, 2021), which was caused by a long wet period in spring (Figure 2). In 2021, as it was mentioned before, freezing temperatures in April damaged grape cluster-shoots and, hence, diminished the yield. On the other hand, no differences were found in pest incidence in August 2021, just before harvest (Table 7). Mulches did not increase the severity of botrytis bunch rot on grape vines compared to Mowing or Tiller, and this is consistent with previous investigations that demonstrated that pest incidence was similar between organic or geotextile mulches and bare soil (Mundy & Agnew, 2002; Hostetler *et al.*, 2007). Nevertheless, the higher water availability provided by mulches favoured grapevines vegetative growth, which is not always desired, as it can influence grape quality (Soltekin and Altındışli, 2021). To this sense, mulches allow irrigation to be reduced if leaf area needs to be regulated, as it is one of the ways to

optimize the composition of grapes in organic vineyards (Horák *et al.*, 2021), enhancing water saving.

While few differences were found in petiole nutrient analysis between mulched treatments and Tiller, a permanent living cover (Mowing) decreased the petiole nutrient status (Table 6), where lower values of N, P, K and B were observed. As other authors already reported (Tan and Crabtree, 1990; Sicher *et al.*, 1995; Tesic *et al.*, 2007), a permanent living cover decrease the nutrient uptake, and Löhnertz *et al.* (2000) observed a decrease in the amino acid concentration in grape juice, attributed to reduced soil moisture and N availability. Conversely, a trend towards a favourable influence of mulching on grape nutrition status was observed in the long-term (28 years) (Morlat, 2008).

Soil chemical characteristics were similar in all treatments in July 2021 (Table 1), and only N-NO₃ concentration was greater in Tiller, probably because N mineralization increased with increasing intensity of soil tillage (Gross *et al.*, 1999; Chatskikh *et al.*, 2008). Nevertheless, it is known that N stock and is increased by no-till in the long-term (Hafif, 2014; Canisares *et al.*, 2021), as well as soil OM if organic mulches are installed (Thomson and Hoffman, 2007), hence improving soil fertility (Ferrara *et al.*, 2012) while protecting from erosion (Mirás-Avalos *et al.*, 2020). All these aspects influence positively on the soil water holding capacity (Oliveira and Merwin, 2011). In our case, initial OM was already high (3.03 %), as vineyard have been historically managed with spontaneous under-vine cover crop that likely favoured OM (Abad *et al.*, 2021a), which is important to avoid soil compaction (Hamza and Anderson, 2005), and after three seasons of experiment similar values of OM were obtained between treatments and when compared to the initial values in 2019. It should be mentioned that other authors observed higher soil compaction under mulching when compared to tillage, in an historically tilled vineyard with low OM (Buesa *et al.*, 2021).

Mulching represents an expensive input in the vineyard. Besides, the amount and availability of material needed, place of origin and transport, could limit its use (Ferrara *et al.*, 2012). Nevertheless, water availability has been widely recognized as the main limiting factor for grapevine productivity under Mediterranean conditions (Keller, 2010). Soil mulching with no-till improve the soil water balance (Buesa *et al.*, 2021) and increase the soil water content, mainly by avoiding water losses by evaporation (Cao *et al.*, 2012; Myburgh, 2013) as it was observed for some specific parameters, like SWP, in our study. Consequently, employing mulches in the under-vine zone may provide substantial water

savings (López-Urrea *et al.*, 2020), as vineyards with mulch tend to suffer less thermal and water stress (Fraga and Santos, 2018). Besides, in the present study, a good weed control and better vine performance was achieved in mulched treatments, avoiding costs of mechanical weeding, and with increased yields. These mulches showed to be agronomic viable strategies for managing weeds without damaging the soil. Moreover, mulches allow vine vegetative growth to be managed and can be considered as a useful strategy that enhance a more sustainable viticulture. Both mulches obtained similar results, so the location of the vineyard and the availability of the material will determine the use of one or the other, suggesting the locally produced one in order to decrease the carbon footprint of transportation, and promote by-product reuse and circular economy.

Acknowledgements

This work has been supported by the Spanish State Research Agency (AEI) and the European Regional Development Fund (ERDF) through the projects AGL2017-83325-C4-2-R and RTI2018-094222-B-I00 (PAgFRUIT), by the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the project 2017 SGR 00646, and also by the Centre for Technological and Industrial Development (CDTI). The first author obtained a PhD grant from the University of Lleida (PhD grants). Second author contract is supported by a Jaume Serra Húnter Programme promoted by Generalitat de Catalunya. We would also like to acknowledge Maria Casamitjana, Bruna Català and Germán Mora for their help in the field work, and the facilities and technical support supplied by the company ALRASA Agraria SL to carry out the field trials in their vineyards.

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CHAPTER 7

General discussion

General discussion

Historically, winegrowers have managed weeds mechanically, and most recently, by repeated use of herbicides. Mechanical weeding provided imperfect but acceptable efficacy, nevertheless when herbicides became available for vineyards, they provided more effective season-long weed control, which was cheaper and easier to use, anyhow leaving the soil bare most of the year. As a result of any of these methods, there was an alarming increase in soil erosion (Merwin *et al.*, 1994; Novara *et al.*, 2011; Steenwerth and Guerra, 2012). Recently, herbicides efficacy has dramatically decreased as many weeds have evolved resistant to them (Heap and Duke, 2018).

The present work provides a complete study of different sustainable strategies that winegrowers can adopt in order to manage weeds, mainly focussing on *Cynodon dactylon* (L.) Pers. and *Conyza bonariensis* (L.) Conq, which are probably the most problematic weeds in irrigated semiarid vineyards. Repeated use of any weeding method is likely to cause a shift in the weed flora to resistant biotypes and tolerant species, hence, integrating or sequencing weed control techniques would help avoid this problematic, and may provide more effective or (and) more economic control strategies (Bond *et al.*, 2001). But, the combination of environmentally friendly techniques to manage weeds while maintaining the grapevine performance and the soil quality is challenging.

Inter-row weed management

Farmers are usually reluctant to maintain any living vegetation in vineyards for fear of yield reduction, loss of wine quality, problems with diseases and pests, and other environmental effects. Nevertheless, several studies support the advantages of implementing cover crops in vineyards (Ibáñez, 2015; Pardini *et al.*, 2002), while maintaining grape yields and even improving wine quality. A continuous soil cover over time has demonstrate to be a key strategy for soil improvement, either physically, chemically, or biologically, while preventing from noxious weeds infestations. The implementation of a cover crop reduces weed development and seed production, not only by competing for light and nutrients (Moonen and Barberi, 2004), but also by the release of allelochemicals (Angelini *et al.*, 1998) and by the interactions of both factors (Brennan and Smith, 2005; Jabran, 201; Bàrberi, 2002; Smith *et al.*, 2001). The effect of the cover crop 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). In this sense, it is important to predict cover crops emergence (Chapter 2) in order to choose the best species depending on the cover crop purpose(s). With the inclusion of temperature (TT), moisture (HTT), photoperiod (PhHTT), and solar radiation (PhSHTT), emergence models for the 18 studied species were successfully developed. The latter two models were more accurate than the former two, and could be applied to a wide range of species in autumn or spring indistinctly, at least, in the same region where they were developed, and for some species even in different geo-climatic regions. This confirms the usefulness of these models as decision support systems tools to predict cover crops establishment, which will contribute to inter-row management in vineyards. The species selected in Chapter 3 were chosen based on the premise of fast emergence, high coverage, and high aerial biomass potential because the main objective was the management of *C. dactylon* in a previously highly infested vineyard. *Cynodon dactylon* is a stoloniferous and rhizomatous perennial grass considered as one of the world's worst weeds (Holm *et al.*, 1977) that seriously competes with the vines for soil resources during summer. Its horizontal development prevents it from being eliminated by continuous shredding. In fact, shredding can create more favourable conditions for the weed as other species are eliminated. In this sense, Valencia-Gredilla *et al.* (2020) reported that the implementation of a cover crop was more effective to control *C. dactylon* infestation than a tillage managed spontaneous vegetation cover, herbicide applications or shredding. Probably, the best strategy to manage *C. dactylon* is the one that combines different techniques. Hence, in Chapter 3, we tried to combine the suppressive effect of a cover crop before *C. dactylon* sprouts in May, with a persistent mulch that delay the weed development by terminating the cover crop with a roller-crimper. Even if in a spontaneous cover, roller-crimper is clearly the best option to achieve that purpose because it provides a consistent mulch linked to the soil by the roots that last longer, acts as a physical barrier and avoids light penetration. The vegetation cover should grow enough biomass to form a dense canopy, and it could be achieved by letting a spontaneous cover crop to grow from autumn, or by seeding vigorous species. In Chapter 3, species such as *Avena strigosa* or *Lolium multiflorum* terminated with roller-crimper were the most favourable combinations, although the overall results showed that the most influencing factor on the final *C. dactylon* coverage was the terminating method by itself rather than the cover crop species. Cover crop-based weed suppression is greatly affected by biomass levels (Mirsky *et al.*, 2013). The residue quantity is more important than the its quality (type of residues), and the increase of the mulch biomass on the soil surface exponentially

decreases weed emergence (Teasdale and Mohler, 2000). Moreover, limiting tillage to only seedbed preparation contributes to minimize the spread of *C. dactylon* in vineyards, as recurrent mechanical interventions can disperse rhizomes and stolons (Abdullahi, 2002; Fernandez, 2003).

Under-vine weed management

If there is a critical area where weed competition needs to be avoided, this is in the under-vine zone, as most of the vine's root system is present in the soil volume covered by the 0.5 m depth and by the perpendicular distance of 0.5 m from plant row approximately (Basso *et al.*, 2003). It is in here where different alternatives are proposed in Chapters 4 and 5, with bioherbicides and mulches, respectively. Bioherbicides were tested on *C. bonariensis*, one of the most problematic weed species throughout the world (Bajwa *et al.*, 2016) and in Spain (Zambrano-Navea *et al.*, 2013), it was chosen because its competitiveness against vines. Besides, the recurrent use of non-selective herbicides has selected herbicide-resistant biotypes of this weed (Urbano *et al.*, 2007), as shown in Chapter 4 with the characterization of the Raimat *C. bonariensis* population, and which was found to be resistant to Glyphosate with a resistance factor of 6. This was confirmed in Trial 3 of Chapter 5, when Glyphosate failed to control *C. bonariensis*, compared to pine mulch in the under-vine zone. The efficacy of the tested bioherbicides was unequal, being the mixture of potassium metabisulfite + pelargonic acid 31%, humic-fulvic acid, and potassium metabisulfite those that obtained high field efficacy throughout the application dates, and were able to maintain an acceptable low *C. bonariensis* cover. Similar to synthetic herbicides, the effect of the tested compounds may rely on dosage, the phenological stage of the target weed, and on the environmental conditions (Urbano *et al.*, 2007; Okumu *et al.*, 2019; Ghorbani *et al.*, 2006; Hallet, 2005), decreasing the sensitivity or injury with increasing growth stages. This aspect has been confirmed by the dose–response curves. When plants grow from BBCH 12–13 to BBCH 14–15, the EC₅₀ doubles in all treatments. In the dose–response experiment, nearly 100% of the biomass reduction was achieved in BBCH 12–13 at some doses in all treatments, compared to the untreated control, demonstrating the potential of these compounds for the control of *C. bonariensis*. *Conyza* species can potentially germinate at any time throughout the year (Zambrano-Navea *et al.*, 2016), and irrigated crops such as drop-irrigated vineyards ease

this process. Thus, the application of a control method (either bioherbicide or synthetic chemical compound) in a homogeneous phenological stage is extremely difficult, and may explain the unequal efficacy obtained in the greenhouse experiments (dose-response curves) and in the field trials. Consequently, although the available bioherbicides are promising compounds for weed control, few have achieved a long-term commercial success in the field (Cordeau *et al.*, 2016), and relying only in bioherbicides to maintain under-vine weeds under control may be difficult. According to our results, bioherbicides may display their full potential when addressed to specific species in early phenological stages, rather than during their widespread use to many species, but are tools that can definitely be in integrated weed management (IWM) strategies.

The other proposed promising alternative is mulching the under-vine zone (Chapter 5 and 6). Organic mulches are known to suppress weed growth through light exclusion by creating a physical barrier (Teasdale and Mohler, 1993), and through the release of allelochemicals (White *et al.*, 1989; Moonen and Barberi, 2006) that may inhibit the germination of some weed seeds. In Chapter 5, straw mulches were capable to maintain low weed rates during the first year, but an important increase of weed cover was observed during the second year (or the third year in *H. vulgare* mulch). On the other side, chopped pine wood and almond shell mulches maintained low percentages of weed cover along the three growing seasons (Chapter 5 and 6). Tillage was effective in maintaining an acceptable weed cover in all trials, as long as three or four mechanical interventions were performed throughout the growing season. However, high weed cover percentages proceeded each tillage event, which implies high competition for resources, while it negatively affects vines (Prosdocimi *et al.*, 2016), because tillage decreases the presence of grapevine roots in the topsoil (Smart *et al.*, 2006). Besides, tillage implies high fuel consumption (Jradi *et al.*, 2018), and deteriorate soils (Steenwerth and Guerra, 2012).

The durability of the mulch is a key factor to achieve successful weed control efficacies, and it is highly related to mulch thickness. Organic mulches need to be at least 10 cm thick to block light and be effective (Lanini *et al.*, 2011). The thickness of organic mulches usually declines by 60 % during the first year, depending on the material (Lanini *et al.*, 2011). For this reason, most straw mulches need to be reapplied every one or two years because of their fast degradation. In windy places, such as Raimat, this replacement of the straw mulch usually must be done yearly. In comparison, chopped pine wood and almond shell mulches last much longer in the soil surface. The large number of small

particles present in the straw mulches result in more space of contact with the soil, which can lead to an early decomposition (Bremer *et al.*, 1991). In Chapter 5, pine mulch maintained at least 80 % of the soil covered for two years in Trials 1 and 2, and the totality of the soil (100 %) after three years in Trial 3, which clearly indicates the better performance of this mulch over time. In Chapter 6, the soil also remained fully covered with the pine mulch and the almond mulch over the three growing seasons.

In a context of glyphosate-resistant weeds like *C. bonariensis*, chopped pine wood mulch is an effective alternative. Glyphosate controlled winter-spring grass weeds in Trial 3 of Chapter 5, but was unable to control *C. bonariensis*, which eventually developed inflorescences and disseminate achenes. In chopped pine wood mulch (and also in almond shell mulch in Chapter 6), only testimonial *C. bonariensis* plants were counted. The total weed cover never exceeded 10 % in these mulches in any of the three seasons and trials, which clearly indicates the efficacy of these mulches to prevent the presence of weeds. The main weed species observed in the mulches was *Convolvulus arvensis*, a vivacious species adapted to many other weed managements as herbicide, tillage or shredding (Cirujeda *et al.*, 2011; Abad *et al.*, 2020; Valencia-Gredilla *et al.*, 2020) and very difficult to control (Tebeau *et al.*, 2017).

Impact of under-vine weed management on agronomic performance of grapevines

In Chapter 6, the effect on vine performance of different under-vine weed management strategies was evaluated. Mulched treatments (chopped pine wood and almond shell) prevented from the increase of soil temperature in the upper soil layers, showing a buffer capacity (Mundy and Agnew, 2002), while soil temperature increase in Tillage and Mowing was greater. On the other side, a sward cover like in Mowing, reduced soil moisture content and increased soil moisture tension (Tesic *et al.*, 2007). This fact was reflected in the mid-day stem water potential (SWP), where two treatment groups were clearly distinguished: the first including mulches and showing 0.1-0.2 MPa higher SWP values than the second, formed by Mowing and Tillage. Thus, mulches improved vine water status. Vine vegetative growth was clearly favored by mulches, mainly in 2019 and 2020, when two groups, showing contrasting growth rhythms, were observed, with mulched vines growing more and showing higher weight values at pruning than in Tillage and Mowing each year. To this sense, mulches allow a reduction in irrigation if vegetative growth needs to be regulated, as it is one of the ways to optimize the grape composition

in organic vineyards (Horák *et al.*, 2021), enhancing water saving.

Mulched treatments also obtained greater yields each year compared to those of the mechanical techniques, confirming the observations from Hostetler *et al.* (2007), Steenwerth and Guerra (2012), and Nguyen *et al.* (2013). Yield increase can be attributed to both the better weed control and higher SWP achieved by mulches each season. Similar conclusions were suggested by Fourie (2011), when mulches were compared with other methods. Furthermore, in Mediterranean conditions, Mirás-Avalos *et al.* (2017) suggested that vine water status is a major factor for grape yield, hence, differences in the SWP between mulches and mechanical treatments would explain differences in yield results. Finally, while petiole nutrient analysis showed similar results between mulched treatments and Tillage, a permanent living cover (Mowing) decreased the petiole nutrient status, with lower N, P, K and B values.

Although mulching represents an expensive input in the vineyard, water availability has been widely recognized as the main limiting factor for grapevine productivity under Mediterranean conditions (Keller, 2010). Soil mulching without tillage improves the soil water balance (Buesa *et al.*, 2021) and increases the soil water content, mainly by avoiding water losses by evaporation (Cao *et al.*, 2012; Myburgh, 2013), as it was observed for some specific parameters (i.e., SWP) in our study. Substantial water savings are provided by mulches (López-Urrea *et al.*, 2020) because vineyards tend to suffer less thermal and water stress (Fraga and Santos, 2018). Besides, a good weed control and better vine performance was achieved in mulched treatments, avoiding costs of mechanical weeding with increased yields. These mulches showed to be viable agronomic strategies for managing weeds without damaging the soil.

General comments

The overall results presented in this Thesis dissertation provide a complete set of alternative strategies for weed management in vineyards. Cover crops terminated with roller-crimper, bioherbicides and mulches are different techniques that are not exclusive, and depending on each situation, they can be combined or used simultaneously in order to manage weeds from an integrated approach, and specifically on two of the most problematic weed species in Mediterranean vineyards, like *C. dactylon* and *C. bonariensis*.

This Thesis becomes a singular contribution to the knowledge of new sustainable

techniques for soil and weed management in vineyards, with impacts on the agronomic performance of grapevines. Simultaneously, it is a starting point for the optimization and search for new methods or their appropriate combination for IWM in vineyards. In this sense, the use of mulches and cover crops, combined with punctual bioherbicide applications, either in the under-vine zone or in the inter-row, are promising future options for weed management in vineyards.

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CHAPTER 8

General Conclusions

General conclusions

From this Thesis dissertation, the following main conclusions emerged:

Cover crops emergence modelling

1. Emergence models were successfully developed for the 18 species studied with potential use as cover crops (*Avena strigosa*, *Bromus catharticus*, *Camelina sativa*, *Fagopyrum esculentum*, *Festuca arundinacea*, *Festuca ovina*, *Hordeum vulgare*, *Lolium multiflorum*, *Lolium perenne*, *Onobrychis viciifolia*, *Phacelia tanacetifolia*, *Plantago coronopus*, *Plantago lanceolata*, *Sinapis alba*, *Trifolium incarnatum*, *Triticum spelta*, *Vicia sativa* and *Vulpia myuros*), either based on TT, HTT, PhHTT or PhSHTT in autumn and spring indistinctly.
2. The validation of the models for four species (*Lolium multiflorum*, *Trifolium incarnatum*, *Vicia sativa* and *Sinapis alba*) out of six with independent data from the south of France reinforces the validity of the models' development method.
3. The accuracy of the models is improved when light is included and, for *Lolium multiflorum*, *Trifolium incarnatum*, *Vicia sativa* and *Sinapis alba*, models can be used widely

Inter-row weed management

4. Sown cover crops (*Avena strigosa*, *Hordeum vulgare*, *Lolium multiflorum*, *Phacelia tanacetifolia*, *Sinapis alba* and x *Triticosecale*) reduce weed community richness when compared with the spontaneous one, which allow control annual winter weeds.
5. The termination method (shredding or roller-crimper) has more influence on *Cynodon dactylon* coverage than the type of cover crop.
6. A good control of *C. dactylon* is achieved with cover crops terminated with roller-crimper when compared to shredding.

Under-vine weed management

7. The herbicidal effect of Acetic Acid 20 % + N32, Potassium metabisulfite + Pelargonic acid 31 %, Pelargonic acid 68 %, Humic-Fulvic acid 87 % and

Potassium metabisulfite on *Conyza bonariensis* plants was confirmed in the greenhouse dose-response experiment. When *C. bonariensis* plants grow from BBCH 12-13 to BBCH 14-15, the EC₅₀ doubled in all treatments, highlighting the importance of the growth stage of the weed.

8. Only the Potassium metabisulfite + pelargonic acid 31%, the Humic-Fulvic acid 87 %, and the Potassium metabisulfite obtained high field efficacy (> 75 % of *C. bonariensis* coverage reduction) throughout most of the application dates, hence, they are able to maintain *C. bonariensis* coverage under control.
9. Organic mulches (*Medicago sativa* straw, *Festuca arundinacea* straw, *Hordeum vulgare* straw, chopped pine wood and almond shell) can control weeds successfully in the under-vine zone as long as mulch cover is high (above 75 %).
10. Depending on the nature of the mulch, the persistence is unequal. Chopped pine wood mulch and almond shell mulch stand out above straw mulches as they achieved high soil cover during, at least, three growing seasons, avoiding weed growth.

Impact of under-vine weed management on agronomic performance of grapevines

11. Mulching (chopped pine wood and almond shell) prevents from an increase of soil temperature in the topsoil, compared with Mowing and Tillage, and improves the vine water status, with higher stem water potential values than mechanical weed managements.
12. Vine vegetative growth is favored by mulches with longer shoots and more pruning weight than Mowing and Tillage, without compromising the sanitary status of the plants.
13. The use of mulches in the under-vine zone increases grape yields compared to mechanical weed management.
14. Mowing decreases the petiole nutrient status, with lower values of N, P, K and B, and soil chemical characteristics remains similar between mulched vines and Mowing, except for N-NO₃ concentration, which is greater with Tillage.

APPENDIX

Published papers



Emergence modelling of 18 species susceptible to be used as cover crops in Mediterranean semiarid vineyards

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ARTICLE INFO

Keywords:

Living mulch
Soil temperature
Thermal time
Hydrothermal time
Photohydrothermal time
Photosolar hydrothermal time

ABSTRACT

Cover Crops (CC) are increasingly appreciated in vineyards because they can provide ecosystems services, such as preventing soil erosion and compaction, increasing soil organic matter or, controlling weeds. Many species from different botanical families can be used depending on the final purpose of the CC, but their successful establishment in Mediterranean semiarid conditions of NE Spain can be challenging. Therefore, it is mandatory to understand and be able to predict the emergence patterns of the chosen species as their success is crucial to achieve a good soil cover. Different models based on thermal time (TT), hydrothermal time (HTT), photohydrothermal time (PhHTT) and photosolar hydrothermal time (PhSHTT) have already been used in crops and weeds for this purpose. In this paper, these four models have been developed for the 18 species susceptible of being CC, some of them being successfully validated with independent data from southern France. Results suggest that, although TT and HTT based models are accurate, their precision is improved when light is included ($R^2 > 0.9$). Models including light could be widespread used in some species as the successful validation with independent data demonstrates. These models considerably contribute to inter-row management in vineyards as decision support systems (DSS) tools to predict CC establishments.

1. Introduction

Vineyards are among the most important perennial crops in Mediterranean climates (Prosdocimi et al., 2016) with 964037 ha cultivated in Spain in 2020 (MAPA, 2020). Most of this cropped area is traditionally managed, which means the inter-row space is managed with tillage, leaving the soil bare most of the year. However, coverless (bare) soil exacerbate erosion, compaction and loss of soil structure, decreases soil organic matter, water-holding capacity and infiltration, while biological activity and soil chemical properties are negatively affected (Durán Zuazo et al., 2006; Ibáñez-Pascual, 2014; Polge de Combret-Champart et al., 2013; Smith et al., 2008), especially in Mediterranean areas, due to their topographic, edaphic and climatic conditions. Soil degradation is combined with the social concern about the food-energy-environment trilemma with the demand to low input and more sustainable agroecosystems (Harvey and Pilgrim, 2011; Horlings and Marsden, 2011). Soil management techniques have undergone important changes over the last decades in other wine regions, from the maintenance of the soil completely free of non-crop plants to the

maintenance of annual or perennial vegetation in the inter-rows (Dastgheib and Frampton, 2000; Pardini et al., 2002). Cover crops (CC) are known to provide several ecosystem services that reduce the problems that tillage causes in the soil, like erosion or a decrease in water infiltration rates (Napoli et al., 2017; Ruiz-Colmenero et al., 2013) among other benefits. Cover crops are also used with varied success to regulate vine vegetative growth and vigour (Dry and Loveys, 1998; Hatch et al., 2011), and improve vine health by reducing the risk of grey mould (Valdés-Gómez et al., 2008). Besides, CC contribute to the control of competitive weeds (Valencia-Gredilla et al., 2020) that cause severe competition during the first few years after planting in areas where vine root growth is limited due to shallow or compacted soil (Haviland et al., 2019). Given this wide range of ecosystem functions of CC, it is important to know the emergence behaviour of the species selected for an optimal establishment and to achieve the benefits provided by CC.

Emergence models have been developed for many plant species, either to improve their establishment, especially in crops (Finch-Savage et al., 1998; Guérif et al., 2001; Porter and Rayner, 1984), or for their management and control, especially in weeds (Bajwa et al., 2015;

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<https://doi.org/10.1016/j.eja.2021.126413>

Received 27 April 2021; Received in revised form 20 September 2021; Accepted 11 October 2021

Available online 2 November 2021

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Royo-Esnal et al., 2020; Sousa-Ortega et al., 2020a). The objective of these models is to be able to describe the emergence based on environmental factors, mainly soil temperature (thermal time, TT) and a combination of TT and soil moisture (hydrothermal time, HTT) so that they can be used as tools for crop or weed management (Forcella et al., 2000; Roman et al., 2000). In some cases, light, as photoperiod and solar radiance, has also been considered, resulting in new units (photo-hydrothermal time, PhHTT; photosolar hydrothermal time, PhSHTT), which give more accurate descriptions of the emergences. Temperature and moisture have worked well in models describing germination of species that show autumn and spring emergence flushes (Royo-Esnal et al., 2010a), so it is expected that it will also work well to model CC, which can be sown in these two seasons. Light, which greatly differs between autumn and spring, may also be an important factor to take into account and may determine the need for developing one model for both seasons or one for each season (Royo-Esnal et al., 2015a, 2015b).

There are few works that have modelled the emergence of CC species, like that from Tribouillois et al. (2018), who underline the importance of soil moisture in the accuracy of these models. For this reason, in this work, the emergence of 18 species that are susceptible to be used as CC in Mediterranean vineyards is studied and their patterns subjected to the above mentioned factors (temperature, moisture and light), with the aim to develop models that can contribute to the decision support systems (DSS) of CC management in vineyards under semiarid conditions of northeastern Spain.

2. Material and methods

2.1. Plant material

The 18 studied species were supplied by commercial seeds companies that produced them in 2018, most of them close to the experimental site (except *Fagopyrum esculentum*, *Plantago coronopus* and *P. lanceolata*) (Table 1).

2.2. Experimental site

The experiment was carried out in autumn 2018 and 2019 and in spring 2019 and 2020 in the experimental fields of the University of Lleida, in northeastern Spain (41°37' N, 0°35' E). Soil texture was 31.25% sand, 38.01% silt, 30.74% clay, with pH of 8.4% and 0.95% of organic matter. According to Köppen-Geiger classification, the climate is cold semiarid (BSk) with an average annual precipitation of 374 mm, and a mean temperature of 14.8 °C (average min of 8.0 °C and average max of 22.4 °C).

Table 1

Species used in the present experiment, their variety (if available), the weight of 1000 seeds, the supplier company and their production site (origin).

Species	Variety	1000 seed weight (g)	Company	Origin
<i>Avena strigosa</i> (L.) Schreb	Saia 6	21.28	Semillas Fitó S.A.	NE Spain
<i>Bromus catharticus</i> (L.) Vahl	Jeronimo	8.92	Semillas Batlle S.A.	NE Spain
<i>Camelina sativa</i> (L.) Crantz	GP204	1.19	Camelina Company S.A	NE Spain
<i>Fagopyrum esculentum</i> Moench	–	27.83	Semillas Silvestres S.L.	South Spain
<i>Festuca arundinacea</i> (L.) Schreb	Fawn	3.05	Semillas Fitó S.A.	NE Spain
<i>Festuca ovina</i> L.	Ridu	0.88	Rocalba S.A.	NE Spain
<i>Hordeum vulgare</i> L.	Meseta	44.7	Semillas Batlle S.A	NE Spain
<i>Lolium multiflorum</i> Lam.	Elirix	1.93	Rocalba S.A	NE Spain
<i>Lolium perenne</i> L.	Sun	1.81	Semillas Fitó S.A.	NE Spain
<i>Onobrychis viciifolia</i> Scop.	Arinda	21.31	Semillas Batlle S.A.	NE Spain
<i>Phacelia tanacetifolia</i> L.	Lilla	1.73	Rocalba S.A.	NE Spain
<i>Plantago coronopus</i> L.	–	0.27	Semillas Cantueso S.L	South Spain
<i>Plantago lanceolata</i> L.	–	1.19	Semillas Cantueso S.L	South Spain
<i>Sinapis alba</i> (L.) Rabenh.	Accent	5.66	Rocalba S.A.	NE Spain
<i>Trifolium incarnatum</i> L.	Contea	6.59	Rocalba S.L	NE Spain
<i>Triticum spelta</i> L.	Benedeto	53.67	Semillas Batlle S.A.	NE Spain
<i>Vicia sativa</i> L.	Libia	49.31	Semillas Fitó S.A.	NE Spain
<i>Vulpia myuros</i> (L.) C.C. Gmelin	–	0.94	Semillas Fitó S.A.	NE Spain

2.3. Weather data

Daily rainfall (l/m²), maximum and minimum air temperatures (°C) and solar radiance (MJ/m²) were obtained from a meteorological station (Lleida - La Femosa) situated 5 km away from the experimental site. All weather data as well as daylight hours can be consulted in the agrometeorological service www.ruralcat.cat. The CC emergence period lasted less than two months in each sowing date, for this reason, in order to show the environmental conditions, the weather data that have been considered is that of the two months immediately following sowing in each season and year.

2.4. Experimental design

A pot experiment was carried out in a randomised complete block design with four replicates. 100 seeds/pot of the 18 species separately were sown and distributed between 0 and 2 cm depth in pots of 19 cm of diameter x 22 cm deep and 6237 cm³. Each pot was filled with soil and buried to simulate field conditions. The upper 4 cm soil layer was previously sterilised at 120 °C during 24 h. Seeds were sown on 5 December 2018 (sowing date was delayed due to inclement weather), 7 March 2019, 16 October 2019 and 26 March 2020. Pots were not irrigated, so that emergence was conditioned mainly by weather conditions. Emergences were sampled every 2–3 days by destructive counts until no more were detected. Due to lack of emergences, data of *F. esculentum* and *P. coronopus* in autumn 2018 and spring 2020, respectively, were excluded for the analysis, because they could bias the interpretation of the results.

2.5. Models development

Data of the autumn and spring emergence periods of the two years were used for the models development. The first model was obtained from the cumulative soil thermal time (TT) (Gupta, 1985).

$$TT = \sum T_i - T_b \quad (1)$$

T is the daily average soil temperature at 1 cm depth and T_b is the base temperature for seedling emergence for each species. TT = 0 when T < T_b.

A second model was developed based on the equation described by Roman et al. (2000), where simulated TT and water potentials (hydro-time, HT) were used to estimate hydrothermal time (HTT):

$$HTT = \sum HT_i \times TT_i \quad (2)$$

where $HT = 1$ when $\psi > \psi_b$, otherwise $HT = 0$; and $TT = T - T_b$ when $T > T_b$, otherwise $TT = 0$. Water potential (ψ) is the daily average water potential in the soil layer at 4 cm depth; ψ_b is the base water potential (in MPa) for seedling emergence (García et al., 2013; Martinson et al., 2007). With these formulas, growing degree days are accumulated only when water potential and temperature conditions were higher than the base water potential and the base temperature. A soil depth of 1 cm was chosen for soil temperature because seeds were buried at that layer; 4 cm depth was chosen for water potential because seedlings must elongate radicles to a certain depth to absorb enough water for emergence (Royo-Esnal et al., 2019). The TT and HTT were estimated using the Soil Temperature and Moisture Model (STM²) (Spokas and Forcella, 2009). The sowing date each season was chosen as the starting point for TT and HTT accumulation; T_b for each species was obtained from literature (see Table 5); and ψ_b was determined by modifying the value until the highest accuracy (R^2) was obtained for the relationship between HTT and cumulative emergence of each species (Royo-Esnal et al., 2010b).

In order to improve model accuracy and compensate for differences in emergence patterns at the four sowing dates, a third model was developed based on HTT corrected by proportional daylight hours and convert it into photohydrothermal time, PhHTT, considering daylight of 24 h = 1, 12 h = 0.5 and 0 h = 0 (Royo-Esnal et al., 2015b). Thus, HTT was multiplied by its corresponding proportional day length as follows:

$$PhHTT = \sum (HTT_t \times D_t) \quad (3)$$

Where HTT_t is the hydrothermal time in day t , and D_t is the proportional day length in day t . Finally, daily solar radiance (SR) was also included in a fourth model, creating the photosolar hydrothermal time (PhSHTT) with the combination of PhHTT with solar radiance (Royo-Esnal et al., 2015b, 2019), estimated as follows:

$$PhSHHT = \sum \frac{HTT_t \times SR_t}{\ln(SR_t) \times 100} \times D_t \quad (4)$$

Where HTT_t is the hydrothermal time in day t , SR_t is the solar radiance in day t , and D_t is the proportional day length in day t . The functional relationship between cumulative emergence and TT, HTT, PhHTT and PhSHTT was described by a three parameter sigmoidal (log-logistic) model as follows:

$$y = \frac{\alpha}{1 + e^{-\left(\frac{x-x_0}{b}\right)}} \quad (5)$$

Where y is the percentage of emergence, is the time expressed as TT, HTT, PhHTT or PhSHTT, and α , b and x_0 , are empirically derived constants: α , is the maximum percentage of the recorded emergence, b is the rate of increase and x_0 is the TT, HTT, PhHTT or PhSHTT required to obtain 50% of maximum emergence. To make this sigmoidal model simpler, was assumed to be 100% for each species in each season. Fitting of the three parameter sigmoidal function for cumulative emergence was performed using SigmaPlot 12.0 (Sistat Software, Inc, San José, CA, USA). The accuracy of models was evaluated with R^2 and the root mean-square error (RMSE) values for each sowing date emergence observations and considering the mean RMSE of the four of them. RMSE is estimated with the following formula:

$$RMSE = \sqrt{1/n \sum_{i=1}^n (x_i - y_i)^2} \quad (6)$$

where x represents observed cumulative percentage seedling emergence, is the predicted cumulative seedling emergence, and is the number of observations (Mayer and Butler, 1993). RMSE provided a measurement of the typical difference between predicted and actual values in units of percentage seedlings emergence. Low RMSE values indicate that emergence model fit had been optimised.

Supplementary data is provided with the fitted models separated by seasons (autumn and spring) for the species that clearly had two differentiated emergence rhythms.

2.6. Emergence model validation

The models of seedling emergence were validated with data published by Tribouillois et al. (2018) with the observed emergence of *A. strigosa*, *L. multiflorum*, *P. tanacetifolia*, *S. alba*, *T. incarnatum* and *V. sativa* in an irrigated experimental field in Auzeville, south of France, (43°31' N, 1°34' E) in August 2012. Weather data was collected from NASA POWER (<https://power.larc.nasa.gov/>) and hydrothermal time was estimated with soil temperature and moisture data from STM². Predicted emergences were superposed on observed seedling emergences, and the differences between them were assessed by root mean-square error predictor (RMSEP), which is calculated with the same formula as (6).

2.7. Statistical analysis

Differences in the total percentage of emergence among seasons (autumn vs spring) and between years (2018–2019 vs 2019–2020) were analysed with two-way ANOVA. If there was any interaction between factors, these were separated and the statistical program automatically applied a Least Significant Difference (LSD) for the comparison between means. Significances were considered at $P < 0.05$ level. When normality and/or homogeneity of variance tests failed, Kruskal Wallis test was performed. These analyses were performed also with Sigmaplot 12.0.

3. Results

3.1. Weather data

The two years differed considerably in temperature and precipitation (Table 2). The 2018–2019 season was colder and much dryer than 2019–2020. Number of rainy days (considered >0.2 mm) also differed between years and seasons (6 in autumn 2018, 12 in spring 2019, 12 in autumn 2019 and 20 in spring 2020) which is reflected in the soil water potential (Fig. 1) as long wet or dry periods. Solar radiation was always higher in spring (19–16.3 MJ/m²) than in autumn (6–10.5 MJ/m²). For modelling purposes, such great natural variability in magnitudes of explanatory variables is highly desirable.

3.2. Seedling emergence

Species showed high variability in their emergence dynamics. Fig. 2 shows the total seedling emergence and its variability within each species. Some species such as *T. incarnatum*, *P. tanacetifolia* and *P. lanceolata* presented high variability while others, like *F. esculentum* and *L. perenne*, had lower variability. Overall, considering all emergence data together, a gradient in total percentage of emergence was observed across the species, ranging from 25% in *F. ovina* up to 70% in *S. alba*.

When total emergence percentage are separated by seasons (autumn or spring), the variability within each species is reduced (Fig. 3). All species showed lower values of percentage of emergence in spring except *F. esculentum*, *P. tanacetifolia*, *A. strigosa*, *T. spelta* and *H. vulgare*. Furthermore, 9 of the 18 species analysed showed significant differences between autumn and spring, while 8 were different between years (Table 3). The highest variability was observed for *T. incarnatum*, ranging from 2.75% in spring 2019–2020 up to 63% in autumn 2018–2019.

3.3. Emergence period

For most species, there were significant differences between the length of the emergence period in autumn and in spring (15 out of 18),

Table 2

Mean temperature (Tm), total precipitation (P), days of precipitation (Pd), and mean solar radiance (SR) of the two months following each sowing date in autumn and spring of the two years, and their corresponding historical average (in brackets) of the range 2007–2016.^a

Year	Tm (°C)		P (mm)		Pd (days)		SR (MJ/m ²)	
	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr
2018–19	5.1 (5.4)	12.1 (18.2)	21.6 (38.2)	26 (82)	6 (11.7)	12 (15.5)	6 (5.6)	19 (18)
2019–20	12.8 (12.3)	12.5 (18.2)	108.4 (83.7)	96 (82)	12 (11)	20 (15.5)	10.5 (9.2)	16.3 (18)

^a Data within the two months after the sowing date; December and January for autumn 2018–19, October and November for autumn 2019–20, March and April for spring of 2018–19 and 2019–20.

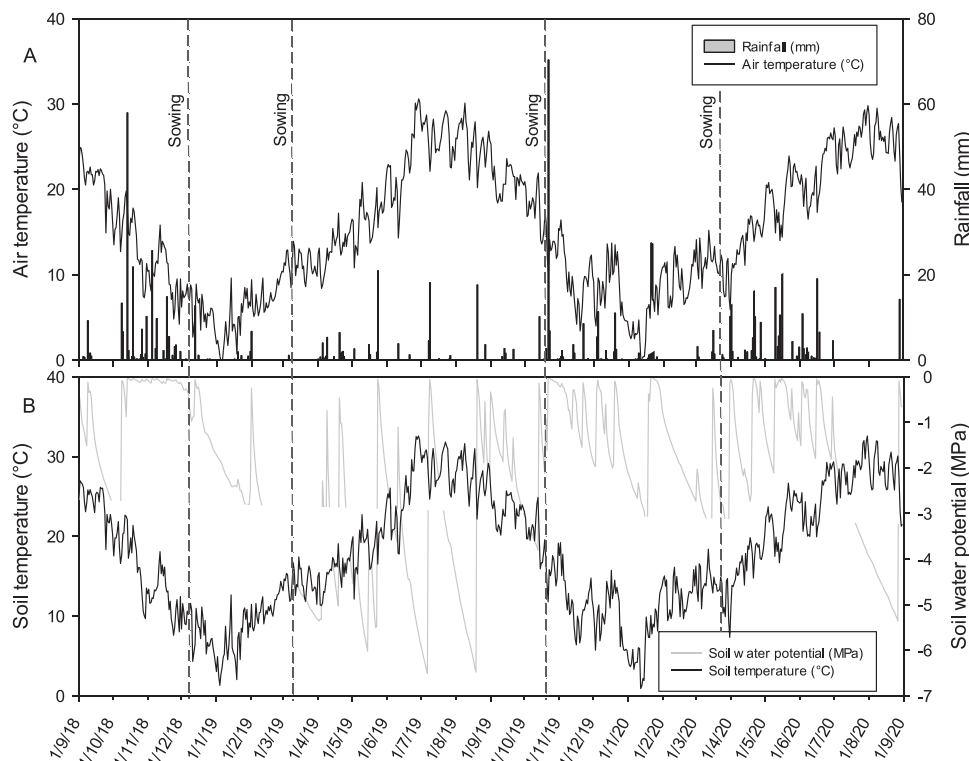


Fig. 1. Mean daily air temperature and precipitation (A) and mean daily soil temperature and soil water potential at 1 cm and 4 cm depth, respectively (B), estimated with the STM² (Spokas and Forcella, 2009). Vertical dotted lines indicate sowing dates.

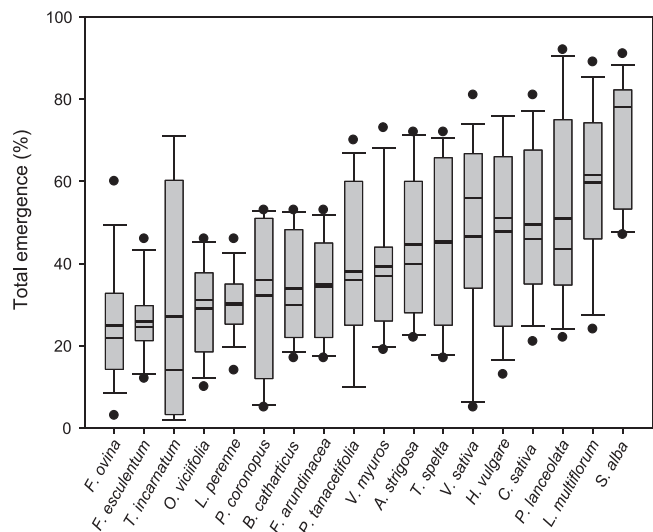


Fig. 2. Total emergence percentage for each species. Vertical bars represent standard errors (SE).

but not between years (7 out of 18) (Table 4, Fig. 4). While there was a range of emergence periods from less than 15 days up to more than 40 days in autumn, the emergence period of all species lasted less than 20 days in spring. This variation of autumn/spring emergence periods, though, was not the same for all studied species. While most of them showed significantly longer emergence periods in autumn, *S. alba* and *F. esculentum* did not, and their emergence periods were similar in both seasons (Table 4). Among those species showing autumn/spring differences for the emergence period, this varied from 25 to 55 days; almost one month of difference between the earliest and the latest species.

3.4. Development of emergence models

The emergence models were calculated using the four series of data (autumn 2018, spring 2019, autumn 2019 and spring 2020). All emergence patterns based in either factor (TT, HTT, PhHTT or PhSHTT) were significantly ($P < 0.01$) adjusted to sigmoidal log-logistic models (Table 5). According to literature, T_b varied from -0.7 °C (*C. sativa*) to 10.0 °C (*F. esculentum*), and the estimated Ψ_b ranged from -3.4 MPa to -1.4 MPa, which can indicate the degree of drought tolerance of each species at emergence. The graphical representations of developed models are shown in Figs. 5, 6 and 7. In these graphs the observed

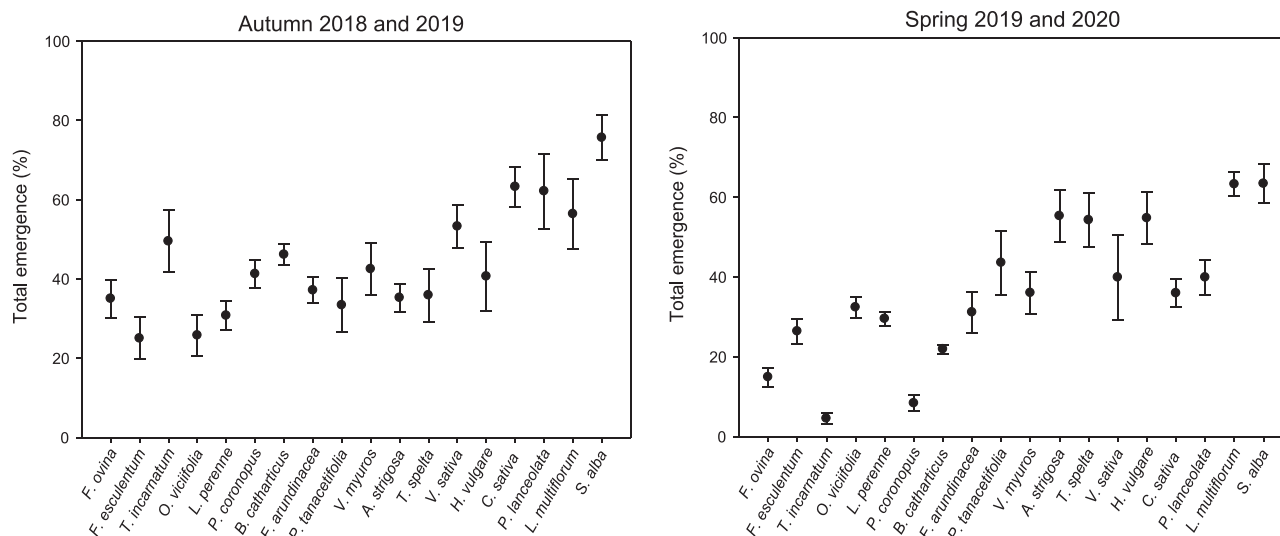


Fig. 3. Total emergence percentage for each species in autumn (left) and spring (right), pooling the data of the two years. Vertical bars represent standard error (SE).

difference between autumn and spring emergence periods can be observed based on the different thermal basis (TT, HTT, PhHTT or PhSHTT). In 17 of the 18 species, both PhHTT and/or PhSHTT based models achieved very good accuracy levels ($R^2 > 0.9$ and $RMSE < 15$). Based on (Royo-Esnal et al., 2012) all models that considered light as day length alone or with solar radiation showed very good ($RMSE = 5-10$) or good ($RMSE = 10-15$) fit, and only in *P. lanceolata*, the accuracy was low ($RMSE = 16.3$). On the contrary, the best accuracy was obtained for *C. sativa*, with an R^2 of 0.98 and a $RMSE$ of 5.3.

3.5. Validation of the models

The validation of the models was carried out for 6 species based on independent data from Tribouillois et al. (2018). Four of the 6 species analysed were successfully validated in, at least, one of the models developed. However, unequal results were found considering that for *L. multiflorum*, *T. incarnatum* and *V. sativa*, the best accuracy in the validation was achieved when light was included, either PhHTT or PhSHTT (best RMSEP of 8.6, 10.5 and 12.1, respectively), while for *S. alba*, the best accuracy was achieved with the less elaborated models (TT and HTT) with a RMSEP of 6.1 and 6.4, respectively. No model could be validated with the independent data for *A. strigosa* and *P. tanacetifolia* ($RMSEP > 35$) (Fig. 8).

4. Discussion

Cover crops fill gaps in cropping systems that would otherwise be occupied by weeds (Liebman et al., 2001; Baraibar et al., 2021). To avoid bare soil where weeds could grow freely, a good establishment of the cover crop is needed, and this is a process conditioned, among others, by its emergence. Soil temperature, soil moisture, soil air quality and light quality are the main environmental factors affecting seedling emergence (Forcella et al., 2000). Weather conditions in the two experimental years were very different, and they also differed quite a bit from the historical average (Table 2). Besides, the two sowing dates in autumn and in spring repeated two years provided a large variability in conditions, resulting in four emerging periods. So from an emergence-modelling perspective, this variability in all those factors contributes to strengthening the models and gives consistency to the results, especially when the majority of the species subjected to the independent data from the south of France (Tribouillois et al., 2018) were successfully validated.

4.1. Emergence percentage

In general, the emergence of all species varied more between seasons within each year rather than between years. Some few exceptions were observed (i.e. *S. alba*). Most of the results in this sense are in accordance with the consulted literature. Grass species, such as *Festuca* spp. and *Lolium* spp., emerged in the range 25–65%, similar or higher than those from Hill et al. (1985) at temperatures from 10 °C to 25 °C; *B. catharticus* showed higher emergence at colder temperatures (autumn) than at warmer temperatures (spring), as observed by Mollard and Insausti (2009), with higher germination rates at 15 °C (35%) than at 20 °C (15%). *Camelina sativa* was highly variable (8.6–40.9%), like for Urbaniak et al. (2008) or Royo-Esnal et al. (2017), this variation in field emergence suggests that it is likely dependent on environmental conditions caused by the seed bed preparation (Urbaniak et al., 2008). Also a high emergence variability was observed for *T. incarnatum* in the present study with values from < 5% to > 50% and similar to those observed by Brooker et al. (2020) (<1–32%). The lack of seedling emergence of *F. esculentum* in autumn 2018 can be explained by its high base temperature (10 °C) (Arduini et al., 2016) that might have prevented its seeds from germinating in December (mean temperatures $< T_b$). The lower emergences observed in the other grass species, *H. vulgare*, *T. spelta* and *A. strigosa* in 2018–2019, was a consequence of seed predation by birds. The lack of emergences of *P. coronopus*, the species with the smallest seeds, in spring 2020 might be explained by the formation of soil crusting in hot days after each rainy period and the inability of its tiny seeds to go through it.

4.2. Emergence models

Most cover crop species considered in this study showed clear differences in the length of the emergence period between autumn and spring sowing dates. This fact, together with the variability of the emergence rates would impede the development of common emergence models for these two seasons, but the estimation of the thermal time (TT), adjusted by soil moisture (HTT), day length (PhHTT) and solar radiation (PhSHTT) significantly improved the overlap of emergence timings. The inclusion of light significantly improved the models for *Thlaspi arvense* (Royo-Esnal et al., 2015a) and *Camelina microcarpa* (Royo-Esnal et al., 2015b) compared to previous models from the same region. Our results showed that for *C. sativa*, *F. arundinacea*, *H. vulgare*, *P. coronopus*, *S. alba* and *T. incarnatum* the achieved accuracy was very good ($RMSE = 5-10$) or good ($RMSE = 10-15$), according to Royo-Esnal

Table 3
Percentages (%) of emergence achieved during the four sowing dates. Results of the ANOVA (or Kruskal Wallis) are also provided with the corresponding F value, significance (P), and interaction between factors (year and season).

Year	Season	A. srigosa	B. catharticus	C. sativa	F. esculentum*	F. arundinacea	F. ovina	H. vulgare*	L. multiflorum	L. perenne
2018–2019	Autumn	39.50	46.50	65.25	–	39.25	38.25	19.75	68.25	24.25
	Autumn	31	45.67	61.25	25	35	31.75	61.50	44.50	37.25
2018–2019	Spring	47.67	20.33	30.75	28.50	44.67	18	43	64.50	32.25
	Spring	61	23	41.125	24.25	21	15.50	66.50	62	26.75
Year	F	P	P	F	F	P	F	F	F	F
	0.115	0.741	0.086	0.776	0.371	0.557	0.007	0.446	0.226	0.162
Season	F	0.021	0.001	0.001	0.012	0.334	10.290	0.008	0.050	0.450
	7.181	60.77	< 0.001	0.917	1.020	0.430	0.124	1.974	1.459	0.250
Year x Season	F	0.154	0.589	0.278	–	–	–	–	–	–
	2.350	0.312	0.589	0.278	–	–	–	–	–	–
Year	O. vicifolia	P. lanacetifolia	P. coronopus	P. lanceolata	S. alba*	T. incarnatum	T. spelta*	V. sativa	V. myuros*	
	13.50	29	45.75	37.75	82.50	63	23	54	48.50	
2018–2019	Autumn	38	37.75	36.75	86.50	36	48.75	52.50	36.50	
	Autumn	34.75	21.67	8.33	35.25	6.25	42.25	13.25	38.25	
2018–2019	Spring	30	60	–	44.50	2.75	66.25	66.50	33.75	
	Spring	F	P	F	F	P	F	F	F	
Year	F	P	P	P	P	P	P	P	P	
	8.577	0.013	0.174	0.174	0.943	0.351	5.594	11.940	0.005	
Season	F	0.073	0.381	0.001	22.010	0.051	48.710	6.513	0.025	
	3.860	0.834	0.381	0.001	4.676	0.051	48.710	6.513	0.025	
Year x Season	F	< 0.001	0.097	–	17.340	0.001	3.321	0.015	0.905	
	18.810	< 0.001	0.097	–	17.340	0.001	3.321	0.015	0.905	

* Non-parametric Kruskal Wallis test. Significant differences at P < 0.05.

Table 4
Emergence duration (days) during the four sowing dates. Results of the ANOVA (or Kruskal Wallis) are also provided with the corresponding F value, significance (P), and interaction between factors (year and season). Species are presented following the order of Fig. 4.

Year	Season	A. srigosa	B. catharticus*	C. sativa	F. esculentum	F. arundinacea	F. ovina	H. vulgare*	L. multiflorum*	L. perenne
2018–2019	Autumn	33	33.50	40.88	–	40.50	43.75	31	35.25	35.50
	Autumn	24	58.67	32.75	11.25	60.75	65.25	22	47.50	63.25
2018–2019	Spring	12.75	22.67	23	15.75	19.50	9.50	20.50	22.75	23.75
	Spring	F	F	F	F	F	12.50	8.67	17.25	14
Year	F	P	P	P	P	P	P	P	P	P
	6.186	0.031	0.062	0.051	0.0709	0.796	42.496	7.325	0.764	0.399
Season	F	< 0.001	0.002	0.002	0.480	0.506	535.860	9.586	0.010	30.662
	38.409	< 0.001	0.002	0.002	0.480	0.506	535.860	9.586	0.010	30.662
Year x Season	F	0.640	0.005	0.362	–	–	24.230	0.135	0.720	5.286
	0.231	0.640	0.005	0.362	–	–	24.230	0.135	0.720	5.286
Year	O. vicifolia	P. lanacetifolia*	P. coronopus*	P. lanceolata*	S. alba	T. incarnatum	T. spelta	V. sativa*	V. myuros*	
	42.50	18	30.50	53	15.25	48.50	35.75	28.75	32.50	
2018–2019	Autumn	60.75	19	30.75	48.25	38.75	27.75	42.75	42	
	Autumn	19.25	7.75	7.67	16.33	9.50	18	13	17.25	
2018–2019	Spring	20.75	10.25	–	17.25	3	6	7.50	10.50	
	Spring	F	F	F	F	F	F	F	F	
Year	F	P	P	P	P	P	P	P	P	
	6.181	0.029	0.693	0.980	0.055	3.974	0.072	4.242	0.062	
Season	F	< 0.001	0.048	0.057	17.180	0.002	84.083	16.547	0.002	
	63.396	< 0.001	0.048	0.057	17.180	0.002	84.083	16.547	0.002	
Year x Season	F	0.057	0.865	–	0.120	0.735	0.159	0.698	0.101	
	4.446	0.057	0.865	–	0.120	0.735	0.159	0.698	0.101	

* Non-parametric Kruskal Wallis test. Significant differences at P < 0.05.

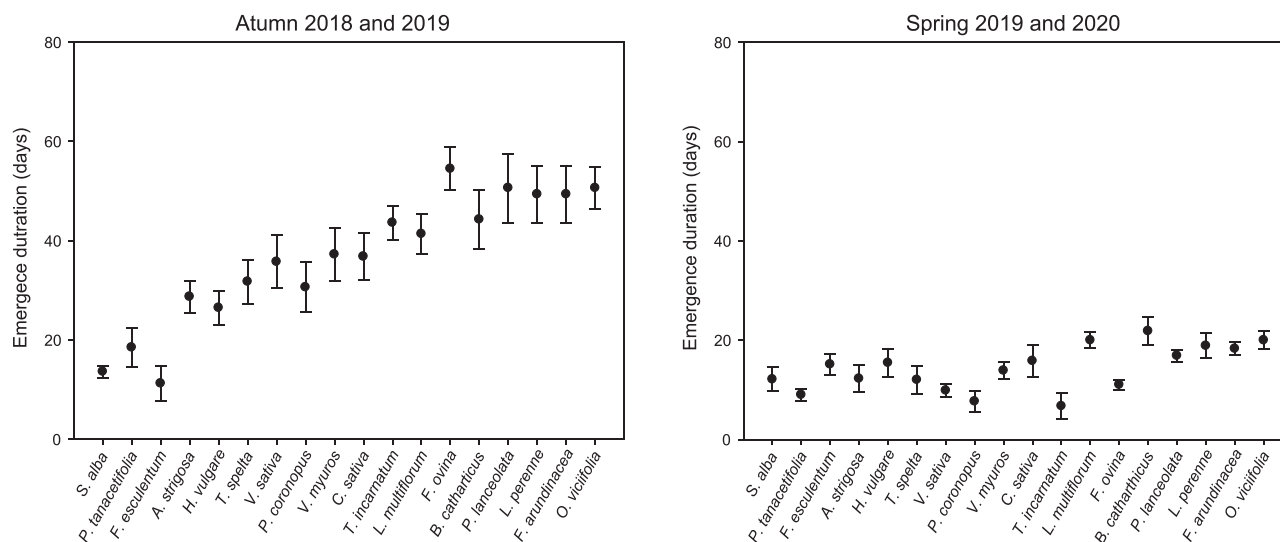


Fig. 4. Emergence duration for each species in autumn (left) and spring (right), pooling the data of the two years. Vertical bars represent standard error (SE).

et al. (2012), in the four developed models. For the remaining species, TT and HTT based models were not accurate enough to predict the emergence. Nevertheless, the incorporation of day length, as well as solar radiation, significantly enhanced the accuracy of the model of the 18 species, either PhHTT or PhSHTT (Figs. 5, 6 and 7) despite the variability in the weather conditions among sowing dates (Table 2), with R^2 values always > 0.9 and $RMSE < 15$ in, at least, one of the models (except for *P. lanceolata*, where the inclusion of light improved the model but with an $R^2 < 0.9$ and $RMSE > 15$). There is limited literature on the inclusion of light to develop emergence models, but it should be considered for species that show autumn and spring flushes, like in our results and in those of Royo-Esnal et al., 2015a and 2015b. Our results show that, there is no need for developing separate models for autumn and spring flushes, as these can be integrated in one single model based on PhHTT or PhSHTT. Some examples of that are *A. strigosa*, *F. arundinacea*, *F. ovina* and *P. tanacetifolia*, where the overlap of the data was good for the TT and/or HTT based models between years but not between seasons. The inclusion of day length and/or solar radiation improved the overlap between years and seasons, enhancing the accuracy of the model (Figs. 5, 6 and 7).

The validation carried out with data from Tribouillois et al. (2018) was successful for the majority of the species tested, mainly when day length and/or solar radiation were included. The climate in Auzeville (southern France), with a stronger Atlantic influence, is very different from the one in north-eastern Spain, and the sowing date in Auzeville was carried out in summer (mid-august). Despite the differences in the sowing timing between localisations, the validation was successful in 4 of the 6 species tested, so that corroborate that temperature, moisture and light are the main factors influencing emergence. Therefore, models including light are potentially useful to be applied in a widespread area. The validation was unsuccessful for only 2 of the 6 species tested (*P. tanacetifolia* and *A. strigosa*), both species with a large spectrum of emergence, which suggest that sensibility to light may differ within some species between Mediterranean and temperate climate as Bell et al. (1995) and Torra et al. (2016) conclude. In this sense, for some species may be necessary to develop independent models for each geo-climatic area, as suggested for *L. rigidum* by Sousa-Ortega et al. (2020b). On the other hand, *S. alba* was successfully validated with the models including only temperature and moisture, which suggest that these two factors are enough to predict accurately its emergence.

How light influences emergence models when seeds are buried is not clear. Direct radiation may inhibit the germination of some seeds, but low irradiances can promote it (Baskin and Baskin, 1998). Seeds were distributed between 0 and 2 cm when sowing, and light apparently

penetrates only the top few millimetres of soil. However, light penetration depth depends on soil particle size, moisture content, and colour (Benvenuti, 1995; Baskin and Baskin, 1998), so a fraction of light may be able to cross the upper soil layer and would be detected by seeds depending on those factors. Another explanation of how seeds detect light is indirectly through soil temperature, light is correlated with temperature (van den Besselaar et al., 2015) and daily temperature variation is detected by seeds in the soil (Ghersa et al., 1992), so it could be that hourly temperature based emergence models could avoid the need of considering photoperiod as proposed by Royo-Esnal et al. (2015b). Hourly temperature is somehow affected by photoperiod itself, and is underlying in the hourly based HTT. Seeds may detect the period of time of the daily temperature fluctuation, so this would be another way of detecting photoperiod without receiving light directly.

4.3. Practical application

An interesting approach of this work is that PhHTT and PhSHTT based models are more accurate than those based on TT and HTT and could be applied to a wide range of species as confirmed by the fact that the obtained RMSE values were below 15 in, at least, one of the first two models for 17 out of 18 species from different botanical families. Models including light can be applied in autumn or spring indistinctly, at least, in the same region where they were developed, and for some species even in different geo-climatic regions. This confirms the usefulness of these models as DSS tools to predict the establishment of CC. This knowledge considerably contributes to inter-row management in vineyards, as species can be chosen depending on the final purpose of the CC (weed control, erosion avoidance, increase of organic matter, control of vine vigour etc.). For example, quick emerging annual species, such as *S. alba*, *C. sativa* or *P. tanacetifolia*, and perennial ones, like *F. arundinacea* or *P. lanceolata* (Figs. 5, 6 and 7), that differ in their emergence rhythm, could be combined in a single sowing date, so that the annual species can cover the soil very fast and reduce weed pressure, while the perennial one is emerging and growing underneath. By the time annual species reaches the end of its life cycle, the perennial could have developed a considerable coverage and continue its growth, and be maintained by mowing. Similarly, models could be used to choose the most appropriate species to quickly cover the soil if erosion is a problem or to match emergence time with vine vigour control. Also, an accurate combination of entomophilous species could provide new sources of pollen for beneficial fauna, which can significantly contribute to the functional flows of the vineyard agroecosystem.

Table 5

Parameters (a, x_0 and b) for the thermal time (TT), hydrothermal time (HTT), photohydrothermal time (PhHTT) and photosolar hydrothermal time (PhSHTT) based models. The R^2 , F value of the model and their significance (P) are also provided, as well as the estimated base water potential (Ψ_b), and the base temperature considered from literature (T_b).

Species	Model	a	x_0	b	R^2	F	P	Ψ_b (MPa)	T_b (°C)	Reference for T_b
<i>A. strigosa</i>	T	100	135.94	19.32	0.76	254.08	< 0.0001	-3.4	4.8	(Tribouillois et al., 2016)
	HTT		135.94	19.32	0.76	254.08	< 0.0001			
	PhHTT		61.07	4.79	0.90	746.22	< 0.0001			
	PhSHTT		3.54	0.42	0.96	1711.70	< 0.0001			
<i>B. catharticus</i>	TT	100	248.12	73.22	0.63	134.79	< 0.0001	-2.1	5.8	(Lonati et al., 2009)
	HTT		224.07	81.03	0.57	106.49	< 0.0001			
	PhHTT		99.40	26.84	0.71	196.32	< 0.0001			
	PhSHTT		4.75	0.81	0.90	745.54	< 0.0001			
<i>C. sativa</i>	TT	100	199.59	27.98	0.93	1097.50	< 0.0001	-3.2	-0.7	(Allen et al., 2014)
	HTT		193.69	34.60	0.91	818.50	< 0.0001			
	PhHTT		84.26	8.44	0.98	3483.90	< 0.0001			
	PhSHTT		4.28	0.99	0.84	409.27	< 0.0001			
<i>F. esculentum</i>	TT	100	75.34	23.54	0.75	146.09	< 0.0001	-1.9	10	(Arduini et al., 2016)
	HTT		57.67	14.02	0.83	240.94	< 0.0001			
	PhHTT		28.24	5.64	0.86	311.32	< 0.0001			
	PhSHTT		1.72	0.25	0.90	440.92	< 0.0001			
<i>F. arundinacea</i>	TT	100	345.17	44.85	0.93	1093.39	< 0.0001	-2.5	3.5	(Sharifiamina et al., 2016)
	HTT		327.29	58.52	0.87	538.47	< 0.0001			
	PhHTT		146.19	19.75	0.97	2745.46	< 0.0001			
	PhSHTT		7.63	1.49	0.83	396.89	< 0.0001			
<i>F. ovina</i>	TT	100	407.31	84.80	0.78	283.19	< 0.0001	-2.1	1.1	(Lonati et al., 2009)
	HTT		366.04	107.50	0.71	193.80	< 0.0001			
	PhHTT		158.74	33.16	0.85	445.54	< 0.0001			
	PhSHTT		7.75	1.09	0.95	1544.85	< 0.0001			
<i>H. vulgare</i>	TT	100	172.05	26.34	0.81	347.35	< 0.0001	-3.4	2.6	(Angus et al., 1980)
	HTT		172.05	26.34	0.81	347.35	< 0.0001			
	PhHTT		74.06	5.07	0.95	1447.86	< 0.0001			
	PhSHTT		4.10	0.64	0.92	888.40	< 0.0001			
<i>L. multiflorum</i>	TT	100	236.67	45.64	0.85	439.42	< 0.0001	-3.2	1.5	(Moot et al., 2000)
	HTT		231.57	44.94	0.84	409.47	< 0.0001			
	PhHTT		105.54	18.36	0.92	865.89	< 0.0001			
	PhSHTT		5.48	0.98	0.92	907.50	< 0.0001			
<i>L. perenne</i>	TT	100	397.91	96.31	0.72	204.77	< 0.0001	-2.3	1.9	(Moot et al., 2000)
	HTT		372.88	117.70	0.65	147.25	< 0.0001			
	PhHTT		162.92	36.78	0.80	323.86	< 0.0001			
	PhSHTT		8.05	1.22	0.95	1397.25	< 0.0001			
<i>O. viciifolia</i>	TT	100	292.34	76.61	0.85	453.27	< 0.0001	-2.7	0	(Tribouillois et al., 2016)
	HTT		272.55	73.47	0.82	360.60	< 0.0001			
	PhHTT		126.33	31.39	0.91	776.31	< 0.0001			
	PhSHTT		6.51	1.64	0.93	1006.39	< 0.0001			
<i>P. tanacetifolia</i>	TT	100	151.90	22.68	0.83	393.72	< 0.0001	-3.4	3.6	(Tribouillois et al., 2016)
	HTT		151.90	22.68	0.83	393.72	< 0.0001			
	PhHTT		66.11	5.78	0.96	1892.97	< 0.0001			
	PhSHTT		3.50	0.54	0.91	755.48	< 0.0001			
<i>P. coronopus</i>	TT	100	158.70	9.23	0.90	562.25	< 0.0001	-1.4	4	(Payne et al., 2018)
	HTT		158.70	9.23	0.90	562.25	< 0.0001			
	PhHTT		73.26	6.34	0.95	1308.43	< 0.0001			
	PhSHTT		3.82	0.73	0.86	392.02	< 0.0001			
<i>P. lanceolata</i>	TT	100	206.23	79.26	0.58	107.74	< 0.0001	-3.4	4	(Payne et al., 2018)
	HTT		206.23	79.26	0.58	107.74	< 0.0001			
	PhHTT		94.44	29.16	0.70	187.53	< 0.0001			
	PhSHTT		4.72	0.90	0.87	537.78	< 0.0001			
<i>S. alba</i>	TT	100	168.14	41.42	0.83	388.38	< 0.0001	-3.4	1.2	(Tribouillois et al., 2016)
	HTT		168.14	41.42	0.83	388.38	< 0.0001			
	PhHTT		76.60	9.37	0.95	1541.81	< 0.0001			
	PhSHTT		3.83	0.82	0.89	667.98	< 0.0001			
<i>T. incarnatum</i>	TT	100	233.64	40.75	0.90	711.00	< 0.0001	-2.3	-0.42	(Baxter et al., 2019)
	HTT		217.82	28.56	0.89	665.77	< 0.0001			
	PhHTT		100.89	19.79	0.92	908.85	< 0.0001			
	PhSHTT		5.36	1.59	0.85	438.25	< 0.0001			
<i>T. spelta</i>	TT	100	172.84	39.11	0.73	214.69	< 0.0001	-3.4	2.6	(Angus et al., 1980)
	HTT		172.84	39.11	0.73	214.69	< 0.0001			
	PhHTT		75.20	8.03	0.88	556.44	< 0.0001			
	PhSHTT		4.16	0.65	0.95	1410.78	< 0.0001			
<i>V. myuros</i>	TT	100	335.95	78.96	0.83	379.71	< 0.0001	-2.1	0.62	(Schermer et al., 2017)
	HTT		271.31	64.26	0.80	318.54	< 0.0001			
	PhHTT		124.57	23.17	0.91	769.40	< 0.0001			
	PhSHTT		6.56	0.98	0.94	1170.89	< 0.0001			
<i>V. sativa</i>	TT	100	248.91	56.96	0.78	284.95	< 0.0001	-3.4	0	(Iannucci et al., 2008)
	HTT		248.91	56.96	0.78	284.95	< 0.0001			
	PhHTT		110.65	17.91	0.89	663.26	< 0.0001			
	PhSHTT		5.66	0.75	0.94	1236.27	< 0.0001			

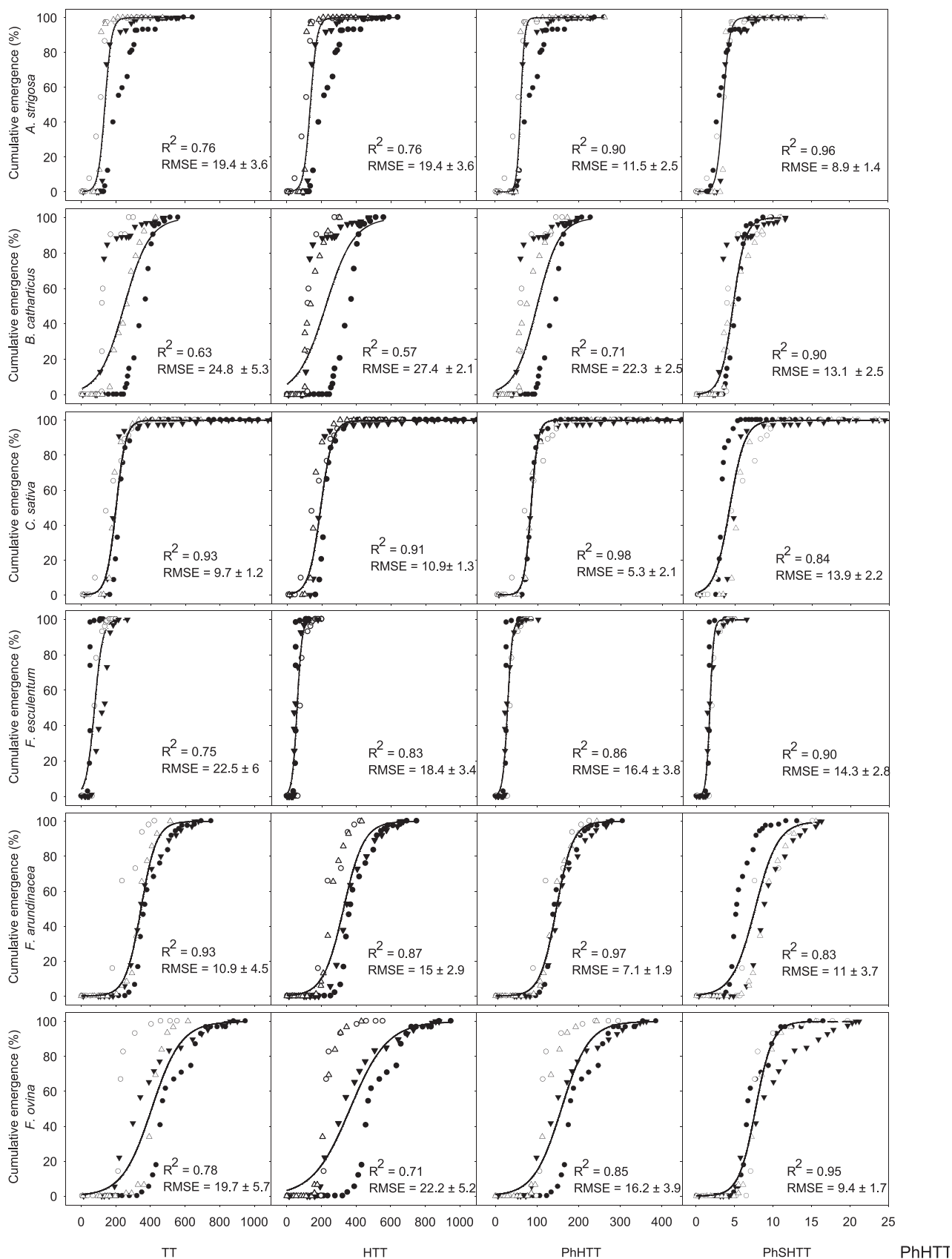


Fig. 5. Cumulative percentage of emergence for each species sown in outdoor pots. Fitted emergences (black lines) are represented for each model based on TT, HTT, PhHTT and PhSHTT. Symbols represents different sowing dates: ●, autumn 2018; ○, spring 2019; ▼, autumn 2019; △, spring 2020. R^2 and RMSE (\pm SE) are also provided.

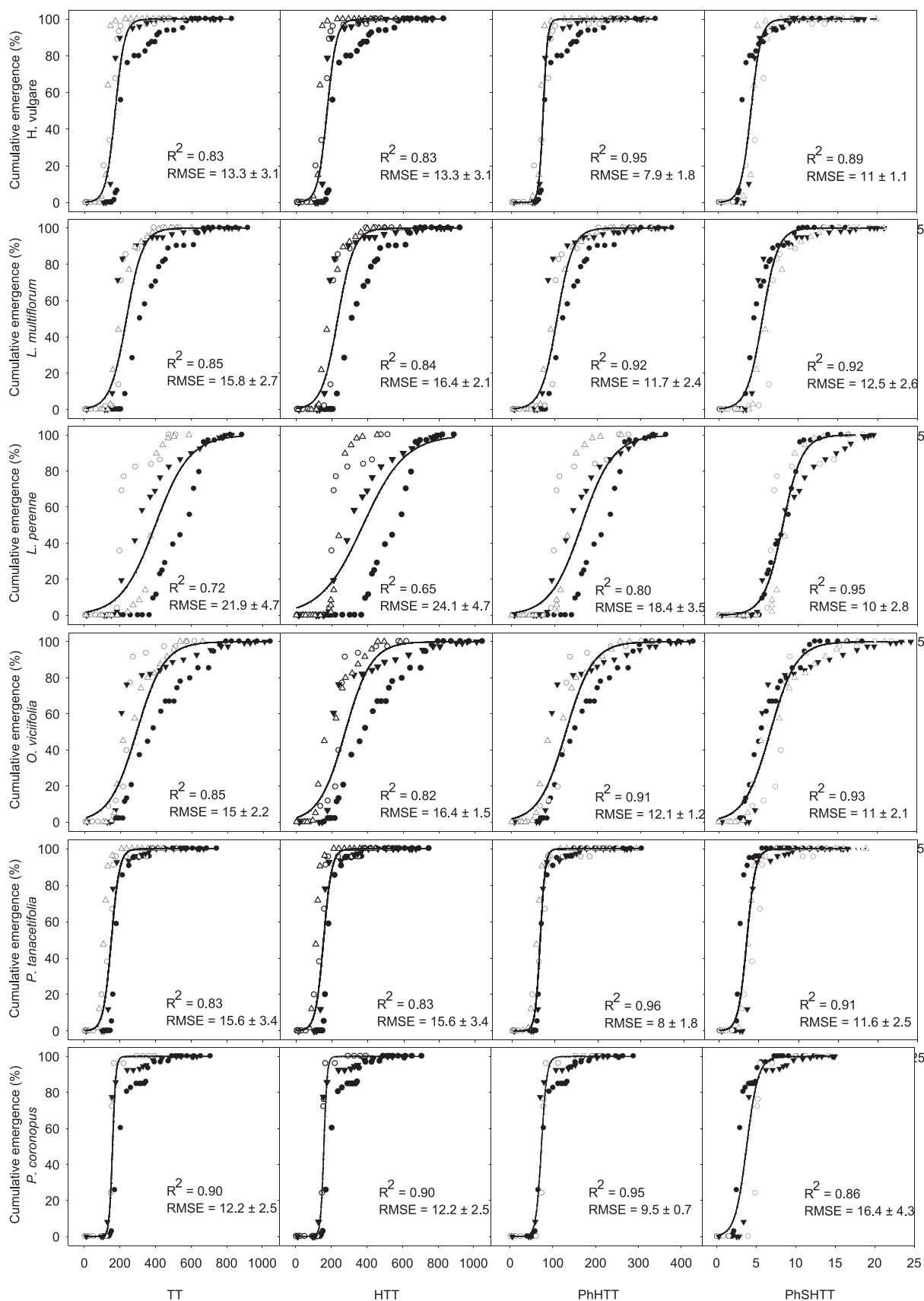


Fig. 6. Cumulative percentage of emergence for each species sown in outdoor pots. Fitted emergences (black lines) are represented for each model based on TT, HTT, PhHTT and PhSHTT. Symbols represents different sowing dates: ●, autumn 2018; ○, spring 2019; ▼, autumn 2019; △, spring 2020. R^2 and RMSE (\pm SE) are also provided.

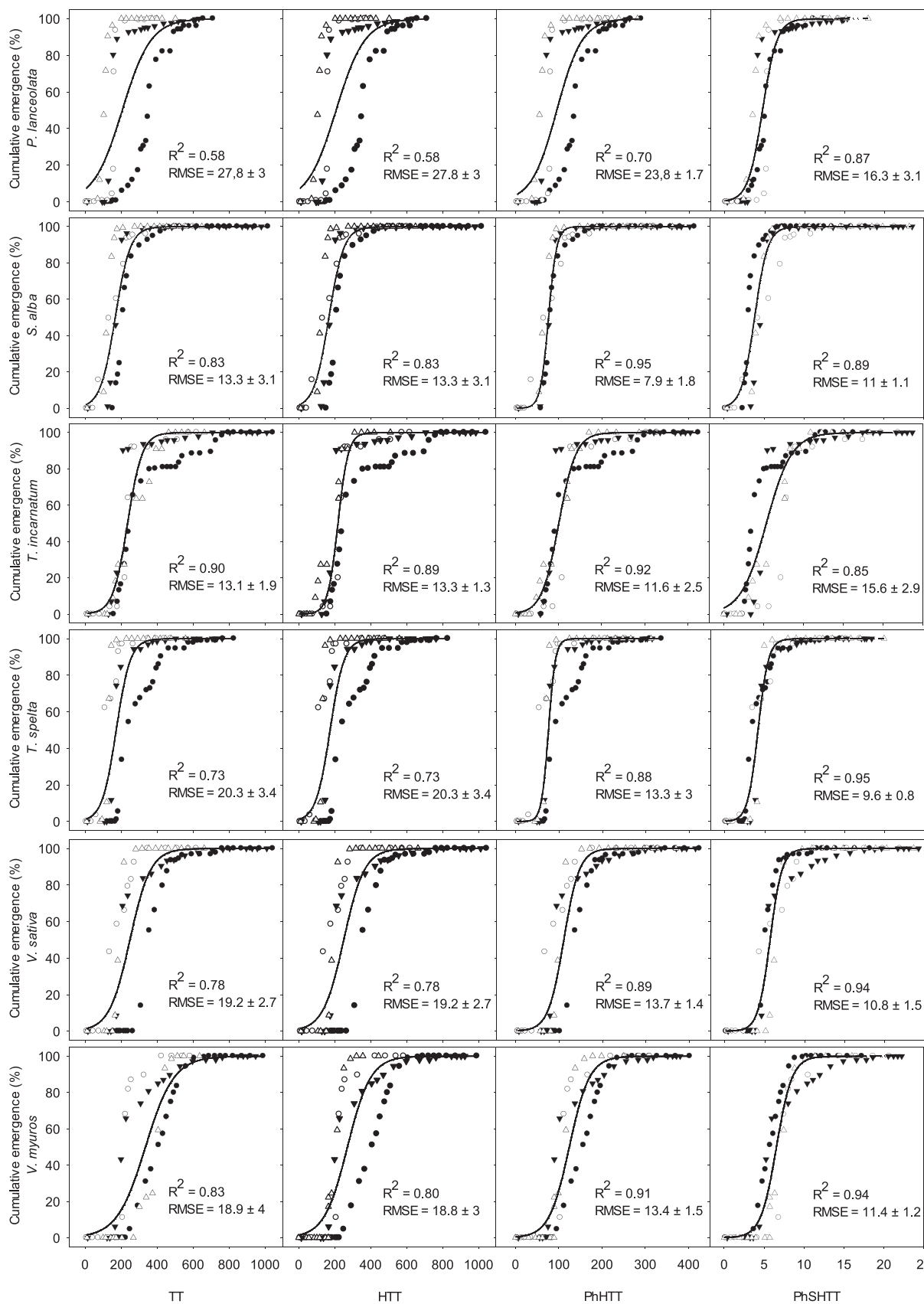


Fig. 7. Cumulative percentage of emergence for each species sown in outdoor pots. Fitted emergences (black lines) are represented for each model based on TT, HTT, PhHTT and PhSHTT. Symbols represents different sowing dates: ●, autumn 2018; ○, spring 2019; ▼, autumn 2019; △, spring 2020. R^2 and RMSE (\pm SE) are also provided.

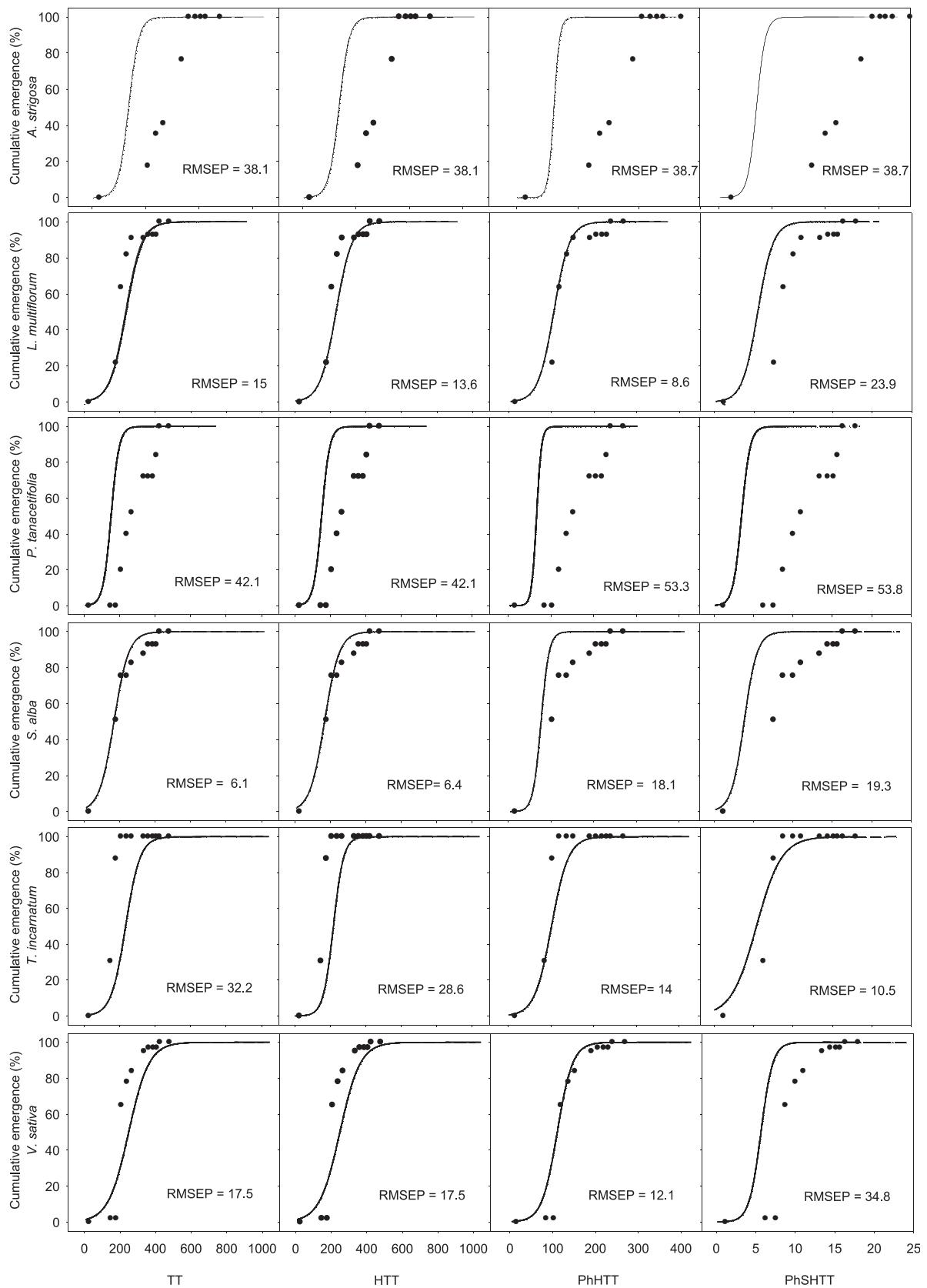


Fig. 8. Validation of the models developed for *A. strigosa*, *P. tanacetifolia*, *S. alba*, *T. incarnatum* and *V. sativa* as a function of TT, HTT, PhHTT and PhSHTT. Black dots (●) represents observed data from [Tribouillois et al. \(2018\)](#); black lines represent predicted emergence by the corresponding model. RMSEP for each validation is also provided.

5. Conclusion

Emergence models were successfully developed for different species with potential use as CC, either based on TT, HTT, PhHTT or PhSHTT. The validation of the models for four species out of six with independent data from the south of France reinforces the validity of the models development method. The accuracy of the models were improved when light was included and, for some species, models could be used widely. These models considerably contribute to inter-row management in vineyards as decision support systems (DSS) tools to predict CC establishments.

CRedit authorship contribution statement

Carlos Cabrera-Pérez: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Jordi Recasens:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing. **Barbara Baraibar:** Writing – review & editing. **Aritz Royo-Esnal:** Data curation, Formal Analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work has been supported by the Spanish State Research Agency, Spain (AEI) and the European Regional Development Fund, EU (ERDF) through the project AGL2017-83325-C4-2-R and also by the project LISA (Low Input in Sustainable Agriculture) financed by ACCIÓ-Generalitat de Catalunya inside the Community RIS3CAT (COTPA). The first author obtained a PhD grant from the University of Lleida (PhD grants). The third author obtained a Beatriu de Pinós post-doc grant from Generalitat de Catalunya. We would also like to acknowledge Maria Casamitjana and Irene de la Fuente for their help in the field work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eja.2021.126413](https://doi.org/10.1016/j.eja.2021.126413).

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Article

Herbicidal Effect of Different Alternative Compounds to Control *Conyza bonariensis* in Vineyards

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Abstract: *Conyza bonariensis* (L.) Cronquist is a widespread noxious weed with high fecundity, associated with no-till systems such as vineyards and other perennial crops in Mediterranean climates. Seeds germinate in staggered flushes, which leads to a great variation in the growth stage between individuals in the same field, and chemical control becomes challenging. Besides, *Conyza* species have evolved resistance to herbicides worldwide, particularly to glyphosate. Even though tillage is expected to provide weed-free fields, it negatively affects vineyards, causing erosion, loss of soil structure and a reduction in organic matter or vine growth (shallow roots can be affected), among other effects. Fuel consumption of this management is also very high because recurrent interventions of in-row tiller are required. In this context, bioherbicides, defined as environmentally friendly natural substances intended to reduce weed populations, are a potential tool for integrated weed management (IWM). In this work, the herbicidal effect of the following six products is tested on a glyphosate-resistant *C. bonariensis* population present in commercial vineyards: T1, mixture of acetic acid 20% and the fertilizer N32; T2, mixture of potassium metabisulfite and pelargonic acid 31%; T3, pelargonic acid 68%; T4, humic-fulvic acid 80%; T5, hydroxy phosphate complex; and T6, potassium metabisulfite. The results showed high field efficacy for T1 and T4 (>80% biomass reduction). For the rest of the products, high efficacy was obtained only in dose–response greenhouse experiments. The present work demonstrates the potential of certain bioherbicide compounds to manage herbicide-resistant weed species, such as *C. bonariensis*. Therefore, bioherbicides could be successfully incorporated into vineyards for IWM.

Keywords: bioherbicides; no-till; conservation agriculture; sustainable weed management; organic viticulture



Citation: Cabrera-Pérez, C.; Royo-Esnal, A.; Recasens, J. Herbicidal Effect of Different Alternative Compounds to Control *Conyza bonariensis* in Vineyards. *Agronomy* **2022**, *12*, 960. <https://doi.org/10.3390/agronomy12040960>

Academic Editor: Fabrizio Araniti and Konstantinos M. Kasiotis

Received: 26 February 2022

Accepted: 13 April 2022

Published: 15 April 2022

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1. Introduction

Conyza bonariensis (L.) Cronquist (hairy fleabane) is one of the most problematic weed species throughout the world [1], and in Spain it is considered one of the most competitive introduced noxious weeds [2] that harms crops and leads to yield loss [3,4], particularly under soil conservation management [5]. In fact, the increase in *C. bonariensis* prevalence has been associated with changes from conventional tillage to minimum tillage or no-till, as reducing soil disturbance favours seed germination and the establishment success of this species [6,7]. Apart from their adaptability to undisturbed crops, *Conyza* species have evolved resistances to herbicides worldwide [8], particularly to glyphosate, which has been widely applied in Spain for weed control in citrus orchards, olive groves, grape vineyards, and others perennial and annual crops [9,10]. Synthetic herbicides are important weed management tools in intensive cropping systems, but the numerous herbicide-resistant weed biotypes and environmental concerns provide limited lifespan to these chemical tools [11]. This situation has been worsened with the lack of new herbicide modes of action discovery in the past few decades [12].

Conyza bonariensis has a high fecundity, producing over 100,000 non-dormant seeds per plant [13]. Seeds germinate in staggered flushes throughout the year, depending on

the environmental conditions, and consequently there is a great variation in the growth stage between individuals in the same field, and chemical control becomes challenging [14], especially in perennial irrigated crops. For example, in Mediterranean vineyards, mainly in those with dry or semiarid climates as is the case in North-Eastern Spain, this weed can be established in high densities in the in-row area of the vine, competing for water and nutrients [15]. This competition is aggravated if glyphosate-resistant biotypes are present in a particular field, because these are more competitive against young vines than glyphosate-susceptible biotypes [16].

Tillage is expected to provide weed-free fields, including *Conyza* species, in Mediterranean vineyards, but it negatively affects vines [17], mainly damaging the young ones, in part, because tillage decreases the presence of grapevine roots in the topsoil [18]. Tillage also causes erosion and a loss of soil structure and reduces the organic matter content [19]. Furthermore, the fuel consumption of this management method doubles the carbon footprint of pesticides or fertilizers [20], because recurrent interventions of in-row tiller along the season are required to effectively manage weeds in vineyards.

Against this background, and considering new challenges related to economic, environmental and social concerns for more sustainable and environmentally friendly weed management [21], the development of alternative weed control tools is mandatory, and especially for *C. bonariensis* in Mediterranean vineyards. Hence, bioherbicides, defined as substances of natural origin intended to reduce weed populations without damaging the environment [22], are a potential tool for integrated weed management. One of the challenges still faced in bioherbicide production is the low herbicidal activity compared to the effects of chemical herbicides [23]. Thus, Bioherbicides are currently underused, and few products have been launched on the market [24]. Nevertheless, the development of new bioherbicides is compelling as these products lag far behind those for pests other than weeds [25]. Furthermore, there are strong needs for any new weed management technology because of the rapid evolution and spread of herbicide resistance, and because weed management is the most difficult (and expensive) pest management problem in organic agriculture [26]. Natural substances face several opponents since there are doubts regarding the registration processes of natural products due to the lack of relevant toxicological data for their use at a commercial scale [27]. Although these concerns might exist, there is evidence that most essential oils and their main compounds are not necessarily harmful to human health [28]. Such natural herbicides are sometimes less hazardous for environmental and human health in comparison to the commercial synthetic herbicides. Some commercial products, such as acetic or pelargonic acid, have already been used as weed control agents. Acetic acid ($C_2H_4O_2$), sold as horticultural vinegar, is not persistent in either soil or water and has a low to medium oral toxicity to most biodiversity. However, it is highly corrosive and so may damage anything it comes into contact with. Pelargonic acid ($C_9H_{18}O_2$) is a saturated fatty acid naturally occurring as esters in the essential oil of *Pelargonium* spp. and can be derived from the tissues of various plant species. Toxicity tests on non-target organisms, such as birds, fish, and honeybees, revealed little or no toxicity [29]. To our knowledge, other products such as a hydroxy phosphate complex and humic-fulvic acid, widely used as organic fertilizers in many crops; potassium metabisulfite, a preservative, antioxidant and bleaching agent in food, especially in acidic foods, such as wine; or N32, a synthetic fertilizer, have never been used as herbicides.

The aim of this study is to assess the mentioned products in order to identify alternative compounds for use as herbicides, which could be incorporated in weed management programs in vineyards, while considering *C. bonariensis* as the main weed. In this study, the suppressive effect of six products on this weed is evaluated in comparison to an untreated control.

2. Material and Methods

The *Conyza bonariensis* population from vineyards located in Raimat, Lleida (NE Spain) was studied. The site is known to have a history of weed-control failures because of field

manager complains about the impossibility to control *C. bonariensis* with glyphosate. Seeds were collected from a treated field with high *C. bonariensis* density and stored during summer 2018 as a potentially herbicide-resistant population.

2.1. Characterisation of the Herbicide Resistance

In autumn 2018, a dose–response experiment was set up with the Raimat population and with a sensitive (SP) population from Argentina, as it was deemed very unlikely to find SP in the region. Seeds were sown in peat and after seven days, seedlings were transplanted to $7 \times 7 \times 8$ cm plastic pots filled with a mixture of silty loam soil 30% (*w/v*), sand 20% (*w/v*), and peat 50% (*w/v*). Four seedlings were transplanted per pot. When populations reached BBCH 12–13 (Weiber et al., 1998), Glyphosate 360 g a.i. L⁻¹ (Roundup; Bayer CropScience, Valencia, Spain) was applied at 90, 180, 360 (1×), 720 and 1440 g a.i. ha⁻¹, with a precision bench sprayer delivering 200 L ha⁻¹ at a pressure of 215 kPa. Seven replicates (pots) were included for each population and dose. Pots were placed in a greenhouse at the University of Lleida (UdL), Spain, and watered regularly. Four weeks after treatment, the above ground part of the plants from each dose was harvested to measure the dry weight. Samples were oven dried at 65 °C for 48 h and weighted with a precision weigher (Mettler Toledo AB54-S, Barcelona, Spain). For the Raimat population, the results obtained for the percentage of reduction for dry weight, with respect to the control, were 10%, 30%, 56%, 65% and 80% at doses of 0.25×, 0.5×, 1×, 2× and 4×, respectively (ED₅₀ = 1.057). On the contrary, the percentages of reduction in dry weight for the SC population were 60% at 0.25×, 80% at 0.5× and 100% at 1×, 2× and 4× (ED₅₀ = 0.176), thereby confirming a resistance factor of 6 in the population from Raimat.

2.2. Bioherbicide Field Trials

A field trial was carried out from February to June in Raimat, Lleida (NE Spain) in an herbicide-managed commercial vineyard (Raventós-Codorníu S.L.). The field trial was repeated in three seasons (2019, 2020, 2021), but the location within the vineyard was changed for each repetition (Table 1). The climatic classification of this area is cold semiarid (BSk) [30], with an average annual precipitation of 342 mm, and annual mean temperature of 14.1 °C (average min of 8.1 °C and average max of 20.7 °C). Weather data were collected from a nearby meteorological station (<https://meteocat.cat>, accessed on 15 September 2021).

Table 1. Field characteristics by season. Vine variety, Caber: Cabernet Sauvignon, Chard: Chardonnay; Coordinates, Lat.: Latitude, Long.: Longitude; Vine spacing; Soil texture; pH, O.M.: Organic matter; Initial infestation level of *Conyza bonariensis*.

Season	Vine	Coordinates ETRS89		Spacing (m)		Soil Texture (%)			pH		Initial
	Variety	Lat.	Long.	Between	Within	Sand	Silt	Clay	pH	O.M.	Infestation
2019	Caber	41°39'26.8" N	0°31'10.3" E	2.7	1.7	59.5	28.1	12.4	8.4	3.18	Low
2020	Caber	41°39'16.5" N	0°30'51.3" E	2.7	1.7	28.4	47.7	24.2	8.4	1.61	High
2021	Chard	41°40'42.9" N	0°27'51.0" E	3	1.5	27.9	38.9	33.2	8.2	2.32	Medium

The trial locations were drip irrigated regularly throughout the growing season and vines were trained as bilateral cordons. The vineyard alleyways were maintained with a spontaneous cover crop that was shredded 2–3 times per season. The soil at this site was classified as a Petrocalcic Calcixercept; the specific field and vineyard characteristics are shown in Table 1, where three different previous levels of *C. bonariensis* infestation are indicated.

The following six treatments were studied by combining different compounds (Table 2) to test their herbicidal effect on *C. bonariensis*: T1, mixture of acetic acid 20% (BioEmpe-20, Bodegas Dinastia S.L., Tomelloso, Spain) and the fertilizer N32 (YaraVita LAST N, Yara Iberian S.A., Madrid, Spain) (70 and 30% *v/v*, respectively); T2, mixture of potassium

metabisulfite (AGROVIN S.A., San Juan, Spain) and pelargonic acid 31% (Finalsan RTU, W. Neudorff GmbH KG, Valencia, Spain); T3, pelargonic acid 68% (Kalina, Comercial Química Massó S.A., Barcelona, Spain); T4, humic-fulvic acid 87% (Herbiz, PRO&Garden, Barcelona, Spain); T5, hydroxy phosphate complex (Xekator, Aldamus Hispania, S.L., Madrid, Spain); T6, potassium metabisulfite (AGROVIN S.A., San Juan, Spain). The herbicidal effect of these compounds occurs through contact and for this reason, their effect is immediate (1–2 days).

Table 2. Compounds tested, application dose and application volume.

Treatment	Compounds	Application Dose	Application Volume (L/ha)
T1	Acetic Acid 20% ⁽¹⁾ +N32 ⁽²⁾	⁽¹⁾ 122.5 L/ha ⁽²⁾ 52.5 L/ha	175
T2	Potassium metabisulfite ⁽¹⁾ +Pelargonic acid 31% ⁽²⁾	⁽¹⁾ 70 kg/ha ⁽²⁾ 17.5 L/ha	500
T3	Pelargonic acid 68%	16 L/ha	200
T4	Humic-Fulvic acid	35 L/ha	700
T5	Hydroxy phosphate complex	15 L/ha	150
T6	Potassium metabisulfite	60 kg/ha	250

Previous essays with T1 and T4 were carried out at the UdL to choose the best application dose (Montull et al., 2019). For T2 and T6, these previous essays were performed by the winery. For T3 and T5, the application doses were chosen according to the manufacturer recommendations.

Each year, a completely randomized design was established with six treatments and four replicates. In 2019 and 2020, the treatments were an untreated control, T1, T2, T3, T4 and T5. In 2021, T5 was excluded due to the low efficacy observed in the previous years, and a new treatment (T6) proposed by the winery was added. Thus, treatments were an untreated control, T1, T2, T3, T4 and T6. The treated area was along the space within three vines (3.0 m or 3.4 m) always with a width of 0.6 m. In 2019 and 2020, treatments were applied four times, between February and May (2019) and from March to May (2020). In 2021, treatments were applied three times, from April to May, when the growth stage of the plants was between BBCH 11–12 (first application) and BBCH 31–32 (last application). All plots were treated the same day in each application with a manual hand sprayer at mid-day on sunny days. A *C. bonariensis* assessment was made before each application to estimate the initial weed coverage, and another one was performed two days after treatment (DAT) to evaluate the herbicidal effect. In July 2020 and 2021, the above ground biomass of *C. bonariensis* plants from each plot was harvested, oven dried at 65 °C for 48 h, and the dry weights were measured.

2.3. Dose–Response Experiment

Seeds of *C. bonariensis* from the Raimat population were sown in peat and, after seven days, seedlings were transplanted to 7 × 7 × 8 cm plastic pots filled with a mixture of silty loam soil 30% (*w/v*), sand 20% (*w/v*), and peat 50% (*w/v*). Four seedlings were transplanted per pot, placed in a greenhouse at the UdL, and watered regularly. The experiment was carried out for T1, T2, T3, T4 and T6 at two different phenological stages (PS) of the weed, namely when seedlings achieved BBCH 12–13 and when they achieved BBCH 14–15. Five replicates (pots) were included for each treatment, PS and dose. Pots were treated with the following doses: (T1) 0, 21.9, 43.8, 87.5, 175 L/ha at BBCH 12–13 and 0, 43.8, 87.5, 175, 350 L/ha at BBCH 14–15; (T2) 0 + 0; 8.75 + 2.19, 17.5 + 4.37, 35 + 8.75, 70 + 17.5, 140 + 35 kg/ha + L/ha, respectively for each compound of the mixture, at both BBCH 12–13 and BBCH 14–15; (T3) 0, 4, 8, 16, 32 L/ha at BBCH 12–13 and BBCH 14–15; (T4) 0, 1.1, 2.2, 4.4, 8.8, 17.6 L/ha at BBCH 12–13 and 0, 2.2, 4.4, 8.8, 17.6 L/ha at BBCH 14–15; (T6) 0, 5, 10, 20, 40, 80, 160 kg/ha at BBCH 12–13 and 0, 20, 40, 60, 80, 100, 160 kg/ha

at BBCH 14–15. Treatments were applied using a manual hand sprayer. Four weeks after treatments, the above ground part of the plants was harvested, oven dried and weighted as in point 2.1, to measure their dry weight.

2.4. Weather Conditions

The highest observed mean temperature (T_m) during the potential emergence period of *C. bonariensis* in Raimat (grey arrows in Figure 1) was in 2020 (14.5 °C), followed by 2021 (14 °C), and it was lowest in 2019 (13.5 °C). Both 2020 and 2021 were also warmer than the historical average (13.5 °C). The years 2019 and 2021 were similar in terms of precipitation during the whole growing season, with 102 mm and 110 mm, respectively, and below the historical average (162 mm), while in 2020 the growth season was very wet (248 mm).

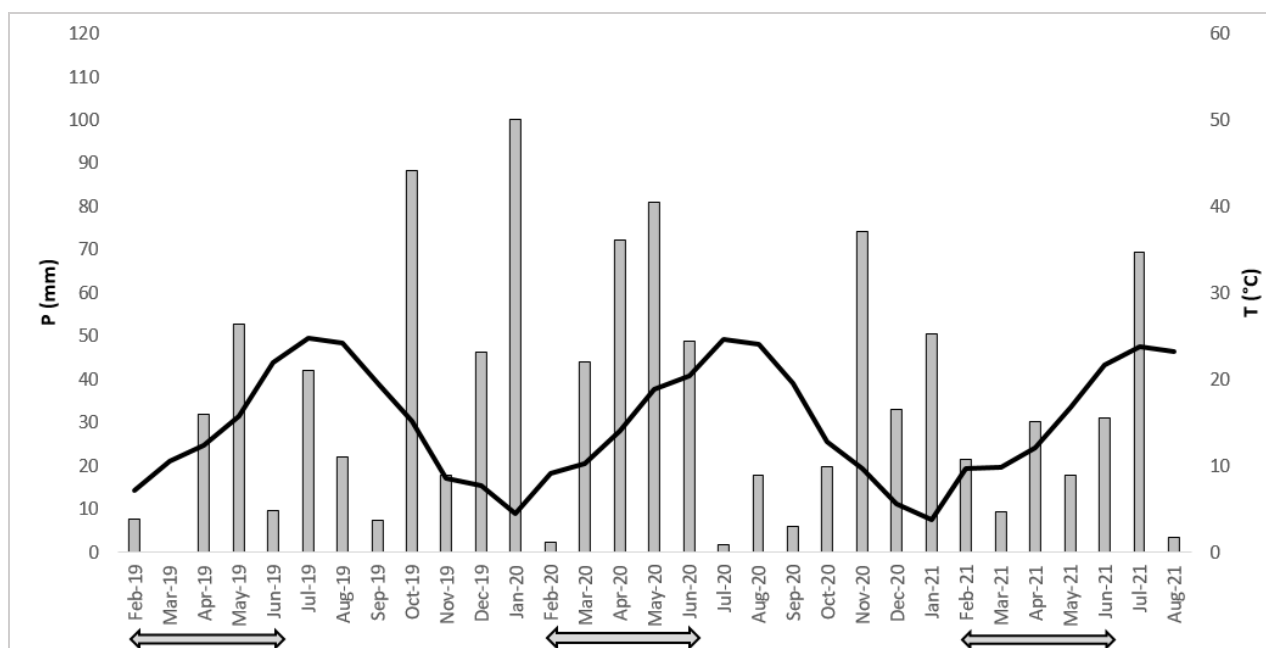


Figure 1. Weather conditions of the experiment period. Grey bars, total monthly precipitation (P); black line, mean monthly temperature (T_m). Arrows represent the growing season each year.

2.5. Statistical Analyses

The field efficacy results of each treatment were expressed as cover reduction after the Henderson–Tilton formula [31]. After testing for normality (Shapiro–Wilk) and homoscedasticity (Leven’s test) requirements for a parametric analysis, both coverage and above ground biomass data were subjected to a one-way ANOVA, followed by multiple comparisons of treatment effects with Tukey’s HSD-test ($p < 0.05$). In the case of heteroscedasticity, the variance was analysed by the Kruskal–Wallis H test. Data from dose–response experiments were analysed using a nonlinear regression model (1). The treatment curve was fitted with a four-parameter logistic function:

$$y = c + \frac{d - c}{1 + \left(\frac{x}{EC_{50}}\right)^b} \quad (1)$$

where y is the response expressed as the percentage of reduction with respect to the untreated control, c is the lower level of the curve, d is the upper level of the curve, b is the slope, EC_{50} indicates the concentration that causes a 50% growth reduction, and x is the treatment dose (independent variable). Data were analysed using JMP Pro 15 (SAS Institute 2010. SAS Campus Drive, Cary, NC, USA. SAS Institute Inc.) and SigmaPlot 12.0 (Sistat Software, Inc., San José, CA, USA).

3. Results

3.1. Field Efficacy Trials

Significant differences were found in all sampling dates between treatments (Table 3). Most of the compounds tested succeeded in decreasing the cover of *C. bonariensis* (Table 4), mainly in 2019, when lower overall cover of the weed was observed, and the maximum values of the untreated plots occurred in June (10.8%). All treatments significantly reduced *C. bonariensis* cover compared to the untreated control, except T5, which showed unsatisfactory efficacy in all application dates (between 13% and 36%). The efficacy of T1 and T2 was very high from April until June (>85%) and, in the case of T4, the efficacy was very high from February until June (>90%), while T3 always ranged between 52% and 77%. In contrast, in 2020, the highest *C. bonariensis* cover was observed, coinciding with the wettest and hottest season, and the untreated plots showed 83% weed cover (on average) by May. Again, lower efficacy was observed in T5, which never exceeded 7.5%; in T1 and T3, the efficacy was also low (15% and 56%, respectively); while in T2 and T4, the efficacy was high and close to 80% or 90% for most sampling dates. This trend repeated in 2021, but T6 was incorporated instead of T5, and efficacy varied between 64% and 85% depending on the application date; T1 and T3 continued to present low efficacy (11% and 36%, respectively). The biomass measured (g/plot) after the last application date in 2021 supported the weed cover results, with the lowest values obtained by T2 (10.4), followed by T4 (45.6) and T6 (53.2), although significant differences were observed only for T2 with respect to T3 (244.0) and the untreated control (393.8), and for T4 and T6 compared to the untreated control (Table 5). In 2020, with higher *C. bonariensis* emergences and cover, no significant differences were found in biomass, although a lower weight was also measured for T2 and T4.

Table 3. Significance of the one-way ANOVA or Kruskal–Wallis test. F/H and *p* values of each sampling date.

	Date	F/H	<i>p</i>
2019	February	4185	0.014
	April	20,963	<0.001
	May	1962	<0.001
	June	21,448	<0.001
2020	March	4358	<0.001
	April	70,444	<0.001
	May	69,285	<0.001
	June	21,488	<0.001
2021	April	31,403	<0.001
	May	20,329	<0.001
	June	22,273	<0.001

3.2. Dose–Response Curves

The equation parameters of the best fitted models, based on the coefficient of determination (r^2) and the EC_{50} values, are shown in Table 6 and represented in Figure 2. The obtained r^2 values were always above 0.8, and most were above 0.9, which indicates the suitability of this function to describe the growth response of *C. bonariensis* to different concentrations of the tested compounds. Biomass reduction was greatly influenced by the phenological stage of the treated plants, while a 100% biomass reduction was achieved in BBCH 12–13 in almost all treatments, although this value was more difficult to reach in BBCH 14–15. On the other hand, the EC_{50} value at least doubled as compared to that of BBCH 12–13 when the population of *C. bonariensis* was in BBCH 14–15.

Table 4. Treatments efficacy in each application date expressed as % after Henderson–Tilton formula. Mean values \pm standard errors of the mean. Different letters indicate significant differences among treatments at $p < 0.05$. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%, T4: humic-fulvic acid, T5: hydroxy phosphate complex, T6: potassium metabisulfite.

2019				2020				2021			
Applic. Date/BBCH	Treat.	Pre-Spray Cover (%)	Cover Reduction (%)	Applic. Date/BBCH	Treat.	Pre-Spray Cover (%)	Cover Reduction (%)	Applic. Date/BBCH	Treat.	Pre-Spray Cover (%)	Cover Reduction (%)
7 February BBCH 11–12	Control	0.6	0.0 \pm 0.0 b	11 March BBCH 11–12	Control	26	0.0 \pm 0.0 c	15 April BBCH 11–12	Control	5	0.0 \pm 0.0 b
	T1	0.5	64.3 \pm 20.6 ab		T1	21	30.4 \pm 7.4 b		T1	3	11.3 \pm 6.6 b
	T2	1	76.8 \pm 16.3 ab		T2	20	37.5 \pm 7.9 b		T2	4	89.6 \pm 3.6 a
	T3	2	76.8 \pm 14.1 ab		T3	19	15.4 \pm 3.1 b		T3	4	13.4 \pm 7.7 b
	T4	2.4	91.7 \pm 4.5 a		T4	21	66.9 \pm 7.3 a		T4	4	90.6 \pm 3.6 a
	T5	1.9	34.5 \pm 5.6 ab		T5	33	2.1 \pm 2.5 c		T6	4	74.6 \pm 7.8 a
16 April BBCH 11–15	Control	3	0.0 \pm 0.0 b	15 April BBCH 11–15	Control	51	0.0 \pm 0.0 d	11 May BBCH 11–15	Control	9	0.0 \pm 0.0 c
	T1	2	87.5 \pm 7.5 ab		T1	34	56.4 \pm 12.6 b		T1	8	28.3 \pm 6.4 b
	T2	2.3	100 \pm 0.0 a		T2	26	91.4 \pm 3.0 a		T2	1	60.0 \pm 10.0 ab
	T3	5.5	73.8 \pm 4.7 ab		T3	36	31.8 \pm 9.6 bc		T3	6	34.7 \pm 8.3 b
	T4	2	100 \pm 0.0 a		T4	32	85.6 \pm 6.1 a		T4	2	75.4 \pm 10.5 a
	T5	4	23.8 \pm 10.3 ab		T5	59	7.2 \pm 2.8 cd		T6	3	85.3 \pm 4.0 a
23 May BBCH 11–31	Control	7	0.0 \pm 0.0 b	7 May BBCH 11–18	Control	64	0.0 \pm 0.0 c	1 June BBCH 11–31	Control	23	0.0 \pm 0.0 d
	T1	1.9	98.3 \pm 1.7 a		T1	38	39.7 \pm 13.0 b		T1	12	10.7 \pm 4.3 cd
	T2	1.1	86.7 \pm 8.1 ab		T2	16	85.5 \pm 5.4 a		T2	2	87.1 \pm 5.3 a
	T3	9.5	53.2 \pm 8.1 ab		T3	34	26.5 \pm 6.9 bc		T3	11	36.3 \pm 15.8 bc
	T4	1.9	97.5 \pm 2.5 a		T4	26	78.5 \pm 7.2 a		T4	3	88.1 \pm 4.7 a
	T5	7.4	36.2 \pm 14.7 ab		T5	66	7.6 \pm 0.4 c		T6	5	64.4 \pm 8.9 ab
13 June BBCH 11–31	Control	10.8	0.0 \pm 0.0 c	20 May BBCH 11–32	Control	83	0.0 \pm 0.0 c				
	T1	0.4	92.5 \pm 7.5 a		T1	49	24.7 \pm 12.0 abc				
	T2	0.3	90.0 \pm 5.8 a		T2	11	81.4 \pm 1.6 a				
	T3	3	52.1 \pm 12 b		T3	44	26.4 \pm 4.7 abc				
	T4	0.9	100 \pm 0.0 a		T4	26	69 \pm 8.5 ab				
	T5	4	13.1 \pm 9.4 c		T5	76	6.6 \pm 0.2 bc				

Table 5. Dry weight (g) biomass of *C. bonariensis* in July 2021. Mean values \pm standard errors of the mean. Different letters denote significant differences among treatments at $p < 0.05$. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%, T4: humic-fulvic acid, T5: hydroxy phosphate complex, T6: potassium metabisulfite.

Treatment	g/Plot	
	2020	2021
Control	308.8 \pm 14.4	393.8 \pm 106.5 a
T1	271.8 \pm 10.0	161.9 \pm 65.5 abc
T2	216.9 \pm 18.9	10.4 \pm 5.2 c
T3	262.2 \pm 41.5	244.0 \pm 103.5 ab
T4	231.5 \pm 30.3	45.6 \pm 18.7 bc
T5	278.1 \pm 47.7	-
T6	-	53.2 \pm 23.6 bc

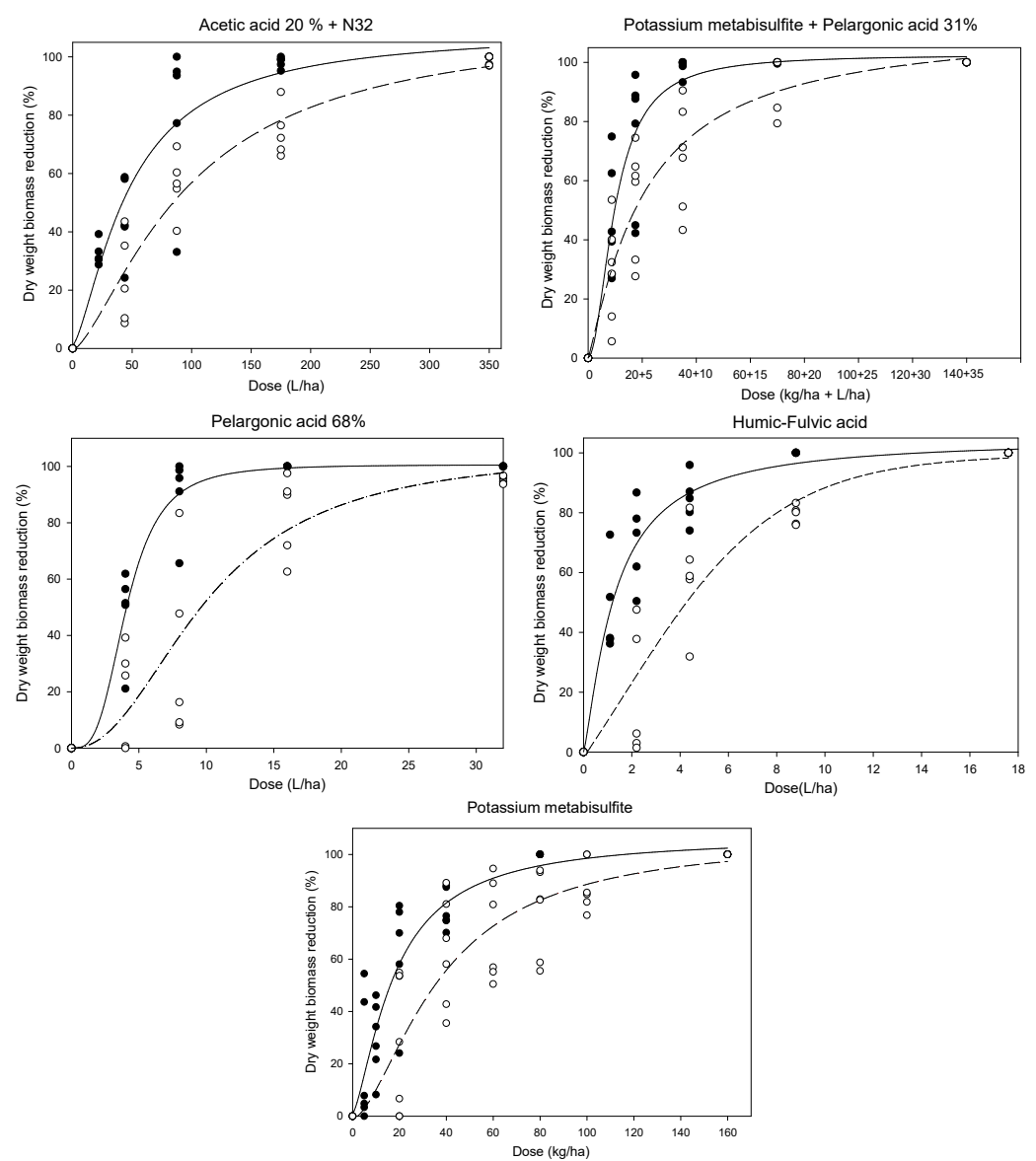


Figure 2. Dose–response curves of T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%; T4: humic-fulvic acid, T6: potassium metabisulfite. Values are presented as dry weight biomass reduction (%) of the no-treated control. Black points (●) and solid lines (—), BBCH 12–13; white points (○) and dashed lines (– –), BBCH 14–15.

Table 6. Parameters of dose–response curves represented in Figure 2. T1: acetic acid 20% + N32, T2: potassium metabisulfite + pelargonic acid 31%, T3: pelargonic acid 68%; T4: humic-fulvic acid, T6: potassium metabisulfite.

Treatment	Compounds	BBCH	r ²	EC ₅₀	Slope (b)
T1	Acetic Acid 20% + N32	12–13	0.900	47.62 (L/ha)	1.44
		14–15	0.940	96.92 (L/ha)	1.45
T2	Potassium metabisulfite + Pelargonic acid 31%	12–13	0.909	10.06 + 2.52 (kg/ha + L/ha)	1.92
		14–15	0.887	21.02 + 5.26 (kg/ha + L/ha)	1.15
T3	Pelargonic acid 68%	12–13	0.954	4.10 (L/ha)	3.25
		14–15	0.827	10.07 (L/ha)	2.2
T4	Humic-Fulvic acid	12–13	0.939	1.30 (L/ha)	1.25
		14–15	0.906	4.12 (L/ha)	2.01
T6	Potassium metabisulfite	12–13	0.892	17.84 (L/ha)	1.43
		14–15	0.812	37.32 (L/ha)	1.64

4. Discussion

The *C. bonariensis* populations from the Raimat vineyards were found to be resistant to glyphosate with a resistance factor of 6. This species is known to easily evolve resistant biotypes as several cases are reported in the literature. For example, Travlos and Chachalis [32] observed 4- to 7-fold resistance levels in *C. bonariensis* growing in Greek perennial crops, including vineyards. Similarly, Urbano et al. [4] established a 7- to 10-fold resistance level in *C. bonariensis* collected from Spanish olive fields, and more recent studies have showed a 27-fold resistance level for this weed in South African vineyards [33]. The cropping systems of the above-mentioned examples are similar to that of this study, and they share common features such as a long and repeated history of glyphosate use, and a lack of crop and herbicide rotation.

According to Bailey [22], bioherbicides are products of natural origin that are useful for weed control, and that can be either living organisms or products derived from living organisms. All the tested compounds fulfil that definition, except N32 (synthetic fertilizer) and potassium metabisulfite. Nevertheless, the last compound was tested because its use in winemaking is very common and, according to the International Chemical Safety Cards (ICSCs), the environmental effects of potassium metabisulfite has no significant effects, according to the current knowledge. Furthermore, T2, T4 and T6 stand out from the rest, as high field efficacy was observed along the application dates (Table 4), which was confirmed with the harvested above ground biomass in 2021 (Table 5), but not in 2020, where emergences were more abundant and constant until July, probably due to a higher initial presence of seeds with the combination of an extraordinarily wet spring. Furthermore, differences in 2020 biomass between high-effective treatments (T2 and T4) and low-effective ones (T1, T3 and T5) could have been diminished because of intraspecific competition of *C. bonariensis* plants in the latter treatments, as higher weed cover was observed but with smaller plants. The efficacy of T1, T3 and T5 was unequal and not always sufficient to maintain low *C. bonariensis* cover. The lack of efficacy of T5 is likely due to a harmless effect on the plant rather than in the applied dose. Conversely, the burning effect of the acetic (T1) and pelargonic (T3) acids are highly effective in early rosette stages of this species; nonetheless, some individuals showed green growth regions in the centre of the rosette after applications, which eventually developed inflorescences and disseminated achenes in the field trials. Pline et al. [34] observed a variation in pelargonic acid efficacy from only 6% up to 65%, depending on the annual weed species and similar to Travlos et al. [35], who reported an efficacy of between <20% up to >90%. Webber III et al. [36] observed good grass control (>80%) and fair (>70%) broadleaf control (without *Conyza* spp.), and Kanatas et al. [37] attributed the low weed control efficacy of pelargonic acid in olive fields to the presence of *C. bonariensis*, which indicates tolerance of this species to this acid,

similar to the observations in the present study. The presence of buds in the *C. bonariensis* taproot allowed for rapid regrowth after clipping [38]. Variations in efficacy are also found in the literature with acetic acid, as Webber et al. [39] observed an efficacy ranging from 4.5% up to 100%, depending on the weed species, when acetic acid at 20% was applied at 187 L/ha. In the current study, acetic acid (T1) obtained good efficacy only in 2019, with low *C. bonariensis* cover (probably due to the low number of emergences) and when all plants could be treated in an early phenological stage.

Similar to synthetic herbicides, the effect of the tested compounds may rely on dosage, the phenological stage of the target weed, and on the environmental conditions [40,41]. In fact, the most effective treatments, potassium metabisulfite (T2) and humic-fulvic acid (T4), obtained better results when applied in April, although in 2019 and 2020 there was a previous application in February and March, respectively, and according to dose–response results (Figure 2, Table 5), it would therefore be expected to be more effective, as *C. bonariensis* rosettes were smaller. This contradictory result can be explained by the weather conditions. In April 2019 and 2020, the temperature was higher than in February 2019 and March 2020, which is known to improve herbicide efficacy [42]. Waltz et al. [43] attributed this enhanced effect with higher temperatures to a change in the epicuticular wax that facilitates the herbicidal effect. This statement would lead to the conclusion that the treatments' effect should improve during spring, but *C. bonariensis* plants that survived the firsts applications of low-effective treatments (T1, T3 and T5) were in an advanced phenological stage by May, so despite the high temperatures, the efficacy was lower, especially in 2020, when there was an abundant emergence of the weed which could hinder droplet contact with the leaves.

The herbicide's efficacy is clearly influenced by the phenological stage of *C. bonariensis* [4,33], with sensitivity or injury decreasing as the growth stage advances. This has been confirmed by the dose–response curves in all treatments (Figure 2). When plants grow from BBCH 12–13 to BBCH 14–15, the EC_{50} doubles in all treatments (Table 5). In the dose–response experiment, nearly 100% of the biomass reduction was achieved in BBCH 12–13 at some dose in all treatments, compared to the untreated control, demonstrating the potential of these compounds to control *C. bonariensis*. Plants showed visible injury ranging from chlorosis, going through necrosis, to eventually complete the wilting of plants. In general, the observed injury symptoms increased with increasing compound concentrations. The same symptoms were observed in the field trials two days after treatments. However, long-term control for *C. bonariensis* is challenging because of its germination and emergence characteristics, with overlapped cohorts along the season. *Conyza* species can potentially germinate at any time throughout the year [44], and irrigated crops such as drop-irrigated vineyards ease this process. For this reason, contrasting reports about the main emergence season for *Conyza* spp. are found in the literature, as it is sometimes considered in terms of winter annuals [38] and at other times in terms of summer annuals [3]. Moreover, Valencia-Gredilla et al. [45] observed the highest germination percentage of *C. bonariensis* at 22 °C, but they also reported that the biotypes from the Lleida region had more germinated seeds at lower temperatures than biotypes from warmer regions. Thus, the application of a control method (either bioherbicide or synthetic chemical) in the homogeneous phenological stage is extremely difficult, and explains differences found in the efficacy between greenhouse experiments of dose–response curves and field efficacy trials.

Consequently, although the available bioherbicides are promising compounds for weed control, few have achieved long-term commercial success in the field [24]. According to our results, bioherbicides may display their potential when addressed to specific species in early phenological stages, rather than during their widespread use to many species. The increase in *C. bonariensis* prevalence had created an urgent need to find alternatives for their control. In this sense, new herbicidal compounds may be incorporated as tools for integrated weed management (IWM). In fact, none of the individual techniques on their own can be expected to provide acceptable control levels, but when combined with other tools, successful results can be achieved [44]. So, the combination of different techniques

such as cover crops, mulching and bioherbicides could facilitate a decrease in *C. bonariensis* infestations in no-till viticulture. It is important to know that in the Mediterranean climate, earlier cohorts of *C. bonariensis* contribute most to the following generations, therefore, they should be preferably targeted when designing control strategies [46]. Predicting the emergence of *C. bonariensis* with already developed models based on climate parameters, such as those from Zambrano-Navea et al. [2], can also contribute to a decision support system for optimum application timing.

Although many naturally occurring materials, such as most of the tested compounds, have herbicidal properties, there is controversy around whether they should be permitted in organic crop production systems [47,48]. Therefore, producers need to know the regulation policies that cover their organic, natural, or sustainable crop production. Finally, given the necessity of reducing the carbon footprint caused by tillage, and the lack of new modes of actions in synthetic herbicides, innovations for bioherbicides are much needed [49], so that they can be successfully incorporated in vineyards for IWM in the short-term.

5. Conclusions

To date, no studies have focused on the herbicide potential of alternative compounds, specifically for *C. bonariensis* in commercial vineyards. The findings of the present study revealed that, despite most of the tested compounds being able to control the weed in the greenhouse dose–response experiment, only the potassium metabisulfite + pelargonic acid 31% (T2), the humic-fulvic acid (T4) and the potassium metabisulfite (T6) obtained high field efficacy throughout the application dates and were able to maintain an acceptable *C. bonariensis* cover.

Author Contributions: Conceptualization, C.C.-P. and J.R.; methodology, C.C.-P. and J.R.; software, C.C.-P.; validation, C.C.-P., J.R. and A.R.-E.; formal analysis, C.C.-P. and J.R.; investigation, C.C.-P. and J.R.; resources, J.R.; data curation, C.C.-P.; writing—original draft preparation, C.C.-P.; writing—review and editing, J.R. and A.R.-E.; visualization, J.R. and A.R.-E.; supervision, J.R. and A.R.-E.; project administration, J.R.; funding acquisition, J.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish State Research Agency, grant number AGL2017-83325-C4-2-R, and by the MACMHER “Grups Operatius” promoted by Departament of Agriculture of the Catalan Government (DARP) in 2018. The first author obtained a PhD grant from the University of Lleida (PhD grants).

Acknowledgments: We would like to acknowledge Maria Casamitjana, Bruna Català and Irene Llobera for their help in the field work carried out, and the facilities and technical support supplied by the company Raventós–Cordorniu to carry out the field trials in their vineyards in Raimat.

Conflicts of Interest: The authors declare no conflict of interest.

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